The Synaptic and Circuit Mechanisms Underlying a Change in Spatial Encoding in the Retina

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http://dx.doi.org/10.1016/j.neuron.2014.02.037

SUMMARY

Components of neural circuits are often repurposed so that the same biological hardware can be used for distinct computations. This flexibility in circuit operation is required to account for the changes in sensory computations that accompany changes in input signals. Yet we know little about how such changes in circuit operation are implemented. Here we show that a single retinal ganglion cell performs a different computation in dim light—averaging contrast within its receptive field—than in brighter light, when the cell becomes sensitive to fine spatial detail. This computational change depends on interactions between two parallel circuits that control the ganglion cell’s excitatory synaptic inputs. Specifically, steady-state interactions through dendro-axonal gap junctions control rectification of the synapses providing excitatory input to the ganglion cell. These findings provide a clear example of how a simple synaptic mechanism can repurpose a neural circuit to perform diverse computations.

INTRODUCTION

The array of neural computations required to explain behavior is far too large to be explained by specialized single-function neural circuits. Instead, the computation performed by a neural circuit often changes as task demands change. Such repurposing has been studied extensively in motor control. Neuromodulators, for example, alter central pattern generator circuits so that common circuit components participate in multiple motor rhythms (Marder and Bucher, 2007). Although similar functional repurposing occurs in circuits throughout the central nervous system, we know much less about the underlying mechanisms.

The optic nerve of the mammalian retina contains the axons of ~20 subtypes of retinal ganglion cells (RGCs) (Masland, 2012), through which all visual information is transmitted to the brain. These same RGCs provide the basis for visually guided behavior under lighting conditions ranging from the darkest night to the brightest day. As the demands of the visual environment change, the computations performed by retinal circuits change correspondingly. Some functional properties of RGCs, like gain (Shapley and Enroth-Cugell, 1984), receptive field size (Barlow et al., 1957), and center/surround ratio (Enroth-Cugell and Lennie, 1975), change with the statistics of the visual environment; other properties have traditionally been considered immutable and correspondingly are often used to classify RGCs into specific types. On versus Off response polarity and direction selectivity are examples of these more stable functional properties, though recent work has disputed the immutability of even these properties (Geffen et al., 2007; Rivlin-Etzion et al., 2012).

Here we show that another property commonly used to classify RGCs—linear versus nonlinear spatial integration of visual signals contained within their receptive field (Enroth-Cugell and Robson, 1966)—can change with the visual environment.

While functional properties of retinal circuits can change rapidly, the underlying circuit wiring is likely fixed over the course of an ~1-hr-long physiology experiment. Thus, rapid functional changes arise from light-dependent changes in the operation of common circuit elements. We find here that tonic input via gap junctions controls the rectification of the dominant excitatory synapse onto RGCs. This tonic input changes with luminance, and the resulting change in synaptic rectification controls whether ganglion cells integrate inputs across space linearly or nonlinearly. More generally, this work illustrates how fine control of the synaptic operating point, in this case via dendro-axonal gap junctions, can control key computational features of a neural circuit.

RESULTS

Spatial Integration Depends on Mean Illumination

We used a flat mount preparation of the mouse retina to characterize how RGCs integrate light inputs across space. By mounting the isolated retina flat in a recording chamber, we could deliver spatially patterned light stimuli to the photoreceptors while measuring the resulting RGC responses. We focused on On alpha RGCs, a physiologically and anatomically well-characterized ganglion cell type (Pang et al., 2003; Murphy and Rieke, 2006; Schwartz et al., 2012).

The spatial dependence of RGC responses was measured using a classic stimulus paradigm designed to characterize cells as linear (“X” cells) or nonlinear (“Y” cells) integrators over space (Enroth-Cugell and Robson, 1966; Victor and Shapley, 1979). A split-field stimulus with regions of equal positive and negative contrast was modulated sinusoidally in time (at 3.75 Hz) so that the light and dark regions changed sides periodically (Figure 1A). When the light and dark regions of the stimulus each
cover exactly half of the receptive field center, linear spatial integration predicts no modulation of the response because responses to the light and dark regions cancel. Nonlinear spatial integration of the same input would result in incomplete cancellation and a response at twice the modulation frequency (a frequency doubled or "F2" response).

RGCs responded at the modulation frequency (an "F1" response) when the border between light and dark regions was far (≥50 μm) from the receptive field center (Figure 1B). These responses were robust at both low (0.25–1 photoisomerizations per rod photoreceptor per second, or R*/rod/s) (Figure 1A, left) and moderate (≥100 R*/rod/s) (Figure 1A, right) luminance. However, when the border was centered on the receptive field, responses were weak or nonexistent at low luminance but strong at moderate luminance (F2 responses; see Experimental Procedures). This change from linear to nonlinear spatial integration was evident in both spike responses and excitatory input currents (Figure 1A). We focused on changes in the spatial...
Integration of excitatory inputs from presynaptic bipolar cells, since inhibitory inputs play a minor role in shaping spike responses of these cells under similar stimulus conditions (Murphy and Rieke, 2006; Schwartz et al., 2012). We quantified nonlinearities in spatial integration from the ratio of the frequency-doubled response to the response at the modulation frequency (i.e., the $F_2/F_1$ ratio); this ratio increased by more than a factor of ten across the luminance range tested (Figure 1B) (see Linsenmeier and Jakiela, 1979 for a comparison with cat RGCs). The change in $F_2/F_1$ ratio with luminance was highly significant ($p = 1 \times 10^{-3}$; Pearson’s correlation test on log-log scale, $n = 19$ cells).

Nonlinear spatial integration underlies many of the computations of RGCs, including sensitivity to second-order motion (Demb et al., 2001), differential motion of object and background (Gollisch and Meister, 2010), and rotations or small translations of texture patterns within the receptive field center (Schwartz et al., 2012). Computations in higher visual areas, like the recognition of form from texture patterns (El-Shamayleh and Movshon, 2011) and the demodulation of spatio-temporal patterns (Rosenberg and Issa, 2011), also depend on nonlinear spatial integration.

We found previously that On alpha RGC responses at high luminance depend on the spatial scale of texture stimuli confined to the receptive field center (Schwartz et al., 2012). The RGC response was modulated maximally by textures containing spatial scales of $\sim 40 \mu m$, much smaller than the $\sim 300 \mu m$ diameter of the full receptive field center. Here, we measured the ability of the same On alpha RGCs to encode information about 2D spatial structure within their receptive fields under different luminance conditions. We presented sets of texture patterns with two different levels of spatial blur that we had previously to elicit different average responses in the RGC at high luminance (Schwartz et al., 2012) (Figure 1C; see Experimental Procedures). At a luminance of 0.5 $R$/rod/s, performance at distinguishing blur levels was not different from chance (55% ± 5% correct; $n = 5$ cells). The same cells performed substantially better at a mean luminance of 100 $R$/rod/s (86% ± 3%; $p = 0.003$). Thus, the change from linear to nonlinear spatial integration confers the On alpha RGCs with sensitivity to fine spatial structure, fundamentally altering the type of information these cells extract from a visual scene. Other computations of these RGCs that depend on nonlinear spatial integration should similarly be impaired or absent at low luminance.

Changes in Signal Rectification Underlie Computational Change

What circuit mechanisms could cause RGC spatial integration to change with luminance? The bipolar cells that provide excitatory synaptic input to RGCs have much smaller receptive fields ($\sim 40 \mu m$) than the RGCs themselves ($\sim 300 \mu m$) (Schwartz et al., 2012), and the On alpha RGC receives synaptic input from hundreds of bipolar cells within its receptive field center. If the bipolar cells report stimulus contrast without rectification (i.e., with equal and opposite responses to positive and negative contrast), inputs from bipolar cells in the dark and light regions of the split-field stimulus would cancel, resulting in linear spatial integration by the RGC. If instead the bipolar cells provide a rectified signal to the RGC, responses from the dark and light regions of the split-field stimulus would not cancel, resulting in nonlinear spatial integration by the RGC (Figures 1C and 1D) (Demb et al., 1999; Schwartz et al., 2012). We have shown previously that excitatory synaptic input to the On alpha RGC is rectified in bright conditions (Schwartz et al., 2012), so we hypothesized that a change in the rectification of bipolar cell input across luminance underlies the observed changes in RGC spatial integration.

To test this hypothesis, we measured changes in RGC excitatory synaptic input elicited by increments and decrements of spatially uniform stimuli across a range of luminances (Figure 2). We focused on responses to the step onset since these reflect the adaptational state of the circuit in the prior period of constant light. At low luminance, ±50% contrast steps elicited approximately equal and opposite responses. At higher luminance, the response profile was markedly rectified, with the positive contrast step eliciting a ~4-fold larger response than the negative contrast step (Figures 2A and 2B). To quantify changes in rectification, we computed a rectification index by dividing the difference between the +50% and −50% contrast responses by their sum (see Experimental Procedures). This index ranges from −1 (negatively rectified) through 0 (nonrectified) to 1 (positively rectified). Rectification increased systematically with increasing luminance (Figure 2C) ($p = 1 \times 10^{-10}$; Pearson’s correlation on log-linear scale, $n = 49$ cells). Changes in rectification could not be explained as simply a change in dynamic range, since a rescaling of the contrast axis did not produce identical contrast-response curves (Figure S1 available online). The transition in rectification occurred across the same luminance range as the change in $F_2/F_1$ ratio (Figure 1B), consistent with changes in synaptic rectification underlying changes in spatial integration (Figure 1D).

Luminance-Dependent Transitions in Relevant Circuit Components

At low luminance, signals traverse the retina primarily through the rod bipolar circuit, while at higher luminance the cone bipolar circuit also contributes to ganglion cell responses (Figure 3A) (Trexler et al., 2005). Signals traversing either circuit must pass through the cone bipolar cell → On alpha RGC synapse, regardless of their origin. The sensitivity of these two circuits in darkness has been explored (Deans et al., 2002; Pang et al., 2007), but their relative contribution at different luminance levels is unclear. Does a transition in signaling between these two circuits contribute to changes in RGC spatial integration? To answer this question, we turned to a retinal slice preparation, allowing better access to bipolar and amacrine cells whose somas reside in the inner nuclear layer (see Experimental Procedures). We then measured signal flow through the rod and cone bipolar circuits across a range of luminances.

The AII amacrine cell receives inputs from both the rod and cone bipolar circuits. Signals from rod bipolar cells are transmitted to the AII amacrine cell via glutamatergic synapses, which contain primarily alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) (Singer and Diamond, 2003; Mørkve et al., 2002). In comparison, signals from the cone bipolar circuit are mediated by connexin36 (Cx36)-containing gap junctions between AII amacrine cell dendrites and On.
cone bipolar cell axon terminals (Figures 3A and 3B) (Deans et al., 2002; Tsukamoto et al., 2001). On cone bipolar cells themselves receive dendritic input from cones via metabotropic glutamate receptors. Thus, input to the AII amacrine cell originating from the rod bipolar circuit can be selectively eliminated by the AMPAR antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione (NBQX), effectively isolating input from the cone bipolar circuit (i.e., the NBQX-insensitive component) (Figure 3B) (Cohen, 2000; Murphy and Rieke, 2008; Manookin and Demb, 2006).

We first recorded the combined signals from both circuits in the AII amacrine cell (in response to +100% contrast steps; voltage clamp) at different luminance levels. We then repeated these measurements in the presence of NBQX to measure signals from the cone bipolar circuit alone (Figures 3C and 3D). These experiments were conducted using retinas from wild-type (WT) and Cx36−/− mice, in which gap junctions in both the inner and outer retina are disrupted (Deans et al., 2002). At a dim background of 0.5 R*/rod/s, NBQX eliminated the entire light response in both WT and Cx36−/− retinas, indicating that rod signals are transmitted primarily through the rod bipolar circuit at this luminance, with little or no contribution from rod or cone signals traversing the cone bipolar circuit (Figures 3C, 3D, and 3F). At 100 R*/rod/s, NBQX again eliminated the entire response in AII amacrine cells from Cx36−/− retinas, but much of the signal in WT retinas remained, indicating that both the rod and cone bipolar circuits normally contributed to the response at this luminance (Figures 3C, 3D, and 3F). The NBQX-insensitive response in WT mice—i.e., the response of the cone bipolar circuit—was half-maximal at a mean luminance of ~5 R*/rod/s (Figure 3E) (similar results for current and voltage responses).

The experiments of Figures 2 and 3 show that the balance of signal flow through the rod and cone bipolar circuits and the rectification of the RGC excitatory synaptic input have a similar dependence on luminance. Thus, a possible explanation for the change in spatial integration is that the rod and cone bipolar circuits encode contrast differently. We tested this hypothesis by measuring responses to positive and negative contrast steps in

Figure 2. Contrast Encoding Changes with Luminance
(A) Excitatory current responses to positive (black) and negative (gray) 50% contrast steps in On alpha RGCs at different luminance.
(B) Representative contrast response functions of excitatory input currents to On alpha RGCs each measured at a different luminance.
(C) Population data for the rectification index of excitatory input (see Experimental Procedures) as a function of background luminance. Open symbols are individual cells (n = 49), and filled symbols are binned means ±SEM. All recordings are from whole-mount preparations.
All amacrine cells and type 6 cone bipolar cells (which provide the dominant excitatory input to the On alpha RGC; Schwartz et al., 2012) at low luminance (0.5 R* / rod/s), where input from the rod bipolar circuit dominates, and moderate luminance (100 R* / rod/s), where both rod and cone bipolar circuits provide sizable input (Figure 4).

Voltage responses of both All amacrine cells (Figures 4A and 4D) and On cone bipolar cells (Figures 4C and 4D) were negatively rectified (i.e., larger responses to negative contrast steps than positive contrast steps) at both low and moderate luminance levels. In comparison, RGC excitatory inputs exhibited near zero rectification at low light levels and became positively rectified as luminance was increased to 100 R* / rod/s (Figures 4C and 4D; see also Figure 2). These data indicate that the change in rectification of the RGC synaptic inputs does not reflect a basic difference in the rectification of the rod bipolar and cone bipolar circuits. Instead, the change in rectification occurs at the synapse between cone bipolar cells and RGCs, a shared element in the two circuits. Such shared elements provide likely sites for interactions of signals from the two circuits.

A Steady-State Interaction between Parallel Circuits

Changes in the steady-state signals observed in Alls, cone bipolar cells, and RGCs provide a clue about the interactions responsible for this luminance-dependent change in RGC encoding and synaptic rectification. On alpha RGCs lost excitatory holding current as luminance increased (Figure 4C) (169 ± 57 pA, n = 5 cells, p = 0.04), indicating a reduction in maintained glutamate release from presynaptic cone bipolar cells. All amacrine cells and type 6 cone bipolar cells hyperpolarized across the same luminance range (ΔVmAII = −2.94 ± 0.14 mV, n = 9 cells, p = 1 × 10−4; ΔVCB6 = −4.32 ± 0.33 mV, n = 6 cells, p = 0.003) (Figures 4A, 4B, and 5G). All amacrine cells in whole-mount and slice preparations exhibited similar behavior, confirming that this aspect of the inner-retinal network had not been compromised by slicing the retina (Figure S2G). Taken together, these data indicate that the entire gap-junctionally coupled

(B) Detailed signaling schematic of the circuit elements and synaptic receptors that mediate transmission within the On alpha RGC circuit. Ox, gap junctions containing connexin proteins; mGluR6, metabotropic glutamate receptor 6; AMPAR, fast ionotropic glutamate receptors.

(C) Responses to +100% contrast steps measured in voltage clamp in an All amacrine cell from both WT (top) and Cx36−/− (bottom) mice before and after bath application of NBQX (10 μM).

(D) All amacrine cell responses across luminance (±NBQX) normalized to the control response at 200 R* /rod/s in wild-type (WT, left) and Cx36−/− (right) retinas. Responses from All amacrine cells in Cx36−/− mice were completely eliminated in the presence of NBQX, confirming a decoupling of the two pathways.

(E) Responses recorded from All amacrine cells in the presence of NBQX in the voltage-clamp configuration (same data as in [D]; closed circles) or the current clamp configuration (open squares) normalized to the response at 200 R* / rod/s. Fits are sigmoidal with the half-max indicated by dotted line. The background eliciting a half-maximal response to 100% contrast steps in the presence of NBQX was 5.1 R* / rod/s, regardless of the recording configuration.

(F) Schematic illustrating the flow of signals through the rod bipolar (purple) and cone bipolar (orange) circuits at two different luminance levels. Error bars in (D) and (E) are SEM across cells. Numbers of cells are indicated in parentheses in panel legends. All recordings are from slice preparations.

Figure 3. Signal Propagation through Parallel Circuits Depends on Luminance

(A) Simplified diagram of the parallel circuits that transmit visual information to On alpha RGCs in mouse retina.
AII-On cone bipolar cell network hyperpolarizes when luminance increases from 0.5 to 100 R*/rod/s. Paradoxically, the cells of interest (On RGCs, On cone bipolar cells, and AII amacrine cells) are all classified as On cells—they depolarize in response to positive contrast. Hence, we were surprised that maintained light exposure hyperpolarized the presynaptic AII-On cone bipolar electrical network and reduced tonic excitatory input to postsynaptic RGCs. In the following paragraphs we explore the mechanistic origin of the steady-state hyperpolarization before returning to its impact on synaptic rectification and spatial integration.

Previous work showed that the transition from low to moderate luminance involves a dramatic reduction in gain of the rod bipolar cell/AII synapse (Dunn et al., 2006; Jarsky et al., 2011; Oesch and Diamond, 2011) and recruitment of the cone bipolar circuit (Figure 3). Specifically, tonic depolarization of the rod bipolar cell reduces synaptic gain whereas hyperpolarization increases gain. Close proximity of the rod bipolar cell → AII synapses and AII → cone bipolar cell electrical synapses suggests that a local change in AII voltage produced by a change in gain of the rod bipolar synapse will be efficiently relayed to cone bipolar axon terminals (Figure 3A) (Tsukamoto et al., 2001). Is this the case? If so, what synaptic mechanisms are involved?

We first compared changes in rod bipolar cell and cone bipolar cell voltage in response to a step in mean luminance from 0.5 to 100 R*/rod/s. While cone bipolar cells slowly hyperpolarized following the luminance increase (Figures 5B and S2A–S2F), rod bipolar cells depolarized and slowly relaxed to steady state (Figure 5E) (Jarsky et al., 2011). Both cell types maintained their polarity as On cells throughout the change in luminance (Figures 5C and 5F), despite their opposing changes in resting membrane potential (平均 = 4.34 ± 0.26 mV, n = 10 cells, p = 3.10^4; AVRB = +7.26 ± 0.49 mV, n = 9 cells, p = 5.310^4)(Figure 5G).

Somatic cone bipolar cell recordings reflect a superposition of axonal input from AIIIs (via gap junctions) and dendritic input through mGluR6 receptors. If dendritic input to rod and cone bipolar cells exhibit similar kinetics, the slow hyperpolarization of the cone bipolar cell following the increase in luminance could reflect slowly decreasing dendritic input and a more rapid axonal hyperpolarization.

Next, we determined how the excitatory input AII amacrine cells receive from rod bipolar cells depended on mean luminance. Steady-state release at the rod bipolar cell/AII synapse was measured using voltage-clamp recordings from AII amacrine cells (in retinal slices). These recordings revealed a decrease in AII holding current with increasing luminance, consistent with a reduction in glutamate release from rod bipolar cells (Figure 5H). Furthermore, bath application of NBQX reduced the AII holding current more at low luminance levels than at higher levels (Figures 6A and 6B)—again consistent with a reduction in glutamate release at the higher luminance level. Recordings from AII amacrine cells in Cx36−/− retina also showed a decrease in NBQX-sensitive current as luminance increased (0.5−500R*/rod/s), conditions under which rod bipolar cells provide their only known excitatory input (i.e., den-dro-axonal gap junctions are absent) (Figure 6B).

Rod bipolar cells depolarized as luminance increased from 0.5 R*/rod/s (Figures 5E and 5G), yet the excitatory current in

![Figure 4. Luminance Alters Rectification at the Cone Bipolar Cell to Ganglion Cell Synapse](image)

(A–C) Responses to +50% (gray) and −50% (black) contrast steps in three different cells types at two different luminance levels (solid lines represent the average response; shaded areas represent the SEM; number of cells indicated in parentheses in [D]).

(D) Rectification index for each cell type at 0.5 R*/rod/s and 100 R*/rod/s (see Experimental Procedures). Error bars represent SEM. Paired t tests were used to compare within-cell data (AII and RGC recordings), and an unpaired t test was used to compare cone bipolar cells that were recorded at 0.5 R*/rod/s or 100 R*/rod/s and to compare cone bipolar cells and RGCs (the symbols *, **, and *** represent p values of <0.05, 0.01, and 0.001, respectively). All amacrine cell and type 6 cone bipolar cell recordings ([A], [B], and [D]) are from slice preparations; On alpha RGC recordings ([C] and [D]) are from whole-mount preparations.
postsynaptic All amacrine cells was reduced (Figures 6A and 6B). Presynaptic depression is known to play a prominent role in controlling gain at the rod bipolar cell → All synapse (Singer and Diamond, 2003, 2006; Dunn and Rieke, 2008), while postsynaptic receptor desensitization contributes minimally (Singer and Diamond, 2003). Extensive studies of the mechanisms underlying presynaptic depression have produced detailed models of glutamate release at this synapse (Jarsky et al., 2011; Oesch and Diamond, 2011) and emphasized prominent roles for vesicle depletion and inactivation of presynaptic CaV channels.

Our experimental conditions were similar to those in Jarsky et al., 2011, allowing us to adopt their physiologically constrained model (see Experimental Procedures; Figures 6C–6E). We used the model to determine how vesicle depletion and CaV channel inactivation influenced the relationship between steady-state synaptic release and mean presynaptic voltage — specifically whether the model could account for the observed reduction in tonic glutamate release with increasing presynaptic depolarization. Presynaptic release rates in this model depend on the probability of release (Pr) and the size and occupancy of the readily releasable pool (RP) of neurotransmitter-filled vesicles. Steady-state release specifically reflects an equilibrium between release from the RRP and replenishment of the RRP from the reserve pool (RP). Furthermore, Jarsky and colleagues found that CaV channels expressed at rod bipolar synaptic release sites became inactivated as mean voltage was increased. They concluded that CaV channel inactivation reduced not only the sensitivity of the synapse to presynaptic voltage but also reduced the effective size of the RRP due to tight nano-domain control of release sites by as few as one CaV channel (Jarsky et al., 2010).

We explored how RRP size and CaV channel inactivation influenced steady-state release using either constant voltage steps or actual time-varying voltage traces recorded from rod bipolar cells as input to the model. At each time step, presynaptic voltage was used to calculate the rate of release based on the Pr curves and vesicle availability (see Experimental Procedures and Figure S4); models were run until release rates reached steady state. In the absence of CaV channel inactivation, increases in presynaptic voltage led to increases in steady-state neurotransmitter release (Figures 6D and 6E). The maximum steady-state release rate occurring under these conditions is set by the rate at which the RRP is replenished. Incorporation of CaV channel inactivation (as in Jarsky et al., 2011) strongly altered the relationship between mean presynaptic voltage and steady-state release. In this more complete model, tonic glutamate release exhibited a nonmonotonic dependence on presynaptic membrane potential (Figures 6D and 6E) that resembled the u-shaped steady-state signals observed in Alls when the range of luminance tested includes darkness (Figure S4C). Experimentally, tonic release from RBCs was maximal near our dim light level of 0.5 R*/rod/sec. Thus, model and experiment together indicate that the depolarization of RBCs produced by increasing luminance from dim to moderate levels (Figure 5G) (see also Jarsky et al., 2011) will reduce tonic glutamate release via vesicle depletion and CaV channel inactivation.

Reduced tonic excitatory input will hyperpolarize the All amacrine cells, and this hyperpolarization will spread to On cone bipolar cell axon terminals via gap junctions. While a decrease in tonic inhibition onto the cone bipolar cell terminal could also contribute to the luminance-dependent hyperpolarization, we found no evidence for such a mechanism using pharmacological manipulations designed to alter inhibition (Figure S3). This is consistent with recent work showing that rectification of the On alpha RGC responses persists in the absence of inhibitory synaptic transmission (Chang and He, 2014). Together these results indicate that adaptation at the rod bipolar → All synapse controls the voltage, and hence synaptic set point, of the cone bipolar → On RGC synapse through dendro-axonal gap junctions.

**Voltage Bias Alters Rectification at the Cone Bipolar Synapse**

The modeling work described above indicates how mechanisms at the rod bipolar output synapse can cause the On cone bipolar synaptic terminal to hyperpolarize with increasing luminance. How does this hyperpolarization regulate rectification of the On cone bipolar synaptic output and RGC encoding (e.g., see Figure 1D)? To answer this question we consider a simplified model of the On cone bipolar cell synapse that predicts output rectification based on the mean and rectification of the presynaptic signals and rectification of the synapse itself (Figure 7).

We modeled synaptic rectification using a probability-of-release curve from the rod bipolar → All synapse model presented in Figure 6 (see Experimental Procedures). The voltage dependence of the presynaptic CaV channels and the high cooperativity of presynaptic calcium sensors together produce a sigmoidal relationship between presynaptic voltage and the rate of glutamate release (reviewed by Neher and Sakaba, 2008) (green curves in Figure 7). As a result, synaptic rectification depends on the mean presynaptic voltage and local curvature of the synaptic input-output function (Figure 7A). For presynaptic voltage signals of equal amplitude and opposite sign (nonrectified presynaptic signals), minimal rectification in synaptic output occurs when the mean voltage equals the midpoint (i.e., V_{1/2}) of the synaptic input-output relationship (Figures 7Ai and 7B, left). This holds true across a range of input voltage signals, since the input-output relationship is symmetric for changes around its midpoint and hence symmetric inputs lead to symmetric outputs. For negatively rectified presynaptic signals (i.e., decrement signals larger than increment signals), the mean presynaptic voltage that minimizes output rectification becomes negative to V_{1/2} (i.e., at a voltage at which synaptic rectification cancels rectification in the presynaptic input signals) (Figure 7B, right).

Using the measured type 6 cone bipolar cell voltage responses at 0.5 and 100 R*/rod/s (Figure 4C) as inputs, we derived the rectification index of the synaptic output for the measured range of mean presynaptic voltages. We first examined the synaptic rectification index for signals observed at low luminance (0.5 R*/rod/s). Because these postsynaptic bipolar cell voltage signals were negatively rectified, the presynaptic resting membrane potential required to reproduce a nonrectified postsynaptic response (i.e., RGC synaptic input) (Figures 4C and 4D) was negative to V_{1/2} (Figure 7D). Using this mean voltage as a baseline, we then introduced the extrinsic voltage bias we measured in Figure 5 (ΔV_{cb} = -4.3 mV) to estimate the synaptic
Figure 5. Steady-State Voltage Changes in the Rod and On Cone Bipolar Circuits as a Function of Luminance
(A) Example of a type 6 On cone bipolar cell reconstructed postrecording in the GJD2-GFP line (Siegert et al., 2009). Scale bar indicates 10 μm.
(B) Changes in mean voltage recorded from type 6 On cone bipolar cells before and after a step in luminance from 0.5 to 100 R*/rod/s. Smaller green symbols are measurements from individual cells (different symbol for each cell) during 1 s preceding a contrast step. Open diamonds are means (error bars are SEM) across cells (n = 6 cells).
(C) Mean responses to positive (black) and negative (gray) contrast steps from an example cell over the time periods indicated in (Ci) and (Cii).
(D) Example of a rod bipolar cell reconstructed postrecording in the GJD2-GFP line. Scale bar indicates 10 μm.
(E) Changes in mean voltage in rod bipolar cells (n = 8 cells) as in (B).
(F) Example rod bipolar cell contrast responses as in (C).
(G) Current clamp measurements of changes in resting membrane potential (relative to 0.5 R*/rod/s) in rod bipolar cells, All amacrine cells, and On cone bipolar cells as a function of luminance (as in [B] and [E]). Each bipolar cell was only tested at two luminance levels (0.5 R*/rod/s and one other level) to minimize the effects of washout. For rod bipolar cells, n is 6, 6, 6, 4, 5, and 4 for the test luminance levels in ascending order.
(H) Changes in the steady-state holding current (relative to 0.5 R*/rod/s) measured in rod bipolar cells and All amacrine cells at different luminance levels. Numbers in parentheses indicate number of cells. All error bars represent SEM. Recordings are from slice preparations except for the All amacrine cell data in (G), which includes data from slice and whole mount (see Figure S2G).
rectification index in response to presynaptic signals recorded at 100 R*/rod/s. This extrinsic hyperpolarization shifted the synaptic output into a more positively rectified state and provided an estimated rectification index of 0.39 (Figure 7D), which agreed well with that measured in the RGC synaptic inputs (0.42) (Figure 4F). This model demonstrates how hyperpolarization of the cone bipolar cell synaptic terminal, originating in part in the rod bipolar circuit, can contribute to the luminance-dependent change in rectification of the excitatory synaptic currents in On alpha RGCs. Rectification of the excitatory inputs in turn controls how the RGC encodes patterns of light within its receptive field (Figures 1C and 1D) (Schwartz et al., 2012).

DISCUSSION

Computation in neural circuits often relies on the integration of the outputs of multiple parallel subcircuits. Here we show that the computation implemented by the circuitry subserving a particular RGC type changes with increasing luminance and that this change in computation is mediated by interactions between two parallel circuits upstream of the RGC. The result is a fundamental change in the stimulus features encoded in the RGC spike response.

Circuit Repurposing Alters Retinal Function

RGCs receive input from hundreds of presynaptic bipolar cells across their dendritic tree, giving rise to the excitatory part of the receptive field center. Integration of the spatial pattern of light within the receptive field center depends on the properties of the cone bipolar cell → RGC synapse. Here we show that changes in the properties of this synapse with background luminance cause a change in how the RGC integrates inputs across space.

At low luminance, mouse On alpha RGCs integrated light near linearly across their receptive field center (Figure 1); this behavior is similar to linear RGCs in other retinas (Enroth-Cugell and Robson, 1966; Schwartz and Rieke, 2011; Rosenberg and Issa, 2011) and supports a simple encoding framework in which each RGC reports the average signal within its receptive field. At higher luminance, On alpha RGCs exhibited nonlinear spatial integration and hence became sensitive to fine spatial structure in images presented in their receptive field center (Figure 1). This nonlinear behavior supports the detection of a variety of spatial
and spatiotemporal features (Demb et al., 2001; Gollisch and Meister, 2010; Gollisch, 2012; Schwartz and Rieke, 2011; Rosenberg and Issa, 2011); sensitivity to these features is well-predicted from models incorporating rectification at the bipolar output synapses and heterogeneous sampling of the bipolar population due to the sparsity of the RGC dendrites (Schwartz et al., 2012).

The change we observe here from linear to nonlinear spatial integration adds to a growing list of stimulus-dependent changes in retinal computations, including changes in the On-Off characteristics of RGCs (Geffen et al., 2007), the direction selectivity of On-Off direction-selective RGCs (Rivlin-Etzion et al., 2012), and the center-surround structure of On alpha RGCs (Farrow et al., 2013). The last of these studies provided a rare link to mechanism, showing that increasing luminance recruited a component of the receptive field surround provided by direct inhibition to the RGC from wide-field amacrine cells.

These studies, together with our findings here, emphasize the breadth of functional repurposing of retinal circuits and the diversity of the underlying mechanisms.

What are the ecological implications of the switch from linear to nonlinear spatial integration with increased luminance? While a complete answer to this question will require considering changes in other retinal circuits, we can make a few speculations here. At low light levels, the loss of texture sensitivity and other computations supported by nonlinear spatial integration may be offset by more efficient integration across the entire receptive field center, which helps average out noise associated with quantal fluctuations in photon absorption and hence improves sensitivity to low contrast signals (Hemila¨ et al., 1998). In brighter conditions, when quantal fluctuations are much smaller relative to the mean rate, integration over the full receptive field center may not be required to achieve adequate contrast sensitivity. Additionally, the linearity of the spike responses in dim conditions allows the On alpha RGC to encode information about both positive and negative contrasts, transmitting both kinds of information to downstream cells. In brighter conditions, when rectification truncates the negative contrast responses, the cell signals almost exclusively to positive contrast changes. Negative contrasts are presumably relegated to the Off RGC pathways, so a downstream neuron would have to receive input

![Image](https://placeHolderImage.com/603x783.png)
from both RGC types to have access to information about both positive and negative contrasts.

**Synaptic Interactions and Specializations at Bipolar Cell Synapses**

Both rod and cone bipolar cells experienced substantial changes in resting membrane potential over the luminance range we explored (Figure 5). With increasing luminance, rod bipolar cells depolarize, but Ca, inactivation accrues and tonic glutamate release declines (Figure 6). This decrease in tonic excitation through the rod bipolar circuit in turn decreases the bias voltage to cone bipolar cells (through dendro-axonal gap junctions with all dendrites), resulting in increased rectification at the cone bipolar cell output synapse (Figure 7).

This synaptic specialization (i.e., axons electrically coupled to the dendrites of other neurons) is not unique to type 6 cone bipolar cell terminals but is instead a common feature of most, if not all, On cone bipolar cell types (Veruki and Hartveit, 2002; Trexler et al., 2001). Consistent with this observation, we found that other On cone bipolar types hyperpolarized with a step from 0.5 R*/rod/s to 100 R*/rod/s (Figures S2A–S2F). This suggests that the operating range of many different cone bipolar cell types will be influenced by the rod bipolar circuit. The luminance-dependent hyperpolarization of the All amacrine cell could also change its tonic glycine release. Consistent with this idea, tonic inhibitory input to Off sustained RGCs, which largely originates from All amacrine cells (Murphy and Rieke, 2008), decreases with increasing luminance (data not shown). A change in tonic glycine release by the All could also alter the operating range of Off bipolar cells.

**Fine Control of Presynaptic Function Alters Neural Computation**

Our work here ties together two broad themes in neural computation. First, modeling work has emphasized the surprisingly complex computational effects that can be achieved by precise tuning of simple mechanisms (Gollisch and Meister, 2010; Priebe and Ferster, 2012). Second, the activity of output neurons in many neural circuits is controlled by convergence of signals from several parallel pathways—a ubiquitous example being converging excitatory and inhibitory inputs. Interactions between signals in such parallel pathways will be a key determinant of circuit computation.

We find that dendro-axonal gap junctions alter the operating point of the primary excitatory input to the RGC and, by doing so, control synaptic rectification and integration of light inputs within the RGC receptive field. This adds to a growing appreciation of the diverse roles gap junctions serve in the retina (reviewed by Bloomfield and Völgyi, 2009). Gap junctions between photoreceptors, for example, route light responses generated in a single rod through multiple photoreceptor synapses and, by doing so, allow effective transmission of the entire rod voltage response through synapses with limited dynamic range (Attwell et al., 1987; Bellem and Copenhagen, 1988; Hornstein et al., 2005; Li et al., 2012). In this case gap junctions help avoid synaptic rectification, rather than control it as we find here. Recent work has also shown that gap junctions between direction-selective RGCs interact with chemical synapses to shape direction selectivity (Trenholm et al., 2013a; Trenholm et al., 2013b); specifically, electrical synapses help compensate for the delays associated with retinal processing and produce a more veridical representation of motion.

Our expanding appreciation of the diverse roles of gap junctions is not limited to the retina. In addition to their common location between dendrites, electrical synapses are also found near synaptic outputs throughout the brain (Schmitz et al., 2001). Gap junctions have been shown to synchronize or desynchronize networks of neurons (Dugue et al., 2009; Vervaeke et al., 2010; Traub et al., 2001a), expand and smooth spatial receptive fields (Elyada et al., 2009), generate persistent firing (Sheffield et al., 2011), and contribute to the initiation of epileptic seizures (Traub et al., 2001b).

Synaptic transmission in other systems, including spiking networks, is similarly highly sensitive to fine changes in presynaptic membrane potential (Awateramani et al., 2005). Parallel pathways could exploit such sensitivity via several mechanisms—including presynaptic voltage bias introduced by gap junctions, as found here, or by tonic presynaptic inhibition. Indeed, a recent study demonstrated that excitability of a population of inhibitory interneurons in the cochlear nucleus is regulated by asymmetrical gap-junctional coupling with excitatory projection neurons (Apostolides and Trussell, 2013). Our work provides a clear link between such changes in synaptic operating point and circuit computation. The components of such circuit interactions—converging parallel circuits, nonlinear synaptic transfer functions, and presynaptic mechanisms that could control synaptic operating point—are common, and hence similar functional changes could be a general feature of neural computation.

**EXPERIMENTAL PROCEDURES**

**Electrophysiology**

Experiments were conducted on whole mount and slice (210 μm thick) preparations taken from dark-adapted wild-type or Cx36−/− C57/B16 mice (Murphy and Rieke, 2006; Sampaio and Rieke, 2004) in accordance with Institutional Animal Care and Use Committee at the University of Washington. Isolated retina was stored in oxygenated (95% O2/5% CO2) Ames medium (Sigma) at ~32°C–34°C, and once under the microscope, tissue preparations were perfused by the same Ames solution at a rate of ~8 mL/min. Isolated retinas were either flattened onto poly-L-lysine slides (whole mount) or embedded in agarose and sliced as previously described (Dunn et al., 2006; Schwartz et al., 2012; Sampaio and Rieke, 2004). Retinal neurons were visualized and targeted for cell-attached and/or whole-cell recordings using infrared light (~950 nm).

Voltage-clamp recordings were obtained using pipettes (for RGCs, 2–3 MΩ; for All amacrine cells, 5–6 MΩ; for bipolar cells, 10–14 MΩ) filled with intracellular solution containing (in mM) the following: 105 Cs methanesulfonate, 10 TEA-Cl, 20 HEPES, 2 QX-314, 5 Mg-ATP, 0.5 Tris-GTP, and 0.1 Alexa-750 hydrazide (~280 mOsm; pH ~7.3 with CsOH). Current clamp recordings were conducted using an intracellular solution containing (in mM) the following: 123 K-aspartate, 10 KO, 10 HEPES, 1 MgCl2, 1 CaCl2, 2 EGTA, 4 Mg-ATP, 0.5 Tris-GTP, and 0.1 Alexa-750 hydrazide (~280 mOsm; pH ~7.2 with KOH). NBOX (10 μM; Tocris) or TTX (0.5 μM; Alamone) was added to the perfusion solution as indicated in Figures 3, 6, and S3. In voltage-clamp recordings, RGCs and bipolar cells were held at the estimated reversal potential for inhibition (~−60 mV) to isolate excitatory synaptic input. Voltage-clamp recordings from All amacrine cells were performed near their resting potential (~−40 mV) to avoid exposing inputs from rod and cone bipolar circuits to nonphysiological driving forces and hence altering the balance of these inputs. Recordings from All amacrine cells in Cx36−/− mice were held at ~60 mV to isolate excitation from rod bipolar cells. On cone bipolar and rod bipolar recordings were kept short (typically <5 min.) to minimize washout effects.
Absolute voltage values were not corrected for liquid junction potentials (K+ based = –10.8 mV; Cs+ based = –8.5 mV).

**Visual Stimuli**

Spatial stimulus patterns were displayed on a 800 x 600 pixel OLED array (eMagin) with a pixel size of either 1.2 μm or 1.8 μm and were focused onto the photoreceptors through the microscope condenser. Split-field stimuli (Figure 1A) were modulated sinusoidally at 3.75 Hz from –80% to +80% contrast. Results were similar across a range of temporal frequencies from 0.5 Hz to 8 Hz (data not shown). Texture stimuli (Figure 1C) were created by applying a 2D Gaussian filter to binary random noise patterns and normalizing the resulting pattern to a uniform distribution of contrasts from –100% to +100%. Patterns were 300 μm squares centered on the receptive field of the RGC and were presented for 0.5 s. Uniform stimuli for measuring RGC contrast-response functions (Figure 2) were circles 300 μm in diameter of various contrasts from –100% to +100% centered on the receptive field of the RGC and were presented for 0.5 s. For all experiments using the retinal slice preparation, full-field illumination (diameter, 560 μm) was delivered to the tissue from LEDs with peak spectral outputs at 470 or 513 nm.

**Analysis**

Response amplitudes were quantified by taking the peak current or voltage during the stimulus presentation in Figures 2–5 or by integrating the current or voltage throughout the duration of the stimulus presentation in Figure 1. F2/F1 ratio (Figure 1B) was computed based on the power of the current traces at the stimulus modulation frequency (F1) and twice the modulation frequency (F2). The noise at each frequency was measured from 2 s of the current trace preceding stimulus onset and subtracted from the power values measured during stimulus presentation. Discriminability of spatial blur patterns (Figure 1C) was determined by a two-alternative forced choice analysis. Each pattern was presented at two different blur values, and discriminability was quantified as the percentage of trials on which the response to the larger blur size exceeded that of the smaller blur size.

Rectification index (RI) (Figures 2, 4, and 7) was defined as

\[
RI = \frac{r_{+50} + r_{-50}}{r_{+50} - r_{-50}}
\]

where \(r_{+50}\) and \(r_{-50}\) were the responses to +50% and –50% contrast. The responses were always of opposite sign, so the index ranged from –1 (complete negative rectification) through 0 (no rectification) to 1 (complete positive rectification).

Two-tailed paired t tests were used to test significance unless otherwise noted. Pearson’s correlation tests were used to determine if there was significant correlation between the data in Figures 1B and 2C. Because the trends were nonlinear in both cases, we used a logarithmic scaling of data on both axes for Figure 1B and only a logarithmic scaling of luminance data for Figure 2C.

**Cell Identification**

For experiments at a fixed luminance (Figures 1A, 1B, and 2), data acquisition began after at least 5 min of exposure to constant luminance. For experiments in which mean luminance was varied (Figure 1C, 3–6, and S1–S3), data acquisition began 50–100 s (depending on cell type) (Figure 5) after a change in mean luminance.

**Modeling**

A stochastic vesicle release model was created in Igor Pro (WaveMetrics) using parameters described in Jarsky et al. (2011). In short, vesicle release and replenishment rates were calculated in 0.1 ms intervals using the voltage-dependent release curves in Figure S4A and a replenishment time constant of 130 ms. For simulations without Ca2+ channel inactivation, the Pr midpoint did not shift with mean voltage. In these simulations we used only the release curve on the far left of Figure S4A. The model used 1,000 release sites and each event (i.e., miniature excitatory postsynaptic current) had an amplitude of –1, a decay time of 1 ms, and a rise time of 0.1 ms. The steady-state Ca2+ channel inactivation (\(I_{Ca}\)) was modeled as a shift and compression of the release curves and a reduction of available vesicles as described in Jarsky et al. (2011) (Figure S4B). Uncorrelated release (B) was also included for mean voltages ≥–48 mV. For simulating the influence of Ca2+ channel inactivation on synaptic release at a mean voltage of –39 mV (a mean voltage not explicitly reported in Jarsky et al., 2011), we derived values for B and \(I_{Ca}\) by fitting the explicit data with a sigmoid and taking values from those fits at –39 mV (Figure S4B). Additionally, the \(V_{r}\) of the release curve used for simulations including Ca2+ channel inactivation at –39 mV was –33.5 mV, a value also extrapolated from their measurements. Differences in experimental conditions—particularly differences in extracellular solutions and uncertainty about the magnitude of RBC voltage changes due to run down during whole-cell recordings—precluded a direct comparison of absolute voltages in our RBC recordings and those in the model.

The generalized model of synaptic rectification in Figure 7 uses a probability release curve (when no Ca2+ inactivation is present) from the rod bipolar cell → AII synapse model presented in Figure 6. The synaptic rectification index in Figure 7 was calculated using the average of the peak amplitudes of the presynaptic voltage responses to positive and negative contrast recorded from type 6 cone bipolar cells (Figure 4B).

**Cell Selection Criteria**

On alpha RGCs were selected for further analysis based on a sensitivity criterion of 10 spikes in response to a +10% contrast step (0.5 s duration) at 100 R°/rod/s. Recordings from slice preparations were performed within ~3.5 hr of retinal dissection, and we specifically targeted neurons that were ≥20 μm below the surface of the slice. These parameters seemed to be particularly important for achieving stable recordings of activity from both the inner and outer retina. Rod bipolar cells were retained for analysis when saturating flashes from darkness produced reliable and robust events both before and after light adaptation. Rod bipolar cell recordings typically lasted 3–4 min. On cone bipolar cells were retained when stable baselines could be achieved within <1 min. Contrast responses in On cone bipolar cells were taken within the first 4 min after break-in at 0.5 and/or 100 R°/rod/s. Individual bipolar cells were tested at a maximum of two luminance levels (0.5 R°/rod/s and one other value). All amacrine cell recordings could be targeted particularly deep in the slice (~40–50 μm) and provided stable, long-lasting recordings (~30 min). One AII amacrine cell from WT retina in Figure 3D was excluded from steady-state pharmacological subtraction analysis in Figure 1B due to an obvious jump in holding current just before switching to the solution containing NBQX.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.02.037.

**ACKNOWLEDGMENTS**

We thank Jon Cafaro, Jeff Diamond, David Perkel, and the anonymous reviewers for helpful comments on earlier versions of the paper and Mike Ahlquist, Mark Cafaro, Shellee Cunnington, and Paul Newman for excellent technical assistance. Support provided by the Helen Hay Whitney Foundation (G.W.S.), HHMI (F.R.), and NIH (EY11850 to F.R.).
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Mechanisms Underlying Change in Spatial Encoding


