Figure S1. Amacrine cell inputs to the wide-field RGCs. (A) The two wide-field RGCs (navy) and four previously published S-cone amacrine cells, which provide M1 ipRGCs with an S-OFF response. Locations of S-cone amacrine cell inputs to the wide-field RGCs are marked in blue [S7] and their rarity indicates they do not alter the wide-field RGCs’ spectral tuning. (B-C) Representative examples of the most common amacrine cell processes presynaptic to the wide-field RGCs, which were likely the axons of a polyaxonal amacrine cell. Synaptic inputs to the left and right wide-field RGCs are marked in blue and green, respectively. Synaptic inputs to M1 ipRGCs (not pictured for simplicity) are marked in red. All scale bars are 20 microns.
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Tissue Preparation
Retinal tissue was obtained from a terminally anesthetized male macaque (*Macaca nemestrina*) monkey through the Tissue Distribution Program at the Washington National Primate Center. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. A block of inferior parafoveal retinal tissue at ~1 mm eccentricity from the fovea center was processed as previously described [S1]. A cross-section of the retina taken with a transmission electron microscope can be found in our previously published work [S2]. Briefly, the eyecup was placed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.4 and, while in this solution, a 1 mm square of retina centered 1.5 mm inferior to the center of the fovea was cut out and fixed overnight at 4°C. The next day, the tissue was washed 5 x 5 min in 0.1M cacodylate buffer, then post fixed in osmium ferrocyanide for 1 h on ice. The tissue was next washed 5 x 5 min in double distilled (dd)H$_2$O at room temperature (RT) and incubated in a 1% thiocarbohydrazide solution for 20 min at RT. The tissue was washed 5 x 5 min in ddH$_2$O and placed in 2% osmium tetroxide for 30 min at RT. Next, the tissue was washed 5 x 5 min in ddH$_2$O, then *en bloc* stained in 1% uranyl acetate (aqueous) overnight at 60°C. The next day, the tissue was washed 5 x 5 min in ddH$_2$O, then *en bloc* stained in Walton’s lead aspartate for 30 min at 60°C. The tissue was again washed 5 x 5 min in ddH$_2$O, dehydrated in ice cold 30%, 50%, 70%, and 95% EtOH, and then allowed to come to RT. This was followed by two changes of 100% EtOH and two changes of propylene oxide. The tissue was next infiltrated in a 1:1 mixture of propylene oxide:Durcupan resin for 2 h, then overnight in Durcupan. The next day, the tissue was given a fresh change of Durcupan for 2 h and then placed in flat embedding molds and polymerized in a 60°C oven for 2 days. The block was then trimmed to approximately 0.5 mm$^2$.

Microscopy
The tissue was imaged using a Zeiss Sigma VP field emission scanning electron microscope equipped with a 3View system and sectioned in the horizontal plane. Tissue preparation and image collection were optimized in signal-to-noise ratio for visualizing small, low contrast features such as synaptic ribbons that have previously been a challenge for serial block-face scanning electron microscopy. The tissue was sectioned horizontally and imaged as a 5 x 5 montage at a resolution of 7.5 nm/pixel. The final volume contained 1893 sections at 90 nm section widths and spanned from the ganglion cell layer through the cone pedicles. Image registration was performed using Nornir (RRID: SCR_003584, [http://nornir.github.io](http://nornir.github.io)) and supplemented with custom MATLAB (Mathworks) code.

Annotation
The serial EM volumes were annotated using the web-based, multi-user Viking software described previously (RRID: SCR_005986, [https://connectomes.utah.edu](https://connectomes.utah.edu))[S3]. Neuronal processes were traced through the sections by placing a circular disc at the structure’s center of mass and linking the disc to annotations on neighboring sections. Synapses annotated with lines connected by 2-3 control points and linked to a parent neuron. Where both were present, the synapse annotations for the presynaptic and postsynaptic neurons were also linked to each other so that the annotated neurons and the specific links between them could be represented and queried as a network.

Data Analysis and Visualization
Data analysis and 3D rendering were performed using SBFSEM-tools, an open-source MATLAB toolbox developed in the Neitz lab (RRID: SCR_017350, [https://github.com/neitzlab/sbfsem-tools](https://github.com/neitzlab/sbfsem-tools)). The annotations for each neuron and the links between annotations were represented as an undirected
graph. By dividing the graph at all nodes of degree greater than 2, segments of connected annotations were obtained and then rendered as rotated cylinders centered at each annotation’s XYZ coordinates and scaled by their radii. To mark locations of synapses in Figure 1E, the coordinates of each synapse annotation were averaged to a single XYZ location. Adobe Photoshop was used to add in color overlays and enhance the contrast of the electron micrograph in Figure 1B. Final figured were compiled in Adobe Illustrator.

Cell Type Identification
Bipolar cell inputs to the wide-field RGCs were identified by the presence of two features across multiple sections: a pre-synaptic ribbon structure and an adjacent post-synaptic membrane density[S4]. Of the 51 bipolar cells presynaptic to the wide-field RGCs, 49 could be classified as either an S-ON bipolar cell, ON midget bipolar cell or DB6 ON diffuse bipolar cell. The remaining two bipolar cells were located at the edges of the volume and could not be reconstructed sufficiently to confirm cell type. The different ON bipolar cell types could be readily distinguished by their morphology and the stratification of their axon terminals within the inner retina[S5]. Further confirmation was obtained by reconstructing the RGCs post-synaptic to each bipolar cell. Each S-ON bipolar cell was confirmed either through contacts with S-cones and/or input to small bistratified RGCs. Each ON midget bipolar cell was confirmed by reconstruction of the post-synaptic ON midget RGC, which had characteristically small, dense dendritic fields that collected the majority of their bipolar cell input from just one or two ON midget bipolar cells[S6]. DB6 bipolar cells could be clearly distinguished from S-ON bipolar cells by their larger dendritic fields, thinner axon terminals and lack of input to small bistratified RGC dendrites.

Small bistratified RGCs were identified by their characteristic morphology (dense branching in sublamina 5 and sparse branches extending to sublamina 2), extensive S-ON bipolar cell input and mosaic tiling of their dendritic fields (Figure 1A). Some small bistratified RGCs are only partially complete as our goal in reconstructing them was to confirm the identity of nearby S-ON bipolar cell terminals. Both the small bistratified RGCs and the wide-field RGCs lacked both conventional and ribbon synaptic outputs, a distinguishing feature of RGCs.

Our classification of the wide-field RGCs as M2 ipRGCs expanded on our previously published criteria for ipRGC identification and reconstructions of M1 ipRGCs[S7]. As described in the text, the wide-field RGCs’ morphology and stratification was consistent with that of M2 ipRGCs (also called inner-stratifying ipRGCs or giant sparse RGCs)[S8-S10] and did not match any other known primate RGC type[S11, S12]. Notably, one of the wide-field RGCs had two axons extending from a major dendrite rather than the standard single axon from the soma, a rare feature described only for M1 and M2 ipRGCs[S9]. Consistent with previous reports, the M2 ipRGCs in our volume received more bipolar cell input than the M1 ipRGCs and synaptic inputs were often found in clusters along the dendrites[S13-S15]. In the past, this bipolar cell input to M2 ipRGCs has been attributed to DB6 bipolar cells. DB6 bipolar cell axons make en passant synapses onto M1 ipRGCs in the OFF layer and markers for synaptic output within DB6 axons terminals in the ON layer have been observed next to M2 ipRGCs dendrites[S16]. We only observed two instances of synaptic input from DB6 bipolar cells (orange circles in Figure 1E), although their ribbon synapses were often found in close proximity to the dendrites of the wide-field RGCs, possibly explaining the earlier results from immunohistochemistry.

The closest alternative correlate to the wide-field RGC is the ON large sparse RGC. However, several lines of evidence make a strong case against the wide-field RGCs in Figure 1C and 1D being ON large sparse RGCs. First, the ON large sparse RGCs stratify closer to sublamina 4, between 60-80% depth within the inner retina (where 0% is the inner nuclear layer and 100% is the ganglion cell layer). The RGCs in our study stratified within sublamina 5 at >90% depth[S11]. Second, the dendrites of both wide-field RGCs extended beyond the edges of the volume; thus, their dendritic field diameter...
far exceeds that reported for ON large sparse RGCs at a similar eccentricity and is more consistent with M2 ipRGCs [S17].

Quantification and Statistical Analyses
The synaptic contacts between S-ON bipolar cells and M2 ipRGCs were analyzed with custom MATLAB code written for SBFSEM-tools (https://github.com/neitzlab/sbfsem-tools). The mean, standard deviation and N are reported in the text.

Data and Code Availability
The Viking View software for visualizing both the dataset and the annotations is freely available (https://connectomes.utah.edu). The 3D reconstructions from Viking Viewer annotations are visualized with SBFSEM-tools, an open-source MATLAB toolbox (https://github.com/neitzlab/sbfsem-tools). Raw data and additional analysis code will be provided upon request by the Lead Contact, Jay Neitz (jneitz@uw.edu).

Author contributions
J.N. and S.S.P. conceived of the project and designed experiments; A.S.B., J.C., M.A.M. and S.S.P. collected and analyzed the data; J.N. and M.N. acquired the volume; M.A.M. and S.S.P. created the figures; S.S.P. wrote the paper with input from A.S.B., M.A.M. and J.N.

SUPPLEMENTAL REFERENCES
S1. Della Santina, L., Kuo, S.P., Yoshimatsu, T., Okawa, H., Suzuki, S.C., Hoon, M., Tsuboyama, K., Rieke, F., and Wong, R.O.L. (2016). Glutamatergic monopolar interneurons provide a novel pathway of excitation in the mouse retina. Curr. Biol. 26, 2070–2077.
S2. Patterson, S.S., Kuchenbecker, J.A., Anderson, J.R., Bordt, A.S., Marshak, D.W., Neitz, M., and Neitz, J. (2019). An S-cone circuit for edge detection in the primate retina. Sci. Rep. 9, 11913.
S3. Anderson, J.R., Mohammed, S., Grimm, B., Jones, B.W., Koshevoy, P., Tasdizen, T., Whitaker, R., and Marc, R.E. (2011). The Viking viewer for connectomics: scalable multi-user annotation and summarization of large volume data sets. J. Microsc. 241, 13–28.
S4. Dowling, J.E., and Boycott, B.B. (1966). Organization of the primate retina: electron microscopy. Proc. R. Soc, London. Ser. B 166, 80–111.
S5. Tsukamoto, Y., and Omi, N. (2016). ON bipolar cells in macaque retina: type-specific synaptic connectivity with special reference to OFF counterparts. Front. Neuroanat. 10, 104.
S6. Kolb, H., and Marshak, D. (2003). The midget pathways of the primate retina. Doc. Ophthalmol. 106, 67–81.
S7. Patterson, S.S., Kuchenbecker, J.A., Anderson, J.R., Neitz, M., Neitz, J. (2020). A color vision circuit for non-image-forming vision in the primate retina. Curr. Biol. 30, 1269-1274
S8. Hannibal, J., Tolstrup, A., Steffen, C., Fahrenkrug, J., and Folke, J. (2017). Melanopsin expressing human retinal ganglion cells: subtypes, distribution, and intraretinal connectivity. J. Comp. Neurol. 525, 1934–1961.
S9. Liao, H.W., Ren, X., Peterson, B.B., Marshak, D.W., Yau, K.W., Gamlin, P.D., and Dacey, D.M. (2016). Melanopsin-expressing ganglion cells on macaque and human retinas form two morphologically distinct populations. J. Comp. Neurol. 525, 2845–2872.
S10. Nasir-Ahmad, S., Lee, S.C.S., Martin, P.R., and Grünert, U. (2019). Melanopsin-expressing ganglion cells in human retina: morphology, distribution, and synaptic connections. J. Comp. Neurol. 527, 312–327.
S11. Dacey, D.M. (2004). Origins of perception: retinal ganglion cell diversity and the creation of parallel visual pathways. In: The Cognitive Neurosciences, M. S. Gazzaniga, ed. (Cambridge, MA: MIT Press), pp. 281–301.
S12. Grünert, U., and Martin, P.R. (2020). Cell types and cell circuits in human and non-human primate retina. Prog. Retin. Eye Res., doi: 10.1016/j.preteyeres.2020.100844
S13. Neumann, S., Haverkamp, S., and Auferkorte, O.N. (2011). Intrinsically photosensitive ganglion cells of the primate retina express distinct combinations of inhibitory neurotransmitter receptors. Neuroscience 199, 24–31.

S14. Schmidt, T.M., and Kofuji, P. (2010). Differential cone pathway influence on intrinsically photosensitive retinal ganglion cell subtypes. J. Neurosci. 30, 16262–16271.

S15. Jusuf, P.R., Lee, S.C.S., Hannibal, J., and Grünert, U. (2007). Characterization and synaptic connectivity of melanopsin-containing ganglion cells in the primate retina. Eur. J. Neurosci. 26, 2906–2921.

S16. Grünert, U., Jusuf, P.R., Lee, S.C.S., and Nguyen, D.T. (2011). Bipolar input to melanopsin containing ganglion cells in primate retina. Vis. Neurosci. 28, 39–50.

S17. Percival, K.A., Martin, P.R., and Grünert, U. (2011). Synaptic inputs to two types of koniocellular pathway ganglion cells in marmoset retina. J. Comp. Neurol. 519, 2135–2153.