Species-specific wiring for direction selectivity in the mammalian retina

Huayu Ding1, Robert G. Smith2, Alon Poleg-Polsky1, Jeffrey S. Diamond1 & Kevin L. Briggman3,4

Directionally tuned signalling in starburst amacrine cell (SAC) dendrites lies at the heart of the circuit that detects the direction of moving stimuli in the mammalian retina. The relative contributions of intrinsic cellular properties and network connectivity to SAC direction selectivity remain unclear. Here we present a detailed connectomic reconstruction of SAC circuitry in mouse retina and describe two previously unknown features of synaptic distributions along SAC dendrites: input and output synapses are segregated, with inputs restricted to proximal dendrites; and the distribution of inhibitory inputs is fundamentally different from that observed in rabbit retina. An anatomically constrained SAC network model suggests that SAC–SAC wiring differences between mouse and rabbit retina underlie distinct contributions of synaptic inhibition to velocity and contrast tuning and receptive field structure. In particular, the model indicates that mouse connectivity enables SACs to encode lower linear velocities that account for smaller eye diameter, thereby conserving angular velocity tuning. These predictions are confirmed with calcium imaging of mouse SAC dendrites responding to directional stimuli.

A thorough understanding of a neuronal circuit requires a detailed anatomical wiring diagram that includes the synaptic connectivity among the component neurons. Even ostensibly subtle connectivity differences during development or between species could underlie substantial changes in circuit behaviour. This is exemplified in the direction selectivity circuit in the mammalian retina, a model neural network that engages just a few well-characterized cell types to compute salient visual information. However, the detailed synaptic connectivity among these neurons, and circuitry differences between species, has not been completely described.

Direction-selective ganglion cells (DSGCs) respond strongly to visual motion in one (preferred) direction but only weakly to motion in the opposite (null) direction1. Bipolar cells provide excitatory synaptic inputs to DSGCs and to densely arrayed SACs, which then inhibit DSGCs (Fig. 1a)2–4. SAC dendrites oriented asymmetrically to a DSGC provide feedforward inhibitory input that establishes DSGC directional tuning4–6. SAC dendrites are themselves directionally selective and release the neurotransmitter GABA (γ-aminobutyric acid) from synaptic terminals at their tips preferentially in response to outward (centrifugal) compared to inward (centripetal) motion relative to their soma6–8. Several mechanisms contribute to direction selectivity within individual SAC dendrites, but the relative importance of the mechanisms is unclear. Proposed intrinsic mechanisms include dendritic morphology9, non-uniform chloride homeostasis10, and active membrane conductances6,11. SAC direction selectivity may also rely on network interactions, such as spatially offset synaptic inputs from particular bipolar cell types12 and reciprocal inhibition between neighbouring SACs8,13–16.

Most anatomical analyses of SAC microcircuitry have been performed in rabbit retina. Sparse electron microscopy reconstructions in rabbit indicated that excitatory and inhibitory synaptic inputs occur along the entire length of SAC dendrites, whereas inhibitory synaptic outputs arise on the distal third2. We explored SAC connectivity in mouse retina using serial block-face scanning electron microscopy17. We discovered a previously unknown asymmetric distribution of inhibitory and excitatory input synapses onto ON and OFF mouse SAC dendrites that is fundamentally different from the connectivity in rabbit retina. We developed an anatomically constrained network model of mouse SAC connectivity that predicts new roles for synaptic inhibition in velocity and contrast tuning and receptive field structure in SACs. Finally, we confirmed these predictions by recording directionally tuned responses in mouse SAC dendrites. Our results indicate that the SAC network has adapted to meet the specific demands imposed by the mouse visual system.

Synaptic inputs are spatially offset

We annotated an ON–OFF DSGC within a conventionally stained serial block-face scanning electron microscopy volume (30 × 210 × 260 μm3) from an adult mouse retina (Extended Data Fig. 1a, b). Neurites forming conventional (inhibitory) synapses (Fig. 1b) onto this cell were back-traced to identify four SACs (2 ON, 2 OFF) located centrally in the data volume (Fig. 1c–e). The morphology of each SAC was fully traced within the data volume and the locations of input and output synapses were annotated. As expected, output synapses arose along the distal third of SAC dendritic trees (Fig. 1f, g). Ribbon-type input synapses (Fig. 1b) from bipolar cells were distributed primarily along the proximal two-thirds of dendrites (Fig. 1f, g). Conventional synapses from amacrine cells (Fig. 1b) were restricted to the initial third of the dendritic trees (Fig. 1f, g). This proximal location of amacrine cell inputs differs from previous reports in rabbit retina that SACs receive reciprocal SAC inputs along their distal dendrites (Extended Data Fig. 1f)2,8,16,18, indicating that SAC connectivity is fundamentally different in mice and rabbits. Next, we identified cells that were presynaptic to the SACs.

Bipolar and amacrine cell types presynaptic to SACs

Recent analysis of contact area shared between different OFF bipolar cell types and OFF SACs suggested a ‘space-time wiring’ presynaptic delay model that supports SAC direction selectivity12. In this model, different bipolar cell types exhibit distinct release kinetics19–21, and
sustained bipolar cells (for example, BC2) provide input more proximally on SAC dendrites than do transient bipolar cells (for example, BC3a). Because our data set allowed us to positively identify synapses, we classified bipolar cell types providing input to ON and OFF SACs (Fig. 2); and we noted several differences compared to the contact-based analysis.

We found synapses onto the OFF SACs from all OFF bipolar cell types (BC1, BC2, BC3a, BC3b, and BC4; Fig. 2a, b and Extended Data Fig. 2) with most input from BC1, BC2, and BC3a (Fig. 2a, b). BC1 and BC3a exhibited segregated radial distributions, potentially supporting a presynaptic space-time wiring model, but BC2 overlapped with both; this overlap, regardless of BC2 response kinetics, would presumably diminish direction selectivity generated in such a model. Space–time wiring may still support direction selectivity in OFF SACs, pending characterization of type-specific OFF bipolar cell response kinetics. Our data suggest that SAC dendrites may simply sample from the available bipolar cells at a particular depth in the inner plexiform layer (IPL) (Fig. 2c), regardless of bipolar cell release characteristics.

If space–time wiring were essential for SAC direction selectivity, one would expect a similar connectivity pattern for ON SACs. Bipolar cell inputs to the ON SACs clustered into four subtypes, corresponding to BC7 and three BC5 subtypes (BC5o, BC5t, BC5i) (Figs 2d, e and Extended Data Fig. 3). We found that type BC7 primarily contacted proximal dendrites, whereas BC5 inputs, collectively, were distributed more distally (Fig. 2d, e). The radial location of synapses correlated with their IPL depth (Fig. 2f). Segregated bipolar cell inputs to ON SACs could support a space–time direction selectivity mechanism, although type BC7 (which we show provide proximal inputs) exhibits transient light responses, counter to the model’s requirements.

Next, we analysed the sources of amacrine cell synapses onto the ON and OFF SACs (Fig. 3a, b and Extended Data Fig. 4). Most inputs originated from neighbouring SACs, identified by their distinctive branching pattern and tight co-stratification with the postsynaptic SACs (Fig. 3a and Extended Data Fig. 4a, b). There was no directional preference in the absolute orientations of presynaptic SAC dendrites. Previous studies hypothesized that SAC direction selectivity could be enhanced if opposing (‘anti-parallel’) SAC dendrites preferentially made reciprocal connections. To test this idea, we measured the relative angle between connected presynaptic and postsynaptic dendrites (Fig. 3c and Extended Data Fig. 5a, b). The distributions of relative angles for both the ON and OFF SACs were significantly skewed towards anti-parallel (180°) wiring (Kolmogorov–Smirnov test, P = 2 × 10⁻⁵; Fig. 3c). We considered whether presynaptic SAC dendrites selectively connect to opposing dendrites or whether the relative angle distribution simply reflects the inter-soma spacing between SACs. We annotated locations where the distal third of presynaptic SAC dendrites passed within 1 μm of the postsynaptic SACs and measured the relative angles between dendrites at each proximity. The proximity-based relative angle distribution was not statistically significantly different from the distribution based on actual synaptic connectivity (Extended Data Fig. 5c, Kolmogorov–Smirnov test, P = 0.18), indicating that the wiring arises primarily from the geometric arrangement of connected SACs. Relative angle was not correlated to the radial distance of each synapse from the soma or the angle at which the SAC was contacted by a bipolar cell (Extended Data Fig. 5d).

Not all inhibitory inputs came from neighbouring SACs. We annotated several apparent wide-field amacrine cells that contributed synapses specifically onto the most proximal dendrites of ON and OFF SACs (Extended Data Fig. 4c, d). Wide-field amacrine cells did not...
co-stratify with SACs, but rather stratified close to the inner nuclear and ganglion cell layers, in contrast to a different population targeting bipolar cell axon terminals presynaptic to DSGCs\textsuperscript{25}. We also found a few synapses from narrow-field amacrine cells, mostly onto ON SACs (Extended Data Fig. 4c)\textsuperscript{25}. Therefore, although most proximal amacrine inputs originate from neighbouring SACs, additional inputs may selectively inhibit perisomatic compartments.

We also quantified the number and types of postsynaptic targets of ON and OFF SAC branches terminating near the centre of the data volume (Extended Data Fig. 6). We traced postsynaptic cells until they could be identified unambiguously as a ganglion cell, SAC, wide-field amacrine cell or bipolar cell. Synapses were formed primarily onto ganglion cells and SACs, with few outputs onto bipolar cells, consistent with findings that bipolar cell terminals are not directionally tuned\textsuperscript{26–28}. ON SACs devoted a higher fraction of outputs to ganglion cells than did OFF SACs, possibly because ON SACs provide inputs to both ON–OFF DSGCs and ON DSGCs.

**Proximal excitation enhances SAC direction selectivity**

Our anatomical data indicate that bipolar cell inputs are restricted to the proximal two-thirds of SAC dendrites and SAC inputs are restricted to the proximal third. Next, we combined computational modelling and physiological imaging to examine how this connectivity pattern affects response properties of SAC dendrites.

We based a single-cell SAC model on an existing passive model\textsuperscript{9} and incorporated measured dendritic diameters and active conductances along the dendrites such that the dendrites and soma both preferred centrifugal motion (Extended Data Fig. 7 and Extended Data Table 1)\textsuperscript{6}. We then constructed a network model comprising one central SAC and six surrounding SACs (Fig. 4a). SAC–SAC synapses were formed when a presynaptic dendrite came within a defined distance of a postsynaptic cell. The inter-soma distance (145 μm) was set to reproduce the relative angle distributions observed anatomically (Extended Data Fig. 5) and the radial distribution of inhibitory synapses (Fig. 4b, upper panel).

We then measured the direction selectivity index (see Methods) at a distal dendritic location (the region of interest (ROI\textsuperscript{*}), Fig. 4d) on the central SAC (Fig. 4a, boxed region). In response to moving bar stimuli, the ROI\textsuperscript{*} preferred centrifugal motion compared to centripetal motion, as expected (Fig. 4d). During centrifugal motion, depolarization of the dendritic tips preceded inhibition from neighbouring SACs. During centripetal motion, inhibition preceded excitation and limited depolarization of the ROI\textsuperscript{*}. We then modified the model to test whether the spatial separation between excitatory inputs and SAC outputs is important for direction selectivity. When bipolar cell inputs were uniformly distributed along SAC dendrites, thereby overlapping with outputs, the ROI\textsuperscript{*} preferred centripetal over centrifugal motion (Fig. 4e). Bipolar cell inputs on distal tips increased surround inhibition during centrifugal motion and caused excitation to lead inhibition during centripetal motion, thereby reducing direction selectivity. This result suggests that restricting excitation to the proximal two-thirds of SAC dendrites establishes a temporal pattern of excitation and inhibition that enhances preference for centrifugal motion.

**Inhibition shapes velocity tuning**

When we simulated rabbit-like connectivity by increasing the inter-soma distances (200 μm) to generate distal SAC–SAC contacts (Fig. 4b, lower graph, 4c), the model still exhibited centrifugal preference. The most obvious distinction between the mouse and rabbit eye is a fivefold difference in diameter (Extended Data Fig. 8a)\textsuperscript{29,30}. Consequently, a 1° visual angle subtends 30 μm on the mouse retina and 150 μm on the rabbit retina. Mouse and rabbit DSGCs respond to similar angular velocities\textsuperscript{31,32} (Extended Data Fig. 8c), suggesting that SACs in both species are also tuned to similar angular velocities. This translates to different linear velocities: 10° s\textsuperscript{-1} motion corresponds to 1,500 μm s\textsuperscript{-1} across rabbit retina, but just 300 μm s\textsuperscript{-1} across mouse retina (Extended Data Fig. 8b).

Both the mouse and rabbit SAC models exhibited direction selectivity at linear velocities above 500 μm s\textsuperscript{-1} (Fig. 4g). At lower velocities,
Figure 4 | Functional consequences of SAC network connectivity. a, c, Compartmental models of mouse (a) and rabbit (c) networks. b, Radial distributions of simulated synapses compared to anatomical reconstructions (rabbit data analysed from ref. 2). d, Schematic of mouse connectivity (top) and simulated responses (bottom) to centrifugal (CF) and centripetal (CP) bar stimuli relative to the location ROI*. Bar location at times $t_1$–$t_6$ indicated by dashed grey lines. Voltage and calcium responses measured at the ROI*; synaptic conductances measured for the central SAC. e, As in d, but with bipolar cell inputs distributed uniformly along SAC dendrites. f, As in d, but incorporating rabbit-like connectivity. g, Simulated velocity tuning curves. The direction selectivity index calculated from [Ca$^{2+}$] at the ROI*. h, i, Simulated responses at 200 μm s$^{-1}$ for mouse and rabbit models, respectively. j, Fluorescence image of an ON SAC filled with OGB1. k, Representative Ca$^{2+}$ transients measured at the varicosity highlighted in j in response to visual stimuli moving at five different velocities (300% contrast). l, Velocity tuning of the direction selectivity index (mean ± s.d.) in $n = 41$ SAC varicosities measured from $n = 3$ ON SACS. Scale bars, 100 μm (a, c), 25 μm (j).

However, direction selectivity in the rabbit model degraded because surround inhibition and central excitation did not overlap sufficiently in time to inhibit centripetal responses as strongly (Fig. 4i). The reduced direction selectivity at lower velocities is consistent with velocity tuning measured in rabbit DSGCs (Extended Data Fig. 8)31. By contrast, the mouse model remained direction selective down to 100 μm s$^{-1}$. The greater spatial overlap of synaptic inputs from neighbouring SACs and bipolar cells in mouse enabled inhibition to coincide with excitation at lower linear velocities during centripetal motion (Fig. 4h). Increasing SAC inter-soma distances to 250 μm, generating tip-to-tip connectivity, further shifted the tuning curve to higher velocities (Fig. 4g).

We tested the prediction from the model by performing two-photon laser scanning microscopy of dendritic calcium from mouse SACs filled with OGB1 in whole-mount retinas (Fig. 4j). Bars of light were swept across SAC receptive fields in eight equally spaced directions at linear velocities ranging from 30–2,000 μm s$^{-1}$; direction selectivity was calculated from calcium transients measured at individual distal varicosities. As the model predicted, mouse SACs remained directionally selective down to at least 100 μm s$^{-1}$ (Fig. 4k, l). These results suggest that SAC circuitry has adapted to conserve angular velocity tuning across species.

**SAC–SAC inhibition expands contrast range**

To encode naturalistic stimuli effectively, SACs must also remain directionally selective over a wide contrast range24,35, a feature predicted by our model (Fig. 5a, b). Simulations suggested that broad contrast tuning requires SAC–SAC inhibition: at high contrasts, blocking inhibition dramatically reduced direction selectivity in simulated SACs due to saturation of postsynaptic responses to both centrifugal and centripetal stimuli (Fig. 5c).

We tested these predictions by imaging SAC dendritic responses to directional motion at different visual contrasts (Fig. 5d–g). Consistent...
with the model, SACs remained directionally selective over different contrast levels and blocking SAC–SAC inhibition with a GABA\(_\text{A}\) receptor (GABA\(_\text{A,R}\) antagonist, SR95531 (25\(\mu\)M), significantly reduced direction selectivity, particularly in response to high-contrast stimuli (Fig. 5g).

**Inhibition shapes SAC receptive fields**

In rabbit retina, most SAC–SAC connections occur between distal dendrites (Extended Data Fig. 1f); consequently, direction selectivity for stimuli restricted to a SAC’s central receptive field relies primarily upon intrinsic dendritic conductances rather than network inhibition\(^6\). In the mouse retina, we found that SACs receive SAC inputs exclusively on their proximal dendrites (Fig. 1), suggesting that direction selectivity within the central receptive field may rely on inhibition from neighbouring SACs.

We explored this first in our mouse network model using a radially expanding or contracting (‘bullseye’) stimulus described previously (Fig. 6a, b)\(^3\). The model exhibited strong centrifugal direction selectivity in response to the bullseye stimulus with inhibition intact, because proximal inhibitory synapses became activated by centrally restricted stimuli (Fig. 6c). Removing inhibition reduced centrifugal direction selectivity over a range of simulated contrasts (Fig. 6c, d). We tested the prediction from the model by imaging dendritic calcium signals evoked by bullseye stimuli restricted to the SAC dendritic arbor (Fig. 6e). Blocking inhibition with SR95531 significantly reduced directional selectivity (Fig. 6f, g), as predicted. SR95531 may also influence presynaptic inhibition of bipolar cell terminals, potentially disrupting bipolar-cell-type-specific release kinetics. If this were the case, however, dendrite autonomous rabbit SAC directional selectivity should also be reduced by SR95531, in contrast to previous reports\(^6,7\).

**Discussion**

When reconstructing wiring diagrams, an important question is what level of detail is required to understand mechanistically how a neuronal circuit performs specific computations\(^36,37\). Our results indicate that seemingly subtle differences in connectivity—such as whether cells receive inputs on proximal versus distal dendrites—can substantially influence neural coding and circuit behaviour. We found that segregating excitatory inputs from synaptic outputs along SAC dendrites helps establish strong centrifugal direction selectivity in a network model of SAC connectivity (Fig. 4e, also see ref. 38). More importantly, comparing wiring diagrams across species revealed a previously unrecognized connectivity difference in direction selectivity circuits of the mouse and rabbit retina (Fig. 1 and Extended Data Fig. 1).

The two species exhibit comparable average SAC dendritic diameters and coverage factors\(^39–42\), suggesting that mouse and rabbit SAC networks theoretically could have been wired similarly. We found instead that the locus of presynaptic inhibition on SACs alters the linear velocity tuning of SAC direction selectivity to compensate for eye size difference and conserve angular velocity tuning across the two species.

---

**Figure 5** | Contrast dependence of SAC to SAC inhibition. a–c. Contrast tuning curve of mouse network model in response to a bar stimulus. Increasing contrast was simulated with stronger bipolar cell depolarization. The maximal conductance of inhibitory synapses in the model was varied. d. Directional tuning of individual SAC varicosities. Vectors indicate preferred direction and the direction selectivity index magnitude. Scale bars, 10\(\mu\)m (upper), 0.5 direction selectivity index (lower). e. Representative Ca\(^{2+}\) transients from individual varicosities under low (100%) and high (300%) contrast (SR: SR95531). f. The direction selectivity index (DSI) of individual varicosities for ON (left: \(n = 201\) ROIs from \(n = 6\) cells; right: \(n = 261\) ROIs from \(n = 10\) cells) and OFF (left: \(n = 193\) ROIs from \(n = 4\) cells; right: \(n = 197\) ROIs from \(n = 9\) cells) SACs. g. The direction selectivity index (mean ± s.d.) following SR95531 application as a fraction of control (paired t-test, Bonferroni correction, \(*P < 0.001\)).

---

**Figure 6** | Receptive field structure of mouse SACs. a, b. The mouse network model (b) was activated with a bullseye stimulus (a) centred on and restricted to the diameter of the central SAC and expanded or contracted to elicit centrifugal or centripetal motion. c. Simulated dendritic Ca\(^{2+}\) at the ROI* in response to centrifugal and centripetal bullseyes (6.7 Hz, 150\(\mu\)m period, 0.05 AU contrast) with inhibition intact (black, grey) or blocked (orange, peach). d. The direction selectivity index versus simulated contrast. e. Fluorescence image of OGB1-filled SAC. f. Representative dendritic Ca\(^{2+}\) transients recorded in response to centrifugal and centripetal bullseye stimuli (2 Hz, 140\(\mu\)m period, 90% contrast). Responses from the ROI in e. g. Scatter plot of \(n = 74\) ROIs from \(n = 5\) ON SACs. SR95531 application significantly decreased the direction selectivity index from 0.46 ± 0.24 (mean ± s.d.) to 0.17 ± 0.14 (paired t-test, \(P = 2 \times 10^{-13}\)). Scale bars, 100\(\mu\)m (b), 25\(\mu\)m (e).
(Fig. 4). Inhibition among SACs also extended their contrast tuning range (Fig. 5): removing inhibition reduced SAC direction selectivity at high-stimulus contrasts, potentially rendering postsynaptic DSGCs blind to directional motion. Proximal inhibition also altered the receptive field structure of mouse SACs compared to previous reports of rabbit SACs (Fig. 6)6,7.

Our simulations effectively guided our physiological experiments, but they underestimated the extensive connectivity of SACs, which actually receive inputs from dozens of neighbouring SACs (Fig. 3). The model also neglects inhibitory inputs to SACs from wide-field amacrine cells and narrow-field amacrine cells and detailed features of the presynaptic bipolar circuitry, important elements to incorporate in future simulations. Other visual stimulus features (for example, size, shape, spatial frequency) also remain to be explored. Nevertheless, the present study exemplifies how connectomic mapping, computational modelling and cellular physiology complement each other to provide new insights into neuronal circuit computations.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 28 December 2015; accepted 27 May 2016. Published online 22 June 2016.

1. Barlow, H. B., Hill, R. M. & Levick, W. R. Retinal ganglion cells responding selectively to direction and speed of image motion in the rabbit. J. Physiol. 173, 377–407 (1964).
2. Famiglietti, E. V. Synaptic organization of starburst amacrine cells in rabbit retina: analysis of serial thin sections by electron microscopy and graphic reconstruction. J. Comp. Neurol. 309, 402–403 (1991).
3. Vaney, D. L., Collin, S. F. P. & Young, H. M. In Neurobiology of the Inner Retina (eds Weiler R. & Osborne N.) 157–168 (Springer, 1989).
4. Briggman, K. L., Helmstaedter, M. & Denk, W. Wiring specificity in the space-time wiring specificity supports direction selectivity in the retina. Nature 469, 402–406 (2011).
5. Hausselt, S. E., Euler, T., Detwiler, P. B. & Denk, W. A dendrite-autonomous mechanism for direction selectivity in retinal starburst amacrine cells. PLoS Biol. 5, e185 (2007).
6. Euler, T., Detwiler, P. B. & Denk, W. Directionally selective calcium signals in dendrites of starburst amacrine cells. Nature 418, 845–852 (2002).
7. Lee, S. & Zhou, Z. J. The synaptic mechanism of direction selectivity in the mouse retina. J. Comp. Neurol. 509, 2466–2475 (2008).
8. Weng, S., Sun, W. & He, S. Identification of ON–OFF direction-selective ganglion cells in the mouse retina. J. Physiol. (Lond.) 562, 915–923 (2005).
9. Tauchi, M. & Masland, R. H. The shape and arrangement of the cholinergic neurons in the mouse retina. Proc. R. Soc. Lond. B 281, 101–119 (1984).
10. Pérez De Sevilla Müller, L., Shelley, J. & Weiler, R. Displaced amacrine cells of the mouse retina. J. Comp. Neurol. 505, 539–546 (2007).
METHODS

No statistical methods were used to predetermine sample size. All n values refer to biological replicates. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

EM tissue preparation. An adult wild-type (C57BL/6) mouse (postnatal day 30 (P30)) was anaesthetized with isoflurane (Baxter) inhalation and killed by cervical dislocation. The eyes were enucleated and transferred to a dish containing carboxygenated room-temperature saline, in which the retinas were disected. All procedures were approved by the local animal committee and were in accordance with the law of animal experimentation issued by the German Federal Government. We used a commercially available saline (Biometra) that was supplemented with 0.5 mM L-glutamine and carboxygenated (95% O2/5% CO2). We hemisected the retina and mounted it on filter paper. The retina was fixed in a solution containing 0.1 M cacodylate buffer, 4% sucrose and 2% glutaraldehyde, pH 7.2 (Serva). The tissue was then trimmed to be 2h at room temperature and then rinsed in 0.1 M cacodylate buffer plus 4% sucrose overnight. A 1 × 1 mm2 region of the retina, approximately half-way between the optic disk and the peripheral edge of the retina, was then excised. The tissue was then stained in a solution containing 1% osmium tetroxide, 1.5% potassium ferrocyanide, and 0.15 M cacodylate buffer for 2h at room temperature. The osmium stain was amplified with 1% thio-carbohydrazide (1h at 50 °C), and 2% osmium tetroxide (1h at room temperature). The tissue was then stained with 2% aqueous uranyl acetate for 12 h at room temperature and lead aspartate for 12h at room temperature. The tissue was dehydrated through an ethanol series (70%, 90%, 100%), transferred to propylene oxide, infiltrated with 50%/50% propylene oxide/Epon Hard, and then 100% Epon Hard. The block was cured at 60 °C for 24 h.

Serial block-face scanning electron microscopy acquisition. The retina (k0725) was cut out of the flat-embedding blocks and re-embedded in Epon Hard, on aluminum sheets. 30,000 serial blocks were scanned using a field emission electron microscope with the retinal plane vertical. The samples were then trimmed to a block face of ~200 μm wide and ~400 μm long. The samples were imaged in a scanning electron microscope with a field-emission cathode (QuantaFEG 200, FEI Company). Back-scattered electrons were detected using a custom-designed detector based on a custom-built current amplifier. The incident electron beam had an energy of 2.0 keV and a current of ~110 pA. Images were acquired with a pixel dwell time of 2.5 μs and size of 13.2 μm × 13.2 μm which corresponds to a dose of about 10 electrons per nm2. Images were performed at high vacuum, with the sides of the block evaporation-coated with a 100–200 nm thick layer of gold. The electron microscope was equipped with a custom-made microtome designed by W. Denk that was previously used to collect retinal serial block-face scanning electron microscopy data. The section thickness was set to 26 nm. 10,112 consecutive block faces were imaged, resulting in aligned data volumes of 4,992 × 16,000 × 10,112 voxels (1 × 5 mosaic of 3,384 × 3,094 images), corresponding to an approximate spatial volume of 50 × 210 × 260 μm3. The edges of neighbouring mosaic images overlapped by ~1 μm. The cutting quality degraded during the course of the experiment, meaning the images in the first half of the data volume (approximately the first 5,000 slices) are of higher quality than the ones coming after. The quality of cut surfaces and neurites could be manually annotated throughout the volume. The imaged region spanned the inner plexiform layer of the retina and included the ganglion cell layer and part of the inner nuclear layer. Cross-correlation-derived shift vectors between neighbouring mosaic images and consecutive slices were used for a global least-squares fit across all shift vectors to align the data sets off-line to subpixel precision by Fourier shift-based interpolation. The data sets were then split into cubes (128 × 128 × 128 voxels) for viewing in KNossOS (http://www.knossostool.org). Skeleton tracing and contact annotation. Skeletons were traced using KNossOS and consisted of nodes and connections between them. Nodes were placed approximately 200 μm apart, and connections were then drawn automatically. The additional files and software (Wavemetrics) and 4D Workshop 4 IDE (4D Systems) to control an LCD mask in front of a collimated LED (405 nm, Thorlabs) with a bandpass filter (BP 405, Thorlabs). The stimuli were projected onto the retina through the objective lens (XLUMPlanFL 20 × 0.95 NA water-immersion, Olympus). Stimulus contrast varied between 100–300%, with the 300% stimulus intensity at ~25 × 104 photons s−1 μm−2 on a background intensity of ~6–10 photons μm−2 s−1. For the bar stimulus, the bar (400 × 400 μm) moved in one of eight evenly spaced directions at a rate of velocities between 0.03–1 mm s−1. The bullseye stimulus was configured as previously described. Each stimulus was repeated 3–5 times and responses were averaged.
calculated by \((PD - ND)/PD\), where ND is the null (or centripetal) and PD is the preferred (or centrifugal) response.

**Statistical analyses.** We included as much of the raw anatomy data as practical in the figures, including neuron and synapse distributions and spatial locations. The identities of neurons presynaptic to SACs were, by definition, blind to the annotator before skeletonization. No reconstructed neurons were excluded from the analysis. For comparing relative angle distributions, we used the non-parametric Kolmogorov–Smirnov test. For dendritic calcium experiments incorporating pharmacology, all measurements were paired (that is, responses at a ROI are reported both before and after drug application). The number of recorded cells was selected to provide typically hundreds of ROIs for comparison and paired \(t\)-tests were used to assess statistical significance. All samples sizes and statistical test results are reported in the figure legends. Statistical tests were performed in MATLAB or GraphPad.

**Code availability.** The Neuron-C simulation language that generated the models described above is available at: ftp://retina.anatomy.upenn.edu/pub/rob/nc.tgz. Included in this distribution is the realistic SAC morphology, the ‘retrim’ retinal circuit simulator that generated the models, and the ‘rsbac_stim_plots_vel’ script that ran multiple model jobs in parallel.

44. Helmstaedter, M. et al. Connectomic reconstruction of the inner plexiform layer in the mouse retina. Nature **500**, 168–174 (2013).
45. Smith, R. G. NeuronC: a computational language for investigating functional architecture of neural circuits. *J. Neurosci. Methods*** 43, 83–108 (1992).
46. Schachter, M. J., Oesch, N., Smith, R. G. & Taylor, W. R. Dendritic spikes amplify the synaptic signal to enhance detection of motion in a simulation of the direction-selective ganglion cell. *PLOS Comput. Biol.* **6**, e1000899 (2010).
47. Pologruto, T. A., Sabatini, B. L. & Svoboda, K. ScanImage: flexible software for operating laser scanning microscopes. *Biomed. Eng. Online* **2**, 13 (2003).
Extended Data Figure 1 | EM data set, additional SAC reconstructions and rabbit connectivity. a. Conventionally stained serial block-face scanning electron microscopy volume of a mouse retina. b. Reconstructed ON-OFF DSGC. c–e. A second reconstructed ON and OFF SAC with annotated synapses locations. f. Annotation of the radial distribution of input and output synapses to and from approximately one-half of an OFF SAC dendritic arbor in rabbit retina. Data analysed from fig. 15 in ref. 2.
Extended Data Figure 2 | Classification of OFF bipolar cells. a, Types 1/2 and types 3/4 separated by IPL depth. b, Types 1 and 2 separate by stratification width and axonal arborization area (convex hull). c, Types 3a, 3b and 4 separate by stratification depth and axonal arborization area.

d, Mosaic patterns and stratification profiles of OFF bipolar cells. e, The number of synapses (mean ± s.d.) each bipolar cell, by type, formed with each SAC. f, Location of bipolar cell synapses onto a second OFF SAC, colour-coded by bipolar cell type. g, The IPL depth of each synapse versus the radial distance relative to the soma.
Extended Data Figure 3 | Classification of ON bipolar cells. a, Type 5 and type 7 bipolar cells separated by IPL depth. b, Types 5o (outer), 5t (thick) and 5i (inner) further subdivide based on IPL depth and stratification width. c, Mosaic patterns and stratification profiles of ON bipolar cells. d, Summary of the number of synapses (mean ± s.d.) each bipolar cell, by type, formed with each SAC. e, Location of bipolar cell synapses onto a second ON SAC, colour-coded by bipolar cell type. f, The IPL depth of each synapse versus the radial distance relative to the soma.
Extended Data Figure 4 | Amacrine cell types presynaptic to SACs. a, b, SACs presynaptic to the second pair of mouse SACs colour-coded by absolute orientation. c, d, Wide-field amacrine cells presynaptic to SACs. e, Narrow-field amacrine cells presynaptic to ON SACs.
Extended Data Figure 5 | Relative angles between presynaptic and postsynaptic SAC dendrites.  

a, Schematic of the relative angle measurement: parallel wiring = 0°, anti-parallel wiring = 180°. 
b, Locations of SAC input synapses colour-coded by relative angle. Grey locations indicate AC synapses that were not analysed. 
c, Cumulative distributions of the relative angles between each presynaptic and postsynaptic OFF SAC dendrite for synapses (black) and proximities (grey). Dashed line indicates a uniform distribution. 
d, Relative angle for each synapse was uncorrelated with the radial distance from the postsynaptic somas ($r = 0.07, P = 0.16$). Scale bar, 50 μm.
Extended Data Figure 6 | Identities of neurons postsynaptic to SAC output synapses. a, Percentage of output synapses formed with different postsynaptic cell types, colour-coded by postsynaptic cell class: ganglion cells (GC) (blue), SACs (red), bipolar cells (BC) (cyan), and wide-field amacrine cells (WAC) (green). b, Locations of 83 annotated output synapses on 1 ON SAC dendrite fragment. c, Locations of 110 annotated output synapses on 2 OFF SAC dendrite fragments. Scale bar, 50 μm.
Extended Data Figure 7 | Single SAC model. a, Dendrite diameters sampled from an ON SAC (grey) and an OFF SAC (black) at different radial distances from their respective somas. b, Single SAC morphology used in all simulations. c, Somatic voltage clamp simulation showed poor space clamp of even proximal dendrites. Voltage traces measured at a different distances (20–150 μm) from the soma. d, Somatic (solid line) and distal dendrite (dashed line) voltage time series in response to an annulus moving centrifugally or centripetally. The addition of active conductances to SAC dendrites (see Extended Data Table 1) rendered somatic voltage recordings directionally selective for centrifugal compared to centripetal stimulation, consistent with electrophysiological measurements. Scale bar, 50 μm.
Extended Data Figure 8 | Velocity tuning of rabbit and mouse direction selectivity circuits. 

a, Schematic of the difference in axial diameters and subtended angle on the retina of rabbit and mouse eyes. 
b, Linear velocity tuning curves from rabbit and mouse ON–OFF DSGCs. 
c, Angular velocity tuning curves from rabbit and mouse ON–OFF DSGCs. Data analysed from fig. 2F of ref. 31 and fig. 1D of ref. 32.
## Extended Data Table 1 | Table of biophysical parameters used in model SACs

### Biophysical parameters for SAC model

| Parameter                                      | Value          |
|------------------------------------------------|----------------|
| Rm (Ω·cm²)                                     | 10,000         |
| Ri (Ω·cm²)                                     | 75             |
| NaV1.8 channel density (S/cm²)                 |                |
| soma                                           | 0              |
| proximal 1/3:                                  | 0              |
| medial 1/3:                                    | 3e¹            |
| distal 1/3:                                    | 3e¹            |
| Kdr channel density (S/cm²)                    |                |
| soma                                           | 3e¹            |
| proximal 1/3:                                  | 2e¹            |
| medial 1/3:                                    | 2e¹            |
| distal 1/3:                                    | 2e¹            |
| L-type Ca²⁺ channel density (S/cm²)             |                |
| soma                                           | 0              |
| proximal 1/3:                                  | 0              |
| medial 1/3:                                    | 1e¹            |
| distal 1/3:                                    | 1e¹            |