Origins of direction selectivity in the primate retina

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From mouse to primate, there is a striking discontinuity in our current understanding of the neural coding of motion direction. In non-primate mammals, directionally selective cell types and circuits are a signature feature of the retina, situated at the earliest stage of the visual process. In primates, by contrast, direction selectivity is a hallmark of motion processing areas in visual cortex, but has not been found in the retina, despite significant effort. Here we combined functional recordings of light-evoked responses and connectomic reconstruction to identify diverse direction-selective cell types in the macaque monkey retina with distinctive physiological properties and synaptic motifs. This circuitry includes an ON-OFF ganglion cell type, a spiking, ON-OFF polyaxonal amacrine cell and the starburst amacrine cell, all of which show direction selectivity. Moreover, we discovered that macaque starburst cells possess a strong, non-GABAergic, antagonistic surround mediated by input from excitatory bipolar cells that is critical for the generation of radial motion sensitivity in these cells. Our findings open a door to investigation of a precortical circuitry that computes motion direction in the primate visual system.

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A bout 60 years ago, neurons in the mammalian nervous system sensitive to the direction of visual motion were described in the primary visual cortex of the cat\textsuperscript{1,2} and in the retina of the rabbit\textsuperscript{3,4}. Thereafter, investigation of the circuit origins and the neural mechanisms for direction selectivity as well as its functional role in motion processing have proceeded on two largely parallel tracks, focusing on diverse motion-processing areas in the primate visual cortex\textsuperscript{5} versus the diverse cell types and circuits in the non-primate mammalian retina\textsuperscript{6,7}. One reason for this dichotomy is that, while a significant fraction of retinal ganglion cells in non-primate mammals show direction selectivity\textsuperscript{8,9}, this fundamental neural signal has not been observed in the primate retina, despite dedicated effort\textsuperscript{10-12}. Moreover, the apparent lack of significant direction selectivity recorded from neurons at the level of the lateral geniculate nucleus, which relays signals from retina to cortex, has suggested that this property must be encephalized in primates, arising initially from neural circuits within primary visual cortex\textsuperscript{13-17}.

The most intensively investigated substrate for direction selectivity is the synaptic connection from starburst amacrine cells to ON–OFF direction-selective ganglion cells (ON–OFF DSGCs) in the mouse and rabbit retina. The ON–OFF DSGCs have characteristic bistratified dendrites that tend to recurve back toward the cell body, and are present as four distinct populations that differ in their direction preference\textsuperscript{8,18,19}. Direction selectivity originates in the distinctive radial dendrites of a single type of retinal interneuron, the starburst amacrine cell\textsuperscript{20,21}, which uniquely co-expresses the inhibitory transmitter GABA and the excitatory transmitter acetylcholine\textsuperscript{22,23}. Strong evidence indicates that selective, spatially asymmetric inhibitory synapses from starburst dendrites to each of the four ON–OFF DSGC types is the basis for direction selectivity in these ganglion cells\textsuperscript{19,24}. In the primate, ganglion cells with dendritic morphology similar to that of ON–OFF DSGCs have been observed previously\textsuperscript{25-27}. Moreover, starburst amacrine cells are also present in the primate retina\textsuperscript{28,29} and the starburst and putative DSGC dendrites appear to costratify in the inner plexiform layer (IPL)\textsuperscript{27,30}. This raises the possibility that direction selectivity is present in the primate retina and perhaps also constructed by circuitry comparable to that in non-primate mammals.

Here, we used a distinctive in vitro preparation of the macaque monkey retina in which the photoreceptor-supporting layers—choroid and retinal pigment epithelium—remain attached to the neural retina, permitting adaptation to a high photopic background that elicits a pure cone photoreceptor-driven response to light\textsuperscript{31}. A unique in vitro photodynamic staining method then permitted a near-complete classification of ganglion cells and the identification of a morphologically distinct, ON–OFF direction-selective ganglion cell type that is coupled via gap junctions to a polyaxonal, ON–OFF spiking amacrine-cell type that is itself direction-selective. For the first time in the primate, we were able to target starburst amacrine cells, characterizing their light response, receptive-field structure, direction selectivity, and connectivity. We found a strong antagonistic surround, raising presynaptically from diverse excitatory bipolar-cell inputs that is critical for the characteristic directionally selective response evoked in macaque starburst cells by radially moving stimuli.

**Results**

**Classification of ganglion cell types and identification of a candidate ON–OFF direction-selective cell population.** Parallel visual pathways originate in morphologically and physiologically diverse retinal ganglion cell populations\textsuperscript{32-34}. In the mouse retina, over 40 functionally distinct ganglion cell types are present\textsuperscript{35-38} with a substantial fraction of them showing direction selectivity\textsuperscript{8}. In the primate retina, by contrast, many fewer ganglion cell types have been identified\textsuperscript{39-42}. A question therefore is whether the account of ganglion cell types in the primate is largely incomplete or whether there is a large species difference in this fundamental aspect of retinal organization. One explanation may be that the abundance of diverse direction-selective types found in non-primate mammals may be absent in the primate. Thus, to identify and characterize a candidate DSGC in the macaque retina a more comprehensive accounting of the dendritic morphologies and spatial densities of the diverse ganglion cell types was required.

To address this problem in the primate—where gene-based cellular reporters are lacking—we used retrograde photodynamic staining\textsuperscript{43}. Injections of the tracer rhodamine dextran were made into the major targets of retinal output: the lateral geniculate nucleus (LGN)\textsuperscript{25,46,47}, the superior colliculus (SC)\textsuperscript{48} and nuclei within the pretectal complex\textsuperscript{49}. After retrograde transport, the retina was dissected and maintained in vitro. Exposure of labeled ganglion cells to high photopic light intensities liberated tracer sequestered in the cell body, which then rapidly diffused throughout the cell to reveal their complete dendritic morphology. By this method, we determined for each ganglion cell population its relative spatial density and fundamental mosaic organization. The spatial densities of 19 morphologically distinct ganglion cell types, determined from the regular overlap of neighboring dendritic trees, accounted for ~97% of the total ganglion cell population in the mid-peripheral retina (Fig. 1; Supplementary Fig. 1; Supplementary Table 1). Only five types (ON- and OFF-midget, ON- and OFF-parasol, and the small bistriated cell; Supplementary Fig. 1) were present at a relatively high density, accounting for the great majority of ganglion cells (~80%) that project to the LGN (parasol cells also project to the SC\textsuperscript{48}). The remaining ~20% of the ganglion cell population comprised a larger number of distinct cell types, each present at relatively low densities (0.5–1.5%) (Supplementary Table 1). One of these low-density types showed recursively branching dendrites that were bistriatified at approximately the same depth as the outer and inner cholinergic bands (Fig. 2d), and in this regard resembled the ON–OFF DSGCs of non-primate mammals\textsuperscript{7}. These cells were retrogradely labeled from tracer injections made into either the LGN or the SC; and were not observed after injections into the pretectum (pretectal olivary nucleus, nucleus of the optic tract, and dorsal terminal nucleus of the accessory optic system; see methods for details) but we cannot exclude the possibility of additional projections to these targets. These recursive bistriatified ganglion cells tiled the retina uniformly with minimal dendritic overlap (Fig. 2a). Thus unlike the non-primate mammal, where four ON–OFF DSGC types with distinct preferred directions form overlapping mosaics and together made up ~12% of the total number of ganglion cells\textsuperscript{49}, we observed only a single mosaic that comprised ~1.5% of the total ganglion cells in the peripheral retina (Fig. 2 and Supplementary Table 1). We also observed a second class of recursively branching ganglion cell present in the same retrogradely labeled retinas (the recursive monostratified cell; Fig. 1; Supplementary Fig. 1) that we have not characterized functionally. These cells showed extensively overlapping and fasciculated dendrites (Supplementary Fig. 2) in the ON layer of the IPL and appeared to comprise at least three separate ganglion cell populations with similar morphology.

The recursive bistriatified ganglion cell type is direction selective. A major advantage of in vitro photostaining was that we could target specific morphologically identified types for physiological characterization\textsuperscript{46,47} in the in vitro retina\textsuperscript{25}. The
recursive bistratified cells transiently depolarized and spiked at both light ONset and OFFset (Fig. 3b) as expected from their depth of stratification in the ON and OFF subdivisions of the inner plexiform layer (IPL). Direction selectivity was found in response to moving bar stimuli (Fig. 3c, d) ($n = 26$ cells; mean ± s.d., $\text{DSI} = 0.82 \pm 0.20$). The relatively low density of the recursive bistratified cells, and their presence as only a single ganglion cell type, likely contributed to their apparent absence in early surveys of primate ganglion cell physiology. The preferred direction was variable in this relatively small sample and foveal location was only roughly estimated, however, cells recorded in nearby locations from the same retina tended to show very similar direction preferences (Fig. 3d). We also note that the sharpness of DS tuning but not the preferred direction varied with the parameter
Fig. 1 Summary of the morphology, stratification and relative spatial densities 19 ganglion cell populations in macaque retina. a Macaque ganglion cells divided into 11 distinctive morphological groups by dendritic structure, dendritic tree diameter, and mosaic tiling (see Supplementary Fig. 1 for details). These morphological groups bear names that arose historically (midget, parasol) or more recently, and related to specific morphological features of newly identified types (e.g., broad thorny ganglion cells show a unique broad dendritic stratification and fine, thorn-covered dendrites. The color inserts show schematically the cell body (small circle) and dendritic arbor of each cell type indicating how some groups (e.g., midget and parasol) have been further subdivided into types based on stratification within the inner plexiform layer (IPL). Percentages indicate estimated % of total ganglion cell density for that group. The number of types within each named morphological group are indicated by the number of cell bodies associated either with dendrites that stratify at different depths in the IPL (or in the single case of the recursive monostratified cells the same depth). This results in a total of 19 ganglion cell populations that together comprise ~97% of the total ganglion cell population in the peripheral retina (see also Supplementary Fig. 1 and Supplementary Table 1).

b Stratification depth in the IPL summarized schematically for all cell types in relationship to the outer and inner choline acetyltransferase (Chat) immunolabeled strata formed by the dendrites of starburst amacrine cells (gray bands) as indicated (see Supplementary Figs. 1 and 3 for details).

c, d Dendritic field diameters of low-density recursive bistratified ganglion cells (purple circles) plotted as a function of retinal eccentricity (n = 122; mean ± s.d. = 327 ± 93; range = 117–557 µm); is large relative to a sample of LGN-projecting parasol ganglion cells, shown for comparison (blue circles). Stratification depth of inner and outer recursive bistratified cell dendrites indicates costratification with the choline acetyltransferase immunolabeled strata (n = 3; see also Supplementary Fig. 1f). Data are shown as mean ± s.d.; n number of cells.

Fig. 2 Dendritic morphology and field size, spatial density, and stratification depth of recursive bistratified ganglion cells in the macaque monkey retina. Recursive bistratified cells comprise ~1.5% of total ganglion cells in the retinal periphery (dendritic field coverage = 1.3; see Supplementary Fig. 1 for details) and were retrogradely labeled and photostained in vitro from injections of rhodamine dextran made into the Lateral Geniculate Nucleus (LGN) and Superior Colliculus (SC). a Camera Lucida tracings of four photostained cells at ~7 mm retinal eccentricity from tracer injections in the superior colliculus; inset, outlines around dendritic perimeters for each of the four cells indicates regular spacing and little dendritic field overlap. b Photomicrograph of a recursive bistratified cell photostained in the in vitro retina after retrograde transport of rhodamine dextran permits precise targeting of this cell type for physiological study. c Dendritic field diameter of low-density recursive bistratified ganglion cells (purple circles) plotted as a function of retinal eccentricity (n = 122; mean ± s.d. = 327 ± 93; range = 117–557 µm); is large relative to a sample of LGN-projecting parasol ganglion cells, shown for comparison (blue circles). Stratification depth of inner and outer recursive bistratified cell dendrites indicates costratification with the choline acetyltransferase immunolabeled strata (n = 3; see also Supplementary Fig. 1f). Data are shown as mean ± s.d.; n number of cells.
of the response that was being measured (spike rate vs total spike count) and the properties of the stimulus (e.g., velocity and contrast) that evoked it but did not explore these parameter spaces in detail here. In the mouse retina, variability in direction preference was linked to retinal topography and in turn to the optic-flow fields generated on the retina as the animal moves through the environment. This question remains to be addressed directly in the primate, but such an optic-flow related spatial geometry predicts that as a population the ON–OFF DSGCs should show varied direction preferences aligned with the flow lines that radiate from the foveal center.

The ON–OFF direction-selective ganglion cell is tracer coupled to a direction-selective, polyaxonal, ON–OFF spiking amacrine-cell type. In some identified recursive bistratified cells, we made additional intracellular injections of the tracer Neurobiotin. Neurobiotin can pass through gap junctions and reveal cell-type-specific patterns of neuronal coupling that are distinctive for a particular retinal microcircuit. In rabbit retina, such injections have shown that one of the four ON–OFF DSGCs is coupled to neighboring ganglion cells of the same type (homotypic coupling). For the macaque recursive bistratified cell, Neurobiotin coupling was not present in neighboring recursive bistratified ganglion cells but was present in a distinctive cell type with a very large cell body that was often displaced into the IPL (Fig. 4a). The morphology, albeit incomplete, as revealed by the tracer coupling strongly suggested that these cells corresponded to the A1 amacrine cell, a previously identified spiking, ON–OFF cell type. A1 cells showed a distinctive, polyaxonal morphology in which multiple, long-projecting axons arose from proximal dendrites and extended in various directions for millimeters within the IPL (Fig. 4f–h). To directly test the hypothesis that A1 cells are coupled to recursive bistratified ganglion cells, A1 cells in the in vitro retina were identified by their distinctive somatic morphology and transient burst of spikes at light ONset and OFFset and were filled by intracellular injection with Po-Pro-1, a cationic fluorescent tracer that passes through gap junctions. Po-Pro-1 revealed coupling patterns similar to that found for Neurobiotin but which could be observed by 2-photon (2 P) fluorescence imaging in the functioning retina in vitro. Po-Pro-1 injections into A1 cells revealed both hetero- and homotypic coupling similar to that previously described, including coupling to a single ganglion cell type with a small cell body (Fig. 4b, c). The Po-Pro-1 labeled ganglion cells corresponded to the recursive bistratified ganglion cell type as hypothesized and showed the expected direction-selective light response (Fig. 4d, e).
Given the link to the recursive bistratified cell and its similar transient, ON–OFF spiking response (Fig. 4j), we tested the A1 cell for direction selectivity. The membrane voltage recorded at the cell body showed directional tuning both for the modulation of the membrane potential (Fig. 4k) and the associated spike discharge (Fig. 4l) in response to moving bar stimuli. A1 cells were filled with the calcium indicator Oregon Green BAPTA-488 (OGB) during intracellular recording (Fig. 4i) and both the long-projecting axons and the thick, spiny dendrites showed stimulus-evoked direction-selective Ca\(^{2+}\) signals (Fig. 4m, n, respectively).

Connectomic reconstruction of A1 cells reveals polarization of synaptic inputs and outputs. The A1 cell’s distinctive axon-like...
processes (Fig. 4f–h) are obvious candidates for spike generation, long-range transmission across the retina, and synaptic output54,58. However, amacrine-cell processes can be both presynaptic and postsynaptic57 and it is unknown how synaptic inputs and outputs are deployed on or from either the spiny dendritic tree or the long axon-like processes, making it difficult to hypothesize about the A1 cell’s role(s) with regard to direction-selective circuitry. Thus, to begin to address this question we initiated connectomic reconstruction of an identified A1 cell (Fig. 5a). After an A1 cell was identified by intracellular recording and dye-filling, the retina was fixed for electron microscopy. Near-Infrared Branding54,58 (NIRB) was then used to burn fiducial marks around the A1 cell body with the 2P laser. After embedding the cell was located by the NIRBed markings and scanning block-face electron microscopy59 (SBFEM) was used to create a retinal volume that included dendritic and axon-like components of the identified A1 cell within the inner plexiform layer (Fig. 5b–h). We found that the thick, spiny dendrites were entirely postsynaptic, and indeed were embedded in a dense plexus of a near-continuous gantlet of inhibitory synaptic inputs from large distinctive varicosities (Fig. 5c, yellow balls, Fig. 5h) with bipolar ribbon synapses targeted to the prominent dendritic spines (Fig. 5d, red balls, Fig. 5h, zoomed inset). Partial reconstructions suggested that the dense inhibitory inputs to A1 dendrites include starburst amacrine cells. By contrast, the axons were entirely presynaptic, with the swellings along the axon’s length making large vesicle-rich presumably inhibitory contacts (Fig. 5c, f–h; Supplementary Fig. 4). The majority output was to bipolar-cell axon terminals, with additional output to ganglion cell dendrites and a minor output to other amacrine processes (Supplementary Fig. 4). Thus, the spiking A1 amacrine appeared entirely polarized, with excitatory and inhibitory inputs to the spiny dendritic tree and inhibitory output arising from the distinctive synaptic boutons that studded the long-projecting, axonal processes.

Morphological identification and direction selectivity of macaque starburst amacrine cells. In non-primate mammals, the starburst amacrine-cell type is the synaptic origin of the direction-selective response observed in ON–OFF DSGCs. Thus, the direction selectivity found in the spiking A1 amacrine could also originate from the primate’s starburst amacrine-cell type. It was therefore critical to identify starbursts in the macaque in vitro retina and to determine whether they show direction selectivity. We identified starbursts displaced to the ganglion cell layer (presumed ON-type), as there are known in non-primate mammals39, by their small, round cell bodies (~8 µm in diameter) and targeted them for whole-cell, patch-clamp recordings. All starbursts were confirmed by their highly stereotyped and characteristic dendritic morphology after dye-filling (either OGB-488 or Alexa fluor 488 or 568) and correlated ON-type light response (Fig. 6a, b and Fig. 7a and Supplementary Fig. 5). Amacrine cells with some morphological features similar to starbursts were observed (Supplementary Fig. 5, l) but distinguished from starburst cells by atypical physiological properties.

A key aspect of starburst cells’ direction tuning is the preference for stimuli that move outward, or centrifugally, from its cell body20,21,60. Therefore, previous studies have used circular gratings centered at the cell body, that either expand (move outward) or contract (move inward) to measure direction selectivity in starburst cells20,61,62. In macaque, the somatic voltage response to such a ‘bullseye’ grating showed a large unequivocal preference for outward motion (Fig. 6c). To measure direction selectivity in the dendrites, as we did for the A1 cells, we again used 2P imaging of calcium transients after cell filling with OGB-488. Dendritic ROIs showed the same striking preference for centrifugal movement (Fig. 6d) as well as broad directional tuning to moving bars or drifting gratings (Fig. 6e–g).

Macaque starburst amacrine cells show center-surround receptive-field structure independent of GABAergic inhibition. In non-primate mammals, an inhibitory surround created by abundant starburst-to-starburst GABAergic synaptic connections has been proposed as a key mechanism for the centrifugal directionality of starburst dendrites60. We thus wanted to further understand the receptive-field spatial structure of starbursts in the macaque to determine if an antagonistic surround was present. Using spots of light of increasing diameter or drifting gratings of increasing spatial frequency, we confirmed that inner starburst cells were ON center (Fig. 7a) and showed clear center-surround organization, well described by the Difference-of-Gaussians (DoG) receptive-field model63,64 (Fig. 7b, c). Annular stimuli were then used to isolate the surround-response component which appeared as a large depolarization at light Offset (Fig. 7d, shaded panel). Surprisingly, this isolated surround-induced OFF-depolarization persisted unattenuated in the presence of the GABAa receptor antagonist SR 95531 (GABAzine) (Fig. 7e, shaded panel). We hypothesized that the surround originates presynaptically in the center-surround organization of the excitatory bipolar cell’s55 input to the starburst. Further support for this conclusion is provided by somatic voltage-clamp recordings showing that synaptic currents evoked by annular stimuli that isolated the surround evoked inward (excitatory) currents (Supplementary Fig. 6).

If the starburst antagonistic surround arises presynaptically via excitatory bipolar input the surround would arise in the outer retinal circuitry, presumably by negative feedback from...
horizontal cells to cones. We therefore tested the effect of HEPES buffer enrichment, which attenuates the surrounds of inner retinal neurons by acting to reduce negative feedback from horizontal cells to cones. By contrast, with bath application of the GABAa receptor antagonist, the starburst surround was largely abolished by increasing the pH-buffer capacity of the Ames’ medium bath solution with the addition of HEPES (pH 7.3; 20 mM) (Fig. 8a, b).

Given a presynaptic, excitatory basis for center-surround receptive-field organization in light-adapted macaque starburst cells, and the abolition of this surround by HEPES buffer enrichment we determined the effect of HEPES on starburst direction selectivity. HEPES dramatically reduced both somatic (Fig. 8c, d) and dendritic (Fig. 8e, f) preference for outward motion. For both the somatic voltage response (Welch’s F (1, 6.748) = 42.535, *p = 0.000382) and the dendritic calcium response (Welch’s F (2, 20.373) = 15.670, *p = 0.000076), the effect of HEPES on responses to high-contrast, inward-moving gratings was primarily to increase their amplitude, with little effect on the response to outward motion. We conclude that, at least for radial motion stimuli, the bipolar cell antagonistic surround is critical for starburst direction selectivity.

To develop insight into how the excitatory surround originating in bipolar cells was critical for radial motion sensitivity in starburst cells, we created a realistic neural model of the starburst receptive field based on the summation of bipolar cells’ center-surround receptive fields to the dendritic tree. To do this, we first needed to estimate the receptive-field sizes of presynaptic bipolar cells as well as the identity and distribution of bipolar-cell synaptic input to the starburst tree. This was especially critical given recent evidence that starburst circuitry can vary...
dramatically even across two well-studied non-primate mammalian species\textsuperscript{69}.

Stationary, contrast-reversing grating stimuli varied in spatial frequency can isolate nonlinear bipolar-cell "subunit" responses transmitted to Y-type ganglion cells by quantifying the second harmonic (F2) response\textsuperscript{70,71}. We hypothesized that transient bipolar inputs to the starburst dendritic tree would likely show nonlinear spatial summation\textsuperscript{48,72}, and found that indeed such a stimulus revealed a second harmonic response in the starburst (Supplementary Fig. 7a) that extended to high-spatial frequencies, implying a small receptive-field center (30-50 µm Gaussian diameter, Supplementary Fig. 7b). We hypothesize that these subunits in the starburst receptive-field center correspond to rectifying bipolar-cell inputs\textsuperscript{60,63}. Consistent with this hypothesis,
the distinctive F2 response to contrast-reversing gratings remained when the surround was attenuated by HEPES buffer enrichment (Supplementary Fig. 7c). Then, with a preliminary model of the starburst and an array of presynaptic bipolar cells, we found that a 30 µm bipolar cell receptive-field diameter could reproduce the real F2 response (Supplementary Fig. 7d). This calibration, along with connectomic data showing the number and type of bipolar cells converging onto the starburst, allowed us to further develop the model of the starburst receptive field.

**Connectomic identification of cone bipolar input layout to a starburst dendritic tree.** Next, to determine the identity and spatial layout of bipolar input to the starburst dendritic tree we again used the NIRB method (Fig. 9a, b) and SBFEM to target a physiologically and anatomically identified starburst for micro-circuit reconstruction. The central 150 µm of an OGB-filled starburst dendritic tree was completely reconstructed (Fig. 9a, g). Inhibitory synaptic outputs, and excitatory and inhibitory synaptic inputs to the starburst dendrites, were identified in high-resolution images and mapped (Fig. 9c–f). Ribbon synapses from bipolar-cell axon terminals were sparsely distributed over the dendritic tree (48 synapses in total) and occurred in the great majority of instances at small spines that extended from the very thin main dendrite (Fig. 9d, e, g inset). These spines usually contained small clusters of vesicles in proximity to the ribbon, but no clear synaptic specialization was apparent.

The complete morphology of the bipolar cells’ axon terminals presynaptic to the starburst were reconstructed, and two anatomically distinct groups were identified. One group had a very small axon terminal with large, bulbous terminal swellings stratified in the IPL at the inner border of the starburst dendrites. The other group showed larger, more highly branched terminals with small terminal swellings stratified more superficially at the outer border of the starburst dendrites (Fig. 9h, i). The starburst dendrites thus traversed a space between these two bipolar axon terminal types, sending short spines either outward or inward to receive ribbon synaptic inputs from both groups. Complete reconstructions of the smaller, more deeply stratified terminals revealed extensive synaptic contact with identified midget (parvocellular LGN projecting) ganglion cells (Fig. 10a–e) and therefore could be unequivocally identified as the midget bipolar-cell type73. By contrast, the larger arbor made extensive synaptic output to an identified parasol (magnocellular LGN projecting) ganglion cell (Fig. 10f–h). Only a single mosaic (single type) of these larger bipolar-cell axon terminals was evident but we refer to this type as DB4/5 because both previously described types (diffuse bipolar types 4 and 5) are now understood to costratify in the IPL, synapsing upon parasol ganglion cells, and showing largely indistinguishable axon-terminal morphology73. Thus, the macaque starburst draws its excitatory input from the major parvocellular and magnocellular visual pathways that transmit sustained vs transient signals, respectively74,75. We determined the spatial layout of the inputs from these two bipolar-cell populations on the starburst dendritic tree (Fig. 9j), and found that the midget bipolar cells were distributed over the entire dendritic tree although tending to dominate proximally, whereas the DB4/5 axon terminals predominated more peripherally on the starburst dendritic tree (Welch’s F (1, 45.580) = 10.033, *p = 0.002744) (Fig. 9j; inset histogram).

Utilizing this information from our connectomic reconstruction, we built a realistic starburst model with 40 midget bipolars, providing a relatively sustained input to the starburst cell, located proximally out to a radial distance of 65 µm. Beyond this distance, midget and DB4/5 bipolar cells in the ratio 1/3 to 2/3, respectively, provided a more transient excitatory input (total of 70 bipolars) (Fig. 9k). Synaptic inhibition was omitted. The model replicated the experimental results, with the starburst cell strongly preferring outward radial motion (Fig. 9i). The model showed in addition that the proximal bipolar inputs to the starburst were, in particular, themselves outward motion-sensitive because their center response was unhindered by subtraction from a delayed surround (Supplementary Fig. 8). When the model was run without the bipolar-cell surround, none of the bipolars were motion-sensitive, and both proximal and distal bipolar cells showed negligible directional differences. This modeling suggested that the radial-grating stimulus generates directional differences in the starburst response based on the fundamental delay of the bipolar receptive-field surround. In models run with a bipolar surround with zero delay, the bipolar-cell responses showed no motion sensitivity, but a modest directional difference was generated in the starburst amacrine cell dendrites, suggesting that a given stimulus condition may engage varied redundant mechanisms for direction selectivity in the starburst amacrine cell. In this regard it is worth noting the critical interaction between surround delay and stimulus velocity. Thus, models with a smaller surround delay (e.g., 20 ms vs. 40 ms) produced a smaller but qualitatively similar outward directional preference in the starburst dendrites, that could be offset by models run with a higher velocity grating (e.g., 400 µm/s vs. 200 µm/s) which produced a larger outward directional preference.

**Discussion**

Direction selectivity appears to start in the primate visual system much earlier than previously recognized. The retinal output has long been considered to be dominated by a few, relatively high-density visual pathways76 that project via the parvocellular and magnocellular LGN to primary visual cortex77. It is now clear that these high-density pathways—linked to the requirements of high-spatial-resolution
Fig. 7 Macaque starburst amacrine cells show center-surround receptive-field structure that is insensitive to GABAa receptor antagonists.

a Starburst whole-cell current-clamp recording (cell imaged in Supplementary Fig. 5e) shows a resting membrane potential ~75 mV on a high photopic background (~10^5 photoisomerizations/cone/sec) and large amplitude depolarizing response (~40 mV) to 0.5 s, 100% contrast, square wave pulse stimulus. With increasing spot diameter (inset at top; 50–720 µm diameter; color coded) the response becomes more transient (black trace, 720 µm diam spot) and the sustained response component is lost. A characteristic feature of the starburst response to this stimulus is a large, spike-like transient that appears as the stimulus is enlarged to fill the receptive-field center.
b, c Starburst spatial tuning (100% sinusoidal contrast modulation of spots of varied diameter at 2 Hz), fit with a center-surround Difference-of-Gaussians receptive-field model (line fit to data; n = 27 cells; center diam, 208 ± 76; surround diam, 360 ± 156); 2D profile of model fit is shown in c; small icon shows starburst soma and dendritic tree at same scale as model.
d Surround response isolated by annular stimuli; increasing the inner diameter of the annulus from 20 to 400 µm (icons at center; top to bottom panels; 2 Hz square wave modulation) eliminates the ON-center depolarization (top panel) and isolates a pure OFF-surround depolarization (bottom panel).
e Surround OFF depolarization is not eliminated by bath application of GABAa receptor antagonist (SR 95331(GABAzine); 10 µM; n = 4; 8 ± 3% reduction).
foveal vision—made it technically difficult to identify the great diversity of relatively low-density ganglion cell types, including discovery of chromatic, achromatic, and non-image-forming pathways. The discovery of a direction-selective ganglion cell in the primate thus necessarily awaited a more complete accounting of these less accessible retinal cell types. Our catalog of 19 different RGC cell types accounts for ~97% of all RGC cells, leaving little margin for additional types. This number is remarkably close to the number of RGC clusters identified in single-cell transcriptional profiling of both macaque retina and human retina, but differs dramatically from the over 40 RGC types/clusters found employing similar methods to mouse retina.

The recursive bistratified ganglion cell was retrogradely labeled from tracer injections into both the LGN and superior colliculus...
and we make the parsimonious assumption that the single anatomically distinct population forming a single mosaic must project to both structures by a branching axon, as is the case for most other ganglion cell types. Only a small fraction (~10%) of LGN and collicular cells show direction selectivity, but this is the expected result for a very low-density retinal output that projects in parallel with a few high-density pathways. We observed a single ON-OFF DSGC population with unity coverage and even though we have accounted for greater than 95% of total ganglion cells (Supplementary Table 1) it remains possible that additional very low-density DSGCs remain to be discovered. It is possible that the known lower density of outer (presumed OFF) starburst cells in human and non-human primate retina could be explained by this relative reduction in ON-OFF DSGCs (1 type vs 4 types in mouse and rabbit). A population of recursive monostratified cells (Supplementary Fig. 1i, 2) are candidates for the ON-D5 types present in non-primate mammals, but the visual physiology, and relationship to the cholinergic plexus for these ganglion cells has yet to be characterized.

The presence of direction selectivity in a spiking, polyaxonal, amacrine cell linked to the recursive bistratified ganglion cell is distinctive but parallels an emerging picture in the non-primate mammal. Direction selectivity in the ON-OFF A1 cell could arise via a gap junction with the ON-OFF DSGC (though we have not observed this feature in our connectomic reconstruction). If this were the case, the dominant axonal output synapse to bipolar-cell axon terminals could contribute to direction selectivity in the latter. Alternatively, a directional signal could flow in the opposite direction, originating in the spiny dendritic tree. There is a striking precedent for such a circuit in mouse retina where a gap junction between polyaxonal amacrine cells and a single type of ON-DSCG underlies a direction-selective response that arises independent of GABAergic inhibition. Regardless of the origin of the direction-selective signal in the A1 cells, its spiking axonal projection suggests that a directionally-tuned inhibitory signal can be transmitted for millimeters across the retina (Fig. 4h), contrasting sharply with the local computation that occurs within semi-isolated electrodendritic compartments of starburst amacrine cells. Moreover, it remains unclear how the direction-selective output is represented in the multiple axonal projections that show distinct origins from separate dendritic sectors.

The neural mechanisms proposed for starburst dendritic direction selectivity include spatially asymmetric wiring of sustained and transient bipolar-cell inputs, GABAergic inhibition, and the electrotonic properties and ion-channel composition of the dendrites. The asymmetric spatial layout of the bipolar-cell inputs from the classic sustained midget-parvocellular pathway and the transient parasol-magnocellular pathway to the macaque starburst bears some similarity to recent findings in the mouse. However, the significance of such a synaptic motif as a mechanism for direction selectivity remains controversial and, necessarily awaits further study in the primate. In the macaque starburst, a very strong surround was present, but surprisingly it was not mediated by the GABAa receptors on starburst dendrites, suggesting that inhibitory network interactions are not essential for light-adapted surround generation in primate retina. Instead, we found that the surround arises presynaptically and is transmitted by the excitatory center-surround receptive field of the bipolar-cell input to starbursts. Moreover, this excitatory surround is a critical element in the generation of starburst direction selectivity evoked by radial gratings, paralleling recent modeling of bipolar cells in mouse retina. Our own initial modeling suggests that the delay of the bipolar surround relative to the center can be utilized to create radial motion preference in the starburst cell dendrites.

Individual dendritic sectors in mouse starburst amacrine cells give rise to direction selectivity in four ON–OFF DSGC types by highly selective asymmetric inhibitory connections. The four DSGC subtypes differ in preferred direction and provide a directional signal that varies systematically with retinal location so as to align with optic-flow fields generated on the retina by the animal’s motion through the environment. In the primate, we have found only a single ON-OFF DSGC type. Outstanding questions thus include how these cells signal direction in relation to the optic flow organized around the foveal center in the forward-facing eyes of a primate, how this single, ON-OFF DSGC type is synthetically linked to the starburst dendritic plexus, and whether removing the bipolar-cell surround alters direction tuning in recursive bistratified ganglion cells. Lastly, our results raise the broader question of how the retinal direction-selective pathway interacts with direction and orientation tuned signals, generated in parallel, by similar circuit motifs within diverse visual areas of primate neocortex.

Methods

Retinal in vitro preparation. Eyes were removed from deeply anesthetized male and female macaque monkeys at the time of death (Macaca nemestrina, Macaca fascicularis, or Macaca mulatta) via the Tissue Distribution Program of the Washington National Primate Research Center and in accordance with protocols reviewed and approved by the University of Washington Institutional Animal Care and Use Committee. A total of 106 retinas acquired from 76 animals were used for this study. The retina was maintained in vitro by dissecting retina-choroid free of the vitreous and sclera in oxygenated Ames’ medium (A1420; Sigma Chemical Co., St. Louis, MO) under light-adapted conditions. The retina-choroid was placed flat, vitreal surface up, in a glass-bottomed superfusion chamber coated with poly-lysine mounted, choroid side down, on the microscope stage. The retina was continuously superfused with Ames’ medium (pH 7.3; oxygenated with 95% O2/5% CO2) and the temperature was thermostatically maintained within the chamber (TC-344B, Warner Instruments) at ~36 °C. The retina was observed under infrared illumination projected through the choroid from the microscope stage to the objective lens.
**Retrograde tracer labeling and in vitro photodynamic staining.** The tracer biotinylated rhodamine dextran was injected into physiologically identified central visual target areas (LGN, 14 animals; pretectum, 8 animals; superior colliculus, 7 animals) in adult macaque monkeys as described previously. In brief, LGN injections were made after mapping LGN borders during recordings of large light-evoked multiunit potentials. Tracer injections were then made in both right and left LGN at multiple depths and anterior-posterior locations with the LGN boundaries. Similarly, SC injections were made in the visual responsive superficial layers at multiple locations. While our histology showed injection sites confined to SC, we cannot exclude tracer spread to pretectal nuclei due to their proximity to the overlying SC. Our pretectal injections were made after recording visual activity in the pretectal olivary nucleus (PON); injections would have likely included the overlying nucleus of the optic tract/dorsal terminal nucleus (NOT/DTN) and would potentially encroach on fibers of passage projecting to the superior colliculus. The relatively large number of animals used for this study reflects progress through multiple experimental goals over an extended time period. For example, as
shown in Fig. 3, the recursive bistriated cells were targeted in retinas where photostained mosaics could be unequivocally identified. After a survival time of 4–7 days, animals were deeply anesthetized, the eyes removed, and the retinas prepared for in vitro physiological recording as described above. Labeled cells were photostained in vitro by brief exposure to epifluorescent illumination which generated the sequestered fluorescence tracing, revealing the complete dendritic morphology of labeled ganglion cells. Following an experiment, retinas were fixed and processed for horseradish peroxidase (HRP) histochemistry using the Vector avidin-biotin-HRP complex. Lightly labeled cells were enhanced in one of two ways. Either photochemically with 0.02% nitro blue tetrazolium (NBT; N-5514; Sigma, St Louis MO) to intensify the DAB reaction product, or by incubating the retinas in biotinylated anti-rhodamine (C# BA-0605, Vector Labs, Burlingame, CA) to intensify the DAB reaction product, or by incubating the retinas in biotinylated anti-rhodamine (C# BA-0605, Vector Labs, Burlingame, CA); antibody design and characterization has been reported previously in detail. Briefly, following enucleation, retinas were dissected free of the eyeball and fixed flat for 2 h in 4% paraformaldehyde. Primary antibody incubation was at 1:100 for 1–4 days and secondary antibody incubation at 1:100 for 1–2 days, both at 4 °C. After rinsing, retinas were processed for HRP histochemistry, intensifying with 0.02% NBT when needed, mounted on slides and coverslipped as described above.

Data analysis of HRP-stained cells. Dendritic field area was determined from high magnification camera lucida tracings of individual cells. The perimeter of a convex polygon drawn around the dendritic tree was measured, and the area was calculated. Effective dendritic field diameter was taken as that of a circle having the same area. The tiling and dendritic tree overlap and thus coverage factor for each cell/mm². We then divided mean dendritic field area in mm² by coverage factor to determine cell density for a given type in cells/mm². This was then expressed as a percentage of the total ganglion cell number at this same area. The tiling and dendritic tree overlap can produce a significant change in relative density. For this reason, our estimate that 97% of the total ganglion cells have been accounted for relies on the finding that midget ganglion cells show a coverage of 1. Near-infrared branding (NIRB) This method permits correlation of light and electron microscopy at the level of single identified neurons. After an A1 amacrine or starburst amacrine had been targeted and identified physiologically and morphologically, the cells were intracellular filled with Alexa fluor 568. The tissue containing the cell was dissected and placed in EM fixative (2.5% glutaraldehyde/2% paraformaldehyde; in 0.1 M cacodylate buffer, 1 h). The tissue piece was then placed on a microscope slide, coverslipped in buffer and returned to the 2P-confocal microscope where the fluorescent amacrine-cell body was located and centered in the field of view with the appropriate filters under episcopic illumination. The cell body was then visualized with the 2P laser and scan line marks made with the focus on the retinal surface/optic fiber layer (linescan mode, 860 nm, power at the entrance to the microscope ~300–350 mW). Scan lines were 40–50 microns in length with scanner running at 40–50 Hz for ~10 s. With these parameters the scanned lines created tissue defects of ~2 μm in the XY and Z dimensions and were easily detectable after plastic embedding during preliminary semithin sectioning. Scan lines detects defects in the retinal tissue are autofluorescent permitting images to be made in which the exact location of the scan lines relative to the fluorescent cell body are recorded; scan line detects detected during subsequent sectioning thus permit a precise localization of the target amacrine cell.
thick sections in an array of 20, 8000 × 8000 pixel (40 × 40 µm) tiles (10% tile overlap) that extended around the NIRBed area. The resulting set of ~16,000 TIFF images were contrast normalized, stitched into composite layers, then aligned into a volume using within layer and across layer procedures available with TrakEM2 software102 (NIH FIJI plugin). All cell and circuit reconstructions were performed using TrakEM2 to first create arbor skeletons; nodes within these skeletons were placed on synaptic structures so that the locations and number of synapses for a given cell (e.g., number and locations of ribbon synapses in an identified midget bipolar-cell presynaptic to a starburst amacrine cell) could be determined. Ground truth volume rendering of selected cells and cell profiles was performed manually using the painting tools available in TrakEM by multiple annotators and proofreaders.

**Electrophysiology and 2-photon imaging.** Retinal ganglion cells were observed using a 25X water immersion Olympus Objective (NA 1.05) under infrared
illumination. Patch pipettes made from borosilicate glass were filled with either Ames’ medium for extracellular “loose” patch recordings or with a K-based solution for whole-cell patch recordings. The pipette filling solution contained (in mM): K-glutamate (120), MgCl₂ (1), KCl (15), NaCl (8), HEPES (10), phospho- creatine (6.6), ATP (4.8), and 0.3 GTP-Na₃, adjusted to pH 7.3 (Osmolarity, ~ 300 mOsm). In most instances 200 µM Oregon Green BAPAT-488, a fluorescent Ca indicator (150 or 200 µM) and/or Alexa fluor 488 or 568 (200 µM) was added to the pipette solution. We estimated a liquid junction potential of ~10 mV and therefore subtracted 10 mV from all membrane potentials intracel- larly recorded in current clamp.

Data acquisition and the delivery of visual stimuli were coordinated by custom software running on an Apple Macintosh computer. Current and spike waveforms were Bessel filtered at 2 or 5 kHz and sampled at 10 kHz. To map the receptive field of the cell, the cell body was first placed at the precise center of the stimulus field. Flashing white squares (2 Hz temporal frequency, 10 or 25 µm wide) were systematically moved in the x and y directions to locate the most sensitive point of the receptive field and determine its approximate size. The location of the maximum spike response was defined as the receptive-field midpoint; and visual stimuli were positioned relative to this point.

Two-photon laser scanning fluorescence imaging was performed with the same 25x objective lens on a Sutter Instruments DS-1000 multiphoton imaging package for the Olympus BX51W1 microscope that was modified to incorporate the output of a digital light projector that provided visual stimuli. Fluorescence was excited by 100-fs pulses at 80 MHz tuned to 955-nm center wavelength from a Ti:Sapphire laser (Spectra Physics Mai Tai HP). Laser intensity was regulated with a Pockels cell (Model 350–80 with model 302 driver, Conoptics) with the power measured at the retina of ~2.5 mW. ROIs for Ca²⁺ activity scanning were 10 × 10 microns (34 × 34 pixels; 3.4 pixels/μm; pixel dwell time, 32 μs, 23.4 Hz frame rate). Fluorescence emission from the laser scanned fields was collected by the objective and delivered to a photomultiplier tube (PMT) (Hamamatsu GaAsP PMT, H10170PA-40) via a dichroic mirror and bandpass filters selective for fluorescein emission at 520 nm. The PMT output signal was digitized and processed using ScanImage, a software application for laser scanning microscopy (Vidrio Technologies). Laser scanning for morphological imaging of neuronal structure was typically done at either 512 × 512 or 1024 × 1024 pixels and image stacks were created at ~1.5 µm z resolution; maximum intensity flattened images were made with NIH FIJI software.

Visual stimulus generation. All visual stimuli used were created either by pro- gramming a computer graphics card (ATI Radeon HD 5770) in OpenGL or using a visual stimulator (VSG5, Cambridge Research Systems) equipped with a toolbox of stimulus presentations. The stimulus video signal drove a digital light projector (Vista X3, Christie Digital Systems) whose output beam was optically relayed into the microscope camera port where a dichroic mirror directed it down the optical axis and into the rear aperture of the microscope objective. The objective focused the stimulus onto the photoreceptor layer of the retina. The irradiance spectra for the microscope camera port where a dichroic mirror directed it down the optical system. The irradiance spectra for the microscope camera port where a dichroic mirror directed it down the optical system. The irradiance spectra for the microscope camera port where a dichroic mirror directed it down the optical system. The irradiance spectra for the microscope camera port where a dichroic mirror directed it down the optical system. The irradiance spectra for the microscope camera port where a dichroic mirror directed it down the optical system. The irradiance spectra for the microscope camera port where a dichroic mirror directed it down the optical system.

Direction selectivity measure. For moving bars or drifting gratings a direction selectivity index (DSI) was calculated as, DSI = (P (Preferred response) – N (Null response))/P (Preferred response), where Preferred response is the maximum response and Null response is the response produced by the stimulus moving in the opposite direction. Responses were either total spike counts (extra- and intracel- lar recorded ganglion cells) or the peak response amplitude for stimulus-evoked dendritic/axonal calcium response in starburst and A1 amacrine cells. For spikes our criteria for direction selectivity was taken as at least a 50% difference in the null vs preferred direction response. For example, a spike cell that responded to the preferred direction stimulus 50% more spikes than to the null direction stimulus would have DSI = 0.50. For the sake of comparison, a typical non-DS ganglion cell type like the midget cell had a mean DSI of 0.043 ± 0.03; n = 4 (ON cell type) corresponding to an insignificant 4% difference in the spike responses evoked by a stimulus moving in the preferred vs null directions. For a stimulus expanding (inward) vs expanding (outward) radially moving stimuli, we used the F1 Fourier value to quantify stimulus preference by taking the inward/outward response ratio. A ratio of 1 indicates equal response amplitudes to both inward and outward movement of the circular grating and a ratio 0.5 indicates that directional selectivity for inward is reduced 50% relative to outward motion.

Statistical analysis and reproducibility. All statistical tests were two sided and parametric. Statistical analyses were performed using SPSS version 28.0. In reporting and interpreting results, both the statistical significance (p value) and substantitive significance (effect size) were used. We used Welch’s ANOVA, and follow-up Games-Howell post hoc test, paired/and unpaired t-tests on the data set as appropriate. The sample sizes were unequal. We also report measures of effect size, such as Hedges’ g and Cohen’s d that reveals the magnitude of the difference between groups, since a p value can only show that a significant effect exists but cannot reveal the size of the effect.

For the examples of light and electron micrographs of retinal cells and synapses (e.g., Figs. 2b, 4a–d and Fig. 5a–g) all images were reproducible and represent examples from multiple samples. For example, the tracer coupling from the A1 amacrine cell to the retrobystiatrit ganglion cell in vitro was reproduced five times with images made. For all electron micrographs shown in Figs. 3, 9 and 10, all synaptic structures were identified (numbers provided in text) and the micrographs represent single examples from this large sample. Additional examples of A1 axon synaptic structures are provided in Supplementary Fig. 4. The starburst amacrine cell shown in Fig. 6a was selected from a large sample of images from all starburst recorded and morphologically identified, with many more examples shown in supplementary Fig. 5. In the cases of our two retinal volumes created for connectomic reconstruction it should be noted that these comprise a single physiologically identified starburst amacrine cell and a single physiologically identified A1 amacrine cell and thus represent single examples of each of these cell types.

Modeling. The dendritic morphology used in the model was digitized from a confocal image stack of a dye-filled macaque starburst cell. The Neuron-C simulator generated a compartmental model of the digitized morphology with a root node comprising a total of 379 compartments. The dendritic diameter at proximal, medial, and distal locations was determined from the charging current trace of an in vitro somatic voltage-clamped macaque starburst cell from a holding potential of ~70 mV, to a +5 mV step. A least-squares fit of the model to the charging curve determined thickness factors for proximal (0.4 μm), middle (1.0 μm), and distal (0.8 μm) dendrite segments, consistent with direct measurements at the ultrastructural level. We modeled the excitatory input to the starburst cell as an array of bipolar cells (n = 70; density, 1900 cells/mm²; average with an inner segment aperture of 9 μm. 1 Td was equivalent to ~30 photo- isomerizations/s/cone³⁸.
spacings –23 μm; regularity index 8). Most of the bipolar cells were given tonic release properties to simulate the midget bipolar-cell type. Beyond a radial distance of 65 μm, two-thirds of the bipolar cells were given a transient release property to simulate the transient release properties of presumed DB4/5 bipolars. Although the DB4/5 bipolars had a more transient release than the midget bipolar cells, the major effect of the DB4/5 transient release in response to sine-wave radial stimuli at a low temporal frequency (0.5 Hz; see Fig. 9I) was to attenuate their input to the starburst cell compared to the more tonic midget bipolar-cell response. Each bipolar cell was simulated as 2 compartments (one for the soma, and another for the axon terminal), and made a synapse onto the closest dendrite of the starburst amacrine cell if it was within a criterion distance (10 μm). Bipolar cells that did not make synaptic contacts were removed. Synaptic vesicle release was modulated by a readily-releasable pool that set the temporal properties of release (midget bipolar: pool size 50, max rate 200/s/DB4/5 pool size 8, max rate 50/s). The bipolar excitatory input synapses to the starburst cell were each given a maximum conductance appropriate to depolarize the starburst to ~60 mV (Gmax, 1500; 5–15 pS per bipolar for typical stimuli). The postynaptic effect of the lower release rate of the DB4/5 bipolars was increased with a greater maximum conductance (Gmax, 3000 pS per bipolar).

To simulate the starburst receptive field and responses to visual stimuli, we gave the bipolar center and surround Gaussian profiles (center diameter 30 μm, surround diameter 120–240 μm, surround/center weight 0.9–0.5) and ran simulated experiments using a variety of visual stimuli, including an expanding series of flashed spots, an expanding series of flashed annuli (outer diameter fixed at 1000 μm), and linear and radial sine-wave gratings. Stimuli were presented to each modeled bipolar cell as a voltage pulse corresponding to the stimulus pattern. The mean intensity and contrast were given as voltages (mean ~50 mV, contrast 4–7 mV). The results from were then fit with a Difference-of-Gaussians (DoG) receptive-field model and compared with the equivalent DoG measurements of starburst amacrine cells from experiments on real cells. Typical Gaussian diameters from model fits for the simulated cells were ~160 μm for the center, and 300–400 μm, weight 0.4–0.7 for the surround.

To provide intuition about the mechanisms involved in starburst direction-selective responses, we included dendritic voltage-gated ion channels (K, Ca-T, NaV) in different combinations. Ion channels were simulated with Markov diagrams taken from the literature and included in the simulator. Potassium channels were modeled as a non-inactivating Kv3 channel106,107. Calcium channels were simulated with a slowly-inactivating T-type channel that approximated the fast activation and slow inactivation kinetics of N/PQ-type channels108,109. Sodium channels were modeled as a NaV1.2 channel or a persistent NaV1.8 channel with slow depolarized activation kinetics108,110,111.

To capture the approximate center-surround dynamics, the bipolar surround Gaussian was simulated with a delay (typically 20–40 ms) from the center Gaussian with a strong integrated weight (0.8–0.9 of the center weight). While we recognize shorter surround delays in some previous models of primate ganglion cell receptive fields112 modeled surround delay was derived from measurements of HEPES-sensitive hyperpolarization response latencies in macaque starbursts. The delay and the strong surround weight allowed the surround to subtract from and reduce the amplitude of the overall bipole-cell response. Outward-moving radial gratings (spatial period 400 μm, velocity 200 μm/s, “contrast” 7 mV) evoked a ~10 mV response in proximal bipolar cells but inward-moving gratings evoked only a ~4 mV response, producing a directional difference of 6 mV (Supplementary Fig. 8). More distal bipolars had a smaller response to outward motion of the grating and a smaller directional difference (0.5 mV). A stationary counterphase grating (2 Hz) centered on the starburst soma at a series of different spatial frequencies evoked a frequency-doubled response comparable to the E2 plots from real data.

### Model biophysical properties

| Channel | Soma | Prox | Medial | Distal |
|---------|------|------|--------|--------|
| Kv3     | 1–3  | 0.2  | 0.2–0.5| 0      |
| Ca-T    | 0.2  | 0.2–2| 3–7   | 6–8    |
| Ca pump Vmax | 2e–6 | 0.5e–1e–6 | 1e–6 | 2e–6 |
| NaV     | 0.2–0.5| 0.2–0.5| 0.2–0.5| 0.2–0.5|
| Rm      | 50e3 Ohm cm²|
| Rs      | 200 Ohm cm²|

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