Supplemental Information

Ambient Illumination Toggles

a Neuronal Circuit Switch in the Retina

and Visual Perception at Cone Threshold

Karl Farrow, Miguel Teixeira, Tamas Szikra, Tim J. Viney, Kamill Balint, Keisuke Yonehara, and Botond Roska
Figure S1. Morphology of PV ganglion cell types, related to Figure 2.

A. *Pvalb*<sup>Cre</sup> × *Thy1<sup>GFP</sup>* retina

B. Morphology of PV cells

C. Quantification of morphology
Figure S1. Morphology of PV ganglion cells. A. Left, in vivo fluorescent image of the eye of a $Pvalb^{Cre} \times Thy^{Stp-EYFP}$ mouse. Right, ex vivo fluorescent image of a whole mount retina from the same mouse line. B. Examples of morphologies of PV cells from each PV cell type. Top panels show maximum image projections of confocal image stacks of neurobiotin filled cells. The two bottom panels show side views highlighting the stratification of dendrites. (dendrites, white; ChAT cells, magenta). Red and yellow lines indicate the position and range within the stack. C. Quantification of the morphology. Left, histogram of the number of confocal microscopy reconstructed cells of each type in our data set. Right, scatter plot of each cell’s dendritic stratification in the inner plexiform layer versus dendritic area. The depth of dendritic stratification was determined using a procedure described before (Munch et al., 2009). Each shaded ellipse spans one standard deviation along each axis. The proximal and distal ChAT bands are defined as 0% and 100% depth.
Figure S2. Details of visual stimuli, related to Figure 1.

A. Normalized emission spectrum of the DLP projector (filled black) used for mouse retina experiments overlaid on the normalized absorption spectra of mouse photoreceptors (Lyubarsky et al., 1999) (black: rod; green: green cone; blue: UV cone).

B. Spectrum of absorbed light for each photoreceptor type.

C. Number of photons per photoreceptor absorbed by each photoreceptor type at the different light levels.

D. Contrast of the stimulus measured across different light levels. The Michelson contrast was 0.9993.

E-G. The
normalized absorption spectrum of rods is overlaid with the emission spectrum of infrared illumination used to visualize cells during experiments (E); to navigate around setup (F), and to dissect the retina (G). These three light sources caused a maximum illumination corresponding to 0.1, 1.2 and 0.05 R*/s.

H. Spectrum of DLP projector (filled black) used for human psychophysics experiments overlaid on absorption spectra of human photoreceptors (black: rod; blue: blue cone; green: green cone; red: red cone).

I. Comparison of light levels used in our experiments for humans and mice with the light levels used by Hood and Finkelstein (1986) (Hood and Finkelstein, 1986) for quantifying human visual performance. Black: light levels when rods are operational; yellow: when cones are operational.
Figure S3. Light pathways for visual stimulation, two-photon imaging, and IR visualization. A, B. Layout of the two-photon microscope. A ~920 nm laser line (red line) from a Mai Tai HP two-photon laser (Newport) was attenuated using polarization optics (Newport) and a Pockels cell (Conoptics, Model 302), and was scanned using mirrors (Cambridge Technologies) mounted on a modified Olympus microscope. The fluorescent signal emitted (green line) was detected with a photo multiplier tube (Hammamatsu, R3896). An infrared camera (Diagnostic Instruments, Spot RT) mounted on the microscope allowed the visualization of the retina during two-photon scanning. To visualize neurons labeled with fluorescent proteins or dyes the retina was illuminated through the condenser with IR light using a DLP projector (V332, PLUS) filtered with an infrared filter (750 +/- 25 nm; Chroma) while simultaneously recording the two-photon fluorescent images. The IR image and scanned two-photon image were fused for visualization. C. Maximum intensity top projection of a two-photon image stack showing EYFP-expressing cells in Pvalb\(^{Cre}\) \(\times\) Thy\(^{Sp}\)-EYFP retina. Top (D) and z-projection (E) of a two-photon stack of images showing an Alexa594 dye-filled PV1 cell. Scale bar on C-E 25 μm.
Figure S4. Response of PV1 cells in dim and bright environments, related to Figure 1.

A. Summary of flashing spot experiments

B. Summary of drifting grating experiments

C. Summary of white noise experiments

Figure S4. Summary of PV1 cell responses in dark and light. A. Summary of PV1 spiking responses. For response consistency the mean firing rate and the individual raster plots from a single experiment are shown. For the background firing rate, latency, peak response and steady-state response the average across all experiments are shown. For background firing rate red is 1000 μm spot, and black represents 400 μm spot. Latency, peak response and steady-state responses refer to 400 μm spot stimulations. Data is plotted as mean +/- sem. B. Summary of drifting grating experiments. Left, example of response of PV1 cell to drifting grating with a contrast of 0.4 and spatial wavelength of either 500 or 4000 μm. Continuous lines show the spike frequency of response. Vertical lines are recorded spikes. Right top, Fourier transform of responses to drifting gratings. Black curves are responses at .26 R*/s. Yellow curves responses at 110 R*/s. Right bottom, contrast sensitivity function of PV1 cell (left). Mean firing during presentation of 500 (black) or 4000 (red) μm gratings (right). C. Summary of spatio-temporal white noise experiments. Left, spatial profile of spike triggered average (STA) of PV1 cell at 0.26 R*/s. and at 110 R*/s. Right, STA of PV1 cells at 0.26 R*/s (black) and 110 R*/s (yellow), spatial profile and time course are shown.
Figure S5. Voltage clamp protocols and example data, related to Figure 4 and 6.

Figure S5. Voltage clamp analysis and examples of PV1 cell inhibitory current traces. A. Current recorded before, during and after the presentation of a spot while clamping the membrane potential of the neuron at different potentials. The light level was 110 R*/s. B. The pre-stimulus current is subtracted from the curves on A. Three time points are indicated by the colored lines. C. Currents from B are linearly fitted at three time-points during the light-response. The colors correspond to the time points indicated by vertical lines of the same color in B. D. The slope of the fitted lines at each time point, representing the stimulus-evoked conductance (g). E. The x-intercept of the fitted lines at each
time point, representing the reversal potential (Vr). 

F. Based on the inhibitory and excitatory reversal potentials of -60 and 0 mV, respectively, the inhibitory and excitatory conductances are separated. In G-L the stimulus was a 1000 mm spot presented at time zero, the first 1.5 s of the response is shown. Each trace is the average of 3 individual presentation of the stimulus from a single recording session. 

G. The inhibitory current recorded in the presence of CPP and NBQX at a light level of corresponding with the switch-OFF state of 0.26 R*/s. 

H. The inhibitory current recorded in the presence of CPP and NBQX at a light level of corresponding with the switch-ON state of 110 R*/s. 

I. The inhibitory current recorded in the presence of CPP, NBQX and Strychnine (Str) at a light level of corresponding with the switch-ON state of 110 R*/s. 

J. The inhibitory current recorded in the presence of CPP, NBQX and Picrotoxin (Pic) at a light level of corresponding with the switch-ON state of 110 R*/s. 

K. The inhibitory current recorded in the presence of CPP, NBQX and APB at a light level of corresponding with the switch-ON state of 110 R*/s. 

L. The inhibitory current recorded in a connexin36 knock out mouse (Cx36^-/-) at a light level of corresponding with the switch-ON state of 110 R*/s.
Figure S6. Monosynaptically restricted virus tracing, related to Figure 5.

A. Strategy 1 (left): G-deleted rabies virus is injected into either the LGN or the superior colliculus (two different projection sites of retinal ganglion cells), while the floxed-AAV (which conditionally expresses G) is injected into the eye where it can infect retinal ganglion cells directly. Strategy 2 (right): G-deleted rabies virus and floxed-herpes virus (which conditionally expresses G) are co-injected into either the LGN or superior colliculus.

B. We performed all viral tracing experiments in the Pvalb\textsuperscript{Cre} × Thy\textsuperscript{Sp-EYFP} or Pvalb\textsuperscript{Cre} × Ai3 mice, where a subset of ganglion cells are labeled green (left panel). If G-deleted rabies expressing a red fluorescent protein is used in a wild-type mouse we see red-labeled ganglion cells (center left panel). If G-deleted rabies is used in a Pvalb\textsuperscript{Cre} × Thy\textsuperscript{Sp-EYFP} or Pvalb\textsuperscript{Cre} × Ai3 mouse we see some ganglion cells labeled both green and red, here indicated as yellow (center right panel). If G is also
provided via either floxed-AAV or floxed-Herpes, we see yellow ganglion cells as well as red-labeled presynaptic amacrine cells. C. Example of transsynaptic tracing of PV1 cells. Maximum image projections of confocal stacks are shown. Left, green channel (Ai3 is the reporter mouse here) shows PV cell bodies. Middle, red channel showing rabies infected cells. Right, overlay of the two channels. Note that the PV1 cell is labeled by both mCherry from rabies and ZsGreen from the Ai3 mouse line, while the connected amacrine cell, A1, is only labeled by mCherry. Note that the processes of the amacrine cells are too thin to be visible at this scale. Scale bar is 50 μm.
Figure S7. Overview image of the viral tracing results, related to Figure 5.

Figure S7. **Overview image in a viral tracing experiment.** Maximum image projection of stitched confocal stacks used to trace connections from an example PV1 cell. The red channel, showing red fluorescent protein expressed by rabies is displayed. The image is flattened from 144 stitched image stacks. A PV1 cell and two connected amacrine cells are highlighted. The processes of amacrine cells are only faintly visible at this scale. Scale bar is 100 \( \mu m \).
Figure S8. Model of the switch, related to Figure 7.

A. Cell types in the circuit of PV1 cells. Electrodes point at positions in the circuit where responses were recorded.

B. Time constant and membrane resistance of PV1 cells.

C. Building blocks of the model.

D. The predictions of the model. The model is described in Experimental Procedures. Each point corresponds to the mean response of the first 500 ms after stimulus onset.
Supplemental Experimental Procedures

Animals

Mice used in our experiments included $P_{valb}^{Cre}\times Thy^{Sp-EYFP}$, $P_{valb}^{Cre}\times Ai9$, $P_{valb}^{Cre}\times Ai3$ and mice in which the $Cx36^{-/-}$ alleles were crossed into $P_{valb}^{Cre}\times Thy^{Sp-EYFP}$ so that PV1 cells were labeled in a homozygous $Cx36^{-/-}$ background. In $P_{valb}^{Cre}$ mice (Hippenmeyer et al., 2005), Cre recombinase is expressed under the control of the parvalbumin locus. In $Thy^{Sp-EYFP}$ mice (Feng et al., 2000), EYFP is expressed from a $Thy1$ promoter in those cells in which the transcriptional stop sequence has been removed by Cre recombinase. In Ai3 and Ai9 mice (Madisen et al., 2009), ZsGreen or tdTomato is expressed from the $CAG$ promoter in those cells where the transcriptional stop sequence has been removed by Cre recombination. $Cx36^{-/-}$ mice are homozygous knockouts for the electrical synapse protein connexin36 (Deans and Paul, 2001). All animal procedures were performed in accordance with standard ethical guidelines (European Communities Guidelines on the Care and Use of Laboratory Animals, 86/609/EEC) and were approved by the Veterinary Department of the Canton of Basel-Stadt.

Preparation of Retinas

Retinas were isolated from mice that had been dark-adapted for 2 hours. Retina isolation was done under infrared illumination in Ringer’s medium (110 mM NaCl, 2.5 mM KCl, 1 mM CaCl2, 1.6 mM MgCl2, 10 mM D-glucose, 22 mM NaHCO3, bubbled with 5% CO2/95% O2, pH 7.4). The retinas were then mounted ganglion cell-side up on filter paper (Millipore) that had a four mm wide rectangular aperture in the center, and superfused in Ringer’s medium at 35–36°C in the microscope chamber for the duration of the experiment. The infrared light used for dissection had its peak power at 850 nm (Figure S2). This resulted in an effective absorption by rods of 0.05 photons absorbed per rod per second (R*/s). Red LED head lamps, with a peak power of 650 nm were used to navigate around the room, and at a distance of 10 cm caused an effective absorption of 1.21 R*/s (Figure S2).

Electrophysiology and Pharmacology

Electrophysiological recordings were made using an Axon Multiclamp 700B amplifier (Molecular Devices) and borosilicate glass electrodes (Sutter Instrument). Signals were digitized at 10 kHz (National Instruments) and acquired using software written in LabVIEW (National Instruments). Data were analyzed offline using MATLAB (MathWorks).
The spiking responses were recorded using the patch clamp technique in loose cell-attached mode with electrodes pulled to between three and five MΩ resistance and filled with Ringer’s medium.

Current recordings were made in whole-cell voltage clamp mode, with electrodes pulled to between five and eight MΩ resistance and filled with 112.5 mM CsCH₃SO₃, 1 mM MgSO₄, 7.8 × 10⁻³ mM CaCl₂, 0.5 mM BAPTA, 10 mM HEPES, 4 mM ATP-Na₂, 0.5 mM GTP-Na₃, 5 mM lidocaine N-ethyl bromide (QX314-Br), 7.5 mM neurobiotin chloride. The pH was adjusted to 7.2 with CsOH. The reversal potential for chloride (E Cl) was calculated to be ~ -60 mV. 13 mV was subtracted from all voltages to correct for the disappearance of the liquid junction potential upon establishing the whole-cell recording. Series resistances of between 10 and 25 MΩ were corrected for offline. Excitatory currents were recorded while holding cell at -60 mV and inhibitory currents were recorded while clamping the cell at 0 mV.

Voltage recordings were made in whole-cell current clamp mode, with electrodes pulled to between five and eight MΩ resistance and filled with 115 mM K gluconate, 1.95 mM KCl, 1 mM MgCl₂·6H₂O, 0.5 mM CaCl₂, 1.5 mM EGTA, 10 mM HEPES, 4 mM ATP-Na₂, 0.5 mM GTP-Na₃ and 7.5 mM neurobiotin chloride. In order to visualize the neurons, in some experiments, either Alexa Fluor 488 or 594 was added to the intracellular solution listed above.

In pharmacological experiments, agents were bath-applied at the following concentrations: 10 μM CPP, 10 μM NBQX, 10 μM APB, 10 μM strychnine, 100 μM picrotoxin. All chemicals were obtained from Sigma-Aldrich, with the exception of APB (Calbiochem), ATP (Labforce), neurobiotin (Vector Laboratories) and Alexa Fluor 488 and 594 (Molecular Probes).

**Bipolar Cell Recordings**

Retinas were mounted ganglion-cell down on filter paper (Millipore). 200 μm thick slices were prepared using a tissue chopper (Stoelting) under infrared illumination. Slices were transferred into a custom-made recording chamber. Bipolar cells were recorded in whole cell voltage clamp configuration, at -60 mV.

**Analysis of Physiological Data**

The firing rate of a neuron was calculated by convolving spike trains with a Gaussian kernel with a standard deviation of 25 ms. 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. Only recordings with a series resistance between 10 and 25 MΩ were used. For voltage clamp recordings, the response to a light stimulus (Figure 4D and E, Figure 6D, E and F) was calculated by taking the mean current during the first 0.5 s after stimulus onset. The early excitatory responses (Figure 6I and J) were calculated by taking the mean current between 50 and 150 ms after stimulus onset.

The spatial integration properties of ganglion cells were evaluated either by comparing their spiking response during the presentation of a spot with the size of the individual cell type’s dendritic field (400 μm for PV1 and PV6 cells; 250 μm for PV2 cells), and the response during the presentation of a spot of 1000 μm such that:

\[
\text{spatial selectivity index (SSI)} = \frac{\text{Response}_{\text{Small}} - \text{Response}_{\text{Large}}}{\text{Response}_{\text{Small}} + \text{Response}_{\text{Large}}}
\]

where the Response was defined as the number of spikes during stimulation. Response is a variable that takes non-negative integers.

Or, during the presentation of drifting gratings, the SSI was calculated by comparing the magnitude of the first harmonic (F1) of the spike frequency response to drifting grating with a temporal frequency of 0.5 Hz and spatial wavelength of either 500 or 4000 μm such that:

\[
SSI = \frac{F1_{500} - F1_{4000}}{F1_{500} + F1_{4000}}
\]

where F1 was calculated by computing the Fourier transform of the spike frequency, and determining the magnitude of the Fourier transform at the temporal frequency of the stimulus. The Fourier transform was calculated using the fast Fourier algorithm in Matlab.

The direction selectivity of a ganglion cell was quantified as the vector sum of the spiking response (number of spikes) of the cell to a bar moving in eight different directions. The Direction Selectivity Index (DSI) is defined as:

\[
DSI = \sum_d \frac{\dot{N}_d}{R_d}
\]
where $N_d$ are vectors pointing in the direction of the stimulus and have a length of $R_d$.

**Targeted Recordings using Two-Photon Microscopy**

Fluorescent cells were targeted for recording using a two-photon microscope equipped with a Mai Tai HP two-photon laser (Spectra Physics) integrated into the electrophysiological setup (Figure S3). To facilitate targeting, two-photon fluorescent images were overlaid on the IR image acquired through the CCD camera. Infrared light was produced using the light from a projector equipped with a digital light processor (DLP) and a 750 +/- 25 nm filter. The resulting light absorbed by the retina corresponded to 0.11 R*/s (Figure S2). In order to target PV1 cells in $Pvalb^{cre} \times Thy^{Sp-EYFP}$ and $Pvalb^{cre} \times Ai9$ we used two anatomical criteria, the size of the cell body and the stratification of the dendrites. In the $Pvalb^{cre}$ mouse line we find three cell types labeled with large cell bodies of >20 µm. Of these, one has dendrites that stratify in the ON lamina. Specifically, the dendrites of the PV1 cell lie between the ganglion cell layer and the proximal dendrites of ON-OFF direction selective cells (PV0). The two strata where the dendrites of ON-OFF direction selective cells stratify were brightly labeled in the $Pvalb^{cre} \times Thy^{Sp-EYFP}$ and $Pvalb^{cre} \times Ai9$ mice. Among the PV cells, PV1 is the only one that arborizes between the proximal dendritic trees of ON-OFF direction selective cells and the ganglion cell layer.

In order to target other cell types in these mouse lines, an image stack was obtained with the two-photon microscope previous to patch-clamp recording. The cells were then targeted based on the size of their cell body and the characteristic morphology of their dendritic trees. The monostratified cells are named in the order in which their dendritic trees terminate in the inner plexiform layer, where the PV1 cells arborize closest to the ganglion cell layer and PV6 cells closest to the inner nuclear layer. PV2 stratified distal to the proximal PV0 labeled strata. See a detailed description of the physiology and morphology of all PV cells in Figure 2 and S1, respectively.

**Visual Stimulation**

Stimuli were generated with a DLP projector (PLUS) at a refresh rate of 75 Hz, controlled with custom software written in MATLAB. The projector produced a light spectrum (Figure S2) that ranged from ~400 nm to ~720 nm. The power produced by the projector was 229 mW/cm² at the retina (Figure S2). Neutral density filters were used to control the stimulus intensity in logarithmic steps. This allowed us to maintain constant contrast at each light level. We calculated contrast as the Michelson contrast:

$$\text{Contrast}_{Michelson} = \frac{\text{Luminance}_{max} - \text{Luminance}_{min}}{\text{Luminance}_{max} + \text{Luminance}_{min}}.$$
For spot stimulations the Michelson contrast was 0.9993, at each light intensity. In all experiments using a spot stimulus the contrast was kept constant, we only changed the mean illumination by neutral density filters. The light intensity of the stimulus, rather than the background, is shown on the figures. The reason for this is that there are conditions when the background is below cone threshold but the stimulus is above cone threshold, and since the switch is instantaneous, this stimulus turns on the switch. When grating stimuli are used the maximum intensity of the grating is shown for the same reason. We express light intensity in photoisomerizations per rod per second (R*/s). Light intensity was measured with a photodiode power meter (Thorlabs), and the spectrum was measured with a spectrometer (Ocean Optics). The photoisomerization rate was computed based on the absorption spectrum of the photoreceptors (Lyubarsky et al., 1999), and a collecting area of 0.5 \( \mu \text{m}^2 \) for rods and 0.2 \( \mu \text{m}^2 \) for cones (Nikonov et al., 2005; Nikonov et al., 2006). The range of light intensities present at the retinal surface, ranged from 0.006 to 1.03 \( \times 10^7 \) R*/s (Figure S2). This range covers the reported ranges of rod and cone visual function in mice (Nathan et al., 2006; Sampath et al., 2005; Umino et al., 2008).

White noise stimuli consisted of a central spot and 8 concentric annuli that were independently assigned a random luminance value each frame, which was drawn from a Gaussian probability distribution with a mean intensity and a standard deviation, such that the Michelson contrast was 0.25. The central spot was 200 \( \mu \text{m} \) in diameter. The concentric annuli had inner diameters of 200, 400, 600, 800, 1000, 1200, 1400 and 1600 \( \mu \text{m} \) and were each 200 \( \mu \text{m} \) thick. The stimulus consisted of 7500 frames and was shown at a frame rate of 15 Hz.

**Classification and Types of Ganglion Cells in PV Mice.**

In \( Pvalb^{Cre} \times Thy^{Ssp-EYFP} \) mice we encountered eight morphological types of ganglion cells (Figure S1). These are named PV0-PV7.

Quantitative morphological classification was based on the depth of dendrites in the inner plexiform layer using the ChAT marked strata as rulers (0 and 100\%) and the area occupied by the dendrites (Manookin et al., 2008; Munch et al., 2009). See Figure S1 for quantitative definition of morphological types. Qualitatively, PV0 cells were bistatified costratifying with the ChAT strata. The rest of cells were monostratified. Proximal from the proximal-ChAT strata are PV1 cells. Just distal from the proximal-ChAT strata are PV2 cells. In between the two ChAT strata are PV3 cells. Just proximal from the distal ChAT strata are the PV4 and PV5 cells. PV4 cells have smaller dendritic area than PV5
cells. PV6 and PV 7 cells are distal from the distal ChAT strata. PV7 cells have smaller dendritic area than PV6 cells.

**Confocal Analysis**

Stained retinas were imaged with a Zeiss LSM 700 confocal microscope. Filled ganglion cells were imaged using a 20x air (NA 0.7) and, a 40x oil immersion (NA 1.2) lens. The mCherry-labeled circuits of PV-positive ganglion cells were imaged with a Zeiss LSM 710 confocal microscope using a 63x (NA 1.4) oil immersion lens. Reconstructions of neurons were made in Neurolucida (MBF Bioscience) and TrackEM2 (ImageJ).

**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 (SADΔG-mCherry) with conditional, rabiesG-expressing replication-defective herpes simplex virus-1 (HSV1); the second used a conditional, rabiesG-expressing adeno-associated virus (AAV) instead of the HSV1 (Figure S6).

G-deleted rabies virus encoding mCherry (Marshel et al., 2010) was supplied by E. Callaway. Rabies virus was harvested from BHK-B19G cells (provided by E. Callaway) and centrifuged (Wickersham et al., 2010).

To create HSV1-EF1a-LoxP-STOP-LoxP-G-2A-EGFP, the EGFP open reading frame (ORF) in the HSV1 vector pR19EF1a-EGFP-WCm (Biovex) was replaced with a sequence of loxP-STOP-loxP followed by the ORF of rabiesG-2A-EGFP. First, the sequence of LoxP sites and G-2A-EGFP ORF was synthesized (DNA2.0) with a combination of EcoR I-BsrGI restriction sites in the extremities. The sequence of rabiesG was taken from pHCMV-RabiesG (Sena-Esteves et al., 2004). The EGFP ORF was removed from pR19EF1a-EGFP-WCm by EcoRI/BsrGI digestion and the synthesized fragment of LoxP-STOP-LoxP-G-2A-EGFP was subcloned into the EcoRI–BsrGI site.

Recombinant AAVs (serotype 7, BIOVEX) were made from a backbone of the vector AAV-EF1a double floxed-hChR2(H134R)-EYFP-WPRE-hGHpA (provided by K. Deisseroth), using the NheI-AscI site; the ChR2 and EYFP ORF was substituted by the ORF of rabiesG taken from pHCMV-RabiesG (Sena-Esteves et al., 2004) using an in-fusion PCR kit (TAKARA). Titer determination was made using real-time PCR (titer: $5.78 \times 10^{12}$ genome copies per ml, determined using real-time PCR).
In the herpes/rabies combination strategy we performed stereotaxic surgery in $P_{valb}^{Cre} \times Thyl^{Sp- EYFP}$ or $P_{valb}^{Cre} \times Ai3$ mice to label ganglion cells projecting to the superior colliculus and the lateral geniculate nucleus (LGN). A cocktail of $10^3$ plaque-forming units of rabies virus and $6 \times 10^4$ plaque-forming units of HSV1 in 20 nl Dulbecco’s modified eagle medium (DMEM) were loaded into pulled-glass pipettes (tip inner diameter of 20–30 μm) and injected into the superior colliculus or LGN using a microinjector (Narishige). Note that LGN infection resulted in many more PV1 cells. In the second strategy AAV particles (1.5μl, $8.68 \times 10^9$ GC) were loaded into pulled glass pipettes and injected into the vitreal space of both eyes of $P_{valb}^{Cre} \times Thyl^{Sp- EYFP}$ or $P_{valb}^{Cre} \times Ai3$ mice. Six days later $10^3$ plaque-forming units of rabies virus was injected into the superior colliculus or the LGN to label ganglion cells projecting to them. Again, LGN infection yielded many more PV1 cells. All rabies, AAV and HSV1 work was carried out under Biosafety level 2 conditions.

The goal of these experiments was to initiate retrograde passage of rabies from PV ganglion cells. Since the conditional AAV or herpes viruses only express rabiesG in Cre-positive ganglion cells, only this subset of ganglion cells are able to infect presynaptically connected cells.

The morphological characterization of wide-field amacrine cells specifically connected to PV1 cell, takes ~1 month for each PV1 cell and we reconstructed three examples of PV1 circuits. With the current tracing protocol (Herpes + rabies from Cre cells, or AAV + rabies from Cre cells), the probability of virus transsynaptic transfer from adult ganglion cells is low, therefore one encounters a number of PV1 cells that are rabies infected but the rabies did not pass to any of the circuit elements.

The practical limitation for reconstructing ganglion-wide-field cell circuits is that the processes of wide-field cells are thin and long (>1 mm) and therefore neither large field/low numerical aperture (NA) nor small field/high NA objectives are capable of capturing the processes of the wide-field cells. In searching for connectivity between wide-field and PV1 cells we performed the following four steps.

The first step was to create a large, stitched 3D image stack that was big enough to capture the PV1 and the wide-field cells (Figure S7). We created a 3D reconstruction of a 2.08 × 2.08 mm piece of retina around a PV1 cell, by creating 144 confocal image stacks with 10% overlap that tile the 2.08 × 2.08 mm retinal space. Each stack has x=1024, y=1024, z=215 pixels, where the size of the z step is 330 nm and the x and y pixel width is 188 nm. The objective has 63x magnification and 1.4 NA. We then stitched these stacks together to create a single 3D digital image of the selected piece of retina.
(11060 × 11060 × 215 pixels). We scanned through every PV1 dendrite to look for thin processes that contacted the dendrites. We identified contact points.

The second step was to confirm each contact point at a higher resolution. We created 3D reconstruction areas around each contact point at higher resolution (see Figure 5 B-D). The x and y pixel widths for this higher resolution were 27 nm and the z step was 166 nm. The size of the digital stack was 2048 × 2048 × 45 pixels.

The third step was the reconstruction of the morphology of the PV1-connected cells. We went back to the original large image stack and traced every 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. Using this procedure we obtained the image as shown in Figure 5A.

The fourth step, which was key to showing connection specificity, was to check if any of the amacrine cells connected to PV1 cells may also contact other PV cells. We walked through all the processes of the PV1-connected amacrine cells to look for connectivity to other PV cells. We accepted that an amacrine cell was specifically connected to PV1 cells if it did not contact any other PV cells. Note that with our current tracing protocol this final step is, in practice, only feasible for displaced amacrine cells connected to PV1 cells (note that the wide-field cells we found connected to PV1 cells were displaced cells) and for amacrine cells which had cell bodies in the inner nuclear layer that were connected to PV6 or PV7 cells. The reason for this is the following: PV1 dendrites occupy the most proximal strata of all PV cells (see Figure S1). If a displaced wide-field amacrine cells is connected to a PV1 cell, it does not cross strata where other PV cell dendrites arborize, and therefore it is relatively easy to rule out connections to other PV cells. The same argument holds for PV6- or PV7-connected amacrine cells that have cell bodies in the inner nuclear layer, since PV6 and PV7 cell dendrites occupy the most distal strata among all PV cells. For other selected PV cells, such as PV3 for example, the processes of both displaced and non-displaced amacrine cells have to pass through strata, which are populated by labeled dendrites of PV cells, which are not PV3. Ruling out connection of an amacrine process to ganglion dendrites as they pass through a retinal layer is only feasible in a few cases.

**Immunohistochemistry**

After the experiments, the retinas were fixed for 30 min in 4% paraformaldehyde in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, and 1.47 mM KH₂PO₄, pH7.4), then washed in PBS for a minimum of one day at 4°C. To aid penetration of the antibodies, retinas were frozen and thawed three
times after cryoprotection with 30% sucrose. All other procedures were carried out at room
temperature. After washing in PBS, retinas were blocked for 60 minutes in 10% normal donkey serum
(NDS), 1% bovine serum albumin (BSA), and 0.5% Triton X-100 in PBS. Primary antibodies were
incubated for seven days in 3% NDS, 1% BSA, 0.02% sodium azide and 0.5% Triton X-100 in PBS.
Secondary antibodies were incubated for 90 minutes in 3% NDS, 1% BSA, 0.02% sodium azide and
0.5% Triton X-100, in PBS. After a final wash in PBS, retinas were embedded in ProLong Gold
Antifade (Molecular Probes).

The following set of primary and secondary antibody combinations were used in experiments in which
we recorded from PV-positive ganglion cells labeled with tdTomato from the Ai9 reporter line.
Primary: goat anti-ChAT and rabbit anti-red fluorescent protein (Millipore). Secondary: donkey anti-
goat IgG conjugated with Alexa Fluor 633; donkey anti-rabbit IgG conjugated with Cy3 (Jackson
ImmunoResearch Laboratories, PA); streptavidin-Alexa Fluor 488 and 4’ ,6-diamidine-2-phenylindole
dihydrochloride (DAPI, Roche Diagnostics). Rabbit anti-red primary antibody binds to tdTomato.

The following set of primary and secondary antibodies combinations were used in experiments in
which we recorded from PV ganglion cells labeled with EYFP from the Thy1Stp-EYFP line. Primary: goat
anti-ChAT, and rat anti-GFP (Nacalai Tesque). Secondary: donkey anti-goat IgG conjugated with
Alexa Fluor 633; donkey anti-rat IgG conjugated with Alexa Fluor 488; streptavidin-Alexa Fluor 568
and DAPI. Rat anti-GFP also binds EYFP.

The following set of primary and secondary antibodies combinations were used in experiments in
which we recorded from PV ganglion cells labeled with ZsGreen from the Ai3 reporter line. ZsGreen
was bright enough to detect cell bodies without antibody labeling. Primary: goat anti-ChAT.
Secondary: donkey anti-goat IgG conjugated with Alexa Fluor 633; streptavidin-Alexa Fluor 568 and
DAPI.

The following set of primary and secondary antibodies combinations were used for staining mCherry
expressing rabies virus-infected retinas. Primary: goat anti-ChAT, rabbit anti-RFP and rat anti-GFP.
Secondary: donkey anti-goat IgG conjugated with Alexa Fluor 633, donkey anti-rabbit IgG conjugated
with Cy3 and donkey anti-rat IgG conjugated with Alexa Fluor 488. Rabbit anti-RFP also binds to
mCherry.
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 two hours 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. This is reported as contrast sensitivity (1/threshold). We repeated this test at spatial frequencies of 0.45, 1.14, 2.80, 4.60, 11.40, 22.80, and 45.60 cycles per degree (cpd) at mean luminance levels of 0.002, 0.02, 0.2, 2.0 and 22 cd/m².

The sine wave images were projected onto a screen with an LCD projector (Epson EH-TW3200) with a refresh rate of 60 Hz and a bit depth of 10 bits for each colour. The projector was enclosed in a light tight box and neutral density filters (Thorlabs) were used to control the brightness of the projected images in logarithmic steps. Stimuli were viewed binocularly with natural pupil at a viewing distance of three meters. The images were rendered on a 1280 × 1028 pixel grid, extending 43.6 × 35° of visual angle. The background luminance was set to the middle of the dynamic range of the display. For the CSF trials 30 possible gratings contrasts were spaced log-linearly from 0.1 to 99.5%. The stimulus sequence began with the presentation of the lowest contrast sinusoidal grating of a particular spatial wavelength. Subjects controlled custom-built software, written in Python, which allowed them to step through the different contrasts and determine their own contrast sensitivity for each grating presented. 11 naive observers and five of the authors participated in the experiment. All observers had corrected-to-normal vision. The human spatial selectivity index (hSSI) was defined as the ratio between the contrast sensitivity at the lowest spatial frequency and the peak contrast sensitivity:

\[
hSSI = \frac{\text{Sensitivity}_{Peak} - \text{Sensitivity}_{Low}}{\text{Sensitivity}_{Peak} + \text{Sensitivity}_{Low}}
\]

The colour discrimination task consisted of a forced choice paradigm, where volunteers were presented two rectangles, one red the other blue, and had to decide which one was red. Each red, blue pair was pseudo randomly selected from a set of five hues of red and five hues of blue. This task was repeated 50 times at each light level.
Model of Switch Circuitry

The model of the switch circuitry consisted of three basic building blocks: the cone bipolar cell terminal (CBT); the switch cell (SC); and the PV1 ganglion cell (PV1). Inputs to the CBT were the weighted light responses of the recorded rod and ON-cone bipolar cells at different background light intensities (Figure 6), and the feedback signal from the SC. The SC was driven by the CBT. The CBT-SC loop was modeled as a coupled differential equation, which controlled the state of CBT and SC. The states CBT and SC were rectified to produce the output of CBT and SC. The bend of the state-output curve was shifted to the right in SC to model spike threshold (Figure S8).

1. \( \dot{x}(t) = -x(t) + W_{rb} \tau b(t) + W_{cb} cb(t) - W_{yx} H(y(t) - T_{sc})(y(t) - T_{sc}) \)

2. \( \dot{y}(t) = -y(t) + W_{xy} H(x(t)) x(t) \)

Where \( x(t) \) represents the state of CBT, \( W_{rb} \) and \( W_{cb} \) are the gains associated with the rod and cone bipolar input, respectively, \( W_{yx} \) is the feedback gain from SC, \( T_{sc} \) models the spike threshold of SC, \( H \) is the Heaviside step function, \( y(t) \) represents the state of SC and \( W_{xy} \) is the gain of the excitatory input from CBT to SC.

The weighted (\( W_e \)) CBT output was the time varying excitatory conductance, \( g_e(t) \), of PV1,

3. \( g_e(t) = W_e H(x(t)) x(t) \)

The weighted (\( W_i \)) SC output was the time varying inhibitory conductance, \( g_i(t) \), of PV1,

4. \( g_i(t) = W_i H(y(t) - T_{sc})(y(t) - T_{sc}) \)

The membrane potential of the PV1 cell was integrated using the membrane equation:

5. \( -C \dot{V}(t) = g (V(t) - V_r) + g_e(t) (V(t) - V_e) + g_i(t) (V(t) - V_i) \)

where \( g \), PV1 membrane conductance in rest, \( C \), PV1 membrane capacitance, and \( V_r \), resting membrane voltage (~-50 mV), were measured (Figure S8). \( V_e \), reversal potential of excitatory currents, was set to 0 mV and \( V_i \), reversal potential for inhibitory currents, was set to -60-70 mV. The spikes were generated from the membrane voltage signal, at membrane depolarization, using a Poisson process. Since \( V_r \) was close to PV1 spike threshold, spikes were initiated even by small membrane depolarization. The free parameters of the model were the gains of the sign preserving and sign
inverting pathways (Figure S8) and the threshold of SC. These parameters were fitted to match the recorded inhibitory, excitatory and spiking responses (see comment at the end of this paragraph about relative weight of inhibition and excitation). The model simulated the responses to two different-sized spots. The first, $400 \ \mu m$ in diameter, covered the entire dendritic field of the ganglion cell and so maximized its excitatory input. The second, $1000 \ \mu m$ in diameter, was larger than the dendritic field of the ganglion cells. The gains of the sign preserving synapses were kept the same for both stimuli. To model wide-field inhibition from SC, the gains of sign inverting, inhibitory, pathways were different between the two stimuli. The difference between these inhibitory gains was determined by fitting the model’s inhibitory conductance to PV1 to the recorded inhibitory conductance evoked by the two stimuli. Note that the experimentally recorded excitatory input to PV1 cells was faster than the inhibitory input (Figure 4F), resulting in few spikes at the onset of the $1000 \ \mu m$ stimulus at daylight conditions (Figure 1A and S4). Model showed similar delay. The delay in the model was caused by the spike threshold of SC. The magnitude of delay was dictated by time course of the bipolar cell responses since this was the slowest component of the system.

In order for inhibitory conductance to achieve the measured reduction in spiking frequency at cone threshold, it has to be $\sim 3$ times larger than excitatory conductance. This is, because during an unclamped voltage response the driving force of inhibition is $\sim 3$ times less than for excitation. Yet, as shown on Figure 4D, the mean inhibition in the first 500 ms after stimulus onset has smaller magnitude than excitation (both measured with the same driving force). There are two reasons for this discrepancy. First, the time course of inhibition and excitation is not the same. Excitation starts with a large transient followed by a smaller sustained component. Inhibition has a similar peak transient, but is delayed compared to excitation (Figure 4C and 4F). The initial large excitatory peak causes a few spikes at stimulus onset (Figure 1A and S4). However, when inhibition is reaching its maximum excitation is already falling and therefore inhibition is larger than excitation. This explains that transient hyperpolarization after the few spikes following stimulus onset. Second, as far as the reduction of spiking in the sustained component of the response, the magnitude of inhibition is likely underestimated in our voltage clamp experiments. When recording inhibitory input we voltage clamp the cell at 0 mV, far from the resting potential. For large cells, such as PV1 cells, this results in space clamp at distal dendrites and the recorded current is a combination of inhibitory outward and excitatory inward current with a net outward current. Therefore the magnitude of the recorded outward current underestimates the current component caused by the inhibitory input. Simulations were carried out in Mathematica.
Statistical Analysis

All measures of statistical difference were performed using a Mann-Whitney $U$ test. In the figures, statistical significant difference is indicated with *, **, or ***, representing $P$ values less than 0.05, 0.01 and 0.001, respectively. All data points are mean +/- s.e.m. The “$n$” in the figures refers to the number of different cells from which recordings were used for the actual figure, or in the case of human experiments the number of individuals.

Supplemental References


