Ambient Illumination Toggles a Neuronal Circuit Switch in the Retina and Visual Perception at Cone Threshold

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SUMMARY

Gradual changes in the sensory environment can lead to abrupt changes in brain computations and perception. However, mechanistic understanding of the mediating microcircuits is missing. By sliding through light levels from starlight to daylight, we identify retinal ganglion cell types in the mouse that abruptly and reversibly switch the weighting of center and surround interactions in their receptive field around cone threshold. Two-photon-targeted recordings and genetic and viral tracing experiments revealed that the circuit element responsible for the switch is a large inhibitory neuron that provides direct inhibition to ganglion cells. Our experiments suggest that weak excitatory input via electrical synapses together with the spiking threshold in inhibitory cells act as a switch. We also reveal a switch-like component in the spatial integration properties of human vision at cone threshold. This work demonstrates that circuits in the retina can quickly and reversibly switch between two distinct states, implementing distinct perceptual regimes at different light levels.

INTRODUCTION

The mammalian visual system operates over a large range of light intensities that challenge it with input regimes in which either individual photons must be gathered to reconstruct the visual scene or salient features need to be extracted from the flux of billions of photons (Hood and Finkelstein, 1986; Rieke and Rudd, 2009). At low light intensities, it collects photons using only the highly sensitive rod photoreceptors, at medium intensities, rod and cone photoreceptors are both at work, while at high intensities, only cones are used. In these three regimes, the visual system gathers information using ~20 discrete visual channels that originate with mosaics of local neuronal circuits in the retina (Masiand, 2001; Wässle, 2004). The neurons that carry the output of these circuits are the ~20 distinct ganglion cell types, each of which highlights a unique feature of the visual scene (Berson, 2008; Dacey, 1994; Farrow and Masiand, 2011; Levick, 1967; Roska and Werblin, 2001). During the transition from starlight to bright daylight conditions, a number of adaptive processes increase the acuity and contrast sensitivity, as well as affect the spatial integration properties of the visual system. These changes have been observed in the retina (Barlow et al., 1957; Bisti et al., 1977; Enroth-Cugell and Robson, 1966; Muller and Dacheux, 1997; Peichl and Wässle, 1983; Rodieck and Stone, 1965), lateral geniculate nucleus (Bisti et al., 1977; Ramoa et al., 1985; Virsu et al., 1977; Wiesel and Hubel, 1966), and visual cortex (Bisti et al., 1977; Ramoa et al., 1985), as well as during visual perception (De Valois et al., 1974; Kelly, 1972; Pasternak and Merigan, 1981; Umino et al., 2008; van Nes et al., 1967).

In the retina, the receptive fields of ganglion cells are organized into center and surround regions, where illumination of the surround reduces the sensitivity of the ganglion cell to center illumination (Barlow, 1953; Kuffler, 1953). Soon after center-surround receptive fields were first described in the retina (Barlow, 1953; Kuffler, 1953), it was noted that in dark-adapted states, the antagonistic surround of some ganglion cells was weak or disappeared completely (Barlow et al., 1957; Bisti et al., 1977; Dedek et al., 2008; Enroth-Cugell and Robson, 1966; Muller and Dacheux, 1997; Rodieck and Stone, 1965). However, other studies have reported that the antagonistic surround is maintained in dark-adapted states (Enroth-Cugell and Lennie, 1975; Troy et al., 1999). These discrepancies have not been resolved, since, with the exception of recordings from X ganglion cells in the cat (Bisti et al., 1977; Enroth-Cugell and Lennie, 1975; Enroth-Cugell and Robson, 1966; Troy et al., 1999), experiments could not reproducibly target an individual ganglion cell type. The neuronal circuitry forming the ganglion cell’s antagonistic surround involves lateral inhibitory signaling pathways that allow adjacent columnar circuits in the retina to interact (Wässle, 2004). These pathways are mediated by horizontal cells in the outer retina and amacrine cells in the inner retina (Cook and McReynolds, 1998; Flores-Herr et al., 2001; Ichinose and Lukasiewicz, 2005; Mangel, 1991; McMahon et al., 2004; Naka and Witkovsky, 1972; Taylor, 1999; Werblin, 1974).
The circuit mechanism underlying the luminance-dependent strength of ganglion cell inhibitory surround, its specificity for certain types of ganglion cells, and whether these changes occur continuously or abruptly across luminance levels have remained in question.

Here we show that the organization of the center and surround of specific types of ganglion cells exist in two discrete states. At low ambient light levels, these ganglion cells have a weak surround, and at higher levels, they have a strong surround. The switch between states is abrupt and reversible, occurring at light levels at which cone bipolar cells are strongly activated. The switch is implemented by the activation of large inhibitory spiking amacrine cells that provide input to ganglion cells. Consistent with the data, we present a model describing how the retina could combine electric transmission and spike threshold to switch inhibition on and off. Finally, we show that human spatial vision can also be reversibly toggled between two discrete states around cone threshold. We discuss the similarities between the luminance-dependent changes in spatial vision and the neuronal responses of the ganglion cells in the retina.

RESULTS

A Switch-like Change in the Receptive Field Structure of a Retinal Ganglion Cell

We performed two-photon laser-targeted patch-clamp recordings from labeled ganglion cells in isolated retinas of transgenic mice in which eight types of ganglion cells express a fluorescent protein (Experimental Procedures, see Figures S1–S3 available online) (Feng et al., 2000; Hippenmeyer et al., 2005; Madsen et al., 2010; Münch et al., 2009). Across eight logarithmic units of light intensity, we presented spots of different sizes to the retina with the same positive contrast, but at different background light levels, while recording either the spiking responses in loose cell-attached mode or voltage responses in current-clamp mode. One cell type, the PV1 cell, responded to small spots at the brighter light levels (Figures 1A and 1B). We found that the application of the GABA antagonist picrotoxin blocked the switch: in the presence of picrotoxin, the responses to the presentation of large spots at these light levels were stable at all brighter light levels. To quantify this luminance-dependent change in PV1 spiking responses, we compared the spiking responses of PV1 cells to the small and large spots using a spatial selectivity index (SSI, defined in Experimental Procedures) across the different background light levels. The SSI is low when the spiking responses to small and large spots are similar and high when the spiking response to small spots is larger than to large spots. We found that the SSI of the PV1 cell fell into one of two regimes: in low light conditions, the PV1 cell had a low SSI, and at higher light levels, the PV1 cell had a high SSI (Figure 1D). The background spiking of the PV cell had a mean of 5.9 Hz and was variable, likely depending on the light adaptation and stimulus history of the recorded cell; however, the variation of background spiking between repetitions recorded from the same cell was low (Figure S4).

The transition from low to high spatial selectivity was abrupt, occurring with full effectiveness in less than 10 s, the minimum time we could probe the cells between the two conditions (Figure 1E). In addition, the transition was reversible: the spiking response could be toggled between two distinct states by shifting the background light levels up and down one log unit (Figure 1F). The change in spatial selectivity is independent of stimulus and contrast, since we observed a similar change for drifting gratings of different spatial frequencies at different contrasts (Figures 1G–1I and S4). Fine resolution stepping through background intensities revealed that the significant change occurs across a change of intensities of 0.07 log units (Figure 1H). Quantifying spiking responses to spatiotemporal white noise stimuli also revealed differences in linear receptive field structure at low and high intensities (Figure S4). Therefore, the spatial integration properties of the PV1 cell shifted abruptly and reversibly at a specific “critical” light level-like a switch. We refer to the state of the circuit as “switch-ON,” when the SSI is high and “switch-OFF” when it is low.

We found that a switch-like change in responses across light levels is not a universal property of retinal ganglion cells. While among PV cells (Figures 2 and S1) two large ganglion cell types, PV1 and PV6, showed an abrupt change in their spatial selectivity around the same background light level (Figures 3A and 3B), other ganglion cell types, most of them with smaller dendritic fields, had either no change in their responses or the responses were continuously changing with increasing background light level (Figures 3C and 3D).

A Large Spiking Inhibitory Neuron Is Activated by the Switch

How does such a strong change in circuit filtering occur at a specific light level? To determine the neuronal and synaptic elements involved, we dissected the circuitry mediating this switch. As a first step, we asked whether inhibitory neuronal elements were required to actively suppress the response of the PV1 cell to the presentation of large spots at the critical light level and above, a likely scenario given the hyperpolarizing responses to the presentation of large spots at these light levels (Figures 1A and 1B). We found that the application of the GABA antagonist picrotoxin blocked the switch: in the presence of picrotoxin, the responses to large spots were similar to the responses to small spots at the brighter light levels (Figures 4A and 4B). Dopamine agonists and antagonists did not influence the switch (data not shown). Therefore, the switch involves the activation of inhibitory elements at a critical light level.

To ascertain whether the inhibitory elements are acting directly on the ganglion cell, we performed a set of voltage-clamp and pharmacological experiments (Experimental Procedures,
Figure S5. We recorded the input currents to PV1 cells at different holding potentials and determined the stimulus-evoked excitatory and inhibitory inputs at switch-ON and switch-OFF circuit states. Our analysis revealed that an inhibitory conductance in the ganglion cell was strongly activated when the switch was toggled ON (Figures 4C and 4D). This inhibitory conductance was blocked with picrotoxin, a GABA antagonist, and TTX, which blocks sodium spikes in the retina, but not by...
strychnine, a glycine antagonist (Figures 4C and 4E). Inhibition was delayed compared to excitation (Figure 4F) and annuli up to 2 mm in diameter were able to activate the inhibitory input at light levels at which the circuit is in the switch-ON state (Figure 4G). The excitatory input to PV1 cells did not show a discontinuous decrease in strength (Figure 4D), suggesting that horizontal cells are not responsible for the switch. Since amacrine cells mediate inhibitory input to ganglion cells, we conclude that the switch involves the activation of GABAergic spiking amacrine cells that can act from a distance and are directly connected to PV1 cells.

To confirm that far reaching amacrine cells directly connect to PV1 cells, we carried out monosynaptically restricted viral tracing using G-deleted rabies virus in which the G protein is supplied to the PV ganglion cells by a conditional adeno-associated (Marshel et al., 2010; Stepien et al., 2010; Wickersham et al., 2010) or Herpes virus (Yonehara et al., 2011)(Figure S6). We reconstructed the transsynaptically labeled amacrine cells around three PV1 cells, each in a different mouse (Experimental Procedures), and found amacrine cells with long processes, some reaching over 1 mm across the retina, connected to PV1 cells (Figures 5, S6, and S7). These “wide-field” amacrine cells, revealed by monosynaptic tracing, are probably the inhibitory cells that are activated by the switch. Note that PV cells other than PV1 also receive input from wide-field cells and, therefore, the PV1 connecting amacrine cells must have special properties that allow the implementation of the switch (Lin and Masland, 2006).
The Implementation of the Circuit Switch

How could inhibition be differentially activated in two different regimes of vision? The retina incorporates two kinds of photoreceptors, rods and cones, which provide the sensory interface for image-forming vision. The more sensitive rods and the less sensitive cones have overlapping light intensity ranges of signaling (Figure S2) and, therefore, three ranges can be defined: vision mediated by rods only, rods and cones, and cones only. In order to determine whether the transition between switch-OFF and switch-ON states corresponds to the transition from vision mediated by rods only to rods and cones, or rods and cones to cones only, we recorded from rod and positive contrast-activated cone bipolar cells in a retinal slice preparation (Figures 6A–6C). We presented the slice with full-field steps of illumination with fixed contrast across different light intensities, incorporating rod only and cone only intensity ranges. The critical light intensity at which the switch was turned on corresponded to those light intensity values in which cone bipolar cells became strongly activated. At this light intensity, rod bipolar cells have already been fully activated. The critical light intensity was within the range reported to activate cones in mice (Nathan et al., 2006; Umino et al., 2008). These experiments are consistent with a view that the activation of cones toggles the switch (see Discussion for an alternative explanation).

Bipolar cells provide excitatory input to both ganglion cells and amacrine cells. How could bipolar cells continuously drive excitatory input to the ganglion cell but independently instruct inhibition through wide-field amacrine cells in a discontinuous, switch-like way? To investigate whether the excitatory input to the PV1 ganglion cell and the inhibitory switch encompassing amacrine cells is mediated by the same or different mechanisms, we blocked glutamate signaling using CPP and NBQX, which are antagonists of the ionotropic glutamate receptors. As expected, the excitation to PV1 cells was blocked. However, at light levels when the switch is ON, the inhibitory input remained, suggesting that the excitatory drive to the amacrine and ganglion cells is acting through a different mechanism (Figures 6D, 6E, and S5). In the presence of NBQX and CPP, the inhibitory current was blocked by APB, which stops the response of those bipolar cells that respond to contrast increments (Figure 6E). As amacrine cells could be driven by electrical synapses rather than chemical synapses (Deans et al., 2002), we created a triple transgenic line in which both alleles of connexin36 were knocked out (Deans and Paul, 2001) and the PV cells were labeled with EYFP. In this knockout animal, we performed the same functional experiments as those that showed the switching filtering properties. Since connexin36 is needed for the rod signals to reach the amacrine and ganglion cells (Deans et al., 2002), there were no inhibitory or excitatory responses at low light levels, as expected. More importantly, the inhibitory input to PV1 cells decreased significantly (Figures 6F and S5) and the spiking responses of the PV1 cell to large and small spots remained similar across higher light intensities (Figures 6G and 6H). These results, taken together with the voltage-clamp recordings (Figures 6D and 6E), suggest that the switching amacrine cells receive excitatory input via electrical synapses incorporating connexin36.

These experiments are consistent with cone bipolar cells providing input to switching amacrine and PV1 cells using different mechanisms but do not explain why the excitatory input to PV1 cells does not show a stepwise increase in strength at the critical light level (Figure 4D). In order to understand this, we examined the time course of the excitation to PV1 cells. The quantification of responses thus far incorporated a long time-scale, using average responses across a 0.5 s time window.
When we quantified excitation in a shorter time window after stimulus onset, the strength of excitation also showed a stepwise increase at the critical light level (Figures 6E and 6J) and a few spikes were detectable transiently after the onset of the light stimulus (Figures 1A and S4). These findings, together with the observed delay between inhibition and excitation (Figure 4F), are consistent with an excitatory input from cone bipolar cell terminals that also shows a stepwise increase at the critical light level but is then silenced after a delay by the action of an inhibitory cell turned on at the same light level. Indeed, the application of picrotoxin and TTX both resulted in an increase of the average excitatory input to the PV1 cell (Figure 4E), suggesting that spiking, GABAergic amacrine cells mediate this inhibition to cone bipolar cells. Note, however, that these increases did not reach the threshold for statistical significance. A possible circuit mechanism explaining the lack of significant increase is the mutually inhibitory interaction between GABAergic and glycinergic inhibitory cells (Roska et al., 1998; Zhang et al., 1997). The blockage of GABAergic inhibition mediated by large spiking GABAergic amacrine cells may have caused an increase of glycinergic inhibition from small amacrine cells (Wässle et al., 2009) that acted on bipolar terminals to inhibit glutamate release. This increase in glycinergic inhibition may have compensated for the expected increase in excitatory input to ganglion cells.

From these experiments, we put together the following model for the circuit switch of PV1 cells (Figure 7). PV1 cells receive inhibitory input from a set of wide-field, GABAergic spiking amacrine cells that we call switch cells. PV1 and switch cells receive excitatory input from cone bipolar cells, either the same or different types. Bipolar cells drive PV1 cells via chemical synapses and the switch cells using electrical synapses (some of their input may also come from chemical synapses). As light levels increase from starlight to daylight conditions, an object with the same contrast evokes increasing activity in cone bipolar cell terminals. The bipolar-to-PV1 cell gain is high (chemical synapse), but the bipolar-to-switch cell gain is low (electrical synapse) and, therefore, the excitatory drive reaches a threshold in PV1 cells, but not the switch cell. An additional factor contributing to the sensitivity of PV1 cells to detect small changes in cone bipolar cell activity is that the resting potential of PV cells is close to their spike threshold (data not shown). At a critical light level, the input to cone bipolar cells suddenly increases, and the cone bipolar cell terminals experience a similar increase in their input. The sharp increase in drive to bipolar terminals leads to a similarly sharp increase in the excitatory drive to switch cells, lifting the voltage above the spiking threshold, resulting in inhibitory input to the PV1 cell. The relative contribution of inhibition and excitation is dependent on the size of the spot stimulus presented. The excitatory input saturates when the size of the spot

(F) Latency between peak of excitatory input and peak of inhibitory input. (G) Excitatory (red) and inhibitory (black) input to PV1 cell responding to annuli with an outer diameter of 2,400 μm and inner diameter ranging from 0 to 2,000 μm (x axis). In this and other figures, inhibition and excitation refer to currents measured at 0 mV and −60 mV, respectively, and these currents, unless indicated, were quantified taking the absolute value of the mean current during the first 0.5 s after stimulus onset (Experimental Procedures). Each data point represents the mean ± SEM.
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is larger than the dendritic field of the PV1 cell, while the inhibitory input continues to increase with increasing spot diameter. This results in a smaller contribution of inhibition for small spots, but for large spots the contribution of inhibition is much larger, significantly decreasing the PV1 cell’s response. As far as the dynamics of the switch circuit, inhibition is delayed compared to excitation, because the switch cell needs time to reach spike threshold, while excitation from bipolar cells is modulated without a threshold. In a brief time window after stimulus onset, before the activation of the switch cell, excitation to PV1 cells shows a similar sharp increase in strength as the time-averaged inhibition. However, the time-averaged excitation does not show a stepwise increase at the critical light level because the switch cells also act at bipolar terminals and dampen the rise in excitation. Note that a chemical synapse is a complex nonlinear filter and therefore the shape and magnitude of excitation in a PV1 cell is probably not the same as the excitation experienced by the switch cell. This is important because excitation to switch cells has to be larger in switch-ON states than in switch-OFF states even at longer timescales, otherwise the switch would turn off. A quantitative model describing the circuit illustrates how the stepwise increase in the strength of inhibition toggles the weighting of center and surround interactions of the PV1 cell (Figures 7C, 7D, and S8).

A Perceptual Correlate of the Retinal Switch

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is there a perceptual correlate of the retinal switch, which toggles the balance of inhibition and excitation in large ganglion cell types of mice around the cone threshold? We investigated the transition of spatial integration properties of the human visual system across the rod only to rod-cone-mediated vision ranges by measuring the contrast sensitivity for gratings of different spatial frequencies (called contrast sensitivity function, Figure 8A) together with the color discrimination abilities at different background light levels of 16 human volunteers. Color discrimination was defined as the maximum of the contrast sensitivity function at a given light level, and a human spatial selectivity index (hSSI) was defined as the ratio between the contrast sensitivity at a given light level, and a human spatial sensitivity was defined as the maximum of the contrast sensitivity function at the lowest spatial frequency and the peak contrast sensitivity. We found that both the acuity and the peak contrast sensitivity increased continuously with increasing light levels (Figure 8B). However, the hSSI increased sharply as the background light intensity crossed a critical luminance threshold, dividing the curve into two regions (Figure 8C). This stepwise change corresponded to a sudden stop in the continuous increase in contrast sensitivity at low spatial frequencies (Figure 8A). The critical light level at which the hSSI increased in a stepwise manner corresponded precisely to the light level at which the volunteers could reliably discriminate between red and blue (Figure 8C). To test whether the sudden jump in hSSI is reversible, we measured the contrast sensitivity function as we increased and decreased the light level above and below the critical light level several times (Figure 8D). The hSSI reliably switched between the low and high values. Therefore, similar to switching on the inhibition in mice, a reversible stepwise change in hSSI corresponded to the light level at which cones are activated, suggesting that the switch circuitry we describe in the mouse is probably conserved in human vision.

DISCUSSION

A Neuronal Circuit Switch

By probing the receptive fields of identified retinal ganglion cells across light levels, we found that PV1 and PV6 cell types, two large ganglion cells, show a step-like change in their spatial integration properties, consistent with the activation of an inhibitory surround. We concluded that the luminance-dependent change in receptive fields of PV1 cells was caused by the activation of surround inhibition from wide-field spiking amacrine cells. The change showed characteristics of a switch: it occurred quickly,
Figure 6. Amacrine Cells Are Driven by Cone Bipolar Cells via Electrical Coupling

(A and B) Responses of bipolar cells, measured under voltage clamp at −60 mV in slice preparation (A, rod bipolar cells; B, cone bipolar cells) to the presentation of full-field stimuli across five log units of light intensity. The Michelson contrast at each light level was 0.9993. Traces are averages across six (A) and five (B) recorded cells, respectively.

(C) Cone bipolar cells become highly active at the critical light level that activates the switch. Black, rod bipolar (RB) cell responses; gray, cone bipolar (CB) cell responses.

(D) Inhibitory input to PV1 cells in the presence of CPP and NBQX. At light levels below 13 R*/s, no inhibitory current is seen in PV1 cells. At light levels of 13 R*/s and brighter, a strong current appears.

(legend continued on next page)
happened at a critical input level, and could be toggled between two distinct states. The critical light level that activated the switch corresponded to light levels at which cone bipolar cells showed a stepwise increase in their responses.

Is the stepwise increase in cone bipolar cell responses a result of the activation of cones or, alternatively, an increase in the response of rods? In the first of these two situations, rod responses are saturated or close to saturation at the critical light level. Therefore, it is the activation of cones that leads to the sudden change in cone bipolar activity. In the second, cones are not yet activated and it is an increase in rod activity acting via rod-cone electrical coupling (DeVries and Baylor, 1995) that leads to the stepwise increase in cone bipolar cell responses.

We made four relevant observations to differentiate between these two scenarios. First, rod bipolar cells, which are driven by rods, are fully activated at light levels below the critical level (Figure 6A). Second, cone bipolar cells are not responsive at light levels below the critical light level including those levels at which rod bipolar cells have reached saturation (Figure 6B). Third, the sustained part of the rod bipolar response, which could not have reached saturation since there were larger responses recorded, decreases at the critical light level (Figure 6C). Fourth, there is only one major increase in the responses of cone bipolar cells across the broad range of intensities tested (Figure 6D).

The activation of cones at the critical light intensity is consistent with these four observations. This interpretation is further supported by the fact that the critical light intensity is within the range reported to activate cones in mice (Nathan et al., 2006; Umino et al., 2008).

The second situation invokes a saturating nonlinearity between rods and rod bipolar cells, as well as a threshold nonlinearity between cones and cone bipolar cells. This model could also account for the first two observations listed above. However, the last two observations are hard to reconcile with this interpretation. The measured decrease in the sustained part of the rod bipolar cell’s response suggests that rod response decreases when the light level is stepped to the critical level. Furthermore, if we assume that it is not the activation of cones that leads to the stepwise increase in cone bipolar responses, then we expect to find a second major increase in the responses of cone bipolar cells when cones are activated at higher light levels. However, our recordings do not show such an increase.
Based on these observations, together with a previous finding that rod-cone coupling in mice is weak during the day when our recordings were performed (Ribelayga et al., 2008), we favor the explanation that the stepwise increase in cone bipolar responses, which leads to switch-ON state, is due to the activation of cones.

In our view, rod activity provides, through the rod-rod bipolar and possibly the rod-cone coupling pathways (Bloomfield and Dacheux, 2001), a constant level of activation at the light levels around the switch. This constant activation, together with the addition of cone activity, enables the combined drive to reach the threshold of amacrine cells. When connexin36 is not present, rod activity does not contribute to the activity of cone bipolar terminals. This may explain the reduced PV1 cell spiking activity at the critical intensity in connexin36 knockout animals. The relative weight of the different rod pathways, which is different in different species (Protti et al., 2005), as well as during day and night (Ribelayga et al., 2008), has probably little influence on the switch since these pathways converge at the cone bipolar terminals.

As one moves from dim to bright environments, adaptive mechanisms in the retina play an active role in enabling vision to continuously function. These mechanisms include adaptive changes in specific synaptic and cell signaling pathways and have been shown to regulate retinal sensitivity depending on the light level (Fain et al., 2001; Green and Powers, 1982; Ichinose and Lukasiewicz, 2007; Pugh et al., 1999; Shapley and Enroth-Cugell, 1984). One form of adaptation is the luminance-dependent changes in electrical coupling between specific cell types including horizontal cells, All amacrine cells, and ganglion cells (Bloomfield and Völgyi, 2004; DeVries and Schwartz, 1989; Hu et al., 2010; Mangel and Dowling, 1985; Ribelayga et al., 2008; Xin and Bloomfield, 1999). Many of these luminance-dependent changes have been associated with light-dependent changes in dopamine release in the retina (Lasater, 1987; Mills and Massey, 1995; Witkovsky, 2004). We found no role for dopamine in effecting the switch of spatial integration properties of the PV1 cell. Instead, we show that the surround of PV1 cells is dependent on the presence of electrical coupling mediated by connexin36. The results of the connexin36 knockout and pharmacology experiments in this work, together with a previous finding that some ON cone bipolar cells express connexin36 (Siegert et al., 2012), suggest that some ON cone bipolar cells are electrically coupled to amacrine cells other than just All (Deans et al., 2002). Our data are consistent with the implementation of a circuit switch that uses a threshold mechanism to turn on and off the antagonistic surround of PV1 cells depending on the strength of the stimulus. Although the proposed circuitry incorporates electrical coupling, it does not rely on adaptive mechanisms affecting the strength of the electrical coupling.

The Relationship between the Retinal and Perceptual Switch

The luminance effects on visual perception of spatial patterns show the same trends in mice, humans, cats, and monkeys.
ganglion cells (Bisti et al., 1977; Dedek et al., 2008; Enroth-Cugell, 1981; Umino et al., 2008; van Nes et al., 1967). With increasing stimulus luminance, contrast sensitivity at each spatial frequency decreases, while peak sensitivity and acuity shift toward higher spatial frequencies. In addition, the relative sensitivity to low spatial frequencies decreases with increasing stimulus intensity (Barlow, 1958; De Valois et al., 1974; Pasternak and Merigan, 1981; Umino et al., 2008; van Nes et al., 1967). While our study agrees with previous reports in regard to the continuous increase in peak sensitivity and acuity, we noted a discontinuous change in the preference for medium low spatial frequencies. This discontinuity occurred at the same light level as the ability to discriminate color and, therefore, at the threshold of cones.

There are similarities between the luminance-dependent changes in the contrast sensitivity of observers and the neuronal responses of the cells in retina. In particular, the corresponding changes in shape of the contrast sensitivity functions of retinal ganglion cells (Bisti et al., 1977; Dedek et al., 2008; Enroth-Cugell and Robson, 1966) and perception (De Valois et al., 1974; Pasternak and Merigan, 1981; Umino et al., 2008; van Nes et al., 1967). Visual spatial processing is thought to be organized into a series of parallel, independent channels in which each is tuned to a different spatial frequency (Blakemore and Campbell, 1969; Watson et al., 1983). In the retina, we found that large, but not small, ganglion cells showed changes in receptive field structure at the critical light level. This could explain the discontinuous increase in contrast sensitivity at low spatial frequencies if these low-frequency channels start specifically with large ganglion cells.

**Potential Benefits of the Switch**

In dim environments, it is necessary to gather as many photons as possible in order to detect objects of interest, while in bright condition one needs to discriminate between objects from the flood of thousands to millions of photons. We found that the change in spatial integration properties occurs only in select ganglion cell types, and occurs over a small luminance change. In light of these findings, we ask why do large ganglion cell types lose their antagonistic surround, and what benefit might the switch-like change in receptive field structure convey for the individual cell, as well as for the mosaic as a whole?

As for the individual cell, we showed that the luminance-dependent changes in the organization of the receptive fields of two large cells (PV1 and PV6) switched at a critical light level, while that of two smaller cells (PV0 and PV2) did not. For some cells, the loss of inhibitory input would eliminate the fundamental response properties that define their function. For example, direction-selective ganglion cells are unable to discriminate direction when their inhibitory inputs are blocked (Caldwell et al., 1978; Fried et al., 2002). For small ganglion cells with center-surround receptive fields, an increase in integration area may not be a significant advantage. However, ganglion cells with large receptive field areas are well designed to detect objects when the photon count is low (low acuity, high sensitivity). For large cells, a loss of antagonistic surround would increase the area from which they could gather photons, making the cell more sensitive to photons arriving within their receptive field. Interestingly, one type of faintly melanopsin-positive cell, M4, has a morphology that is similar to PV1 cells (Ecker et al., 2010; Estevé et al., 2012). If the two cell types are indeed the same, an intriguing possibility is that during evolution, a class of melanopsin cells acquired input from a special type of wide-field amacrine cell that conferred to it new spatial processing properties.

The loss of antagonistic surround may also have benefits for the mosaic as a whole. The contrast sensitivity of the rod pathways is thought to be lower than that of the cone pathway. This leads to a sparser encoding of the visual scene in low light levels forming contiguous blank neuronal representations in the rod pathways. An increased overlap between neighboring cells’ receptive fields would allow the ganglion cell mosaic to interpolate between neighboring high-contrast features (Cuntz et al., 2007; Seung and Sompolinsky, 1993). This difference in contrast sensitivity between rod and cone pathways may explain why the transition between the two circuit states is switch-like and not continuous.

We found that the change in spatial integration properties of PV1 cells occurs over a small luminance change (0.07 log unit), as compared to the more than three log unit range of intensities typical of many natural scenes (Geisler, 2008; Mante et al., 2005; Rieke and Rudd, 2009). In addition, the spatial integration properties of the PV1 cell could be toggled quickly as the light level was switched above and below the threshold light level. The circuit we propose would allow each ganglion cell of a single mosaic to individually set their spatial integration properties instantaneously, depending on the local luminance level of the scene. This would allow the mosaic to multitask in a spatially structured manner, simultaneously performing different computations in separate portions of the visual field.

**EXPERIMENTAL PROCEDURES**

**Animals**

Mice used in our experiments included PvalbCre x ThyStp-EYFP, PvalbCre x Ai9, and mice in which the Cx36+/− alleles were crossed into PvalbCre x ThyStp-EYFP so that PV1 cells were labeled in a homozygous Cx36+/− background.

**Preparation of Retinas**

Retinas were isolated from mice that had been dark adapted for 2 hr. Retina isolation was done under infrared illumination in Ringer’s medium. The retinas were then mounted ganglion cell-side up on filter paper that had an aperture in the center and were superfused in Ringer’s medium at 35°C–36°C for the duration of the experiment.

**Electrophysiology and Pharmacology**

The spiking responses of PV1 cells were recorded using the patch-clamp technique in loose cell-attached mode. Current recordings were made in whole-cell voltage-clamp mode. During voltage-clamp recordings, excitatory and inhibitory synaptic currents were separated by voltage clamping the cell to the equilibrium potential of chloride (−60 mV) and unselective cation channels (0 mV), respectively. Voltage recordings were made in whole-cell current-clamp mode; bipolar cells were recorded in whole-cell voltage-clamp configuration, at −60 mV in 200-μm-thick slices.

**Analysis of Physiological Data**

The firing rate of a neuron was calculated by convolving spike trains with a Gaussian kernel with an SD of 25 ms. For voltage-clamp recordings, the response to a light stimulus was calculated by taking the mean current during the first 0.5 s after stimulus onset. The early excitatory responses

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were calculated by taking the mean current between 50 and 150 ms after stimulus onset.

**Monosynaptically Restricted Circuit Tracing**

Two different strategies were used to achieve monosynaptic restriction of virus infection: one used a combination of G-deleted rabies virus encoding mCherry with conditional, rabies G-expressing replication-defective herpes simplex virus-1 (HSV1); the second used a conditional, rabies G-expressing adeno-associated virus (AAV) instead of the HSV1. In the herpes/rabies combination strategy, we injected the superior colliculus or the lateral geniculate with a cocktail of rabies virus and HSV1. In the second strategy, AAV particles were injected into the vitreal space of both eyes. Six days later, rabies virus was injected into the superior colliculus or the lateral geniculate nucleus (LGN). Anatomical tracing of labeled cells was done on a large, stitched three-dimensional (3D) image stack big enough to capture the PV1 and the wide-field cells. We created a 3D reconstruction of a 2.08 x 2.08 mm piece of retina around a PV1 cell, by creating 144 confocal image stacks with 10% overlap. We identified contact points with the PV1 cell within this image and confirmed each contact point using a higher-resolution reconstruction around each contact point. The x and y pixel widths for this higher resolution were 27 nm and the z step was 166 nm. We went back to the original large image stack and traced each cellular process that contacted the PV1 ganglion cell dendrite back to their cell bodies, and then we further traced all the processes that emerged from those cell bodies.

**Psychophysical Experiments**

In order to assess the spatial integration properties of human vision at different light levels we measured the contrast sensitivity function (CSF) of human volunteers at five different light levels after a period of 2 hr of dark adaptation. To measure the CSF of each volunteer, we determined the minimum contrast at which a Gaussian-windowed vertical sinusoidal grating could be detected. The hSSI was defined as the ratio between the contrast sensitivity at the lowest spatial frequency and the peak contrast sensitivity. The color discrimination task consisted of a forced choice paradigm, in which volunteers were presented two rectangles, one red and the other blue, and had to decide which one was red. The psychophysical experiments were performed according to institutional guidelines.

**Statistical Analysis**

All measures of statistical difference were performed using a Mann-Whitney U test. In the figures, statistical significant difference is indicated for p values less than *p < 0.05, **p < 0.01, and ***p < 0.001, respectively. All data points represent mean ± SEM. The “n” in the figures refers to the number of different cells included for retinal recordings, or in the case of human experiments, the number of individuals.


**SUPPLEMENTAL INFORMATION**

Supplemental Information includes eight figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.02.014.

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