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Origins of direction selectivity in the primate retina

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Abstract

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^{1,2}. In primates, by contrast, direction selectivity is a hallmark of motion processing areas in visual cortex^{3,4}, but has not been found in the retina, despite significant effort^{5,6}. 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 poly-axonal amacrine cell and the starburst amacrine cell, all of which show direction selectivity. Moreover, we found unexpectedly 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 new door to investigation of a novel circuitry that computes motion direction in the primate visual system.

Introduction

About 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^{7,8} and in the retina of the rabbit^{9,10}. 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 cortex⁴ versus the diverse cell types and circuits in the non-primate mammalian retina^{11,12}. One reason for this dichotomy is that, while a significant fraction of retinal ganglion cells in non-primate mammals show direction selectivity^{2,13}, this fundamental neural signal has not been observed in the primate retina, despite dedicated effort^{6,14,15}. 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 cortex¹⁶⁻²⁰.

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. These cells have characteristic bistratified dendrites with a recursive branching pattern, and are present as four distinct populations that differ in their direction preference^{2,21,22}. Direction selectivity originates in the distinctive radial dendrites of a single type of retinal interneuron, the starburst amacrine cell^{23,24}, which uniquely co-expresses the inhibitory transmitter GABA and the excitatory transmitter acetylcholine^{25,26}. 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^{22,27}. In the primate, ganglion cells with dendritic

morphology similar to that of ON-OFF DSGCs have been observed previously²⁸⁻³⁰, and starburst amacrine cells are also present in the retina^{31,32}. This raises the possibility that direction selectivity is present in the primate retina and perhaps also computed 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 light³³. 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 connectome. Unexpectedly, we found a distinct antagonistic surround, arising 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

The recursive bistratified ganglion cell type shows ON-OFF direction selectivity

Parallel visual pathways originate in morphologically and physiologically diverse retinal ganglion cell populations³⁴⁻³⁶. In the mouse retina, over 40 functionally distinct ganglion-cell types are present³⁷⁻⁴⁰, with a substantial fraction of them showing direction selectivity². In the primate retina, by contrast, many fewer ganglion-cell types have been identified^{29,41,42}.

To recognize and characterize a candidate ON-OFF DSGC, a more comprehensive accounting of the dendritic morphologies and spatial densities of the diverse ganglion-cell types in the macaque retina was required.

To address this problem in the primate – where gene-based cellular reporters are lacking – we applied a singular method called retrograde photodynamic staining²⁸. Injections of the tracer rhodamine dextran were made into the major targets of retinal output: the lateral geniculate nucleus (LGN), the superior colliculus (SC) and the pretectum. 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 complete dendritic morphology. By this method, we determined for each ganglion-cell type its relative spatial density and fundamental mosaic organization. We then targeted specific morphologically identified types for physiological characterization^{43,44}. The spatial densities of 19 ganglion-cell types, determined from the regular overlap of neighboring dendritic trees of the same type, accounted for ~97% of the total ganglion-cell population in the mid-peripheral retina (extended data Figs.1-3; extended data Table 1). Only five types (ON- and OFF-midget, ON- and OFF-parasol, and the small bistratified cell; extended data 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). The remaining ~ 20% of the ganglion-cell population comprised a large number of distinct and diverse cell types, each present at relatively low densities (0.5-1.5%) (extended data Table 1). One of these low-density types showed recursively branching dendrites that were bistratified at approximately the same depth as the outer and inner cholinergic bands (Fig. 1a,f, extended data Fig. 4), and in this regard resembled the ON-OFF DSGCs of non-primate mammals¹². These cells were

retrogradely labeled from tracer injections into both the LGN and SC, and they tiled the retina uniformly with minimal dendritic overlap (Fig. 1b). Unlike the non-primate mammal, where four ON-OFF DSGC types with distinct preferred directions form overlapping mosaics and together make up ~12% of the total number of ganglion cells⁴⁵, we observed only a single mosaic that comprised ~1.5% of the total ganglion cells in the peripheral retina (extended data Fig. 1f, Table 1). We also observed a second class of recursively branching ganglion cells present in the same retrogradely-labeled retinas (the recursive monostratified cell; extended data Figs. 1i) that we have not characterized functionally. These cells showed extensively overlapping and fasciculated dendrites (extended data 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 bistratified cells transiently depolarized and spiked at both light ONset and OFFset (Fig. 1c) 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. 1d, e) ($n = 26$ cells; mean $\pm$ sd, DSI = 0.82 ± 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 physiology⁵. The preferred direction was variable in this relatively small sample. In a recent study in the mouse retina variability in direction preference was linked to retinal location and in turn to the optic flow fields generated on the retina as the animal moves through the environment. We do not know the precise location of the recorded direction-selective cells relative to the fovea and horizontal and vertical meridians, so the significance of this variability in the primate remains to be determined.

The ON-OFF direction-selective ganglion cell is tracer-coupled to a direction-selective, poly-axonal, 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 microcircuit⁴⁶. 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)⁴⁵. For the macaque recursive bistratified cell, Neurobiotin coupling was not present in neighboring recursive bistratified ganglion cells but appeared in a distinctive cell type with a very large cell body that was often displaced into the IPL (Fig. 2a). 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 type⁴⁷⁻⁴⁹. A1 cells showed a distinctive, poly-axonal morphology in which multiple, long-projecting axons arose from proximal dendrites and extended in various directions for millimeters within the IPL (Fig. 2f-h and i). To directly test the hypothesis that A1 cells are coupled to recursive bistratified ganglion cells, we targeted A1 cells in the *in vitro* retina as previously described⁴⁸ and made intracellular injections of the fluorescent nuclear tracer Po-Pro-1. Po-Pro-1 passed through gap junctions and revealed coupling patterns similar to that found for Neurobiotin but which could be observed by 2-photon (2P) fluorescence imaging directly in the functioning retina *in vitro*⁵⁰. Po-Pro-1 injections into A1 cells revealed both hetero- and homotypic coupling similar to that previously described⁴⁸, including coupling to a single ganglion-cell type with a small cell body (Fig. 2b,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. 2d, e).

Given the link to the recursive bistratified cell and its similar transient, ON-OFF spiking response (Fig. 2j), 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. 2k) and the associated spike discharge (Fig. 2l) in response to moving bar stimuli. A1 cells were filled with the calcium indicator Oregon Green BAPTA-488 (OGB) during intracellular recording (Fig. 2i) and both the long-projecting axons (Fig. 2m) and the thick, spiny dendrites (Fig. 2n) showed stimulus-evoked direction-selective Ca^{2+} signals.

Connectomic reconstruction of A1 cells reveals polarization of synaptic inputs and outputs

The A1 cell's distinctive axon-like processes (Fig. 2f-h) are obvious candidates for spike generation, long-range transmission across the retina, and synaptic output^{49,51}. However, amacrine-cell processes can be both pre- and postsynaptic⁵² 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. We therefore initiated connectomic reconstruction of an identified A1 cell (Fig. 3a) to begin to address this question. After an A1 cell was identified by intracellular recording and dye-filling the retina was fixed for electron microscopy. Near-Infrared Branding^{49,53} (NIRB) was then used to burn fiducial marks around the A1 cell body with the 2-photon laser. After embedding the cell was located by the NIRBed markings and scanning block-face electron microscopy⁵⁴ (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. 3b-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. 3c, yellow balls, Fig. 3h) with bipolar ribbon synapses targeted to the prominent dendritic spines (Fig. 3d, red balls, Fig. 3h, 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. 3c, f-h; extended data Fig 5). The majority output was to bipolar-cell axon terminals, with additional output to ganglion-cell dendrites and a minor output to other amacrine processes (extended data Fig. 5). 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.

Macaque starburst amacrine cells show radial direction selectivity that requires an antagonistic bipolar-cell surround

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 others have in non-primate mammals²³, 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. 4a, extended data Figs. 6 and 7). Cells with some

morphological features similar to starbursts were observed (extended data Fig. 6m, n) 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 body^{23,24,55}. 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 cells^{23,56}. In macaque, the somatic voltage response to such a 'bullseye grating' showed surprisingly strong outward directional preference (Fig 4b). To measure direction selectivity in the dendrites, as we did for the A1 cells, we again used 2-photon imaging of calcium transients after cell filling with OGB-488. Dendritic ROIs showed the same striking preference for centrifugal movement (Fig. 4c) as well as broad directional tuning to moving bars or drifting gratings (extended data Figure 8).

In non-primate mammals, an inhibitory surround created by abundant starburst-to-starburst GABAergic synaptic connections has been implicated as a key mechanism for the centrifugal directional preference of starburst dendrites⁵⁵. 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 (extended data Figure 7a) and showed clear center-surround organization, well described by the Difference-of-Gaussians (DoG) receptive-field model^{57,58} (Fig. 4d). Annular stimuli were then used to isolate the surround-response component which appeared as a large depolarization at light OFFset (Fig. 4e left panel, shaded) that persisted unattenuated in the presence of the GABA_A receptor antagonist SR 95531 (GABAzine) (Fig. 4e, right

panel, shaded). By contrast, the 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. 4f). HEPES has been shown to attenuate the surrounds of inner retinal neurons by acting in the outer retina to reduce negative feedback from horizontal cells to cone photoreceptors⁵⁹⁻⁶¹, supporting the conclusion that the surround originates presynaptically in the center-surround organization of the excitatory bipolar cell's⁶² input to the starburst. Consistent with this conclusion in a somatic voltage-clamp recording from a single starburst the synaptic currents evoked by annular stimuli that isolated the surround were clearly excitatory (extended data Fig. 7b).

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 and dendritic preference for outward motion (Fig. 4g-h). 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's 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 species⁶³.

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^{64,65}. We hypothesized that transient bipolar inputs to the starburst dendritic tree would likely show non-linear spatial summation^{66,67}, and found that indeed such a stimulus revealed a second harmonic response in the starburst that extended to high spatial frequencies, implying a small receptive-field center (30-50 μm Gaussian diameter, extended data Fig. 9a, b). We hypothesize that these subunits in the starburst receptive-field center corresponded to rectifying bipolar-cell inputs^{60,63}. Consistent with this hypothesis, the distinctive F2 response to contrast-reversing gratings remained when the surround was attenuated by HEPES-buffer enrichment (extended data Figure 9c).

Next, to determine the identity and spatial layout of bipolar input to the starburst dendritic tree we again used the NIRB method (Fig. 5b) and SBFEM to target a physiologically and anatomically identified starburst for microcircuit reconstruction. The central 150 μm of an OGB-filled starburst dendritic tree was completely reconstructed (Fig. 5a, g). Inhibitory synaptic outputs, and excitatory and inhibitory synaptic inputs to the starburst dendrites, were identified in high-resolution images and mapped (Fig. 5c-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. 5d, e, and 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. 5h, 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 (extended data Fig. 10) and therefore could be unequivocally identified as the midget bipolar-cell type⁶⁸. By contrast, the larger arbors made extensive synaptic output to an identified parasol (magnocellular LGN-projecting) ganglion cell (extended data Fig. 10). 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 co-stratify in the IPL, synapsing upon parasol ganglion cells, and showing largely indistinguishable axon-terminal morphology⁶⁸. Thus, the macaque starburst draws its excitatory input from the major parvocellular and magnocellular visual pathways that transmit sustained vs transient signals, respectively^{69,70}. We determined the spatial layout of the inputs from these two bipolar-cell populations on the starburst dendritic tree (Fig. 5j), and found that the midget bipolar cells were distributed over the entire dendritic tree although tending to dominate on the proximal , 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. 5j; 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 75 μm . Beyond this distance, an equal number of midget and DB4/5 bipolar cells provided a more transient excitatory input (total of 70 bipolars) (Fig. 5k). Synaptic inhibition was omitted. The model replicated the experimental results, with the starburst cell strongly preferring outward motion (Fig. 5l). 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 (extended data Fig. 11). 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 modelling 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 the model, when linear gratings or bars (i.e., non-radial gratings) were presented moving across the complete starburst dendritic arbor with intact bipolar center and surround, the bipolar-cell responses showed no sensitivity to motion, suggesting that a given stimulus condition may engage varied redundant mechanisms for direction selectivity in the starburst amacrine cell.

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 pathways⁷¹ that projected via the parvocellular and magnocellular LGN to primary visual cortex⁷². It is now clear that these high-density pathways – linked to the requirements of high-spatial-resolution foveal vision – made it technically difficult to identify the great diversity of relatively low-density ganglion-cell types, including novel chromatic^{36,73}, achromatic⁴³, and non-image-forming pathways⁴⁴. 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. The direction selectivity previously recorded in the accessory optic system/pretectum⁷⁴, a retinal target, was thought to arise from feedback projections from visual cortex. While such projections may still contribute importantly, our results suggest a primary role of direct retinal projections^{75,76}. The recursive bistratified ganglion cell was retrogradely labeled from tracer injections into both the LGN and superior colliculus. Only a small fraction (~10%) of LGN and collicular cells show direction selectivity^{77,78}, 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 (extended data Table 1) it remains possible that additional very low density DSGCs remain to be discovered. A population of recursive monostратified cells (extended data Figure 1i, 2) are candidates for the ON-DS types present in non-primate mammals¹², but the visual physiology of these ganglion cells has yet to be characterized.

The presence of direction selectivity in a spiking, polyaxonal, amacrine type linked to the recursive bistratified ganglion cell is distinctive, but parallels an emerging picture in the non-primate mammal^{11,63,79,80}. 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 latter⁸⁰. 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-DSGC underlies a direction-selective response that arises independent of GABAergic inhibitory circuitry⁸¹. 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. 2h), contrasting sharply with the local computation that occurs within semi-isolated electrotonic dendritic compartments of starburst amacrine cells²⁴.

The neural mechanisms proposed for starburst dendritic direction selectivity include spatially asymmetric wiring of sustained and transient bipolar-cell inputs⁸²⁻⁸⁴, starburst-to-starburst GABAergic surround inhibition⁵⁵, and the electrotonic properties⁸⁵ and ion-channel composition of the dendrites⁵⁶. 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^{82,83}. However, the significance of such a synaptic motif as a mechanism for direction selectivity remains controversial⁸⁶ 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 GABA_A receptors on starburst dendrites, suggesting that inhibitory network interactions are not essential for light-adapted surround generation in primate retina⁸⁷. Instead, we found that the surround arises presynaptically and is transmitted by the excitatory center-surround receptive field of the bipolar cell⁶² 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 retina⁸⁸. 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 connections²². 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 environment². In the primate we have found only a single ON-OFF DSGC type. Major outstanding questions include how these cells signal direction in relation to the optic-flow organized around the foveal center in the forward-facing eyes of a primate⁸⁹⁻⁹¹, how this single, ON-OFF DSGC type is synaptically linked to the starburst dendritic plexus, and whether removing the bipolar-cell surround alters direction tuning in recursive bistratified ganglion cells. Overall, our results suggest that the distinctive design of the direction-selective circuit in the primate is more in line with the unique specializations of a smart retina⁹² than the encephalization⁹³ of this fundamental neural computation.

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 in vitro retinal preparation has been previously described^{73,94}. In brief, the retina was dissected free of the vitreous and sclera in oxygenated Ames' Medium (A1420; Sigma Chemical Co., St. Louis, MO) under light-adapted conditions. The retina-RPE-choroid was placed flat, vitreal surface up, in a superfusion chamber mounted on the microscope stage. The retina was continuously superfused with Ames' medium (pH 7.3; oxygenated with 95% O₂/5% CO₂) and the temperature thermostatically maintained (TC-344B, Warner Instruments) at ~36°C.

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 previously⁴³. 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 photo-stained in vitro by brief exposure to epifluorescent illumination, which liberated the sequestered fluorescence tracer, 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) prior to HRP processing. Retinas were mounted flat using either an aqueous solution of glycerol and polyvinyl alcohol or Fluoromount-G (Cat. #17984-25; Electron Microscopy Sciences, Hatfield, PA), coverslipped, and stored refrigerated.

Melanopsin immunohistochemistry. Ten adult macaque retinas were processed for immunohistochemistry using a purified rabbit IgG against human/monkey melanopsin N-terminal (hNA) and biotinylated goat anti-rabbit (BA-1000; Vector Labs, Burlingame, CA); antibody design and characterization has been reported previously in detail⁹⁵. Briefly, following enucleation, retinas were dissected free of the eyecup and fixed flat for 2 hr 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, intensified 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 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 ganglion-cell type was taken from clusters of photo-stained ganglion cells of the same type forming orderly mosaics. 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 eccentricity (625 cells/mm²; 8 mm temporal retina)⁸⁹. For large field low density ganglion cells these estimates are little influenced by small variations in coverage. However, for the midget ganglion cells, which show an exceptionally high spatial density, a small change in dendritic field 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 (extended data Fig. 1b) which has also been shown in detail for the human retina⁹⁶.

475

476 **ChAT immunocytochemistry.** Ganglion cells were first targeted for intracellular staining
477 using high resistance patch pipettes ($> 10\text{ M}\Omega$) with 2% Neurobiotin (Vector Laboratories)
478 and 100 μM Alexa594 (Invitrogen). After cell filling retinal pieces were fixed in 4%
479 paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, (PB) for 40 min. Immunocytochemical
480 labeling was performed by using the indirect fluorescence method. Retinal pieces were
481 incubated 4-6 days at 4°C with goat anti-choline acetyltransferase (ChAT) antibodies
482 (AB144P, Millipore) at a dilution of 1:200 with 5% normal donkey serum, 1% bovine serum
483 albumin, and 0.5% Triton X-100 in PB. After washing in PB, secondary goat antibodies
484 conjugated to Alexa488 (Invitrogen) together with Alexa568-conjugated streptavidin
485 (Invitrogen) to visualize Neurobiotin were applied for 3-4 hrs at room temperature at a
486 dilution of 1:500.

487

488 **Light microscopy and image analysis.** Image stacks of whole-mounted retina were taken
489 with a FV1000 confocal microscope (Olympus) equipped with an argon and an He-Ne laser,
490 using a UPlanSApo 60x/1.35 oil immersion objective at 1,600 x 1600 pixels and a z-axis
491 increment of 0.25 μm and processed with NIH FIJI software. The analysis of the
492 stratification depths of ganglion-cell dendrites and their position relative to the ChAT bands
493 were usually performed at four different sites per ganglion cell's dendritic tree where the
494 outer and inner borders of the inner plexiform layer (IPL) were determined and defined as
495 0% or 100% IPL depth, respectively. Subsequently, the stratification depth of the ganglion-
496 cell dendrites and the inner and outer ChAT bands were measured at the same location.
497 Vertical projections of image stacks from whole-mounted retina were created with Amira
498 software (Thermo Fisher).

499

Near Infrared Branding (NIRB). This method permits correlation of light and electron microscopy at the level of single identified neurons⁵³. 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 cells was dissected and placed in EM fixative (2.5% glutaraldehyde/2% paraformaldehyde; in 0.1M cacodylate buffer, 1 hr). 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 sec. With these parameters the scanned lines created tissue defects of ~ 2 μ m in the XY and Z dimensions and were easily detectable after plastic embedding during preliminary semithin sectioning. Scan lines 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 defects located during subsequent sectioning thus permit a precise localization of the target amacrine cell.

Serial Block-Face Scanning Electron Microscopy (SBEM) and circuit reconstruction.

After NIRBing the tissue containing the target cell was then rinsed thoroughly in cacodylate buffer, pH 7.4 (0.1M), and incubated in a 1.5% potassium ferrocyanide and 2% osmium tetroxide (OsO₄) solution in 0.1 M cacodylate buffer for 1 h. After washing, the tissue was placed in a freshly made thiocarbohydrazide solution (0.1 g in 10ml double-distilled H₂O heated to 60°C for 1 h) for 20 min. After another rinse, the tissue was incubated in 2% OsO₄ for 30 min. The samples were rinsed again and stained en bloc in 1% uranyl acetate

overnight at 40°C, washed, and stained with Walton's lead aspartate for 30 min. After a final wash, the retinal pieces were dehydrated in a graded alcohol series and placed in propylene oxide for 10 min. The tissue was then plastic embedded (Durcupan, 44610, Sigma Aldrich). After NIRB lines were located during semithin sectioning the tissue block was trimmed, gold-coated by standard methods, and mounted in a Volumescope SEM (Thermo Fisher). The block face was scanned at 5 nm xy resolution (incident electron beam energy, 2.2 keV, pixel dwell time of 1.5 μ s) after each of ~800, 50 nm thick sections in an array of 20, 8000x8000 pixel (40x40 μ m) 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 software⁹⁷ (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-gluconate (120), MgCl₂ (1) KCl (15), NaCl (8), HEPES (10), phosphocreatine, 2K (7), ATP-Mg (4), and 0.3 GTP-Na (0.5), adjusted to pH 7.3 (Osmolarity, ~ 300 mOsm). In most instances 200 μ M Oregon Green BAPAT-488, a

550 fluorescent Ca indicator (150 or 200 μ M) and/or Alexa fluor 488 or 568 (200 μ M) was added
551 to the pipette solution. We estimated a liquid junction potential of ~ 10 mV and therefore
552 subtracted 10 mV from all membrane potentials intracellularly recorded in current clamp.
553
554 Data acquisition and the delivery of visual stimuli were coordinated by custom software
555 running on an Apple Macintosh computer. Current and spike waveforms were Bessel filtered
556 at 2 or 5 kHz and sampled at 10 kHz. To map the receptive field of the cell, the cell body
557 was first placed at the precise center of the stimulus field. Flashing white squares (2 Hz
558 temporal frequency, 10 or 25 μ m wide) were systemically moved in the x and y directions to
559 locate the most sensitive point of the receptive field and determine its approximate size. The
560 location of the maximum spike response was defined as the receptive-field midpoint; and
561 visual stimuli were positioned relative to this point.
562
563 Two-photon laser scanning fluorescence imaging was performed with the same 25x
564 objective lens on a Sutter Instruments DF-Scope multiphoton imaging package for the
565 Olympus BX51WI microscope that was modified to incorporate the output of a digital light
566 projector that provided visual stimuli. Fluorescence was excited by 100-fs pulses at 80 MHz
567 tuned to 935-nm center wavelength from a Ti:Sapphire laser (Spectra Physics Mai Tai
568 HP). Laser intensity was regulated with a Pockels cell (Model 350–80 with model 302
569 driver, Conoptics) with the power measured at the retina of ~ 2.5 mW. ROIs for Ca^{2+} activity
570 scanning were 10x10 microns (34x34 pixels; 3.4 pixels/ μ m; pixel dwell time, 32 μ sec, 23.4
571 Hz frame rate). Fluorescence emission from the laser scanned fields was collected by the
572 objective and delivered to a photomultiplier tube (PMT) (Hamamatsu GaAsP PMT,
573 H10770PA-40) via dichroic mirrors and bandpass filters selective for fluorophore emission at
574 520 nm. The PMT output signal was digitized and processed using ScanImage, a software

application for laser scanning microscopy (Vidrio Technologies). Laser scanning morphological imaging of neuronal structure was typically done at either 512x512 or 1024x1024 pixels and image stacks were created at $\sim 1.5 \mu\text{m}$ z resolution; maximum intensity flattened images were made with NIH FIJI software.

Visual stimulus generation. All visual stimuli used were created either by programming 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 configurations. 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 red, green, and blue primaries were measured with a spectroradiometer (PR705, Photo Research); peak wavelengths and integrated photon fluxes were 636, 550, and 465 nm and 5.7×10^7 , 4.9×10^7 , and 3.3×10^7 photons/s/ μm^2 , respectively. Light from these primaries were combined and with added calibrated filtering provided quantal catch rates for the long- (L) and middle- (M) wavelength sensitive cone photoreceptor types (comprising 90-95% of the cones) of $\sim 6 \times 10^5$ photons/s/ μm^2 . These quantal catch rates are in the mid to high photopic range and we have previously shown that rods are in saturation at these light levels³³. As previously quantified, given the uncertainties of the size of the cone aperture that strongly influence the efficiency of photoisomerization, we consider our estimates of quantal catch as very conservative. The intensity of the stimuli used in studies of human vision can also be expressed in units of retinal illuminance, or Trolands (Td) which scales by effective pupil size. We previously

calculated that for a peripheral cone with an inner segment aperture of 9 μm , 1 Td was equivalent to ~30 photoisomerizations/s/cone³³.

Direction selectivity measure. For moving bars or drifting gratings a direction selectivity index (DSI) was calculated as, $\text{DSI} = (\text{P (Preferred response)} - \text{N (Null response)}) / \text{P (Preferred response)}$, 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 intracellular recorded ganglion cells) or the peak response amplitude for stimulus evoked dendritic/axonal calcium response in starburst and A1 amacrine cells. For contracting (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. 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 substantive 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 when 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.

Modelling. The dendritic morphology used in the model was digitized from a confocal image stack of a dye-filled macaque starburst cell. The Neuron-C simulator⁹⁸ generated a compartmental model of the digitized morphology with a compartment length of 0.05 λ for a total of ~300 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), medial (0.2 μm), and distal (0.8 μm) dendrite segments⁸⁶, 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/ mm^2 ; 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 75 μm , half of the bipolars were given a transient release property to simulate the transient release properties of presumed DB4/5 bipolars. 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 were each given a maximum conductance appropriate to depolarize the starburst to -60 mV (G_{max} , 1500; 5-15 pS per bipolar for typical stimuli). The postsynaptic effect of the lower release rate of the DB4/5 bipolars was increased with a greater maximum conductance (G_{max} , 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 diam 30 μm , surround diam 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 diam 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 $\sim$ -50 mV, contrast 4 - 7 mV). The results from these models 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 $\sim$ 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 channel^{99,100}. Calcium channels were simulated with a slowly-inactivating T-type channel that approximated the fast activation and slow inactivation kinetics of N/P/Q-type channels^{101,102}. Sodium channels were modeled as a NaV1.2 channel or a persistent NaV1.8 channel with slow depolarized activation kinetics^{101,103,104}.

To capture the appropriate 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). The delay and the strong surround weight

672 allowed the surround to subtract from and reduce the amplitude of the overall bipolar-cell
673 response. Outward-moving radial gratings (spatial period 400 μm , velocity 200 $\mu\text{m/s}$,
674 "contrast" 7 mV) evoked a ~ 10 mV response in proximal bipolar cells but inward-moving
675 gratings evoked only a ~ 4 mV response, producing a directional difference of 6 mV
676 (extended data Fig 11). More distal bipolars had a smaller response to outward motion of
677 the grating and a smaller directional difference (0.5 mV). A stationary counterphase grating
678 (2 Hz) centered on the starburst soma at a series of different spatial frequencies evoked a
679 frequency-doubled response comparable to the F2 plots from real data.

680

681 **Model Biophysical properties**

682 Channel	Soma	Prox	Medial	Distal	
683 Kv3	1-3	0.5-2	0.2-0.5	0	mS/cm ²
684 Ca-T	0.2	0.2-2	3-7	6-8	mS/cm ²
685 Ca pump Vmax	2e-6	0.5-1e-6	1e-6	2e-6	mA/cm ²
686 NaV	0.2-0.5	0.2-0.5	0.2-0.5	0.2-0.5	mS/cm ²
687 R _m	50e3 Ohm-cm ²				
688 R _i	200 Ohm-cm				
689					

690 **Data availability**

691 The data that support the findings of this study are available from the corresponding authors
692 upon reasonable request.

693

694 **Code availability**

695 The Neuron-C simulation language was used to generate the models described in this
696 study. This code is available at: <ftp://retina.anatomy.upenn.edu/pub/rob/nc.tgz>. Included in
697 this distribution is the realistic SAC morphology, the "retsim" retinal circuit simulator for

model generation and the "rsbac_primate" script file that ran multiple model jobs in parallel. The scripts for the models are in the subfolder nc/models/sbac_primate. Neuron-C compiles and runs under Linux and Mac OSX. An Ubuntu Linux image that runs under virtualbox (<http://www.virtualbox.org>) is available at <ftp://retina.anatomy.upenn.edu/pub/ubuntu.vdi.zip>.

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Author Contributions

Conceptualization: D.M.D., P.B.D., Y.J.K., R.G.S., J.B.T.; Methods and Technique development: D.M.D., P.B.D., O.S.P., F.V.; Programming and modeling: R.G.S., J.W., J.B.T., O.S.P.; Connectomics data analysis: Y.J.K., D.M.D., O.S.P.; Other anatomical analyses: J.D.C., H.R.J. and B.B.P., and C.P.; Physiological experiments: Y.J.K., D.M.D., O.S.P. and J.D.C.; Retrograde tracing experiments: D.M.D., P.D.G., J.D.C. and F.R.R.; Antibody generation: K.-W.Y.; Data analysis and figure generation: Y.J.K., P.B.D., O.S.P., and D.M.D.; Writing and editing: D.M.D., Y.J.K., P.B.D., J.B.T., R.G.S., F.V., P.D.G., K.-

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724

725 **Additional Information**

726 The authors declare no competing financial interests. Correspondence and requests for
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728

729 **Figure legends (main)**

730 **Figures 5**

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733

734 **Figure 1. The recursive bistratified ganglion cell type of the macaque monkey retina**735 **is ON-OFF direction-selective and forms a single anatomical population. a, Dendritic**736 **morphology of a recursive bistratified type in macaque retina retrogradely photo-stained**737 **from rhodamine dextran tracer injections placed in the LGN. b, Four photo-stained**738 **neighboring recursive bistratified cells tile the retina with little dendritic overlap indicate a**739 **single anatomical ganglion-cell population (Inset: polygons traced around neighboring**740 **dendritic trees shown in b). c, ON-OFF voltage response recorded intracellularly in whole-**741 **cell, current-clamp. d, Polar plot shows direction-selective tuning of a recursive bistratified**742 **cell (moving bar, 100X600 μm ; 2000 $\mu\text{m}/\text{sec}$, DSI = 1.0). e, Preferred direction was variable**

743 (n = 26 cells; mean $\pm$ sd, DSI = 0.82 ± 0.20). **f**, The recursive bistratified cells' inner and
744 outer dendrites costratified with immunolabeled Choline acetyltransferase (ChAT) bands
745 created by starburst amacrine-cell populations (see also extended data Fig. 4). See also
746 extended data figure 1f for further quantification of recursive bistratified morphology.

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748

Figure 2. The recursive bistratified ON-OFF DSGC is tracer-coupled to the A1 cell, a distinctive, polyaxonal, ON-OFF spiking amacrine-cell type that also shows direction

selectivity. a, A recursive bistratified cell (cell body, arrowhead) filled intracellularly with Neurobiotin shows tracer-coupling to an amacrine-cell type (arrows) with the somato-dendritic morphology of the well characterized A1 amacrine. **b**, A1 amacrine cell in vitro after intracellular dye fill with OGB and Po-Pro-1 showing excitation of the OGB (488 nm) fluorescence. **c**, When Po-Pro-1 fluorescence is excited (420 nm) a Po-Pro-1 tracer-coupled ganglion cell is evident in the field (blue-arrow). **d**, OGB filling of a ganglion cell Po-Pro-1 tracer coupled to another A1 amacrine corresponds to the recursive bistratified type described in Fig. 1. **e**, Direction-selective response (intracellularly recorded spikes, DSI = 0.5) of the recursive bistratified ganglion cell shown in d. **f**, Camera-lucida tracing of an A1 amacrine cell illustrating the proximal spiny dendrites and the origins of four axon-like processes from these dendrites (red arrowheads). **g**, The axon-like processes are more sparsely branching and extend widely beyond the dendritic tree. **h**, At lower magnification the wide-spreading extent of the axon-like arbor (black) relative to the dendritic tree (red) is shown (note scale bar at bottom = 1 mm) for a second A1 cell (Neurobiotin fill); f-g drawings modified from the senior author's previously published anatomical descriptions of the A1 cell^{47,48}. **i**, 2P-confocal image of an OGB fill of an A1 cell in vitro illustrates how the axonal component (pseudo-colored blue-green) is distinguished from the main dendrites for the purpose of functional calcium imaging. **j**, The A1 cell responds transiently at light ONset and OFFset with large 60 mV spikes arising at the leading edge of large 20+ mV membrane potential depolarizations. **k-l**, Direction-selective response for both membrane voltage peak to peak amplitude (mV) and spike count for the same cell in response to a sinusoidal drifting grating (**k**) (4Hz drift rate, 200 μ m cycle period; 100% contrast; n = 5; mean $\pm$ sd DSI = 0.7 $\pm$ 0.21) and total spike count (**l**) (n = 12, mean $\pm$ sd DSI = 0.47 $\pm$ 0.34). **m, n**, Direction selectivity to moving bar stimuli in the optically recorded calcium response at A1 cell axonal and dendritic locations. **m**, ROI at an axonal swelling, stimulus, bar 500w x 1000h; velocity

777 1000 $\mu\text{m/s}$; contrast 100%, DSI = 0.63. Plotted data are the amplitude of initial peak of the
778 stimulus evoked change in fluorescence indicated by blue shading ($n = 11$ ROIs, mean $\pm$ sd
779 DSI = 0.47 ± 0.10 , range = 0.28 – 0.63). **n**, ROI on a secondary dendrite, Bar 100w x 700h,
780 velocity 8000 $\mu\text{m/s}$ contrast 60%, DSI = 0.56. ($n = 9$ ROIs, on 2 cells; mean $\pm$ sd DSI =
781 0.41 ± 0.15 , range = 0.22 – 0.63).

782

783

Figure 3. The spiking ON-OFF A1 cell shows complete polarization of synaptic inputs and outputs. **a**, Proximal dendritic morphology of a physiologically identified A1 amacrine cell, dye-filled (OGB+Alexa 468) and imaged in vitro by 2-photon confocal microscopy; arrowhead points to the same dendritic location in **a**, **b** and **h**. **b**, Ultrastructure of primary dendrites of A1 cell (tan fill) shown in **a**, with cell body protruding into the inner plexiform layer (IPL) confirms identification of this cell in EM tissue volume; partial view of NIRBED line at lower left (arrow). **c**, A1 cell dendritic shaft (tan) receiving an inhibitory synapse

793 (yellow, red arrowhead). **d**, A1 cell dendritic spine (tan) receives a ribbon synapse (red
794 arrowhead) from a bipolar-cell axon terminal (pink). **e, f**, A1 cell axon-like process (teal)
795 synapses on a bipolar-cell axon terminal (blue, red arrowheads) at 2 different locations;
796 synapses from bipolar cells to A1 amacrine-cell axon-like processes were not observed. **g**,
797 A1 cell axon-like process synapses (teal, red arrowhead) on an amacrine cell process
798 (yellow). **h**, Reconstruction of proximal dendrites and axon segment for A1 cell shown in **a**,
799 illustrates that synaptic inputs from bipolar-cell ribbon contacts (red balls) were made
800 primarily to the dendritic spines that arose from dendritic shafts (inset lower left). By contrast
801 dense inhibitory synaptic inputs from other amacrine cells were made directly upon the
802 dendritic shafts. Location of amacrine inputs to one segment of a secondary dendrite
803 (between arrows) is indicated by the yellow ball structures. Reconstruction of a segment
804 from the axon-like arbor (teal process at upper left) reveals synaptic output from varicosities
805 primarily to bipolar-cell axon terminals (blue balls) but also to an amacrine-cell process
806 (green ball) and to a ganglion-cell dendrite (magenta ball).

807

808

Figure 4. Starburst amacrine cells in macaque retina show center-surround receptive-field structure and direction selectivity that is abolished by HEPES buffer enrichment.

a, Starburst amacrine cell targeted for patch-clamp recording in the in vitro macaque monkey retina and filled with the calcium indicator Oregon Green Bapta-488 (OGB). **b**, Somatic voltage, intracellular recording, shows selectivity to radial outward motion using an expanding (outward, green trace) or contracting (inward, black trace) radial grating stimulus; 2 stimulus cycles shown (100% contrast; 0.24 cycles/degree of visual angle; 0.5 Hz temporal frequency; $n = 6$; inward/outward = 0.26 ± 0.17). **c**, Calcium response, 2-photon optically imaged ROIs at terminal dendrites also show selectivity to radial outward motion; 3 stimulus cycles shown ($n = 36$ ROIs; inward/outward = 0.32 ± 0.18). **d**, 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; $n = 27$; center diam, 208 ± 76 ; surround diam, 360 ± 156); inset shows 2D profile of model fit; small icon shows starburst soma and dendritic tree at same scale as model. **e**, Surround response isolated by annular stimuli; left side; increasing the inner diameter of the annulus from 20 to 400 μm (icons at center; top to bottom panels; 2Hz square wave modulation) eliminates the ON center depolarization (top panel) and isolates a pure OFF-surround depolarization (bottom panel); right side, surround OFF depolarization is not eliminated by bath application of GABA_A receptor antagonist (SR 95331(GABA_Azine); 10 μM ; $n = 4$; $8 \pm 3\%$ reduction). **f**, By contrast HEPES buffer enrichment of Ames' medium greatly attenuates the surround OFF depolarization (20 mM HEPES; pH 7.3; $n = 6$; $83 \pm 41\%$ reduction); stimulus protocol as shown in **e**, except that temporal modulation is 1 Hz. **g**, HEPES buffer enrichment greatly attenuates the preference for outward motion of radial grating stimuli; stimulus protocol as given in **b**; somatic voltage response to inward motion is increased with HEPES bath application (20 mM HEPES; pH 7.3); histogram at bottom plots inward/outward response

835 ratio for control and HEPES application (paired t-test: $t(2) = -6.986$, $*p = 0.010$, *Cohen's d* =
836 3.76). **h**, Calcium response, 2-photon optically imaged ROI at terminal dendrite; stimulus
837 protocol as in **c**; 6 stimulus cycles shown. HEPES application again increases response to
838 inward motion and eliminates outward motion preference. Histogram at bottom plots
839 inward/outward response ratio for control, HEPES and GABAazine application. HEPES
840 (Games-Howell post hoc test: $*p = 0.000285$, *Hedges' g* = 2.54) but not GABAazine (Games-
841 Howell post hoc test: $p = 0.984$, *Hedges' g* = 0.05) eliminates the outward directional
842 preference for radial grating stimuli.

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Figure 5. Connectomic reconstruction reveals spatial layout of distinct bipolar-cell types for an identified macaque starburst amacrine cell. **a**, Dendritic morphology of a physiologically identified starburst cell, dye-filled (OGB+Alexa 468) and imaged in vitro by 2-photon confocal microscopy; arrowhead points to a hairpin loop in a secondary dendrite (see also **g**). **b**, After tissue fixation, the starburst cell was located by epifluorescence and the NIRB method was used to burn fiducial marks (boxed area) in the optic fiber layer around the identified starburst cell (teal dot indicates location of starburst cell body). **c**, High ultrastructure resolution enables unequivocal identification of excitatory and inhibitory synapses. Starburst synaptic output from peripheral dendrite (teal fill; red arrowhead) to ganglion-cell dendrite. **d**, **e**, Two examples of bipolar cell (tan fill) ribbon synaptic input to short spines (teal fill, red arrowhead) that extend from the starburst cell main dendrites. **f**, Inhibitory synaptic input to a proximal starburst dendrite (teal fill; red arrowhead). **g**, Reconstruction of inhibitory synaptic inputs (yellow balls) and bipolar-cell ribbon excitatory synaptic inputs (red balls) to a portion of the starburst dendritic tree (arrowhead points to distinct hairpin loop in dendritic tree indicated in **a**). The reconstructed axon terminals of two presynaptic bipolar cells are shown (gold fills); one of these terminals (and its associated piece of starburst dendrite) is enlarged and rotated (dotted lines) with ribbon synapses indicated with the red balls within the semi-transparent axon terminal. Inset zooms to show further how a spine arises from the starburst dendrite to receive a single ribbon synapse from this axon terminal. **h**, Complete reconstruction of two anatomically distinct populations of bipolar-cell types in synaptic contact with the starburst tree; smaller axon terminals (gold) are identified as midget bipolar cells and larger blue terminals are identified as diffuse bipolar type 4/5 (DB 4/5) by their synaptic connections to midget and parasol ganglion cells respectively (see extended data Figure 10). **i**, Zoomed view of all midget bipolar (gold) and DB 4/5 (blue) synaptic contacts on a portion (light yellow boxed area in **h**) of the

reconstructed starburst dendritic tree. **j**, Left, spatial locations of all midget (gold balls) and DB4/5 ribbon synapses (blue-balls) on the starburst tree. Histogram plots distance of bipolar-cell axon terminal from the starburst cell body showing that midget bipolar cells extend from proximal to distal and DB 4/5 dominate more peripherally on the starburst dendritic tree (midget bipolar, $n = 25$, mean $\pm$ sd distance from starburst soma = 34.5 ± 23.6 μ m, DB 4/5, $n = 23$, mean $\pm$ sd distance from starburst soma = 54.3 ± 19.6 μ m; these mean spatial distributions differ significantly, unpaired t-test: $t(46) = -3.1433$, $*p = 0.0029$, *Cohen's d* = 0.91). **k**, Realistic biophysical model of distribution of bipolar input to a complete macaque starburst dendritic tree based on anatomical data (gold – midget bipolar cells; blue – DB 4/5 bipolars). The model starburst received only excitatory input from simulated bipolar cells (5 - 15 pS/synapse; $n = 70$). Each bipolar cell had a center-surround receptive field (center = 30 μ m diam; surround = 120 μ m diam, integrated weight 0.5 - 0.9). **l**, Calcium level due to depolarization in distal dendrite shows a robust directional difference (compare to Figure 4b). The stimulus was an outward/inward moving radial sine wave grating (spatial period 400 μ m, velocity 200 μ m/s, "contrast" 7 mV, see Methods).

889 **Figure legends (extended data)**

890 **Figures 11, Tables 1**

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Extended data Figure 1. Morphology, spatial densities and stratification depth of 19 proposed ganglion-cell types in the macaque monkey retina. A near complete atlas of the ganglion-cell types reveals recursive bistratified ganglion cells are present as a single population and a small fraction of the total ganglion cells that project to both the LGN and superior colliculus. **a**, Parasol cells comprise ~16% of total ganglion cells (coverage: inner cells 1.9; outer cells 1.6) and project to the lateral geniculate nucleus (LGN), superior colliculus (SC) and pretectum (Pt). Left, photomicrograph of two photo-stained cells tracer labeled from injections in the superior colliculus (arrowheads, cell bodies; HRP histochemistry). Center, tracings of the adjacent dendritic trees of the two cells shown on the left (arrowheads, axons; inset at bottom, Outlines of the overlapping dendritic fields of 5 neighboring outer parasol cells ~6 mm from the fovea. Right, dendritic field diameter plotted against retinal eccentricity ($n = 438$ cells; mean $\pm$ sd = 206 ± 65 μ m diam; range = 30.5 - 400 μ m) and mean dendritic stratification measured in wholemount retina and plotted as percentage depth in the IPL (inner cells, $n = 36$; outer cells, $n = 20$) and relative to choline acetyl transferase (ChAT) immunostained bands (see extended data Fig. 3 and Methods).

b, Midget ganglion cells include inner (ON-center) and outer (OFF-center) stratifying types that comprise ~58% of total ganglion cells (coverage: inner cells 1.1; outer cells 1.1) and project to the LGN. Left, photomicrograph of three photo-stained inner midget cells labeled from injections in the LGN (HRP histochemistry). Center, tracings of the cells shown in a; reveals near perfect tiling of retinal surface with little dendritic overlap (arrows indicate axons); inset at bottom, outlines of the overlapping dendritic fields of eight neighboring inner midget cells. Right, dendritic field diameter plotted against retinal eccentricity (red, $n = 181$; mean $\pm$ sd = 84 ± 41 ; range = 4.9 - 195.5 μ m diam); parasol cells (blue) shown for comparison). Mean dendritic stratification measured relative to ChAT bands (inner midget/parasol cell pairs, $n = 4$; outer midget/parasol cell pairs, $n = 4$). **c**, Small bistratified

920 cells make up ~6% of total ganglion cells (coverage = 1.6) and project to the LGN. Left,
921 photomicrograph of a small bistratified cell intracellularly injected with Neurobiotin (HRP
922 histochemistry), with the focus on the inner dendritic arbor. Center, tracings of three
923 neighboring small bistratified cells photo-stained from tracer injections in the LGN (HRP
924 histochemistry); arrowheads indicate axons. Inset at bottom, outlines of the overlapping
925 dendritic fields of the three cells. Right, dendritic field diameter plotted as a function of
926 eccentricity (orange; $n = 191$; mean $\pm$ sd = 178 ± 76 ; range = 52 - 388 μ m) with parasol cells
927 (blue) shown for comparison; mean dendritic stratification measured relative to ChAT bands
928 and parasol stratification in wholemount retina and plotted as percentage depth in the IPL
929 (parasol/small bistratified pairs: $n = 11$). **d**, Broad thorny cells form a single population that
930 comprises ~1.5% of total ganglion cells and project to the LGN, SC and pretectum. Left,
931 photomicrograph of a broad thorny cell at 6.8 mm eccentricity in inferior retina, intracellularly
932 injected with Neurobiotin and processed for HRP histochemistry. The cell showed tracer-
933 coupling to a population of small-bodied amacrine cells. Center, tracing of the same cell.
934 Arrowhead indicates axon; inset, outlines of the overlapping dendritic fields of 4 neighboring
935 broad thorny cells ~5 mm from the fovea, retrogradely labeled from tracer injections in the
936 SC (coverage = 1.2). Right, dendritic field diameter (green) plotted as a function of
937 eccentricity (mean dendritic field diam $\pm$ sd = 331 ± 102 μ m; range = 146 - 586 μ m; $n = 67$)
938 and shown with parasol cells (blue) for comparison. Mean dendritic stratification measured
939 relative to starburst amacrine-cell dendrites (ChAT, gray bands) in wholemount retina and
940 plotted as percentage depth in the IPL ($n = 7$). **e**, Narrow thorny cells form inner and outer
941 stratifying populations that comprise ~3% of total ganglion cells and project to the LGN, SC
942 and pretectum. Left, photomicrograph of an outer narrow thorny cell located at 6.5 mm
943 eccentricity in inferior retina, intracellularly injected with Neurobiotin and processed for HRP
944 histochemistry. Arrow indicates the cell body. Center, tracing of the same cell shown in A.

945 Arrowhead indicates axon; inset, outlines of the overlapping dendritic fields of 3 neighboring
 946 outer narrow thorny cells retrogradely labeled from tracer injections in the SC (coverage:
 947 inner cells 1.0, outer cells 1.0) Right, dendritic field diameter (gold) plotted as a function of
 948 eccentricity and shown with parasol cells (blue) for comparison; mean dendritic field
 949 diameter $\pm$ sd = $331 \pm 119 \mu\text{m}$; range = 163 - 682 μm ; n = 59. Mean dendritic stratification
 950 measured relative to ChAT bands (inner narrow thorny: n = 1; 4 dendritic locations; outer
 951 narrow thorny: n = 1, 4 dendritic locations). **f**, Recursive bistratified cells comprise ~1.5% of
 952 total ganglion cells (coverage = 1.3) and project to the LGN, superior colliculus and
 953 pretectum. Photomicrograph of a recursive bistratified cell intracellularly injected with
 954 Neurobiotin (HRP histochemistry). The cell shows tracer coupling to multiple amacrine-cell
 955 populations. Center, tracings of four cells photo-stained from injections in the superior
 956 colliculus ~7 mm eccentricity; inset, outlines of the overlapping dendritic fields of the same
 957 four cells. Right, dendritic field diameter plotted as a function of eccentricity (n = 122; mean
 958 $\pm$ sd = 327 ± 93 ; range = 117 - 557 μm). Mean dendritic stratification measured relative to
 959 parasol stratification in wholemount retina and plotted as percentage depth in the IPL
 960 (recursive bistratified/parasol cell pairs, n = 3). **g**, Sparse monostратified cells form inner and
 961 outer stratifying populations that comprise ~2% of total ganglion cells and project to the LGN
 962 and the SC. Left, photomicrograph of an outer sparse monostратified cell retrogradely
 963 labeled from tracer injections in the SC and reacted for HRP histochemistry (arrowhead, cell
 964 body). Center, tracings of two neighboring inner sparse monostратified cells located ~3.7
 965 mm from the fovea in superior retina. inset; outlines of the overlapping dendritic fields of four
 966 neighboring outer sparse monostратified cells located at 11.5 mm eccentricity, (coverage:
 967 inner cells 1.5, outer cells 1.5). Right, dendritic field diameter (purple) plotted as a function
 968 of eccentricity (mean dendritic field diameter $\pm$ sd = $447 \pm 143 \mu\text{m}$; range = 93 - 706 μm ; n =
 969 78) and shown with parasol cells (blue) for comparison. Mean dendritic stratification

970 measured relative to starburst amacrine-cell dendrites (ChAT bands) in wholemount retina
971 and plotted as percentage depth in the IPL (inner cells, $n = 1$; outer cells, $n = 3$). **h**, Large
972 bistratified cells make up $\sim 3\%$ of total ganglion cells (coverage: ~ 2) and project to the LGN.
973 Left, photomicrographs of a large bistratified cell intracellularly injected with Neurobiotin
974 (HRP histochemistry). Focus is on the inner dendritic arbor. Center, tracing of the same cell
975 shown in photomicrograph (outer dendritic arbor is shown in red, arrowhead indicates axon);
976 inset, outlines of the dendritic fields of two neighboring large bistratified cells with
977 overlapping dendritic fields. Right, dendritic field diameter plotted as a function of
978 eccentricity ($n = 46$, mean $\pm$ sd = 365 ± 92 , range = 206 - 595 μm diam) Mean dendritic
979 stratification measured relative to parasol stratification (parasol/large bistratified cell pairs: n
980 = 5). **i**, Recursive monostratified cells comprise $\sim 4.2\%$ (3 populations; single population:
981 $\sim 1.3\%$) of total ganglion cells (coverage: single population 1.2; 3 populations 3.5) and
982 project to the LGN and pretectum. Left, photomicrograph of photostained cells labeled from
983 tracer injections in the pretectum (HRP histochemistry). Center, tracing of a recursive
984 monostratified cell 8.9 mm from the fovea (arrowhead, axon); inset, outlines of the
985 overlapping dendritic fields of 3 neighboring recursive monostratified cells ~ 6.8 mm from the
986 fovea. Right, dendritic field diameter plotted as a function of eccentricity ($n = 39$, mean $\pm$ sd
987 = 323 ± 77 , range = 188 - 480 μm diam). Mean dendritic stratification measured relative to
988 parasol stratification (recursive monostratified/inner parasol cell pairs, $n = 3$). **j**, Giant
989 melanopsin cells form inner and outer stratifying populations that comprise $\sim 1\%$ of total
990 ganglion cells and project to the LGN, SC and pretectum. Left, photomicrograph of a giant
991 melanopsin cell (arrowhead) intracellularly injected with Neurobiotin (HRP histochemistry).
992 The cell shows Neurobiotin tracer-coupling to several populations of amacrine cells (smaller
993 cell bodies surrounding the ganglion cell). Tracing of an inner giant melanopsin
994 immunolabeled and processed for HRP histochemistry. Bottom, outlines of the overlapping

dendritic fields of 5 neighboring inner giant cells located at 10.5 mm eccentricity (coverage: inner cells 1.5, outer cells 2.0). Right, dendritic field diameter (red circles) plotted as a function of eccentricity (dark blue, mean dendritic field diameter $\pm$ SD = 740 ± 219 μ m; range = 202 - 1316 μ m; n = 276) and shown with parasol cells (blue circles). Mean dendritic stratification measured relative to starburst amacrine-cell dendrites (ChAT, gray bands) in wholemount retina and plotted as percentage depth in the IPL (inner giant cell: n = 2; outer giant cell: n = 2). **k**, Smooth monostratified cells comprise ~2.5% of total ganglion cells (coverage: inner cells 1.4; outer cells 1.4) and project to the LGN, superior colliculus and pretectum. Left, photomicrograph of two smooth cells intracellularly injected with Neurobiotin (HRP histochemistry). The smooth cells are tracer-coupled to a population of small-bodied amacrine cells. Tracings of eight inner smooth cells ~7 mm from the fovea, labeled from tracer injections in the superior colliculus (HRP histochemistry); inset, outlines of the overlapping dendritic fields of the eight cells. Right, dendritic field diameter plotted as a function of eccentricity (n = 102, mean $\pm$ sd = 419 ± 110 , range = 138 - 634 μ m diam). Mean dendritic stratification measured relative to parasol stratification (inner smooth/parasol cell pairs, n = 4; outer smooth/parasol cell pairs, n = 20). For all panels: GCL, ganglion-cell layer; IPL, inner plexiform layer; INL, inner nuclear layer.

Extended data Figure 2. Recursive monostratied cells show fasciculating dendrites and at least 3 multiple overlapping cellular mosaics. **a**, Photomicrograph of a patch of photo-stained cells tracer labeled from injections in the pretectum and reacted for HRP histochemistry. The dendrites of the cell bodies labeled 1 thru 4 and other nearby cells (cell bodies outside of image) form a dense plexus of co-fasciculated processes. Right, magnified view of boxed area in **a**. Arrows indicate 5 examples of co-fasciculation. Numbers correspond to the dendrites' cells of origin as shown in **a**. **b**, The highly overlapping and cofasciculating dendrites of cells 1-3 appear to be components of three separate ganglion-cell mosaics (red dotted line boxed area corresponds to location of image shown in **a**)

1024 illustrated by the tiles shown in pink (cells 2, 4), gray (cells, 3,6-8) and green (cells 1, 5),
1025 traced around additional neighboring dendritic trees with minimal overlap.

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1029 **Extended data Figure 3. Summary of the morphology of 19 ganglion-cell types in**
1030 **macaque retina.** This figure summarizes the data shown in extended data Figure 1. Camera
1031 lucida tracings of examples of each of the morphological groups (e.g., midget, parasol, etc.).
1032 Small colored insets indicate number of types associated with each morphology (inner and
1033 outer midgets, red, etc.). Stratification for all types relative to the ChAT bands is indicated at
1034 bottom.

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Extended data Figure 4. Stratification levels of distinct ganglion-cell types relative to ChAT-labeled starburst amacrine cells. a-d, Upper panels show maximum intensity projections from confocal image stacks of ganglion cells injected with Neurobiotin in whole-

1042 mounted macaque retina. Neurobiotin was visualized by dye-coupled streptavidin and the
1043 specimens were double-labeled with antibodies against choline acetyl transferase (ChAT).
1044 Lower panels show z-reconstructions (vertical views) from different regions of the ganglion
1045 cells' dendritic fields together with ChAT staining. **a**, Dendrites of inner parasol cells (green)
1046 costratify with the inner ChAT band (red). Dendrites of an outer parasol cell (bottom panel)
1047 show an inversed pattern of stratification levels relative to the outer ChAT band. **b**, The
1048 outer (upper and middle panel) and inner (bottom panel) midget cells' dendritic ramifications
1049 overlap with the ChAT bands, but are broad and also extend deep (inner midgets) or
1050 superficial (outer midgets) to the ChAT bands. **c**, Inner dendrites of a small bistratified cell
1051 stratify within a wide region largely deep to the inner ChAT band. The outer dendritic tier
1052 ramifies largely at the level of the outer ChAT band. **d**, The dendritic ramifications of a broad
1053 thorny cell occupy the central third of the IPL in space between the outer and inner ChAT
1054 bands.
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Extended data Figure 5. Origin of A1 axon at an axon hillock like structure and the presynaptic nature of the A1 axonal processes. a, A1 cell partial reconstruction (gold; see also Figure 3, main text) illustrating the origin of one axon from a thick proximal dendrite (teal, boxed area, axon hillock). Inset, boxed area zoomed at lower left; at its point of origin the axon swells and is densely packed with mitochondria; the axon shows a constant

diameter and lacks all synaptic input as it extends distally; the first synapse appears distal to the axon hillock (boxed area, zoomed inset; first synapse after axon hillock). Axonal processes co-stratify with the dendritic tree and are numerous; two additional examples are shown; these processes do not receive synaptic input and make synaptic output primarily to cone bipolar axon terminals (8 axon segments; 73/127 synapses; 57.5 % of total) and to a lesser extent with presumed ganglion-cell dendrites and dendritic spines (36/127 synapses, 28% of total; 18 additional synapses, 14%, either contacted other amacrine cell or unidentified processes). **b**, Red boxed panels show synapses on a ganglion-cell dendrite and a bipolar axon terminal from shorter axon segment (red arrow). **c**, Blue boxed panels show examples of synaptic output from points along longer axon segment (blue arrow) onto both bipolar axon terminals and presumed ganglion-cell dendrites. All postsynaptic bipolar-cell axon terminals were identified by ribbon synapse content and ganglion-cell dendrites were inferred by reconstruction and lack of synaptic output.

Extended data Figure 6. Distinctive dendritic morphology of inner starburst amacrine cells in macaque monkey retina. **a**, Starburst cell radially symmetric dendritic morphology. The thin primary and secondary dendrites and distinct varicose dendritic terminals create a nearly circular dendritic tree, a feature common to starburst cells of other non-primate mammals (max intensity z-projection from confocal stack; OGB-488 intracellular fill). **b**, Magnified view of boxed area (red dotted line) illustrates the characteristic extremely thin primary and secondary dendrites and the thicker, complex varicose terminal dendrites of this starburst cell. **c-k**, Panels illustrate starburst cell morphological variability and the relative constancy of dendritic tree diameter across much of the peripheral retina (6-10 mm retinal eccentricity; OGB filled electrodes are evident in some images). All cells impaled and physiologically identified as inner, ON-center starbursts that displayed the same basic light response as illustrated in extended data Figure 7. **l**, Physiologically identified starburst cell at ~3 mm eccentricity shows a smaller dendritic tree (about 150 μm diam). **m-n**, Two examples of amacrine cells with radial symmetric dendritic morphology and a dendritic tree size similar to that of starburst cells but were identified as distinct 'non-starburst' amacrine-cell types due to multiple features of the light response (e.g., extremely sustained ON response) that were not shared by all other starbursts.

Extended data Figure 7. Some basic features of the macaque starburst cell photopic light response. **a**, Starburst whole-cell current-clamp recording (cell imaged in extended data Fig. 6g) shows a resting membrane potential ~ 75 mV on a high photopic background ($\sim 10^5$ photoisomerizations/cone/sec) and large amplitude depolarizing response (~ 40 mV) to 0.5 sec, 100% contrast, square wave pulse stimulus. With increasing spot diam (inset at top; 50-800 μm diam; color coded) the response becomes more transient (black trace, 800 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**, Macaque ON-center starburst cells receive a strong excitatory input at light OFF. Top, inset, an annular stimulus was used to

1109 measure receptive-field spatial structure in which annulus inner diam (I.D.) was increased
1110 from 40 to 450 μm ; stimulus was square wave modulated at 1 Hz temporal frequency. When
1111 the annulus inner diam was small (black and red traces) a large transient depolarization
1112 appears at light ONset and an equally large, more sustained depolarization appears at light
1113 OFFset. As annulus inner diam increases to encompass the receptive-field center (~ 300
1114 μm) the ON component diminishes (green thru yellow traces) but the OFF field, larger in
1115 diam than the center, remains present. Traces are shifted vertically for illustration purposes.
1116 **c**, In voltage-clamp mode, both the ON and OFF responses (inset at bottom right; annulus
1117 inner diam is fixed at 80 μm for all traces) appear as large, inward excitatory synaptic
1118 currents at negative holding potentials (V_{hold} , -80 to 0 mV, color coded). The ON component
1119 approaches equilibrium at the cation reversal potential (0 mV hold, green trace), but a small
1120 OFF inward current remains present.

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Extended data Figure 8. Starburst cell dendritic direction selectivity to bar and

grating stimuli. In addition to centrifugal direction sensitivity to radial grating stimuli (Fig. 4)

macaque starburst cell dendrites also showed direction selectivity to both moving bars and

drifting square wave gratings. **a**, Max intensity z-stack of OGB-488 filled starburst *in vitro*;

locations of 7 dendritic ROIs are indicated by the yellow circles; arrows indicate preferred

direction and arrow length indicates direction-selective strength (peak amplitude, mean of 3

1130 stimulus cycles to drifting square wave gratings (0.47 cycles/deg, 0.5 Hz) at 8 orientations; n
1131 = 7 ROIs; 0.29 ± 0.20 ; range = 0.15 - 0.58 DSI). **b**, Polar plots of the optically imaged
1132 calcium response of 2 ROIs (5 and 6) from the cell shown in **a**. for two ROIs from two
1133 different starburst cells. **c**, Examples of two additional polar plots for dendritic ROIs for two
1134 different cells using drifting bars (bar w x h = 200 x 700 μm , 4000 $\mu\text{m}/\text{sec}$) to measure
1135 directional tuning (n = 12 ROIs from 7 cells; mean $\pm$ sd = 0.37 ± 0.17 , range = 0.12 - 0.76
1136 DSI).
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1140 **Extended data Figure 9. Characterization of small excitatory non-linear subunits in**

1141 **the starburst receptive-field center.** **a**, Counterphase modulated square-wave grating

1142 (left, inset) (100% contrast) elicits a large somatic starburst voltage response at 4 Hz, twice

1143 the 2 Hz stimulus frequency (response to 2.8 cycles/degree (cpd) grating spatial frequency).

1144 The Fourier amplitude of the second harmonic (F2) is 5.4 mV with little response at the

1145 fundamental stimulus frequency, F1 Fourier amplitude = 0.3 mV. **b**, Left, plot of F2

1146 amplitude with increasing stimulus spatial frequency (black data points), the F2 response
1147 extends to high spatial frequencies; F1 values are plotted for comparison (grey data points).
1148 Right, the F2 data are well fit (red line) by a single Gaussian (diam $\sim 35 \mu\text{m}$; $n = 7$; 55 ± 18).
1149 **c**, Left, addition of HEPES to bath to eliminate surround response (red, HEPES, black,
1150 control) has little effect on the F2 response to counterphase modulated gratings indicating
1151 that the F2 response component reflects summation of bipolar-cell receptive-field centers. **d**,
1152 F2 response to counterphase square wave gratings is reproduced in model starburst cell;
1153 the bipolar-cell center size (Gaussian diam = $30 \mu\text{m}$) used to produce these data was used
1154 in the starburst simulations of Fig. 5 and the bipolar-cell responses of Extended Data Fig.
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Extended data Figure 10. Connectomic reconstruction identifies midget and DB 4/5 cone bipolar types as presynaptic to the starburst amacrine. a, Reconstructed starburst amacrine cell (teal; see Fig. 5, main text) showing 3 cone bipolar cells with large axon terminals (blue) and three cone bipolars with small axon terminals (gold). The three large blue bipolar terminals are all presynaptic to the starburst. The two most proximal small gold bipolar terminals are also presynaptic to the starburst cell; the distal small bipolar overlaps but does not synapse with the starburst. Hairpin turn in starburst dendrite denoted by black arrowhead (see Fig. 4 also). **b,** partial reconstruction of an inner (ON-center) parasol ganglion cell (orange; identified by uniquely large soma diameter) receives synaptic input from the 3 large (blue) bipolar terminals (~ 5 synapses/bipolar, 16 ribbon synapses total). Since inner parasol cells receive their major synaptic input from diffuse bipolar (DB) cell types 4 and 5 we identify this bipolar type as DB 4/5 (these two types are not easily distinguishable (see main text). Hairpin loop in starburst amacrine (teal, black arrowhead) is created by wrapping around the primary dendrite of this parasol cell. **c, d,** Two examples of ribbon synapses from the DB 4/5 bipolars onto the dendrites of the parasol cell shown in **b**. **e,** Complete reconstruction of a midget ganglion cell within the starburst dendritic field (midget cell identified by its uniquely small dendritic field size at this retinal eccentricity). The small gold bipolar-cell axon terminals are enmeshed by the ganglion-cell dendrite and provide profuse synaptic input to this midget ganglion cell (25, 34, 27 ribbons synapses each from the three midget bipolar cells shown; this midget ganglion cell received a total of 405 ribbon synaptic inputs; the vast majority, ~90% were derived from midget bipolar cells) identifying them as midget bipolar cells. **f,** Zoomed view of boxed area in e illustrates the close association of midget bipolar and midget ganglion cell for the two bipolars that also contact the starburst amacrine. **g, h,** Two examples of ribbon synapses from midget bipolar (gold) to midget ganglion-cell dendrites (purple).

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1186 **Extended data Figure 11. Bipolar-cell responses are motion sensitive due to the delay**

1187 **between center and surround evoked signals.** **a.** Model of starburst amacrine cell with
 1188 the locations of its presynaptic bipolar cells. Gold dots, midget bipolar cells; light blue dots,
 1189 DB4/5 bipolar cells. The starburst amacrine dendrites are gray and its soma is the centrally
 1190 located dark blue circle. An array of bipolar cells was generated to produce a nearest
 1191 neighbor distance of 23 μm and a regularity index of 8. Those bipolar cells in the array
 1192 within 10 μm of a starburst dendrite were considered to make a synaptic connection. To
 1193 account for the differential distribution of midget and DB 4/5 bipolar cells (Fig 5) the density
 1194 of midget bipolar inputs to the starburst was reduced beyond 75 μm from the soma and DB
 1195 4/5 bipolars inserted where midgets had been removed. Modeled voltage responses of **(b)** a
 1196 proximal bipolar cell (cell 1 in **a**) and **(c)** a peripheral bipolar cell (cell 2 in **a**) to radial moving
 1197 square-wave gratings (spatial period, 500 μm , velocity 500 $\mu\text{m}/\text{s}$, "contrast" 7 mV) centered
 1198 on the starburst soma in both outward and inward directions. **d** and **f.** Response of the
 1199 proximal **(d)** and peripheral **(f)** bipolar cells to their respective center receptive-field
 1200 components. **e** and **g.** Response of the proximal **(e)** and peripheral **(g)** bipolar cells to their
 1201 respective receptive-field surround components. Responses of bipolar cells near the

starburst soma are sensitive to the direction of motion. The response to outward motion is greater than the response to inward motion. This was not the case for the peripheral bipolar cell. This difference in behavior between proximal and peripheral bipolar cells results from the time difference in engagement of the center and surround components of their receptive fields for the two conditions, outward and inward motion. The receptive fields comprised Gaussian blurred center and surround components (center: diam 30 μm ; surround: diam 120 μm , integrated weight 0.7-0.9 of center weight, delay 40 ms). The preference of the proximal bipolar cells for outward-moving stimuli was transmitted to the postsynaptic starburst cell which therefore also responded to the same radial grating with a preference for outward motion as shown in Fig 5. These modeling results are consistent with a recent report of sensitivity to motion in bipolar cells⁸⁸.

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Summary of ganglion cell types in macaque retina

Ganglion cell morphological type		% of total ganglion cell population*	Central projections	Some physiological properties
1	Midget inner	27.0	LGN parvo 3-6	Achromatic/chromatic L vs M cone opponent
2	Midget outer	31.0		
3	Parasol inner	8.0	LGN magno 1, 2 Superior colliculus Pretectum	Achromatic L+M cone input S cone input controversial
4	Parasol outer	7.8		
5	Small bistratified	6.1	LGN konio 3, 4	S ON; L+M OFF opponent
6	Recursive monostratified (3 inner populations)	1.4	LGN Pretectal area (NOT?)	Possible correlate of ON direction selective ganglion cells
7		1.4		
8		1.4		
9	Narrow thorny inner	1.5	LGN Superior colliculus Pretectum	Unknown
10	Narrow thorny outer	1.5		
11	Smooth inner	1.3	LGN Superior colliculus	Achromatic L+M cone input
12	Smooth outer	1.3		
13	Sparse monostratified inner	0.9	LGN Superior colliculus	L+M ON; S OFF opponent details unknown
14	Sparse monostratified outer	0.9		
15	Recursive bistratified	1.5	LGN Superior colliculus	ON-OFF direction selective ganglion cells
16	Large bistratified	1.5	LGN	S ON opponent details unknown
17	Broad thorny monostratified	1.3	LGN Superior colliculus Pretectum	"Local edge detector"
18	Giant melanopsin inner	0.5	LGN Superior colliculus Pretectal area, PON, SCN	Sustained ON response S OFF; L+M ON opponent Strong rod input Intrinsically photosensitive Intraretinal axon collaterals
19	Giant melanopsin outer	0.6		

*Total ganglion cell density is from Wässle et al., 1989, for temporal retina ~ 8 mm from the fovea.
Individual cell type densities were determined as described in Methods.

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1233 **References**

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