An offset ON-OFF receptive field is created by gap junctions between distinct types of retinal ganglion cells

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In the vertebrate retina, the location of a neuron’s receptive field in visual space closely corresponds to the physical location of synaptic input onto its dendrites, a relationship called the retinotopic map. We report the discovery of a systematic spatial offset between the ON and OFF receptive subfields in F-mini-ON retinal ganglion cells (RGCs). Surprisingly, this property does not come from spatially offset ON and OFF layer dendrites, but instead arises from a network of electrical synapses via gap junctions to RGCs of a different type, the F-mini-OFF. We show that the asymmetric morphology and connectivity of these RGCs can explain their receptive field offset, and we use a multicell model to explore the effects of receptive field offset on the precision of edge-location representation in a population. This RGC network forms a new electrical channel combining the ON and OFF feedforward pathways within the output layer of the retina.

Receptive fields (RFs) are a foundational concept in sensory neuroscience. The RF of a sensory neuron is shaped by the properties of its synaptic inputs from connected neurons. In the early visual system, retinotopic maps define a strict correspondence between the location of a cell’s dendrites and its RF location in visual space1. RGCs, the output cells of the retina, form dendritic mosaics that tile retinal space and have corresponding RF mosaics that tile visual space2-2. Superimposed on the mosaic organization of retinal neurons is the division into ON and OFF channels, which respond to light increments and decrements, respectively. The ON and OFF pathways diverge at the first synapse in the visual system, the output of the photoreceptors, and they reconverge in multiple locations, including in ON-OFF RGCs that increase their firing for both increments and decrements. In the mouse, where they are best characterized, RGCs comprise greater than functionally, morphologically and transcriptionally distinct types3-8. All previously identified ON-OFF RGC types have aligned ON and OFF RFs9-13, and they all receive excitatory synaptic inputs from both ON and OFF bipolar cells. Inputs from ON and OFF bipolar cells are formed either at two distinct dendritic strata in the inner plexiform layer14 or at a single stratum in the middle of the inner plexiform layer where ON and OFF bipolar cell terminals overlap15.

We report on an RGC type that breaks both of these conventions. These RGCs have a systematic spatial offset between their ON and OFF RF subfields. The RGCs do not receive ON and OFF input from bipolar cells on spatially offset dendrites. Instead, the RF offset arises from a new circuit composed of gap junctions with several RGCs of a single, different functional type. While RFs with offset ON and OFF subfields result in a modest amount of direction selectivity and orientation selectivity for certain stimuli at the level of single RGCs, modeling demonstrates a large enhancement in the encoding of edge position within a population of RGCs. Our multicell model reveals that offset ON and OFF RF subfields could help a population of RGCs encode edge position with precision down to 0.6 degrees of visual angle, less than 12% of the RF diameter of a single RGC.

Results

F-mini RGCs have both ON and OFF responses. F-mini RGCs were recently identified as two different cell types, F-mini-ON and F-mini-OFF, based on their expression patterns of several transcription factors, their unique morphologies and their light responses16. F-mini RGCs are the second and third most numerous RGC types in the mouse retina, together constituting 13% of RGCs. We recorded light responses from functionally identified F-mini RGCs in dark-adapted mouse retina (Methods) and later confirmed their identity by morphological analysis (Fig. 1a-c) and immunohistochemistry (IHC) (Extended Data Fig. 1). Unlike in the initial reports, we found that both F-mini RGC types responded to both light increments (ON) and decrements (OFF) for small spots and moving bars (Fig. 1d-k and Discussion). We focused primarily on F-mini-ON RGCs in search of a circuit mechanism for the ON-OFF responses.

To explore the robustness of the ON-OFF responses in F-mini-ON RGCs, we adapted the retina to different mean luminances across the range from scotopic to photopic. We found robust ON and OFF responses across this range (Fig. 1j). We also measured the contrast response functions of F-mini-ON RGCs on a photopic background and found similar contrast sensitivity for the ON and OFF responses (Fig. 1k).

The ON and OFF RFs of F-mini-ON RGCs are systematically displaced despite aligned dendritic strata. We mapped ON and OFF RFs in F-mini and other RGCs using a stimulus consisting of small spots of positive and negative contrast17. We identified a consistent spatial offset between the ON and OFF RF subfields in F-mini-ON RGCs (Fig. 2). F-mini-ON RGCs had an ON-OFF offset of 38±14 μm (n=9 cells; Fig. 2d), greater than that of control RGCs, which had an offset of 25±15 μm (n=14 cells; P=0.047).
Fig. 1 | F-mini-ON and F-mini-OFF RGCs have both ON and OFF light responses. a,b, Images of F-mini-ON (magenta) and F-mini-OFF (cyan) RGCs from fixed tissue. All cell images are shown with the dorsal direction on the retina toward the top. Targeted RGCs were traced and colored, overlaid on gray. Morphology was consistent in all images (n = 40; 20 cells). c, Stratification profiles of an F-mini-ON and an F-mini-OFF RGC from our cell fills (colored lines) and from the data in the Eyewire museum (black lines). Dashed lines indicate ON and OFF choline acetyltransferase bands. Shading shows the stratification regions of ON (yellow) and OFF (gray) bipolar cells (BCs) in the inner plexiform layer (IPL). d, F-mini-ON RGC in current-clamp recording responding to the onset and offset of a positive contrast spot from darkness to 200 rhodopsin isomerizations per rod per second (R°·rod·s−1). e, F-mini-ON RGC responding to the leading and trailing edge of a positive contrast moving bar (140 μm × 500 μm, 1000 μm·s−1, 200 R°·rod·s−1). f,g, Same as d and e for an F-mini-OFF RGC. h, Spike counts in F-mini-ON RGCs responding to positive contrast spots of varying diameters. Mean onset responses are in yellow; mean offset responses are in black. The shaded region is the s.e.m. (n = 172 cells). i, Same as h for F-mini-OFF RGCs (n = 85 cells). j, Spiking responses of F-mini-ON RGCs to a flashed spot at varying mean luminance, showing the variations in ON and OFF responses to light level (n = 3 cells; spot diameter: 130 μm). k, Spiking responses of F-mini-ON RGCs to onset of flashed spots of varying contrast from the background luminance. Data are the mean ± s.d. (shaded area) across cells (n = 8).

We found a significant asymmetry in the offset of the F-mini-ON RGCs but not control RGCs (all mean ± standard deviation (s.d.); one-sample two-tailed t-test). The vertical component of the offset in F-mini-ON RGCs was always ventralward (OFF ventral to ON; −30 ± 13 μm, P = 9.2 × 10−5; Fig. 2c), with a horizontal component not significantly distributed to one side (−7.5 ± 24 μm, P = 0.37). Control ON–OFF RGCs lacked a systematic vertical or horizontal displacement (vertical: −4.0 ± 18 μm, P = 0.42; horizontal: −3.7 ± 23 μm, P = 0.56). The distribution of offset directions was uniform for control ON–OFF RGCs (P = 0.93; Rayleigh test) and highly non-uniform for F-mini-ON RGCs (P < 0.001, Fig. 2c).

We also quantified the fractional overlap (intersection area/union area) between the ON and OFF RIs and found it to be lower in F-mini-ON RGCs than in other ON–OFF RGCs (n = 9 and 14
Fig. 2 | RF ON and OFF subfields measured by flashed spots are spatially offset. a, Average responses of an F-mini-ON RGC to 30-μm spots of positive (orange) or negative (black) contrast at the indicated positions. b, c, Interpolated ON and OFF spatial RFs from the data in a. White and black crosses mark the ON and OFF centers of mass, respectively. d, Population data showing the fractional overlap of the OFF RF relative to the ON RF for F-mini-ON RGCs (magenta; n = 9) and other ON-OFF RGCs (black; n = 14; Methods). e, Polar histograms showing the offset angle between the ON RF center of mass and the OFF RF center of mass for F-mini-ON RGCs (magenta) and other OFF-OFF RGCs (gray). The vertical component of the offset in F-mini-ON RGCs is ventralward (OFF ventral to ON). f, Image of the cell from a–c overlaid with its ON and OFF RF contours. g, Average OFF RF for F-mini-ON RGCs aligned to the center of mass of each ON RF at the origin. Crosses are the centers of mass for individual cells (n = 9). Scale bar is the same in a–c, f and g. Box plots in d show the maximum, 75th percentile, median, 25th percentile and minimum values.

cells; P < 0.01, two-sample t-test; Fig. 2d,e). A smaller sample of F-mini-OFF RFs also showed diffuse ON and ventrally displaced OFF subfields (Extended Data Fig. 2).

We sought to determine whether the RF offset might be explained by an offset within the dendritic area of an F-mini-ON RGC. RF locations in visual space are derived from bipolar cell and amacrine cell inputs arriving at the dendrites of the RGC. So, a spatial offset in the dendritic area could create a spatial offset in the RF. We used the Eyewire museum electron microscopy reconstruction RGC dataset and measured the center of mass of the dendrites within ON and OFF bipolar cell layers for all bistriated RGC types. The population of F-mini-ON RGCs showed well-aligned dendritic strata, similar to other bistriated RGCs (Fig. 3 and Extended Data Fig. 3). Thus, F-mini-ON RGCs have RFs with spatially offset ON and OFF subfields, with the OFF subfield consistently ventral of the ON subfield, and this result is not simply explained by displacement of their dendritic strata.

F-mini-ON and F-mini-OFF RGCs are directly coupled through gap junctions. Many RGCs make gap junctions with other RGCs of the same type or with amacrine cells, and these electrical synapses can affect RF properties. Since dendritic morphology alone could not explain the ON–OFF RF offset in F-mini-ON RGCs, we sought to test whether gap junctions contributed to this unusual RF property. We filled individual F-mini-ON RGCs with the gap-junction-permeable tracer Neurobiotin, delivered during whole-cell patch-clamp recordings. In addition to the patched cell, Neurobiotin labeled several surrounding cells in the ganglion cell layer with a distinct morphology. We confirmed that the coupled cells were RGCs by the presence of an axon extending toward the optic nerve, and we confirmed their identity as F-mini-OFF RGCs by their light stimulus responses, their morphology and the presence of both of the transcription factors forkhead box protein P1 and P2 (FOXP1 and FOXP2; Figs. 1 and 4 and Extended Data Fig. 1).
Gap junctions between F-mini RGCs were not only bidirectionally permeable to Neurobiotin (Figs. 1a, b and 4) but also to the larger fluorescent molecule Alexa Fluor 488 (Fig. 5a). This dye allowed us to identify coupled cells in live tissue by two-photon (2P) excitation and record their light responses sequentially or simultaneously (Fig. 5b). F-mini-ON RGCs were coupled to 3.8±1.3 RGCs (n=13 cells) and F-mini-OFF RGCs to 4.0±3 RGCs (n=3 cells; Fig. 5c). In every case where we attempted to classify a cell directly coupled to an F-mini RGC, it was an F-mini RGC of the ‘other’ type. For F-mini-ON RGC injections, all coupled cells tested by IHC were FOXP1+FOXPO2+, the molecular identity of F-mini-OFF RGCs (n=19 cells). All coupled cells measured in live retina had the morphological signature (ventrally directed, compact and OFF-stratifying dendrites) and/or the physiological signature (primarily transient OFF response and strong surround suppression) of F-mini-OFF RGCs (n=54 cells). Similar experiments in F-mini-OFF RGCs revealed F-mini-ON RGCs verified by IHC as FOXP1+FOXPO2+ (n=14 cells) or by physiology and morphology (n=24 cells). Regions of dendritic contact between F-mini-ON and F-mini-OFF RGCs did not show immunoreactivity for antibodies against three connexin proteins known to exist in the inner retina, Cx36, Cx45 and Cx30.2 (Extended Data Fig. 4), so the identity of the connexin at these gap junctions remains an open question.

Having found a dense network formed by heterotypic coupling of RGCs, we sought to test for functional connectivity. We performed paired whole-cell current-clamp recordings aided by 2P visualization of Alexa Fluor 488. Both depolarizing and hyperpolarizing current injections were transmitted between the coupled cells, and the resulting voltage transfer was symmetric, the hallmarks of a non-rectifying electrical synapse (Fig. 5d–f; P>0.19 for both hyperpolarizing versus depolarizing currents, and injections into F-mini-ON versus F-mini-OFF RGCs). A coupling coefficient expresses the fraction of the voltage change in one cell that is transmitted to the coupled cell. We measured a coupling coefficient of 0.14±0.08 (range 0.05–0.31, n=11 cell pairs) between F-mini-ON and F-mini-OFF RGCs (Fig. 5f), comparable to the strongest coupling coefficients reported in the inner retina, between amacrine cells (0.25) or between RGCs of the same type (0.14). Pharmacological block of gap junctions with meclofenamic acid (MFA) decreased the coupling coefficient to 0.04±0.03 (n=4 cell pairs; P=0.0056, paired-sample one-tailed t-test; Fig. 5f,g) and also reduced noise correlations (P=0.0068, paired-sample one-tailed t-test; Extended Data Fig. 5). While the reduction in noise correlations in MFA could, in principle, be due to a number of factors, including indirect coupling, multiple lines of evidence (dye coupling with no intervening amacrine cells, paired recordings and additional experiments described below) support the claim that
heterotopic coupling between F-mini RGCs is direct. Thus, F-mini-ON and F-mini-OFF RGCs are not only dye coupled, but they are also capable of passing substantial amounts of current through their gap junctions, which could potentially mix ON and OFF pathways directly at the level of the RGCs.

OFF responses in F-mini-ON RGCs arise from coupled F-mini-OFF RGCs. To determine how gap junctions with F-mini-OFF RGCs affect the light responses in F-mini-ON RGCs, we sought to measure light responses from the same F-mini-ON RGCs with and without coupling. We used two different manipulations to uncouple F-mini-ON RGCs from their electrical network: pharmacological gap-junction block with MFA and physical ablation of coupled RGCs.

Our first strategy to isolate F-mini-ON RGCs from their coupled network used MFA, which decreases gap-junction coupling (Fig. 6f,g). We recorded from F-mini-ON RGCs in current-clamp mode and stimulated the retina with positive contrast spots and moving bars (Fig. 6a,b). MFA was bath applied, and we observed that the OFF responses were selectively decreased (all mean ± s.d.; n = 6 cells, paired-sample one-tailed t-tests). We compared the OFF:ON ratio in these RGCs and found that MFA selectively decreased OFF responses. The OFF:ON spiking ratio decreased from 0.22 ± 0.24 to 0.00 ± 0.00 (P = 0.034; Fig. 6c). The OFF:ON subthreshold peak response ratio decreased from 0.70 ± 0.27 to 0.02 ± 0.15 (P = 0.0011; Fig. 6d). To evaluate the effect of MFA on the ON pathway, we compared ON responses before and after MFA. The ON spiking decreased from 10.0 ± 1.73 to 5.29 ± 2.23 spikes (P = 0.0074; Fig. 6e), whereas the ON subthreshold peak responses did not (25.2 ± 3.69 to 24.0 ± 2.52; P = 0.28; Fig. 6d). The reduction in ON spike count despite an unchanged light-evoked depolarization can be attributed to a hyperpolarizing baseline shift (from −61 ± 2.37 mV in control to −66.2 ± 5.64 mV in MFA; P = 0.011), likely caused by nonspecific effects of MFA. Non-F-mini RGCs showed a moderate reduction of spiking in MFA, consistent with reduced contrast sensitivity, but the ON and OFF pathways were affected similarly (n = 3; P > 0.05 in all cases; Extended Data Fig. 6).

Our second strategy to isolate F-mini-ON RGCs from their coupled network used physical ablation, which had the advantage of increased specificity. Using Alexa Fluor 488 fluorescence under 2P illumination, we were able to visualize the F-mini-OFF RGCs coupled to a targeted F-mini-ON RGC. In the ablation procedure, we recorded responses to positive contrast spots and moving bars in F-mini-ON RGCs (Fig. 6g,h) in current-clamp mode before and after destroying the coupled cells by membrane rupture with sharp microelectrodes (Fig. 6e,f) shows partial ablation; in general, all connected cells were ablated). We compared the OFF:ON ratio in these RGCs and found that ablation selectively decreased OFF responses (all data show the mean ± s.d. from n = 6 cells; paired-sample one-tailed t-tests). The OFF:ON spiking ratio decreased from 0.53 ± 0.20 to 0.00 ± 0.00 (P = 0.00062; Fig. 6i). The OFF:ON subthreshold peak response ratio decreased from 0.92 ± 0.11 to 0.18 ± 0.12 (P = 0.00013; Fig. 6j). To evaluate the effect of ablation on the ON pathway, we compared ON responses before and after ablation. The ON spiking decreased slightly from 11.1 ± 2.35 to 8.87 ± 3.15 (P = 0.06; Fig. 6i). The ON subthreshold peak responses decreased slightly from 26.1 ± 5.33 to 22.5 ± 5.12 (P = 0.043; Fig. 6j).

The reduction in ON responses is significant but small, and might be caused by a decrease in second-order connectivity, that is, input current from F-mini-ON RGCs connected to neighboring F-mini-OFF RGCs.

Results from both approaches demonstrated that F-mini-ON RGCs receive ON input through canonical chemical synaptic pathways and receive OFF input through a noncanonical pathway involving gap junctions with F-mini-OFF RGCs.

The spatial arrangement of coupled F-mini RGCs and their dendritic fields can account for the ON-OFF RF offset in F-mini-ON RGCs. With this knowledge of the different circuits responsible for the ON and OFF components of the RFs of F-mini-ON RGCs, we returned to our observation of the spatial offset between the ON and OFF RF subfields in search of a mechanism. The distinctive asymmetric morphology and connection pattern of F-mini RGCs offered an important clue. In the ventral and central retina, where we performed our measurements, F-mini-ON RGCs have dorsally directed dendrites, and F-mini-OFF RGCs have ventrally directed dendrites, relative to their somas. We also observed in F-mini-ON RGCs that coupled soma positions were located dorsally to the filled soma. Using confocal microscopy images of dye-filled F-mini-ON RGCs, we observed that their dendrites generally lie dorsally to the dendrites of coupled F-mini-OFF RGCs (Fig. 7a). A measurement of this offset could explain the offset RF position. However, the dendritic tips of coupled cells are generally not well filled by intracellular dye, so single-cell fills are not well suited for this measurement, and complete fills by manual dye filling is experimentally prohibitive. So, we sought to model this combined dendritic offset using accessible imaging morphology datasets (Fig. 7).

To construct this model, we created two datasets. For each dye-filled F-mini-ON RGC, we measured the positions of neighboring brightly labeled (first-order) connected somas (Fig. 7d; n = 50 coupled soma positions from 13 injected F-mini-ON RGCs). Next, for both F-mini-ON and F-mini-OFF RGCs, we measured a polygon around the dendrites within their ON and OFF layer stratifications, respectively (Fig. 7e; n = 38, 12 cells). With the knowledge that the neighboring somas were F-mini-OFF RGCs, we could combine these datasets. We randomly generated combinations of measured soma offsets, F-mini-ON polygons and F-mini-OFF polygons.
Fig. 5 | F-mini-ON and F-mini-OFF RGCs are electrically coupled to each other by gap junctions. a. An example illustrating heterotypic network connectivity. The F-mini-OFF RGC labeled 'O' was filled with Alexa Fluor 488 (cyan), revealing seven coupled somas (white). b. Cell-attached recordings from each of the labeled somas shown in a. Cells 1-6 show clear signs of being neighboring F-mini-ON RGCs. The soma of cell 7 is dimmer and is likely a second-order connected F-mini-OFF RGC. c. Distribution of the number of dye-coupled cells observed in Neurobiotin and Alexa Fluor 488 cell fills of F-mini-ON and F-mini-OFF RGCs; n = 13 and 3 cells. d. Average voltage traces from an RGC pair in which one F-mini RGC was injected with current (top row) and the coupled F-mini RGC of the opposite type (bottom row) showed a response. Current injections were +50 pA (lighter traces) and −50 pA (darker traces). e. Voltage change relationship across the electrical synapse in both directions for the pair in d. f. Distribution of the coupling coefficient (slope of line in e) for all recorded pairs, in control conditions (top) and in the presence of MFA (bottom). g. Example of MFA abolishing voltage deflection, showing voltage in F-mini RGCs (for −50 pA injection in coupled cell) in control conditions and in the presence of MFA (green). Image in a is a composite of a maximum projection image of the F-mini-OFF dendrites in cyan with a maximum projection image of the ganglion cell layer in white. Cell 'O' in b was recorded in current-clamp mode. Box plots in c show the maximum, 75th percentile, median, 25th percentile and minimum values.

(Fig. 7c). The resulting ON-OFF RF subfield center-of-mass offset was on average 26-µm long ventrally (Fig. 7f), similar to the dorsalventral offset we measured in F-mini-ON RGCs (Fig. 2g; −30 ± 13 µm). This result demonstrates that the spatial structure of the F-mini RGC network is sufficient to account for the ON-OFF RF offset we observed in F-mini-ON RGCs.

The RF structure of F-mini-ON RGCs can account for their weak direction and orientation selectivity. What visual features are represented by an RF structure with offset ON and OFF subregions? We first tried to answer this question at the level of single RGCs using a model that captured both the spatial structure of the ON-OFF RF center of F-mini-ON RGCs and suppression by their RF surround (Extended Data Fig. 7a,b and Methods). The search for a component of visual stimulus space that is encoded by an RGC type has many avenues. Direction selectivity, which is an asymmetry in the response spike count or rate with visual motion direction, has been reported in F-mini-ON RGCs.25 We also measured mild direction selectivity in F-mini-ON RGCs only for slowly moving stimuli, a behavior the model replicated (Extended Data Fig. 7c,d). Direction preference was broadly distributed with no apparent relationship to retinal position (Extended Data Fig. 7g). We also found mild orientation selectivity in F-mini-ON RGCs with the presentation of drifting gratings (Extended Data Fig. 7e). In a version of our single-cell model that matched the axis of elongation of the F-mini-ON RFs, we were able to predict a similar degree of orientation selectivity to our measurements (Extended Data Fig. 7f). However, neither of these properties provides a satisfying explanation for this unique RF structure, since other specialized RGCs encode movement direction27 and orientation28,29 with much greater specificity and robustness over parameters like speed (Extended Data Fig. 7c,e).

A multicell model of F-mini-ON RGCs demonstrates that systematic ON-OFF offset RFs can aid in the encoding of edge position.
Next, we constructed a multicell model to explore whether offset ON–OFF RFs could provide an encoding benefit in the population that is less apparent at the single-cell level. Specifically, we tested whether a population of RGCs with consistently offset ON–OFF RFs is more precise in representing the position of a dark–light edge than a population of RGCs with either overlapping ON–OFF RFs or randomly offset RFs (Fig. 8). Our model used (1) overlapped ON and OFF RFs, (2) larger RFs resulting from offsetting the same ON and OFF subfields or (3) RFs with the same overall size but with offset ON and OFF subfields, as measured in F-mini-ON RGCs (Fig. 8a). The two offset models used either a consistent ventral offset between ON and OFF subfields or a random distribution of offset directions, consistent with our measurements from F-mini-ON and control ON–OFF RGCs, respectively (Fig. 2d,e). All other aspects of the five models were identical. We presented our five RF models with edge stimuli at various orientations and spatial locations to generate a response lookup table for each.

To generate a population of RGC responses, we simulated many of the above RGCs simultaneously. Cell positions were generated as a noisy hexagonal grid based on the measured density of F-mini-ON RGCs (250 cells in an area of 1 mm²). Gaussian noise with a magnitude consistent with our spike data from F-mini-ON RGCs was added to each response. In each instantiation of the multicell model, the random seeds that defined position jitter and response noise were varied. To evaluate performance, we created a simple position decoder on the output responses of the model cell population (Methods). We computed the decoded position as the center of mass of the RFs from model RGCs responding over a cut-off threshold (8 ± 1.5 cells, mean ± s.d.) with each RGC weighted by its response strength. Error was measured as the distance between decoded position and the true stimulus center.

RGC models having offset ON–OFF RFs with consistent direction had lower error than those with overlapped or randomly offset RFs in representing the position of an edge stimulus (Fig. 8d–f). Along the vertical axis of separation of the RF subfields, F-mini-ON
RGC model was 40% better than the overlapped RF model at representing the position of horizontal edges (Fig. 8d), particularly the vertical component perpendicular to the edge (Fig. 8e). This improvement came with no decrease in horizontal position decoding performance (Fig. 8f), but with a trade-off in performance for edges having a contrast offset perpendicular or opposed to the RF offset. The F-mini-ON RGC population model was able to represent the position of an edge with precision down to 0.6 degrees of visual angle (Fig. 8f), less than 12% of the RF diameter (2σ) of a single F-mini-ON RGC. The improvement of the offset models relative to the overlapped RF model was robust across a broad range of cell densities, RF sizes and noise amplitudes (Extended Data Fig. 8).

**Discussion**

Collectively, our results demonstrate that the F-mini-ON RGC mixes a canonical ON input via chemical synapses with a noncanonical OFF input via gap junctions with F-mini-OFF RGCs to create an RF with spatially offset ON and OFF subfields. The offset is consistent with the asymmetric morphology and connectivity of F-mini RGCs (Fig. 7). Our multiecell model shows that the ON–OFF RF offset can improve the precision with which F-mini-ON RGC populations represent the position of an edge (Fig. 8). A causal link between this proposed role in encoding edge location and a specific behavior will require selective genetic access to F-mini RGCs. With advances in molecular profiling of RGCs, the tools for such a study are on the horizon.

This report follows on from the work of several groups investigating both of these RGC types in the mouse. Descriptions of their electrophysiological responses to visual stimuli have varied in approach and findings. In the byewire dataset, Bae et al. found both ON and OFF calcium responses in F-mini-ON RGCs to a moving bar. The F-mini-OFF is labeled in the PV-IR mouse line (type PV7). Using that line, Farrow et al. found both ON and OFF responses to small spots in F-mini-OFF RGCs. Working with a Fox2-IR mouse line, Roussou et al. identified only primary polarity responses in F-mini RGCs. Although we found the ON–OFF responses to be robust across a range of light levels and contrasts (Fig. 1j,k), differences in light adaptation state or other recording conditions may have caused this discrepancy.
Heterotypic RGC coupling was also recently identified in the guinea pig using multielectrode array recordings and morphological tracing with Neurobiotin. The coupled RGCs in the guinea pig study were both ON sustained cells—one of them was the ON alpha—so heterotypic coupling in that system does not mix ON and OFF signals. Nonetheless, the discovery of heterotypic RGC coupling in two different circuits in two different species suggests that it might be a conserved motif in the mammalian retina, augmenting our understanding of the organization of information in RGC populations.

Notably, F-mini-ON RGCs are bistratified even though we found no evidence of OFF bipolar cell input. Perhaps the OFF dendritic stratum is used primarily for making gap junctions with F-mini-OFF RGCs or chemical synapses with amacrines. This adds to a growing list of RGC types with dendrites in the outer half of the inner plexiform layer with no apparent OFF bipolar cell input: sustained suppressed-by-contrast, ON delayed, ON orientation selective (both horizontal and vertical), OFF orientation selective (horizontal and vertical) and M1 intrinsically photosensitive RGCs. The size of this list suggests a reevaluation of the dogma that the stratification of an RGC alone is sufficient to predict the polarity of its bipolar cell inputs.

Recent work has shown that mice use vision to capture small, quickly moving prey objects, like crickets, so perhaps precise edge localization with F-mini-ON RGCs plays a role in this behavior. While our multicell model used flashed stimuli, a
similar mechanism could, in principle, aid in the precise localization of moving objects by the joint firing of coupled F-mini RGCs. It has long been known that direct electrical coupling between RGCs is important for synchronizing spikes on the timescale of several milliseconds29,30, and tight spike synchrony has been shown to enhance transmission at retino-geniculate synapses46. Synchronous firing among RGCs has been proposed as a mechanism for improving the fidelity of the population code for direction selectivity31 and binding visual objects across space4. Precise timing between the spikes of coupled RGCs has recently been proposed as a mechanism for object localization with resolution much higher than predicted by the RF size of a single RGC32. Estimating the position of a moving object is, of course, a more complex computation because neural lag can be conflated with object speed. A subset of direction-selective RGCs uses homotypic gap junctions to help normalize this lag12,19. Simultaneous recordings from larger populations of F-mini RGCs could test our model for the localization of static edges (Fig. 8) and reveal whether this network could play a role in the localization of moving objects.

Since the RF offset in F-mini-ON RGCs is along the vertical axis, our model showed enhanced object localization preferentially along this axis (Fig. 8). It will be interesting to see whether future behavioral experiments reveal that mice show more precise object localization along the vertical axis than along the horizontal axis of the visual field. Since rodents use compensatory eye movements to maintain the orientation of their eyes relative to the horizon33, a potential advantage of precise localization along the vertical axis is that it would provide information about changes in distance: approaching objects have increasing space between their dorsal and ventral edges with time, while receding objects have decreasing space. The direction of the ON–OFF asymmetry is also interesting as it relates to rodent visual ethology. Dark-below-light edges in retinal space were represented best by our model F-mini-ON RGC population (Fig. 8d). This corresponds to dark-above-light edges in visual space, consistent with the special behavioral relevance of ‘looming’ dark objects in the upper visual field34.

The dynamics of the F-mini RGC network are another target for future studies. The strength of gap junctions in the retina, including those between RGCs, can be altered by neurotransmitters27,28,45. If the F-mini RGC network is modulated, this could change its function with sensory or behavioral context. Finally, comparisons with other species will provide information about the evolution and function of this particular heterotopic RGC circuit. FOXP2 is also found in a subset of RGCs in the ferret46 and macaque retina45, so it is possible that the F-mini RGC circuit has a homolog in humans.

Online content
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Methods

Animals and electrophysiology. The retinas of wild-type mice (C57BL/6), The Jackson Laboratory were used for all experiments. The mice were dark adapted overnight and killed by cervical dislocation in accordance with all animal care standards provided by Northwestern University's Institutional Animal Care and Use Committee. Lighting in animal facilities was kept on a 14/10-h cycle, with lights on at 06:00. Typical retina in vitro times were 12,000 through 19:00. For all experiments, mice of either sex (approximately 69% male) and of ages postnatal day (P) 3–90 were used; no differences in results were observed sex or age. Eyes were dissected in oxygenated Ames’ medium at 32°C. Dissections were performed in complete darkness using infrared (900 nm) illumination and photoconverters. In the experimental rig, retinas were mounted in a shallow dish, below a microscope objective and above a digital projector, in oxygenated Ames’ medium from Sigma-Aldrich at 32°C at a flow rate of 2 ml/min. Two electrodes on head-stage amplifiers were mounted on micromanipulators on either side. Cell-attached and current-clamp experiments were performed as previously described.

Microscopy. Two microscopes were used to visualize cell morphology. The in vitro microscope, a Scientific SlicerScope Pro 6000, used 980 nm illumination from a Spectra-Physics MaiTai Laser steered by a Galvo scanner for 2P excitation and infrared visualization. The software used was ScanScope version 1.3 by Scientifica in LabView (http://stoga.foldable.co/) and operated by the scanner. The condenser 488 and 568 hydrazides from Invitrogen (A10436 and A10437), the latter of which was found to be not gap-junction permeable and was used for single-cell imaging. Microscopy was continued on fixed retinas, which were stained with antibodies and fluorescent dyes for IHC. The fixed-tissue microscope was a Nikon A1R microscope equipped with a 1.0 NA oil immersion objective. Imaging was performed with the NorthWestern Center for Advanced Microscopy. In Figs. 1a–c and 7a, neurons were traced using Simple Neurite Tracer (ImageJ/Fiji). Stratification analysis in Fig. 1c used cholined acetyltransferase layers to computationally flatten retinal neural morphology. In Fig. 4, individual image channels from confocal microscopy were contrast adjusted and de-speckled with a 3×3 median filter to improve clarity, then projected at maximum intensity through 7 μm of depth.

Light stimulation. Spatiotemporal light patterns were focused on the photoreceptors of the in vitro retina. The light patterns were generated by a computer-controlled galvo display. DLP LightCrafter 5500 from Texas Instruments, illuminated by a blue LED at 457 nm (peak wavelength after optics), integrated by EKB Technologies. This input supported a resolution of 1,140 × 912 pixels, operating at 60 Hz, with frames modulated to an 8-bit intensity depth. Overall light intensity was modulated using neutral density filters (Thorlabs) and calibrated regularly. Measured intensity values were converted to R0 ± R0.5. Light stimuli were generated by MATLAB software packages; Schwartz Lab protocols (https://github.com/Schwartz-AlaLaurab-Lab/sa-labs-extension) interfacing with the Symphony 2 Data Acquisition System (https://symphony-das.github.io) drawing via the graphical interface. The laser was centered at a 32° at a distance of 40 μm. A triangular grid of 30-μm spacing was used for F-mini RGCs. Larger spacing and spot sizes of up to 80 μm were used for RGCs with larger RRs and lower sensitivity to small spots. Voltage responses to individual spots were separated, and peak values were averaged to generate a value for each position. These values were displayed on the grid locations to create a 2D RF strength map. The center of mass of the map area above the 80–85th percentile of response strength was used to generate offset vectors for comparison to the model. The RF overlap index (Fig. 14) uses the proportion of overlap relative to the total area of the RF within the 80th percentile of response strength. Control ON–OFF RGCs were of types 12P, 12H, 1H2 and ON–OFF DS, which exhibit similar ON–OFF transient responses to small spots.

Cell identification. Somas in the ganglion cell layer were surveyed in cell-attached mode using a set of basic light stimuli: flashing small contrast steps in 160-μm spots, spots of varying sizes and moving bars. F-mini RGCs could be identified by their characteristic responses to these stimuli. Once an F-mini RGC was identified, it was dye filled and recorded to verify type and collect data. Pairs were generally identified sequentially as follows: (1) identify F-mini ON/OFF RGC by its light response, (2) fill with Alexa Fluor 488, confirming typology by morphology, (3) use dye to dye-fused coupled F-mini ON/OFF RGCs and (4) patch and record original and dye-filled cells. Sequential filling of coupled F-mini RGCs with Alexa Fluor 488 allowed for identification of large networks of >10 cells. Neurobiotin in a single-F-mini RGC labels second-order connected cells more dimly than first-order ones, in fixed tissue, allowing for similarly large network identification.

Pharmacology. MFA (Sigma-Aldrich, M4531) was bath applied at 100 μM to block gap junctions (Figs. 2 and 3 and Extended Data Figs. 5 and 6). Electrophysiology results in MFA conditions were 5–15 min during application. MFA washed out incompletely, so no data after application was reported.

Ablation. Neighbor ablation is a physical technique for neuronal inactivation where a micropipette is used to rupture the cellular and nuclear membranes of the dye-illuminated somas. This causes the membrane of the entire cell to dissociate, and stops it from having a membrane potential or transmitting and receiving signals within the dendrites. Neuronal death was confirmed by Alexa Fluor 488 in 2P imaging. Some responses continued for one stimulus epoch before being silenced. Where incomplete network ablation occurred, changes to response properties were partial. These data were not used further.

Immunohistochemistry. Target neurons were filled with Neurobiotin (Vector Laboratories, SP-1150) at 3% wt/vol and 280 μM Os in our standard potassium aspartate internal solution 35. For whole-mount IHC, retinas were fixed after the in vitro period in 4% paraformaldehyde for 15 min, then rinsed in phosphate buffer, washed in saline, and fixed for 2–3 days in 4% paraformaldehyde plus 0.1% Triton X-100 from Sigma-Aldrich. Primary antibodies and their dilutions for connexin typing were CX30.2 to rabbit (1:200) from B. Novich and FOXP2 to rabbit (1:200) from Millipore (ABE73), Primary antibodies and their dilutions for connexin typing were CX30.2 to rabbit (1:200) from Invitrogen (40–7000), CX66 to rabbit (1:250) from Invitrogen (51–6200) and CX43 to mouse (1:200) from Invitrogen (41–5800). Retinas were soaked with primary antibodies in normal donkey serum + Triton X-100 from Sigma-Aldrich (T8787) for 3 d at 4°C. Secondary antibodies/fluorophores were (for topology) donkey anti-rabbit Alexa Fluor 568 (1:500) from Life Technologies (A10042), goat anti-guinea pig Alexa Fluor 647 (1:400) from Abcam (ab51087) and (for connexins) donkey anti-rabbit Alexa Fluor 647 (1:500) from Abcam (ab175470), donkey anti-rabbit Alexa Fluor 488 (1:500) from Jackson Immunoresearch (711–055–15), donkey anti-mouse Cyanine Cy3 (1:500) from Jackson Immunoresearch (715–165–150); and (for both) Streptavidin DyLight Conjugate 488 (1:500) from ThermoFisher (21852). Retinas were soaked in secondary antibodies in normal donkey serum + Triton X-100 for 1 h at 4°C. Retinas were mounted on glass slides in Fluoromount Aqueous mounting medium from Sigma-Aldrich (F4688) and stored at −20°C.

Stratification offset analysis. Offsets are measured as a vector from proximal inner center of mass to distal outer center of mass, which, in most RGCs, is ON to OFF dendrites. The mean and s.d. are shown by red crosses. All figure data is from the Eyewire database, exported via the Eyewire museum mesh tool. Meshes were flattened and offset by eye.

Morphological receptive field model. Two types of datasets were used. RFs relative to soma location of F-mini ON and F-mini OFF RGCs were estimated using the area between the tips of the dendritic fields (n = 38 F-mini ON-ON cells; n = 12 F-mini OFF cells). These were outlined manually using 2P or confocal image stacks. Locations of F-mini RGCs in coupled networks were traced from images of dye-filled somas to create maps of soma locations (n = 11 networks). Random combinations of network soma locations, F-mini ON dendrite fields and F-mini OFF dendrite offsets were generated 5,000 times and averaged to generate a mean OFF RF relative to ON RF (Fig. 7f). The model ignores any possible interdependence of F-mini ON and F-mini OFF RGC dendritic fields (meta-mosaic) and assumes that F-mini OFF RGCs receive OFF input via bipolar cells at their dendritic tips.

Single-cell RF model. A computational model (Extended Data Fig. 7a) was used to generate single-cell responses to an edge, moving bar and drifting grating stimulus. The model simulated four pathways of input to a single RGC: ON and OFF, excitation and inhibition for each. Excitation was modeled as a small 2D Gaussian function of direct excitation with a larger 2D gaussian function subtracted to model presynaptic inhibition. Direct inhibition was modeled as a larger 2D gaussian function. The visual stimulus was multiplied by the spatial RFs, then those signals were integrated across space and temporally filtered by convolutions. Temporal filter kernels were parameterized curves with values extracted from typical F-mini ON–OFF voltage–clamp light-step responses, over a 3 s simulation time. A semi-rectifying nonlinearity was applied to each ON and OFF subfield, then the responses were summed. The OFF delay relative to the ON delay was estimated from spike latencies. RF sizes and surround strength were adjusted manually to match F-mini ON responses to spots of multiple sizes. This model is meant to explore RF maps concepts analytically over many variables and is not meant to precisely emulate recorded RGCs.
Multicell decoding model. The multicell model composed responses from many single-cell RF instances and decoded them using their center of mass. Gaussian noise was added to each of the modeled cellular responses. The decoded location of the stimulus was compared to the true location to generate an error value. Trials of randomly placed cells and stimulus were used, with 500 trials for each parameter configuration. Cellular RF centers were laid out on an equilateral triangular grid with a Gaussian jitter of $\sigma = 10 \mu m$. RF strengths were integral normalized across shape and position. The percentage parameters used a density of 250 RGCs per $mm^2$, a noise of 2 arbitrary units, but similar to spiking output and a stimulus angle of 0 (horizontal, ON upper). RGC responses fell to baseline outside of the model region, which had an area of 0.36 mm$^2$ with a 600-μm side length. The stimulus was placed, uniformly at random, within the central 300 x 300μm square region. The edge stimulus was a 150-μm long edge of positive and negative 100% contrast, falling off in a linear gradient above and below the edge for 150μm. Rousso et al. found a range of densities of between 100 and 350 (RGCs per mm$^2$ for F-mini-ON RGCs. Eyewire museum's patch of retina, the E2198 dataset, has a density for F-mini-ON RGCs (anatomical type 63) of approximately 240 RGCs per mm$^2$. Decoder input cell activity threshold was 0.3 times the highest response, resulting in 8.5 ± 1.5 s.d. RGCs at the default parameters.

Analysis and statistics. Analysis was performed with a custom MATLAB software package. Figures were generated in Igor 8.0 from WaveMetrics. All data are reported as the mean ± s.d. unless otherwise noted. No statistical methods were used to determine the sample sizes, but our sample sizes are similar to those reported in previous publications. In general, data distribution was assumed to be normal but this was not formally tested; data points are shown on the figures. Data collection was not randomized due to the nature of the experiments. Data collection and analysis were not performed blind to the conditions of the experiments. Cells were excluded from analysis if confidence in typology was insufficient. All data points shown are individual RGCs or cell pairs presented as the mean of three or more repeated stimulus presentations. Box plots in figures show the maximum, 75th percentile, median, 25th percentile, and minimum values. Comparisons of statistical significance were performed with a paired or unpaired Student's t-test, as appropriate, unless otherwise noted. Direction and orientation selectivity indices were calculated as the normalized magnitude of the vector sum of the responses across directions or orientations. For measurements of coexistence overlap, RGCs were traced using Neurite Tracer in Fiji software. RF ellipticity was measured using the 80th percentile response contour, finding the longest line contained within that (a), then the longest such line perpendicular to that line (b). The ellipticity is then lengths $a/b$. All threshold membrane potential measurements (RF maps and Fig. 6d) were normalized to a pre-stimulus baseline mean value and used a spike removal low-pass filter of a 100 Hz cutoff frequency.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Data for ganglion cell typology in the mouse is available at https://RGCTypes.org/. A subset of the datasets that support the findings of this study are available at https://github.com/SchwartzNU/ProjectData_Femiini. The remainder of the datasets are available from the corresponding author upon reasonable request.

Code availability

References

Acknowledgements
We thank the entire Schwartz Laboratory group for discussions, advice and support. We thank R. Novich for generously providing the FOXP1 antibody. Imaging work was performed at the Northwestern University Center for Advanced Microscopy, generously supported by a National Cancer Institute cancer center support grant (P30 CA06553) awarded to the Robert H. Lurie Comprehensive Cancer Center. Multiphoton microscopy was performed on a Nikon A1R multiphoton microscope, acquired through the support of the National Institutes of Health (NIH: 1S10OD010398-01). This work was supported by grants from the NIH National Eye Institute (F31 EY02593 and T32 EY025202) and an NIH Director’s New Innovator (DP2) award (EY026770).

Author contributions
S.C. and G.W.S. performed the experiments. S.C. analysed data and constructed models. S.C. and G.W.S. designed research and wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41593-020-00747-8.
Supplementary information is available for this paper at https://doi.org/10.1038/s41593-020-00747-8.
Correspondence and requests for materials should be addressed to G.W.S.
Peer review information Nature Neuroscience thanks Bart Borghuis, Stuart Trenholm, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.
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Extended Data Fig. 1 | Coupled cells are immunoreactive for F-mini RGC markers. Images of the ganglion cell layer in a patch of retina in which a single F-mini-ON RGC was filled with Neurobiotin (magenta arrowhead). Left panel shows the Neurobiotin channel, with three brightly labelled coupled cells (white arrowheads) and three dimly labelled cells that likely represent second-order connections (magenta asterisks). Middle panel shows the same region with immunoreactivity for FOXP1, which labels F-mini-OFF RGCs, but does not label F-mini-ON RGCs5. Right panel shows immunoreactivity for FOXP2, which labels both F-mini RGC types. This experiment was performed on five F-mini RGC networks in four retinas: four F-mini-ON RGCs and one F-mini-OFF RGC injected. Three networks were stained for FOXP2 and FOXP1; two networks for FoOXP2 only. Neurobiotin labeled 9.0 ± 6.4 somas per retina, and was found in varying amounts in neurons; indicating first and second order connectivity. FOXP2 was present in 43 of 45 RGCs that were labeled with Neurobiotin. Coupled cells from these networks that could be morphologically identified by using the visible primary dendrites, and all showed the expected patterns of FoxP1 expression. 8/8 F-mini-ON RGCs were FOXP1 negative and 14/14 F-mini-OFF RGCs were FOXP1 positive.
Extended Data Fig. 2 | Example RF maps from F-mini-ON and F-mini-OFF RGCs. Receptive field maps of peak response to 40 μm flashed spots over the RF area, averaged over 2 or 3 repeats. a, A GJ coupled F-mini-ON and F-mini-OFF recorded simultaneously. b, Another such RGC pair. c, Two unconnected F-mini-ON RGCs. d, Two unconnected F-mini-OFF RGCs. On all plots, the cross markers are at the center of mass of responses over the 80th percentile (ON, white; OFF, black). Color scale is in mV change from baseline. All scale bars are 100 μm.
Extended Data Fig. 3 | Alignment between ON and OFF strata of bistratified RGCs. Offset values in μm from each bistratified RGC in Eyewire by type, followed by Eyewire anatomical type name in parentheses. Offsets are measured as a vector from proximal/inner COM to distal/outer COM, which in most RGCs is ON to OFF dendrites. Mean and SD of offsets are shown by red crosses. All figure data is from the Eyewire dataset, exported via the Eyewire Museum mesh tool. Meshes were flattened and offset computationally with parameters fit by eye to maximize flatness.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Immunohistochemistry for three types of Connexin at RGC contact points shows negative results. Three connexins were evaluated for presence at the regions of contact between an F-mini-ON and multiple F-mini-OFF RGCs, n = 1 of each experiment. a, b, Full depth maximum intensity projection images of a Neurobiotin-filled F-mini-ON RGC (magenta), the connected F-mini-OFF RGCs (cyan), and a cell of unclassified type due to insufficiently filled dendrites (yellow). Tracing, segmentation, and masking were performed manually. Image brightness was scaled separately by cell type for illustration here but not for analysis. c, d Thin projection images of regions in orange squares in a, b showing an example RGC crossing point with yellow square for spatial reference. Stack depth is 3.5 μm. e–g, The same region and depth as in c, d, showing the IHC channels for the three connexin proteins. h, Quantification of overlap between connexin images and RGC contact region masks. Values are similar before and after a 90 degree rotation of the connexin image. Points mark the overlap of the single F-mini-ON RGC with each F-mini-OFF RGC in the image.
Extended Data Fig. 5 | Noise correlations between F-mini-ON and F-mini-OFF RGCs. a, Traces from a simultaneously recorded pair of F-mini-ON (magenta) and F-mini-OFF (cyan) RGCs in current clamp in darkness (no stimulus). b-e, Example cross correlation of the simultaneous voltage from the cells in a. Brown trace is for shuffled trials. Shaded regions are SEM across trials. Time shift is F-mini-ON - F-mini-OFF (positive values are F-mini-ON earlier). b, Results in darkness. c, Results in darkness in the presence of MFA. d, Results under randomly moving object light stimulation. e, Results under the same light stimulation in the presence of MFA. f, Population data showing peak cross-correlation in control and in MFA. Values in MFA are significantly lower than corresponding values in control (n = 4 cell pairs, p = 0.0068, paired-sample one-tailed t-test). g, Full width at half max and h, time shift (right) of cross correlation peak in control conditions. Error bars in f-h are SEM across cell pairs and points are each cell pair. i, Relationship between cross-correlation peak and coupling coefficient in darkness measured from current injections as in Fig. 2e-h. Box plots in f,g,h show maximum, 75th percentile, median, 25th percentile, and minimum.
Extended Data Fig. 6 | MFA does not selectively eliminate OFF responses in non-F mini RGCs. a, Example of an ON-OFF direction selective RGC responding to the onset and offset of a dark spot from a mean luminance of 2000 R"/rod/s in control conditions (black) and in MFA (green). b, Population data of spike counts and c, subthreshold potential responses to an OFF light step as in a for 3 ON-OFF DS RGCS. Baseline voltage level shift mean in control RGCs was −59.9 to −61.8 mV (n = 3 cells). Box plots in b,c show maximum, 75th percentile, median, 25th percentile, and minimum.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | A single cell model generates responses similar to those observed in F-mini-ON RGCs. a, Diagram of single cell receptive field offset model showing the parameters for each of four RGC input component pathways. b, Responses of the model to flashed spots of varying sizes showing a qualitative match of surround properties to F-mini-ON RGCs as seen in Extended Data Fig. 2a. c, Measured direction selectivity mean in F-mini-ON and ON-OFF DS RGCs, varying over speed (error bars are SD). Individual F-mini-ON RGCs are shown in gray (n = 103 F-mini-ON and n = 279 ON-OFF DS). d, Model response DSI over object speed showing similar DSI magnitude and low-speed preference properties to measured responses. e, (upper) Orientation selectivity of the population of F-mini-ON RGCs. Dashed lines are published means for OS and control RGCs56,72. (lower) Distribution of OS preference angle. f, Moving bar DS preference angle distribution across retina space of F-mini-ON RGCs. Blue = left eye, green = right eye. D,V,N,T denote dorsal, ventral, nasal, and temporal, respectively.
Extended Data Fig. 8 | Multi-cell model results are robust over several parameters. 

a. Illustration of the difference of gaussians RF map used in the single cell model, with an ellipse at the central 2\alpha contour. 

b. Diagram of RF offset and scaling properties in the model: the diameter (D) and the offset ratio (F) between ON (magenta) and OFF (cyan) sub-fields. 

c. Heatmap of vertical position error (for horizontally oriented stimuli) across models with a range of RF size (D) and RF offset ratio (F). Black and magenta points are the parameters used in the following panels and those in Fig. 5d-f. 

**Absolute Error** 

d. Absolute error, e. vertical error change ratio, and f. horizontal error change ratio for the three RF models across a range of cell density. 

**Vertical Error Change** 

g. Absolute error, h. vertical error change ratio, and i. horizontal error change ratio for the three RF models across a range of noise values.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐ The statistical test(s) used AND whether they are one- or two-sided
  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
☐ A description of all covariates tested
☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
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☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Commercial:
MATLAB (Mathworks) 2018a, SciScan (Scientifica) 1.3, LabView (National Instruments) 2016
Open source:
Symphony Data Acquisition System 2, Stage 2, Custom stimulus protocols on GitHub: https://github.com/Schwartz-AlaLaurila-Labs/sa-labs-extension

Data analysis

Commercial:
MATLAB (Mathworks) 2019b, Igor Pro (Wavemetrics) 8.0.3
Open source:
Fiji 2.0.0, Custom MATLAB analysis code on GitHub: https://github.com/SchwartzNU/SymphonyAnalysis and https://github.com/SchwartzNU/ProjectData_JMini

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data and code that support the findings of this study will be available at https://github.com/SchwartzNU/ProjectData_Fmini

Cell type classification data (basic electrophysiology) is available at RGCTypes.org.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Sample sizes were chosen to be similar to those reported in previous publications (below) and were not statistically predetermined. Data for cell typology (basic electrophysiology) uses the Schwartz Lab shared data set, which has a progressively increasing sample size with time, and which is similar in size or greater in size than that used in other contemporary ganglion cell typology projects (below)

Sample size references:


Typology size references:


Data exclusions

Data were excluded from our analysis if an evaluation was made that the health or recording condition of the experiment was poor, or if the confidence in typology was poor. This exclusion occurred at the initial cell recording stage, prior to the RF map, MFA, or ablation experiments.

Replication

All experiments were repeated multiple times in multiple (>5) animals with consistent results, providing replication. Experimental conditions were designed to provide consistency to ensure the reproducibility of results.

Randomization

Wherever possible, stimulus conditions were randomized during electrophysiology experiments. Randomization helped to reduce the effects of temporal correlations. In several cases (MFA application, ablation, and experiments at high light levels), the experimental manipulation was non-reversible, so separate control experiments were performed in different retinas. The overall order of these non-reversible experiments relative to their control counterparts was random as was selection of animals (all wild-type and sex and age matched) for different experimental groups.

Blinding

Blinding was not relevant in any of our experiments. There were no subjective analysis steps that could be influenced by knowledge of the experimental condition. The same analysis code was applied to all data.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
Materials & experimental systems

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Methods

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Antibodies

**Antibodies used**

Primary antibodies for cell typology were FOXP1 to guinea pig (1:10,000) from Prof. Bennett Novitch48 and FOXP2 to rabbit (1:200) from Millipore (ABE73)[49]. Primary antibodies for connexin typology were Cx30.2 to rabbit (1:50) from Invitrogen (40-7400)[50], Cx36 to rabbit (1:250) from Invitrogen (51-6230)[51], and Cx45 to mouse (1:200) from Invitrogen (41-5800)[52]. Secondary antibodies/fluorophores were (for typology) donkey anti-rabbit Alexa Fluor 568 (1:500) from Life Technologies (A10042), goat anti-guinea pig Alexa Fluor 647 (1:400) from abcam (ab150187), (for connexins) donkey anti-rabbit Alexa Fluor 568 (1:500) from abcam (ab175470), donkey anti-rabbit Alexa Fluor 647 (1:500) from Jackson ImmunoResearch (711-605-152), donkey anti-mouse Cyanine Cy3 (1:500) from Jackson ImmunoResearch (715-165-150), and (for both) Streptavidin Dylight Conjugate 488 (1:500) from Thermo Science (21832).

**Validation**


Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**

Mouse strain C57BL/GJ from Jackson Labs, either sex, ages P30-P90

**Wild animals**

No wild animals were used

**Field-collected samples**

No field collected samples were used

**Ethics oversight**

All animal procedures were performed in accordance with all animal care standards provided by Northwestern University’s Institutional Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.