Spatially displaced excitation contributes to the encoding of interrupted motion by the retinal direction-selective circuit

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Abstract

Spatially distributed excitation and inhibition collectively shape a visual neuron’s receptive field (RF) properties. In the direction-selective circuit of the mammalian retina, the effects of strong null-direction inhibition of On-Off direction-selective ganglion cells (ON-OFF DSGCs) on their direction selectivity are well-studied. However, how excitatory inputs influence the On-Off DSGC’s visual response is underexplored. Here, we report that the glutamatergic excitation of On-Off DSGCs shows a spatial displacement to the side where preferred-direction motion stimuli approach the soma (the ‘preferred side’). Underlying this displacement is a non-uniform distribution of excitatory conductance across the dendritic span of the DSGC on the preferred-null motion axis. The skewed excitatory RF contributes to robust null-direction spiking during RF activation limited to the preferred side, a potential ethologically relevant signal to encode interrupted or discontinuous motion trajectories abundant in natural scenes. Theoretical analysis indicates that such differential firing patterns of On-Off DSGCs to continuous and interrupted motion stimuli may help leverage synchronous firing to signal the spatial location of a novel moving object in complex, naturalistic visual environments. Our study highlights that visual circuitry, even the well-defined direction-selective circuit, exploits different sets of neural mechanisms under different stimulus conditions to generate context-dependent neural representations of visual features.

Introduction

How do sensory systems convert sensory inputs into behaviorally relevant neural signals? This question has been extensively investigated in the early visual system,
where a neuron’s responses to a set of parameterizable visual stimuli can be systematically probed to reveal a cell’s receptive field (RF) properties in space and time. It has been increasingly appreciated that context-dependent neural mechanisms dynamically shape neuronal RFs even at the earliest stage of visual processing in the retina. The transformation from the visual input to a retinal ganglion cell’s spiking output is influenced by spatiotemporal patterns of stimuli in both the RF center and surround regions, highlighting the necessity of using diverse, ethologically relevant visual stimuli for delineating RF properties and for ultimately understanding neural coding of the animal’s natural environment (Chiao and Masland, 2003; Demb et al., 1999; Deny et al., 2017; Huang et al., 2019; Ölveczky et al., 2003; Takeshita and Golisch, 2014; Turner et al., 2018).

Direction-selective ganglion cells (DSGCs) in the mammalian retina are well-studied for their motion direction selectivity. A DSGC fires maximally to visual stimuli moving across its RF in its preferred direction and is inhibited from firing by stimuli moving in the opposite, null direction (Barlow and Hill, 1963; Barlow and Levick, 1965). The direction-selective spiking of the DSGC is largely attributed to the strong null-direction inhibition that the DSGC receives from the starburst amacrine cell (SAC) (Briggman et al., 2011; Euler et al., 2002; Fried et al., 2002; Lee and Zhou, 2010; Taylor and Vaney, 2002; Wei et al., 2011; Yonehara et al., 2011). The most studied DSGC type, the On-Off DSGC, prefers motion in one of the four cardinal directions (Oyster and Barlow, 1967; Sabbah et al., 2017). They have bistratified dendritic arbors in the On and Off sublamina of the inner plexiform layer (IPL) to extract motion directions of bright and dark signals, respectively (Figure 1A) (Famiglietti 1983; He and Masland, 1998; Kittila and Massey, 1997).

The RF of On-Off DSGCs has been studied with conventional visual stimuli such as stationary and moving spots, bars, and gratings. For motion stimuli that traverse across the entire RF, On-Off DSGC responses remain direction-selective over a broad range of contrast, luminance, speed, and background noise levels (Barlow and Levick, 1965; Chen et al., 2016; Grzywacz and Amthor, 2007; Lipin et al., 2015; Sethuramanujam et al., 2016; Sivyer et al., 2010). However, motion stimuli restricted to the distal RF subregion on the preferred side (defined as the side from which the preferred-direction moving stimulus approaches the RF) can elicit non-directional firing (He et al., 1999; He and Masland, 1998; Rivlin-Etzion et al., 2011; Trenholm et al., 2011). Based on the responses to moving stimuli presented to different subregions of the DSGC RF, the cell’s RF structure can be viewed as consisting of multiple ‘DS subunits’ and a ‘non-DS zone’ at the edge of the preferred side. However, the neural mechanisms underlying the modular and heterogenous RF subunits of On-Off DSGCs have not been elucidated. Furthermore, the functional significance of this fine RF structure is not clear.
In this study, we investigated the spatial RF structure of the mouse On-Off DSGC subtype that prefers motion in the posterior direction of the visual field (pDSGC). We found that the pDSGC spiking RF is skewed towards the preferred side of the cell for both stationary and moving stimuli, even in the absence of SAC-mediated inhibition. Combining anatomical and functional analyses, we found a spatially non-uniform glutamatergic excitatory conductance that contributes to this spatial displacement. As a result of the displaced RF, moving stimuli that only activate the preferred side of the pDSGC RF trigger robust firing during either preferred or null direction motion. Theoretical analyses of the On-Off DSGC population response allow us to speculate about the ethological relevance of the displaced RF in processing complex natural scenes, suggesting that it can allow for better estimation of object location when a moving object emerges from behind an occluder. We term this type of motion ‘interrupted motion’ to distinguish it from more standard constant motion stimuli. This phenomenon might also allow synchronous firing from different sub-types of DSGCs to serve as a useful alarm signal in complex scenes.

**Results**

**Glutamatergic excitation of the On-Off pDSGC is spatially asymmetric relative to the soma**

To investigate the spatial distribution of excitatory inputs to On-Off pDSGCs, we targeted pDSGCs in the Drd4-GFP transgenic mouse line to record their On spiking and synaptic responses to the light onset of flashing stationary bright spots presented to the periphery of the DSGC RF. These spots were 110 µm in diameter and centered 165 µm from the soma of the recorded DSGC (Figure 1B, schematic). Measurement of the average radius of pDSGC On dendrites (88.1, SEM 13.9 µm, from 25 cells, see Methods) indicates that the spot mostly covered areas beyond the dendritic span of these cells. For each cell tested, we also presented a moving bar stimulus to confirm its directional tuning to posterior-direction motion in the visual field.

The spot stimulus delivered to the pDSGC RF periphery uncovered an asymmetric RF organization: the maximal spiking was triggered by the spot shown on the preferred side of the cell, and the minimal response was triggered to the spot shown on the null side (Figure 1B), indicating a spatial displacement of this aspect of the RF relative to the soma. To quantify the extent of this RF displacement, we calculated a region selectivity index (RSI) defined as the normalized difference of the maximum response (Max) and the response to the spot presented in the opposite region (Opp) (see Methods). pDSGCs exhibit a robust spatial bias of their spiking RFs to the preferred side (Figures 1C “Control” and S1A).
Figure 1. Drd4-GFP labeled pDSGCs have spatially asymmetric glutamatergic receptive fields.

(A) Schematic showing types of presynaptic neurons to an On-Off DSGC and the neurotransmitters they use. (B) Top: Example On spiking responses of a pDSGC to four different peripheral spots presented around dendritic span. Bottom: Individual (gray) and mean (black) pDSGC On spike counts evoked by spots presented at different locations (25 cells). (C) Top left: Pairwise comparison of mean spike counts in regions evoking the maximum number of spikes (Max) and the opposite region (Opp) in the control condition (25 cells). Top right: Polar histogram of Max region locations aligned to the preferred-null motion axis. Radius indicates number of cells. Bottom: Same as in top but experiments performed in DHβE + Gabazine (18 cells). (D) Left: Example On EPSC responses to spots shown in the regions evoking the strongest EPSCs (Max) and the opposite region (Opp) in control (top) and in DHβE + Gabazine (bottom). Middle: Pairwise comparisons of EPSC amplitude and charge transfer to spots presented in the Max region and Opp region (Control: 15 cells; DHβE + Gabazine: 24 cells). Right: Polar histograms of Max region locations determined by EPSC charge transfer aligned to the preferred-null motion axis. Radius indicates number of cells. Summary statistics are mean ± SEM, ***p < 0.001.
Because a well-documented asymmetry in the direction-selective circuit is the asymmetric inhibition from SACs to DSGCs, we next tested whether the displacement of the pDSGC spiking RF is eliminated by blocking SAC inputs. We perfused the retina with the nicotinic antagonist DHβE and the GABA$_A$ receptor antagonist gabazine to block these inputs. Under this condition, we still observed a spatial asymmetry in pDSGC spiking activity evoked by the flashing spots (Figures 1C, S1A and S1B, “DHβE and Gabazine”), indicating a spatial displacement of glutamatergic excitation of the pDSGC in the absence of SAC influence.

To directly measure the strength of excitatory inputs to pDSGCs at different stimulus locations, we performed whole-cell voltage clamp recordings of excitatory postsynaptic currents (EPSCs) evoked by peripheral flashing spots. Consistent with the pattern of pDSGC spiking activity, spot-evoked EPSCs show a spatial bias towards the preferred side (Figures 1D and S1C, “Control”). Isolation of the glutamatergic component of the EPSC by the addition of DHβE and gabazine confirms the persistence of the spatial asymmetry, indicating that the glutamatergic excitation of the pDSGC is not isotropic but is spatially displaced relative to the soma (Figures 1D, S1B and S1C, “DHβE and Gabazine”). This pharmacological manipulation also rules out potential confounding influences of asymmetric inhibitory inputs from SACs on pDSGC EPSCs during voltage clamp recordings due to space clamp.

We investigated the Off responses in tandem with the On responses and found the same RF displacement in all conditions (Figures S1D – S1F). We focused on the On response for the remaining experiments.

**Non-uniform excitatory conductance across the preferred-null motion axis contributes to the asymmetric glutamatergic RF**

An asymmetric excitatory RF of a retinal neuron may result from asymmetric dendritic arbors and/or an asymmetric distribution of excitatory synaptic inputs across its dendritic field. To examine the relationship between pDSGC dendritic morphology and glutamatergic inputs, we performed two-photon imaging of dye-filled dendritic arbors after recording glutamatergic EPSCs evoked by the peripheral flashing spot stimulus described above (Figures 2A and 2B). Consistent with previous studies, On dendritic arbors of pDSGCs do not exhibit a salient or consistent bias relative to the cell’s preferred motion direction. The total dendritic length or the number of dendritic branching points does not significantly differ between the preferred and null sides of the pDSGC dendritic field (Figures 2C, 2D and S2A). This apparent randomness of dendritic arbor distribution relative to the cell’s preferred motion direction contrasts with the previously reported mouse On-Off DSGC subtype preferring motion in the superior
Figure 2. pDSGC dendritic morphology does not show a spatial bias towards the preferred side

(A) Traced On layer dendritic morphologies of pDSGCs aligned to their preferred-direction motion.

(B) Example morphology of a pDSGC cell divided into eight sectors for calculating normalized dendritic length vector. (C) Left: Normalized vector sum of dendritic length aligned to pDSGCs’ preferred direction motion. Right: Pairwise comparison of dendritic length on the preferred vs null sides of each cell (26 cells, p = 0.51). Blue represents example cell in B. (D) Left: Normalized vector sum of branch points aligned to pDSGCs’ preferred direction motion. Right: Pairwise comparison of branch points on the preferred vs null side (26 cells, p = 0.68). Blue represents example cell in B.
direction that have dendritic arbors strongly displaced to the null side of the soma (Kay et al., 2011; Trenholm et al., 2011).

Although pDSGC dendritic fields are not strongly skewed towards the preferred side of the soma, a quantitative comparison between pDSGC dendritic arbor distribution and the distribution of their glutamatergic EPSCs has not been performed. We found a non-random skew of the pDSGC dendritic length and number of branch points towards the regions corresponding to spots evoking the strongest glutamatergic EPSCs (Figure S2B). However, this structural bias is not correlated with the bias in EPSC strength (Figure S2C), suggesting that dendritic arbor density alone cannot fully explain the displacement of the pDSGC glutamatergic RF.

To further explore the relationship between the pDSGC glutamatergic RF and its dendritic distribution, we performed another set of experiments to obtain a more complete RF map. First, the preferred direction of each cell’s spiking activity was determined by loose cell-attached recordings using a moving bar stimulus with no synaptic blockers. Next, peripheral flashing spots were used to estimate the spatial displacement of the pDSGC glutamatergic RF in the presence of nicotinic and GABAergic receptor antagonists (DHβE + Gabazine) as described above. Then, a smaller, 20 µm diameter bright stationary spot was repeatedly flashed at random locations within a 11-by-11 220 µm square grid centered on the pDSGC soma. A heatmap of glutamatergic EPSC charge transfer evoked by the small flashing spot was generated and overlaid with the reconstructed dendritic arbors for each pDSGC (Figure 3A).

RF mapping with small flashing spots also revealed a spatial displacement of glutamatergic EPSC distribution that aligns well with the displacement determined by larger spots presented to the pDSGC periphery (Figure 3B). Similar to the displacement pattern revealed by stimulation of the RF periphery, the strongest glutamatergic EPSCs are preferentially located on the preferred side of pDSGC somas (Figure 3C). Notably, for most cells, the center of the glutamatergic EPSC RF is displaced from the center of the dendritic field (Figure S3A – S3B), indicating additional mechanisms underlying the glutamatergic RF displacement apart from the dendritic arbor distribution.

We further examined the strength of the glutamatergic input across the pDSGC. On dendritic field (Figure 3D). We normalized the EPSC charge transfer by the total dendritic length in a circle with a 60 µm diameter centered on each small flashing spot. This provides an estimate of the strength of glutamatergic inputs per unit dendritic length, or “EPSC density”, at each stimulus location. When we calculated the density along the axis of maximal glutamatergic RF displacement, we found that the EPSC
Figure 3. Non-uniform glutamatergic synaptic excitation across pDSGC dendritic span contributes to skewed excitatory receptive field.

(A) Left: Schematic for small spot RF mapping experiment in DHβE + Gabazine. Middle: Example heat map. Right: Preferred motion direction of the example cell. (B) Vector sum plot of EPSC charge transfer center of mass determined by peripheral spot stimulus. (C) Spatial locations showing centers of mass of glutamatergic excitatory charge transfer obtained from experiments illustrated in A aligned to each cell’s preferred motion direction (15 cells). (D) Example EPSC responses to spots presented to a pDSGC along the maximum-opposite axis of glutamatergic RF displacement. (E) Left: “EPSC current density” (i.e. ratio of charge transfer per dendritic length) versus distance from soma along the maximum-opposite axis of glutamatergic RF displacement (16 cells, ***p < 0.001) as well as the orthogonal regions. Right: Summary plot of glutamatergic charge transfer as a function of total dendritic length centered around the flashing spot for spots shown more than 50 µm away from the soma (16 cells, ***p < 0.001). (F) Same as E but along the preferred-null motion axis (15 cells, charge transfer/dendritic length vs distance from soma **p = 0.0026, charge transfer vs dendritic length ***p < 0.001).
density on the displaced side of the dendritic field is larger than the corresponding region on the opposite side (Figure 3E, left). From 50 µm away from the soma, spots on the side corresponding to the maximum displacement (Maximum) yield stronger EPSCs than spots on the opposite side (Opposite), while controlling for the same dendritic length (Figure 3E, right). This heterogeneity in EPSC density across the dendritic span persists when comparing EPSC responses along the preferred-direction motion axis of the cell (Figure 3F). Analysis of the peak amplitudes showed similar results as charge transfer (Figures S3C – S3D).

Next, we asked what mechanisms could underlie the non-uniform excitatory conductance across the dendritic span, focusing on the influence of bipolar cell inputs onto the DSGCs. A potential explanation for stronger excitatory responses to stimuli presented on the preferred side would be a higher concentration of bipolar cell ribbon synapses on the preferred side. To quantify the distribution pattern of ribbon synapses across the DSGC dendritic field, we performed a connectomic analysis of ribbon input sites on three On-Off DSGCs tuned to the horizontal motion axis (Figure 4A) using a previously published serial block-face scanning electron microscopy (SBEM) dataset (Ding et al., 2016). The preferred direction of the traced DSGCs was determined by the orientation of starburst inputs onto these cells (Figure 4B). The posterior-anterior axis was estimated by several cell types traced in the same block whose dendritic arbors are strongly biased in the orthogonal superior-inferior axis including Jam-B RGCs (Kay et al., 2011) and F-RGCs (Rousso et al., 2016). We found no marked gradient in ribbon density across the preferred-null motion axis of the three cells (Figures 4C – 4E, S4A – S4B), suggesting that the gradient in EPSC density across this axis is not determined by the density of bipolar inputs.

In addition to ribbon density, we also examined the time course of glutamatergic EPSCs in the presence of DHβE and Gabazine along the axis of maximal glutamatergic RF displacement and along the preferred-null motion axis. We found no significant differences in the latency, rise and decay times in EPSC waveforms across the axis of maximal glutamatergic RF displacement in response to the small flashing spots (Figures 4F – 4H, left panels). In addition, we did not detect a monotonic change in glutamatergic EPSC kinetics along the preferred-null motion axis in DHβE and Gabazine (Figures 4F – 4H, right panels) or in DHβE only (Figures S4C and S4D). Similar glutamatergic EPSC waveforms across the pDSGC dendritic field contrast with a previous study of On DSGCs reporting a gradient of EPSC temporal kinetics from slow/sustained to fast/transient corresponding to different bipolar cell subtypes along the preferred-null motion axis to implement a Hassenstein-Reichardt-Detector-like mechanism for their direction selectivity (Matsumoto et al., 2019).
Figure 4. Bipolar cell ribbon synapse density and glutamatergic EPSC kinetics do not systematically vary along the axis of RF displacement.

(A) Ribbons from On bipolar cells on an example On-Off DSGC. (B) Starburst amacrine cell inputs onto example On-Off DSGC. Red arrows indicate the direction and location of the SAC inputs on the DSGC dendrites. (C) Density map of bipolar ribbon synapses for 3 example On-Off DSGCs with estimated preferred directions along the posterior-anterior axis (see Methods). The soma location is indicated by the white spot in the center. (D) Bipolar ribbon heat map for the 3 example cells, respectively. Number in each square indicates the number of ribbons detected in that square. (E) Quantification of ribbon density across the preferred-null axis, respectively. The soma location is at 0. (N.S. = null side, P.S. = preferred side). (F) Left: Summary of latency of glutamatergic EPSC responses along the maximum-opposite axis (Max = maximum glutamatergic EPSC region, Opp = opposite region)(16 cells, p = 0.75). Right: Summary of latency along the preferred-null motion axis (N.S. = null side, P.S. = preferred side)(16 cells, p = 0.88). (G) Same as in F, except for rise time (10% - 90%). (Left: p = 0.12, right: p = 0.21, 16 cells). (H) Same as in F, except for decay time (90% - 30%). (Left: p = 0.25, right: p = 0.26, 16 cells).
Null-direction response emerges during partial activation of the displaced pDSGC RF

Because On-Off DSGCs are thought to be dedicated to encoding object motion, we next asked how the displaced excitatory RF of the pDSGC revealed by the stationary flashing stimuli contributes to motion processing. We reasoned that because conventional simple motion stimuli such as moving bars and drifting gratings traverse the entire RF of the pDSGC, SAC-mediated inhibition would exert a dominant influence on pDSGC spiking by strongly suppressing the pDSGC null-direction response. However, when motion trajectories are more complex, as often occurs in the natural environment, the displaced excitatory pDSGC RF may confer additional characteristics to the cell’s motion encoding. Therefore, we investigated how the direction-selective circuit would process interrupted motion because moving objects in the natural environment often pass behind occluders or start moving after being stationary.

To examine if the displaced excitatory RF of the pDSGC plays a role in encoding interrupted motion, we created an occluded motion stimulus where a moving bar disappeared behind a central occluder 220 µm in diameter (Figure 5A). The occluder covered the dendritic span of the pDSGC and a substantial portion of its RF center. In contrast to the full-field motion stimuli, the occluded motion stimulus caused both preferred and null-direction On spiking responses (Figures 5B and S5A). These responses are only evoked when the bar travels in the preferred side, which corresponds to the displaced side of the pDSGC RF (Figure 5B, middle row). In contrast, no spiking response is evoked when the bar moves across to the null side beyond the occluder (Figures 5B, bottom row, S5C). To test whether this regional difference is due to the asymmetric wiring between SACs and DSGCs, we blocked the cholinergic and GABAergic transmission with DHβE and gabazine and saw that the regional difference persisted (Figures 5C and S5B). Consistent with the spiking pattern, the EPSC responses also reflected this regional asymmetry (Figure 5D).

Moreover, we blocked GABA<sub>C</sub> receptor activity, glycine receptor activity, and gap-junction coupling with TPMPA, strychnine and MFA, respectively, and found that the spatial asymmetry of the spike response remains during the occluded motion stimulus (Figures S5C – S5E). Based on these pharmacology results, we conclude that the asymmetric glutamatergic RF of the pDSGC contributes to robust spiking in both null and preferred directions when the preferred side of the RF is activated by the occluded motion stimulus.
Figure 5. Displaced excitatory receptive field contributes to null-direction responses in the preferred region. (A) Full-field moving bar and occluded bar stimuli. (B) Example On spiking responses of a pDSGC to full-field bar (top) and occluded moving bar in the preferred (red) and null (black) directions. (C) Top: Mean spike counts of pDSGCs to the full-field moving bar (full-field) and the occluded moving bar on the preferred side and null side (48 cells). Bottom: Mean spike counts in DHβE + Gabazine to the occluded bar stimulus in the region evoking the maximum spiking (Max) and the opposite region (Opp) (13 cells). (D) Mean pDSGC EPSC peak amplitude and charge transfer in the region evoking the maximum response (Max) and the opposite region (Opp) in the control (top, 19 cells) and DHβE + Gabazine (bottom, 13 cells) conditions. (In DHβE + Gabazine: Pref Dir – Max vs Pref Dir – Opp amplitude **p = 0.0028, Pref Dir – Max vs Pref Dir – Opp charge transfer **p = 0.0012). (E) Top: Example directional tuning curve of GCaMP6 signal of a pDSGC. Bottom: Example tuning curve of an aDSGC. (F) Top left: Example GCaMP6 fluorescence traces of a pDSGC for the full-field moving bar in the preferred (red) and null (black) directions. Bottom left: Example GCaMP6 traces of the cell for the occluded bar stimulus. Top right: Normalized amplitude of the posterior-prefering cell null-direction response during the full-field bar and the occluded bar (12 cells). Bottom right: Normalized area of the null-direction response during the full-field bar and the occluded bar (12 cells, **p = 0.0035). (G) same as in F, for aDSGCs. (12 cells, normalized amplitude *p = 0.013, normalized area *p = 0.019). Summary statistics are mean ± SEM, ***p < 0.001 except where specified otherwise.
Since there are two On-Off DSGC subtypes that are tuned to the opposite directions along the posterior-anterior axis, we investigated if the anterior-direction-selective DSGC (aDSGC) exhibits the same null-direction response pattern to the occluded motion stimulus as the pDSGC. To identify both DSGC subtypes, we performed calcium imaging of GCaMP6-expressing RGCs in another transgenic mouse line carrying Vglut2-IRES-Cre and floxed GCaMP6f (Figure S6A) during the full-field moving bar stimulus. We then used an online analysis to identify aDSGCs and pDSGCs based on the directional tuning of their calcium signals to the anterior and posterior motion directions (Figures 5E and S6B – C). Next, we centered the occluded motion stimulus on individual aDSGC and pDSGC somas and performed calcium imaging during the occluded motion stimulus. Consistent with pDSGC spiking activity, a significant null-direction calcium response of the pDSGCs was evoked when the occluded moving bar traveled across the receptive field to the preferred side of the RF (Figures 5F and S6D). Notably, aDSGCs also exhibited a null-direction response to the occluded motion stimulus that is similar to that of pDSGCs (Figure 5G and S6D), indicating that aDSGC RFs are also displaced to the preferred side.

The above results show that continuous motion interrupted by a stationary occluder in the center of the pDSGC’s RF causes unexpected null-direction spiking as the bar emerges from behind the occluder into the preferred side of the pDSGC RF. However, this occluded motion stimulus cannot distinguish whether the interruption itself or only the start position of the emerging bar was necessary for the null-direction response. Therefore, we created a stimulus where a growing edge of a moving bar would emerge at different locations along the preferred-null motion axis of the cell (Figure 6A). For the null-direction moving bar, there is an increase in both the spike number and the firing rate as the starting position of the moving bar is located past the soma on the preferred side of the pDSGC RF (Figures 6A – 6C). Thus, the emergent growing edge caused null-direction spiking of pDSGCs in a similar pattern as the moving bar emerging behind the central occluder. These results illustrate that the null-direction response of the pDSGC during the occluded motion stimulus is dependent on the position in the receptive field from which the moving edge emerges, not the previous motion approaching the occluder.

Null-direction responses of DSGCs during partial activation of their RFs can be useful for decoding object location

Because of their displaced RFs, pDSGCs generate robust spiking activity in both the preferred and null directions when a moving object appears and starts moving on the preferred side of the cell’s RF, as when an object emerges from behind an occluder positioned over the cell’s soma. We asked whether this prima facie aberrant signaling
Figure 6. Null-direction response is dependent on start position of emerging bar
(A) Schematic of moving bars emerging from different locations along the DSGC’s preferred-null motion axis and example spiking responses. The soma location is at 0. Vertical dashed lines on the schematic indicate the positions of the emerging leading edge of the moving bar. (B) Mean spike counts (top, null direction: -330 vs 55 *p = 0.012, -330 vs 110 **p = 0.008) and (C) firing rates (bottom, null direction: -330 vs 0 *p = 0.014) to bars emerging from different locations along the preferred-null axis (10 cells). Summary statistics are mean ± SEM, ***p < 0.001 except where specified otherwise.
can have a functional role relevant to the behavioral goals of the organism. We hypothesize that partial RF activation of a DSGC at a given region in visual space may provide precise information about the spatial position of a moving object along an interrupted motion trajectory. In particular, if a moving object emerging from behind an occluder activates the preferred side of an On-Off DSGC’s RF, this DSGC would generate a null-direction spiking response together with the preferred-direction response of a nearby On-Off DSGC subtype preferring the opposite motion direction (Figure 7A). Such synchronous spiking activity between DSGCs of opposite preferred directions at the location of the occluder could yield a stronger and more localized spatial signal at the population level in response to interrupted motion.

To test how the null-direction response during interrupted motion might enhance downstream estimation of stimulus position, we simulated the spiking responses of pDSGCs in a one-dimensional model consisting of On-Off DSGC subtypes that prefer opposite motion directions on the posterior-anterior axis (aDSGCs and pDSGCs, Figure 7A). One hundred cells of each subtype were arranged along the preferred motion axis of both groups such that the spatial positions of the two subtypes were uncorrelated. We simulated a bright edge moving along this axis in a single direction at a constant velocity under both uninterrupted and interrupted motion conditions and analyzed the On response to the edge. The mean spiking response of each DSGC was modeled as a rectified sine wave with parameters obtained from our experimental data (Figures 7B–7D and S7), and sub-Poisson trial-to-trial variability was introduced to the mean spiking response on each trial (Figure S7D). The spatial positions of the DSGCs were shuffled in each simulation block to allow us to average over different spatial arrangements of the RFs.

Using a population vector decoder (see Methods), we compared the scenario in which none of the DSGCs were occluded (full-field) with the scenario in which a single occluder was placed over a DSGC that prefers the direction opposite to the motion direction of the moving edge (occlusion). The occluded DSGC’s spiking response to an edge moving in its null direction certainly degrades direction estimation and appears to be a defect of the direction-selective circuit. Nevertheless, the coincidence of this null-direction response from a pDSGC and the preferred-direction responses of neighbor aDSGCs that prefer motion in the anterior direction reduces the error in the population estimate of the edge’s spatial position by ~15 percent (Figure 7E). The synchronous firing may, itself, also be a useful alarm signal to trigger attention downstream of the retina, independent of the precise position information it might additionally convey. In conjunction with other retinal cell types in the mouse that encode the location of moving objects, On-Off DSGCs may contribute to fine spatial discrimination during interrupted motion in complex natural scenes.
Figure 7. Null-direction spiking of On-Off DSGCs during the occluded motion stimulus improves position estimation.

(A) Schematic of one-dimensional DSGC population model along with example spike trains for two pDSGCs (red trace showing their preferred-direction responses) and an aDSGC (black trace showing its null-direction response). An occluder 220 μm wide (shaded bar) was centered on the soma of the aDSGC.

(B) Example half sine wave fits (green) to PSTHs obtained from experimental data (left: full-field, 73 cells; right: occlusion, 69 cells).

(C) Fits to probability distributions of spike response onset times (left: full-field, 73 cells; right: occlusion, 69 cells).

(D) Linear correlation between spike response duration and spike response onset time (left: full-field, 73 cells; right: occlusion, 69 cells).

(E) Root-mean-square error in estimating the position of the light bar’s leading edge for full-field (black) and occluded (blue) motion.
Discussion

Our study reveals a new form of asymmetry in the direction-selective circuit: a spatial displacement of glutamatergic inputs to the preferred side of the On-Off pDSGCs due to a non-uniform distribution of synaptic conductances across the pDSGC dendritic span. The impact of this displaced excitation on DSGC spiking is demonstrated by using moving stimuli with interrupted trajectories, a feature abundant in natural scenes. In contrast to full-field continuous motion which maximizes the contribution of SAC-mediated null-direction inhibition, occluded motion stimuli reduce the contribution of SAC-mediated inputs to allow the excitatory receptive field to dominate the spiking response in a non-directional manner when only part of the RF is stimulated. Therefore, an On-Off DSGC’s response is dynamically shaped by visual stimulus-dependent interactions of excitatory and inhibitory synaptic circuitries and may flexibly represent motion direction or motion location in a context-dependent manner.

The non-isotropic excitation of the pDSGC alludes to a more sophisticated set of wiring and signaling mechanisms from the bipolar cell population (Franke et al., 2017). A detailed explanation of the glutamatergic RF displacement awaits future studies. The gradient of glutamatergic current density across the pDSGC dendritic span may arise from a number of possible scenarios including varying strengths of individual glutamatergic synapses, heterogeneous membrane properties across pDSGC dendritic field, and contribution from Vglut3+ amacrine cells (Kim et al., 2015; Lee et al., 2016). Although our study focuses on the On component of the direction-selective circuit, we observed a similar pattern of RF displacement for the pDSGC Off response, suggesting similar mechanisms operating in the Off layer of the IPL.

We combined experimental recordings and theoretical analysis to obtain insights into the nature of the visual information encoded by On-Off DSGCs during interrupted motion. Previous theoretical analysis has addressed the encoding of motion direction by DSGCs at the population level (Fiscella et al., 2015; Kühn and Gollisch, 2019; Zylberberg et al., 2016). In this study, we explored a new hypothesis that On-Off DSGC population activity contains information about both the direction and the location of a moving object. When a moving object emerges from an occluder, the null-direction response of an On-Off DSGC is synchronized with the preferred-direction responses of On-Off DSGCs preferring the opposite direction with overlapping spatial RFs. Since synchronized activity among RGCs has been shown in multiple species to encode ethologically relevant visual information (Ishikane et al., 2005; Schwartz et al., 2007), it is possible that the highly localized and synchronized firing of oppositely tuned DSGC types may render the object emerging from behind the occluder more salient by enhancing positional information. For instance, when a predator moves suddenly from...
behind an occluding object, a mouse may prioritize information about the predator's
position over its direction of movement. The encoding of both motion direction and
spatial location may be particularly useful in the superior colliculus (SC) where On-Off
DSGC inputs are targeted to the superficial layer (Huberman et al., 2009; Shi et al.,
2017), as the colliculus supports threat evaluation and visually guided eye and head
movements (Ito and Feldheim, 2018).

It is likely that other ganglion cell types, such as W3-RGCs and HD-RGCs (Kim
et al., 2010; Zhang et al., 2012; Jacoby and Schwartz, 2017), also participate in fine
spatial discrimination and motion encoding. However, because no other ganglion cell
types are known to project to the same regions of the dorsal lateral geniculate nucleus
dLGN) and the SC as On-Off DSGCs (Huberman et al., 2009; Kay et al., 2011; Sanes
and Masland, 2015; Shi et al., 2017), they may not participate in the same visual
processing tasks as On-Off DSGCs or may provide complementary information about
spatial location of moving objects. The synchronous firing of two typically asynchronous
On-Off DSGC subtypes may, in and of itself, form a useful attention signal downstream
of the retina. Elucidating the roles of diverse RGC types in motion encoding requires a
thorough understanding of visual signal transforms along pathways and how the visual
system instructs visually guided behavior.

Together, our study demonstrates how a visual neuron’s response is determined
by stimulus-dependent assembly of circuit components, and thereby provides insights
into the neural basis of context-dependent visual encoding of complex natural
environments.

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Competing Interests
All authors would like to report no competing interests.
Methods

Animals

Chat-IRESCre mice (129S6-Chat<sup>tm2(cre)Lowl/J</sup>) and floxed tdTomato mice (129S6-Gt(Rosa)26Sor<sup>tm9(CAG-tdTomato)Hze/J</sup>) were acquired from the Jackson Laboratory. Drd4-GFP mice were originally developed by MMRRC (http://www.mmrrc.org/strains/231/0231.html) and were backcrossed to the C57BL/6 background. These lines were crossed so that both pDSGCs and SACs were labeled. Vglut2-IRESCre mice and floxed Ai95(RCL-GCaMP6f)-D mice were acquired from Jackson Laboratory and were crossed and used for calcium imaging experiments. Mice of ages P21 – P60 of either sex were used. All procedures regarding the use of mice were in accordance with the University of Chicago Institutional Animal Care and Use Committee and with the NIH Guide for the Care and Use of Laboratory Animals and the Public Health Service Policy.

Whole-mount retina preparation

Mice were dark adapted for 1 hour and then were anesthetized with isoflurane and euthanized by decapitation. Retinas were isolated at room temperature in oxygenated Ames’ medium (Sigma-Aldrich, St. Louis, MO) under infrared illumination. The retinas were separated into dorsal and ventral halves and were mounted ganglion-cell-layer up on top of a ~1.5 mm<sup>2</sup> hole in a small piece of filter paper (Millipore, Billerica, MA). The orientation of the posterior, anterior, inferior, and superior directions were noted for each piece. During the experimental day, the mounted retinas were kept in darkness at room temperature in Ames’ medium bubbled with 95% O<sub>2</sub>/ 5% CO<sub>2</sub> until use (0 – 8h).

Visual stimulation

A white organic light-emitting display (OLEDXL, eMagin, Bellevue, WA; 800 X 600 pixel resolution, 60 Hz refresh rate) was controlled by an Intel Core Duo computer with a Windows 7 operating system and was presented to the retina at a resolution of 1.1 μm/pixel. Visual stimuli were generated using MATLAB and the Psychophysics Toolbox, and were projected through the condenser lens of the two-photon microscope focused on the photoreceptor layer.

For the peripheral spot experiments, stationary spots of size 110 μm diameter were presented 165 μm away from the soma of the recorded pDGSC for 2 seconds. At this distance, these spots were presented outside of the dendritic span of the majority of recorded pDSGCs. These spots were presented in 8 different locations pseudorandomized around the recorded pDSGC, with 3 repetitions each.
For moving bar experiments, a bright moving bar with dimensions 110 μm (width) X 880 μm (length) and speed 330 μm/s moved in 8 different pseudorandomized directions across the receptive field of the pDSGC, with 3 – 6 repetitions each. The occluded bar stimulus was the same as the moving bar stimulus except for the addition of a central occluder with a diameter size of 220 μm which obscured the movement of the bar. The size of the occluder was large enough to obscure the entire dendritic span for the majority of recorded pDSGCs.

For the emergent moving bar experiments where the bar emerged from different locations of the receptive field, a bright moving bar with dimensions 110 μm (width) X 440 μm (length) and speed 330 μm/s could start moving pseudorandomly from 13 different locations across the receptive field (Figure 5). The bar could move in the preferred or null direction of the recorded cell.

For the receptive field mapping experiments, bright spots with a 20 μm diameter were presented across the receptive field of the recorded pDSGC. They were shown for 3 – 4 repetitions with a duration of 400 ms in a random sequence within an 11 X 11 grid, covering a total area of 220 X 220 μm². To examine the kinetics of EPSCs, bright spots with either a 20 μm diameter (for DHβE and gabazine experiments) or a 60 μm diameter (for DHβE experiments) were shown across the receptive field in a random sequence within an 11 X 11 grid for 3 – 4 repetitions. Spots were shown with a duration of 400 ms.

The light intensity for bright peripheral spot experiments, the moving bar and occlusion experiments, and the receptive field mapping experiments was ~1.6 X 10^4 R*/rod/s.

Targeting cells for electrophysiology
Cells were visualized with infrared light (>900 nm) and an IR-sensitive video camera (Watec). DSGCs were targeted with the aid of two-photon microscopy in Drd4-GFP mice. Cell identity was confirmed physiologically by extracellular recordings of responses to moving bars and/or by filling the cell with 25 μM Alexa 594 (Life Technologies) to show bistratified dendritic morphology. Because the tissue was aligned on the filter paper, we confirmed that the preferred direction of the spiking responses to moving bar aligned well with the anatomical posterior (visual field coordinate) or nasal (retinal coordinate) direction.

Quantification of dendritic morphology
The ON layer of each cell was isolated from a z-stacked image in ImageJ. To calculate the radius, a contour of the dendritic span was made by drawing a boundary around the dendritic tips, and the area within the contour and resulting equivalent radius was calculated. For calculating dendritic length, the On layer from the z-stack projection was
traced in NeuronStudio. The two-dimensional trace was then exported to MATLAB. Dendritic length was calculated in MATLAB using a custom-written code. We subdivided the cell into eight sectors for analysis. Sectors extended out from the soma and were aligned to the spot stimulus such that each spot was at the center between each sector's radial boundaries. When computing normalized vector sums (Figures 2B – C, S2A – S2B), total dendritic length in each sector was normalized by the total dendritic length of the cell.

**Connectomic analysis**

The previously published SBEM dataset was used (Ding et al., 2016). Three On-Off DSGCs along the putative anterior-posterior motion axis were identified by their bistratified dendritic morphology and costratification with SAC processes. The anterior-posterior axis of the tissue is estimated by the dendritic orientation of several morphologically distinctive RGC types that are known to align the dorsa-ventral axis, such as Jam-B RGCs (Kay et al., 2011) and F-RGCs (Rousso et al., 2016). The preferred directions of On-Off DSGC were estimated by weighting the orientation of SAC dendritic inputs onto the DSGC dendrites, since SAC dendrites oriented in the null direction of the DSGC preferentially form GABAergic synapses with DSGCs. Ribbon synapses from On bipolar cells onto DSGC dendrites were manually annotated. All analyses were performed by tracing skeletons and annotating synapses using the Knossos software package (https://knossostool.org/).

To measure local ribbon density across the DSGC dendritic field, the dendritic span was divided into individual squares with 10 x 10 μm (Figure S4) or 20 x 20 μm (Figure 4) dimensions. Total dendritic length in each square was calculated in Matlab with a custom-written code. The total number of ribbon synapses in each square was also quantified. Ribbon density was calculated by dividing the ribbon synapse number by the total dendritic length in the appropriate squares.

**Electrophysiology recordings**

Recording electrodes of 3 – 5 MΩ were filled with a cesium-based internal solution containing 110 mM CsMeSO4, 2.8 mM NaCl, 4 mM EGTA, 5 mM TEA-Cl, 4 mM adenosine 5'-triphosphate (magnesium salt), 0.3 mM guanosine 5'-triphosphate (trisodium salt), 20 mM HEPES, 10 mM phosphocreatine (disodium salt), 5 mM N-Ethyllidocaine chloride (QX314), filled with a cesium-based internal solution containing 110 mM CsMeSO4, 2.8 mM NaCl, 4 mM EGTA, 5 mM TEA-Cl, 4 mM adenosine 5'-triphosphate (magnesium salt), 0.3 mM guanosine 5'-triphosphate (trisodium salt), 20 mM HEPES, 10 mM phosphocreatine (disodium salt), 5 mM N-Ethyllidocaine chloride.
(QX314), and 0.025 mM Alexa 594, pH 7.25. Retinas were kept in oxygenated Ames’ medium with a bath temperature of 32 – 34 °C.

Data were acquired using PCLAMP 10 and a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 4 kHz and digitized at 10 kHz. Light evoked EPSCs were isolated by holding cells at -60 mV after correction for the liquid junction potential (~10 mV).

To isolate the contribution of synaptic inputs, a host of pharmacological agents were perfused in the bath during electrophysiology recordings. 8 μM Dihydro-b-erythroidine hydrobromide (DHβE; from Tocris, Cat#2349); 10 μM SR 9551 hydrobromide (gabazine; from Tocris, Cat #1262); 100 μM Meclofenamic acid sodium salt (MFA; from Sigma-Aldrich. Cat#M4531); 1 μM Strychnine (from Sigma-Aldrich, Cat#S0532); 50 μM TPMPA (from Tocris, Cat#1040).

Data analysis of electrophysiological recordings
Spiking data from loose-patch recordings were analyzed using custom protocols in MATLAB. The number of spikes evoked by the On response to the peripheral spots were quantified in MATLAB and averaged across 3 repetitions in 8 spatial locations. Light-evoked EPSC responses to peripheral spots were obtained as well, and 3 repetitions of EPSC traces were averaged to obtain the mean peak amplitude and charge transfer response to each condition. The RSI is determined by (Max - Opp)/(Max + Opp) where Max is the number of spikes, peak amplitude or charge transfer in the region of maximal activation using the peripheral spot stimulus, and Opp is the response in the region directly opposite to the Max region.

For the receptive field mapping experiments, the light-evoked EPSCs in response to spots sized 20 μm in diameter were obtained and averaged across 3 – 4 repetitions. The center of mass of the receptive field was determined using the EPSC charge transfer or amplitude at each square in the 11 X 11 grid. The dendritic length was calculated at each point on the RF stimulus grid by summing the dendritic length in a circle 60 μm in diameter centered on the square of interest. For maximum vs. opposite region analysis and the preferred side vs null side analysis (Figures 3E and 3F), we considered the EPSCs in two 5 X 5 subsets of the total grid located opposite of each other. The preferred-null side of each cell was determined by spiking responses to the full-field moving bar, and the maximum-opposite axis was determined by EPSC charge transfer responses to the peripheral spot experiment performed in DHβE and gabazine. The distance from soma at each grid location was defined as the distance between the center of the square and the soma center.
To estimate the spatiotemporal profile across the maximum-opposite or the preferred-null axis for cells in DHβE and gabazine, the light-evoked EPSCs in response to small spots sized 20 µm in diameter were obtained and averaged across 3 – 4 repetitions (Figures 4F – 4H). To estimate the spatiotemporal profile across the preferred-null axis for cells in DHβE only, the light-evoked EPSCs in response to small spots sized 60 µm in diameter were obtained and averaged across 3 repetitions (Figures S4C – D). For both experiments, EPSC parameters of latency, rise time (10 - 90%), and decay time (90 - 30%) were quantified. The preferred-null axis of each cell was determined by spiking responses to the full-field moving bar, and the maximum-opposite axis was determined by EPSC charge transfer responses to the peripheral spot experiment performed in DHβE and gabazine. The parameters of latency, rise time, and decay time were averaged across the squares at equal distances along the cell’s preferred-null axis or maximum-opposite axis.

Spiking data evoked by the On and Off responses to the moving bar and occlusion stimuli were quantified in MATLAB using 3 – 6 repetitions in 8 different directions. Spiking data evoked by the bar starting in different positions were quantified in MATLAB across 3 repetitions in the preferred and null directions.

**Calcium imaging in posterior- and anterior-preferring On-Off DSGCs**

Genetically encoded calcium indicator GCaMP6f was expressed in all RGCs by crossing Vglut2-IRES-Cre mice (JAX 016963-Slc17a6tm2(cre)Lowl) and floxed Ai95(RCL-GCaMP6f)-D mice (JAX 028865-Gt(ROSA)26Sortm95.1(CAG-GCaMP6f)Hze/J). GCaMP6f fluorescence from isolated retinas was imaged in a customized two-photon laser scanning fluorescence microscope (Bruker Nano Surfaces Division). GCaMP6 was excited by a Ti:sapphire laser (Coherent, Chameleon Ultra II, Santa Clara, CA) tuned to 920 nm, and the laser power was adjusted to avoid saturation of the fluorescent signal. Onset of laser scanning induces a transient response in RGCs that adapts to the baseline in ~3 s. Therefore, to ensure the complete adaptation of this laser-induced response and a stable baseline, visual stimuli were given after 10 s of continuous laser scanning. To separate the visual stimulus from GCaMP6 fluorescence, a band-pass filter (Semrock, Rochester, MA) was placed on the OLED to pass blue light peaked at 470 nm, while two notched filters (Bruker Nano Surfaces Division) were placed before the photomultiplier tubes to block light of the same wavelength. The objective was a water immersion objective (60x, Olympus LUMPlanFl/IR). Time series of fluorescence were collected at 15–30 Hz.

We performed an initial direction selectivity test to identify posterior- and anterior-preferring On-Off DSGCs. We recorded GCaMP6f fluorescence from RGC somas within a 75-µm X 75-µm field of view while presenting a full-field moving bar visual stimulus (a bright moving bar 110 µm (width) X 880 µm (length) moving at 330 µm/s across a 660...
µm circular mask diameter along 8 different directions). At the onset of each moving bar sweep, a TTL pulse was triggered by the visual stimulus computer and recorded by the imaging software to correlate GCaMP6f signals with the direction of each moving bar. Immediately following acquisition of each time series stack, custom-written MATLAB scripts were used to extract fluorescence over time data from time-series images and sort calcium transient by direction of the moving bar. For each RGC soma, raw GCaMP6f fluorescence traces and tuning curves were plotted. On-Off DSGCs were identified by their characteristic singular-lobe directional tuning curves, DSI values ≥ 0.3, and two fluorescence peaks time-locked to the leading (On) and trailing edge (Off) of the moving bar. On-Off DSGCs with preferred directions along the posterior-anterior axis were then selected for further imaging.

Once an On-Off DSGC of interest was identified, the visual stimulus was centered to the soma of that cell and a new field of view was drawn to enclose this cell and some background with no GCaMP6f fluorescence. Full-field and occlusion moving bar visual stimulus were presented to the cells as described above (8 directions, 3-4 repetitions). Time series data was collected and subjected to offline analysis.

**Imaging analysis for calcium imaging**

Analysis was performed using ImageJ and MATLAB. Regions of interest (ROIs) corresponding to DSGC soma and background were manually selected in ImageJ. The fluorescent time course of each ROI was determined by averaging all pixels within the ROI for each frame. The fluorescence of the background region was subtracted from the raw fluorescent signal of the soma ROIs at each time frame. The visual stimulus included a 3–4 second intersweep interval between the end of one sweep and the start of another. Fluorescence intensities during these intersweep intervals were used to create a baseline (F0) trace for each ROI by fitting either a single- or two-term exponential decay function. Fluorescence measurements were then converted to ΔF/F0 values by calculating ΔF=(F−F0)/F0 for every datapoint. The transformed traces were then smoothed using an average sliding window of 4 datapoints. ΔF/F0 traces were clipped, sorted by visual stimulus direction (0, 45, 90, 135, 180, 225, 270, and 315 degrees), and averaged over 3-4 trials. Prior to further analysis, ROIs were subjected to a response quality test QI = Var[Avg. Resp]/Avg(Var[R(t)]) ≥ 0.45 to ensure consistency across trials. Responses to the full-field and occlusion moving bars were broken up into On and Off components according to the circular mask entrance and exit times of the leading and trailing edge, respectively. Peak, area ΔF/F0, and time of peak values were calculated for On, Off, and the full trace along all 8 directions. Direction selectivity index (DSI), vector sum, and preferred direction were calculated for both On and Off components.
**Statistical analysis**

Grouped data are presented as mean ± SEM. The Kolmogorov-Smirnov test was used to test data for normality. Student’s t-test was used for statistical comparisons of paired samples in Figures 1 and 2. One-way analysis of variance was performed on grouped data in Figures 5 and 6 and subjected to Bonferroni correction.

For the EPSC/dendritic length vs distance from soma experiments, we performed linear regression analysis using an additional categorical predictor variable indicating the maximum-opposite or the preferred-null side. The p-value associated with interaction term (distance*region) in the resulting model was used to determine whether the slopes are significantly different between the two regions. For the EPSC vs dendritic length experiments, we again performed linear regression with an additional categorical predictor variable indicating the maximum-opposite or preferred-null region. The p-value associated with the categorical predictor in the resulting model was used to determine whether the y-intercepts were significantly different between the two regions. The number of branches in each square of the grid was determined by a custom MATLAB code.

For the kinetic analyses of EPSC parameters of latency, rise time, and decay time, we performed linear regression analysis to determine whether a statistically significant linear relationship exists between the distance from the soma and each EPSC parameter.

For all data sets, \( p < 0.05 \) was considered significant. \( *p < 0.05; \ **p < 0.01; \ ***p < 0.001. \)

**Experimental parameters for population model**

Experimental data for the spiking response to full-field (73 cells) and occlusion (69 cells) stimuli were obtained. 3 – 6 repetitions were obtained for each full-field or occlusion protocol. The baseline firing rate for each repetition was obtained by binning the spiking response in 25-ms time bins and taking the maximum firing rate during a silent period where no stimulus was displayed, and the baseline firing rate was averaged across all repetitions.

To model the spiking response of the pDSGCs, we binned the spikes evoked by the On response to the motion stimulus in 25-ms time bins and plotted the PSTHs for all 8 motion directions. Then, we fit a rectified sine wave to the PSTH of each pDSGC. We defined the threshold for above-baseline firing to be 4 SD above the baseline firing rate. The onset of the spiking response was determined by the time bin at which the firing rate exceeded the threshold and was immediately followed by a second above-
threshold bin (Rate change method; Levakova et al., 2015). We inspected the spiking response onset times returned by our detection algorithm and manually adjusted the spiking response onset times for 3 out of 91 pDSGCs to match the experimental data. Onset times of spiking responses to the full-field bar moving in the preferred direction ± 45 degrees were similar to those of the preferred-direction response (Figure S7A, left). Likewise, onset times of spiking responses to the occlusion stimulus moving in the null direction ± 45 degrees were similar to those of the null-direction response (Figure S7A, right). Therefore, we included onset times of spiking responses to motions in the directions ± 45 degrees from the preferred-null motion axis in our analysis. Four-parameter beta distributions were fit to histograms of spiking response onset times for preferred- and null-direction motions (Figure 7C). The four parameters included two shape parameters and two parameters that specify the minimum and maximum of the distribution’s range.

To determine the offset of the spiking response, we used the algorithm for identifying spiking response onset. Unlike the protocol for determining spiking response onset, however, the detection algorithm started from the most recent time bin and traversed backwards in time. The offset of the spiking response was defined to be the time bin at which the firing rate exceeded the threshold in two out of three consecutive time bins. For each pDSGC, we calculated the spiking response duration by finding the difference between the spiking response onset and offset times. Furthermore, we calculated the linear correlation between spiking response duration and onset (Figure 7D). Figure S7B shows that using only spiking responses to motion along the preferred-null motion axis and using spiking responses to motion along the preferred-null motion axis as well as motion in the directions 45 degrees away from the preferred-null motion axis yielded consistent results. We also calculated the linear correlation between peak firing rate and spiking response onset, but the correlation was not significant for the full-field protocol (Figure S7C).

**Motion direction tuning curves**

We computed motion direction tuning curves for all pDSGCs exposed to the full-field moving bar stimulus (73 cells) using the CircStat toolbox in MATLAB developed by P. Berens (2009). The height of each tuning curve was given by the total spike count evoked during the presentation of the full-field moving bar stimulus. We fit a Gaussian function to the histogram of the tuning curve widths (Figure S7E). Figure S7F shows all the normalized motion direction tuning curves. The heights of the tuning curves were rescaled by dividing by their peaks and the widths of the tuning curves were rescaled by dividing by their angular deviation, which is the square-root of twice the circular
The normalized tuning curves were fit to a one-term Gaussian model (Figure S7F).

One-dimensional population model

In our computational model, we arranged two populations (left motion-preferring and right motion-preferring) of DSGCs in a horizontal one-dimensional array. Each population had 100 cells, and the nearest neighbor distance distribution within each population was Gaussian, with a mean of 39 μm and a SD of 16 μm (Huberman et al., 2009). The spatial positions of the DSGCs between the two populations were uncorrelated.

Each DSGC’s mean spiking response to the moving edge was modeled as a rectified sine wave. The amplitude of the sine wave was given by the peak firing rate, while the period and phase were determined by the spiking response duration and onset time, respectively. For each spatial arrangement of DSGCs, we sampled peak firing rates directly from our experimental data. We sampled spiking response onset times from our four-parameter beta distributions (Figure 7C) and determined the spiking response durations by finding the linear correlation between the two (Figure 7D).

Noise was introduced into the preferred directions of the DSGCs so that they were not all perfectly aligned with the left/right motion axis. To determine the degree of jitter in a DSGC’s preferred direction, we sampled from a uniform distribution ranging from -14.1 degrees to +14.1 degrees, where 0 degrees represented a preference for motion directly along the left/right motion axis (Fiscella et al., 2015).

To determine the motion direction tuning width of each simulated DSGC, we sampled circular variances from a Gaussian distribution fit to the histogram of circular variance of the tuning curves from our experimental data (Figure S7E). We scaled the collapsed tuning curve (Figure S7E) by the sampled tuning width and peak firing rate to obtain the tuning curve. We used the motion direction tuning curve to adjust the peak firing rate of the spiking response according to the jitter in the preferred direction alignment.

We simulated the DSGC population response to a moving edge traveling from left to right at a constant speed of 330 μm/second. To simulate the occlusion protocol, we introduced an occlusion placed over a left motion-preferring DSGC whose position in space was fixed (approximately the 50th left motion-preferring DSGC). For each spatial arrangement of DSGCs, the simulation was repeated 100 times. The spiking response of each DSGC was discretized in time. At each 25-ms time bin, the firing rate given by the rectified sine wave fit was converted to a mean spike count. The number of spikes generated by a DSGC was obtained by sampling from a Gaussian distribution with this...
mean and a sub-Poisson, constant variance of 0.4. The sub-Poisson noise was
determined from our experimental data by analyzing the variance of the spiking
responses to 6 repetitions of the full-field moving bar (10 cells) and the occlusion
stimulus (9 cells) (Figure S7D). The spatial positions of the DSGCs were shuffled in
each simulation block for a total of 1000 blocks with 100 repetitions in each block.

A population vector (Dayan and Abbott, 2001) was used to decode the spatial position
of the moving bar’s leading edge. The position estimate \( \hat{x} \) at a given time was the firing
rate-weighted average of DSGCs’ spatial positions

\[
\hat{x} = \frac{\sum_i r_i \bar{x}_i}{\sum_i r_i}
\]

where \( r_i \) is the firing rate of the \( i^{th} \) cell and \( \bar{x}_i \sim N(x_i, w_i^2) \) where \( x_i \) is the soma position
and \( w_i \) the RF width of the \( i^{th} \) cell. The RF width was taken to be the 1 SD boundary of
the Gaussian center profile (Chichilnisky and Kalmar, 2002). RF widths were obtained
by scaling the dendritic field radii by 1.25. Dendritic field radii were obtained by sampling
from Gaussian distribution with \( \mu = 88 \mu m \) and \( \sigma = 14.8 \mu m \). In the population vector
decoder, we added 130 \( \mu m \) to the soma position of the occluded DSGC to compensate
for the lag of the null-direction response to the occlusion stimulus. Errors are reported
as the root mean-square-error in the position estimate (Figure 7E).

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