Dendrite morphology minimally influences the synaptic distribution of excitation and inhibition in retinal direction selective ganglion cells

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ABSTRACT

Throughout the nervous system, the organization of excitatory and inhibitory synaptic inputs within a neuron’s receptive field shapes its output computation. In some cases, multiple motifs of synaptic organization can contribute to a single computation. Here, we compare two of these mechanisms performed by two morphologically distinct direction selective retinal ganglion cells (DSGCs): directionally tuned inhibition and spatially offset inhibition. Using drifting stimuli, we found that DSGCs that have asymmetric dendrites exhibited stronger directionally tuned inhibition than symmetric DSGCs. Using stationary stimuli to map receptive fields, we found that DSGCs with both symmetric and asymmetric dendrites exhibited similar spatially offset inhibition. Interestingly, we observed that excitatory and inhibitory synapses for both cell types were locally correlated in strength. This result indicates that in the mouse retina, dendritic morphology influences the amount of tuned inhibition attained through asymmetric wiring but does not dictate the synaptic organization of excitation relative to inhibition.

SIGNIFICANCE STATEMENT

Neural circuit function is dependent on the detailed organization of excitatory and inhibitory synapses onto dendrites. Here we use a classic neural circuit - the direction selective circuit of the retina - to assess how changes in dendritic shape impact the synaptic organization. We find the direction selective cells of the retina that have asymmetric dendrites have similar synaptic organization to those that have symmetric dendrites, indicating the the shape of dendrites does not dictate the final computation of the neurons.

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INTRODUCTION

Detecting the direction of moving stimuli is an essential part of sensory processing. In the mouse visual system, direction selectivity is first observed in the retina, where direction selective ganglion cells (DSGCs) fire many action potentials in response to motion in their preferred direction, and few to no action potentials in response to the opposite, or null, direction. Direction selective computations occur across many layers of the mammalian visual system from DSGCs of the retina, to the retino-recipient neurons of the dorsal lateral geniculate nucleus (dLGN) of the thalamus (Liang et al., 2018; Marshel et al., 2012), thalamo-recipient layer 4 neurons and intracortical circuits of the visual cortex (Rasmussen et al., 2020; Rossi et al., 2020). Additionally, direction selectivity has been shown to arise in nonvisual areas like the mouse whisker somatosensory cortex (Laboy-Juárez et al., 2019) and in the primary auditory cortex (Ye et al., 2010; Zhang et al., 2003).

Retinal direction selectivity is mediated primarily by inhibition through two non-mutually exclusive mechanisms. The first mechanism mediating direction selectivity is based on directional tuning of inhibition, where the amount of inhibitory input onto a DSGC is greater for null direction motion than for preferred direction motion. In the mammalian retina, this asymmetric inhibition is provided by starburst amacrine cells (SACs), where the combination of SAC centrifugal directional tuning (Ding et al., 2016; Gavrikov et al., 2006; Hausselt et al., 2007; Vlasits et al., 2016), and DSGC-SAC asymmetric wiring (Briggman et al., 2011; Rosa et al., 2016; Wei et al., 2011; Yonehara et al., 2011) ensures maximal spike suppression in response to null direction motion, compared to preferred direction motion. Though the role of asymmetric inhibition in tuning the direction selective circuit has been well established in the mouse and rabbit (Fried et al., 2002; Grama & Engert, 2012; Morrie & Feller, 2015; Taylor & Vaney, 2002; Wei et al., 2011; Yonehara et al., 2011), its dependence on the morphology of DSGCs has been relatively unexplored.

The second mechanism is based on spatially offset inhibition - a term used to describe when excitatory and inhibitory receptive fields are spatially offset from each other such that, preferred direction motion elicits an excitatory response before the stimulus enters the inhibitory receptive field, thus a temporal delay is introduced into the inhibitory response. During null direction motion, the stimulus enters the inhibitory receptive field before it enters the excitatory receptive field, thus a temporal delay is introduced to the excitatory response and the inhibitory input effectively suppresses spiking output. This is the classical mechanism postulated to underlie direction-selective responses in both the retina (Fried et al., 2002; Yonehara et al., 2011) and in the visual cortex (Hubel & Wiesel, 1959, 1962; Priebe & Ferster, 2005; Rossi et al., 2020). Several studies have revealed temporal delays, consistent with spatially offset inhibition, play a role in the DS computation of the mouse retina (Hanson et al., 2019; Pei et al., 2015; Ding et al, 2021). Recently, we used receptive field mapping to show that a population of asymmetric, ventral preferring DSGCs (vDSGCs) have both tuned inhibitory inputs and spatially offset inhibition, though neither of these circuit contributions were impacted by dramatic changes in the dendrite orientation due to dark-rearing (El-Quessny et
al., 2020). However, how the inhibitory receptive fields compare to excitatory receptive fields in DSGCs with symmetric versus asymmetric dendrites is not known.

Anatomical studies indicate that ON-OFF DSGCs exhibit a uniform distribution of GABA_A receptors on their dendrites (Auferkorte et al., 2012; Sigal et al., 2015; Bleckart et al., 2018), while functional studies indicate that SACs whose somas are located on the null side of a DSGC provide stronger inhibitory drive than SACs located on the preferred side of DSGC asymmetric wiring (Wei et al. 2011, Morrie et al. 2015; Lee et al., 2010). Here, we compare the organization of excitatory and inhibitory receptive fields of two subsets of DSGCs that have distinct morphologies. The first is the subset of ventral motion preferring DSGCs (vDSGCs), which have asymmetric dendrites that are oriented toward their preferred direction (Trenholm et al., 2011), a configuration which contributes to their direction selectivity in the absence of inhibitory input (El-Quessny et al. 2020). The second is the subset of nasal motion preferring DSGCs (nDSGCs), which have symmetric dendrites that are not oriented in any particular direction (Rivlin-Etzion et al., 2011). Multielectrode array data has shown that the spiking output of both DSGC subtypes possesses similar directional tuning under bright stimulus conditions (Yao et al., 2018). Here, we combine morphological reconstructions with whole cell voltage clamp recordings to show that asymmetric vDSGCs have sharper tuning of inhibition relative to symmetric nDSGCs. Additionally, we map the receptive fields of both DSGC subtypes, using stationary stimuli, and show no difference in the spatial offset of inhibition relative to excitation despite distinct dendritic morphologies.

METHODS

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice used in this study were aged from p30-60 and were of both sexes. Animals used in experiments had not previously been involved in other experiments or exposed to any drugs. Animal health was monitored daily and only healthy animals were used in experiments. To target ventral preferring DSGCs, we used Hb9::GFP (Arber et al., 1999) mice, which express GFP in a subset of ventral preferring DSGCs which have asymmetric dendrites (Trenholm et al., 2011). To target nasal preferring DSGCs, we used Trhr::GFP (Rivlin-Etzion et al., 2011). All experiments involved recording from 1-7 cells from at least 2 animals of either sex. All animal procedures were approved by the UC Berkeley Institutional Animal Care and Use Committee and conformed to the NIH Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy, and the SfN Policy on the Use of Animals in Neuroscience Research.

METHOD DETAILS

Retina Preparation

Mice were anesthetized with isoflurane and decapitated. Retinas were dissected from enucleated eyes in oxygenated (95% O2/5% CO2) Ames’ media (Sigma) for light responses or ACSF (in mM, 119 NaCl, 2.5 KCl, 1.3 MgCl_2, 1 K2HPO4, 26.2 NaHCO3, 11D-glucose, and 2.5 CaCl_2) for paired recordings. Retinal orientation was determined as described previously (Wei...
et al., 2010). Isolated whole retinas were micro-cut at the dorsal and ventral halves to allow
flattening, with dorsal and ventral mounted over a 1–2 mm² hole in nitrocellulose filter paper
(Millipore) with the photoreceptor layer side down and stored in oxygenated Ames’ media or
ACSF until use (maximum 10 h). All experiments were performed on retinas in which dorsal-
ventral orientation was tracked.

Visual Stimulation

For visual stimulation of DSGCs, visible light (420–530 nm) were generated using a computer
running 420-520 nm light through a digital micro-mirror device (DLI Cel5500) projector with a
light emitting diode (LED) light source generated using MATLAB software with the
Psychophysics Toolbox. Visual stimuli are focused on the photoreceptor layer using a condenser
in the DMD path to the chamber.

Moving stimuli

To measure the directional tuning of synaptic currents onto DSGCs, drifting bars of positive
contrast on a grey background (96% Michaelson’s contrast) were presented (velocity = 250
µm/s, length =600 µm width =350 µm over a 700 µm radius circular mask) in 8 block shuffled
directions, repeated 3 times, moving along the long axis of the bar. Each presentation lasted 6 s
and was followed by 3 s interstimulus interval of grey background. For these moving stimuli, the
illumination radius on the retina was 1.4 mm to limit modulation of DSGC responses by
inhibitory wide-field amacrine cells (Chen et al., 2016). A 20X water-immersion objective
(Olympus LUMPlanFl/IR 360/1.0 NA) was used to target cells for voltage clamp recordings,
which were simultaneously acquired using methods described below.

Static stimuli for receptive field mapping

To map excitatory and inhibitory receptive fields of DSGCs, positive contrast square stimuli (30
x 30 µm) were flashed over a grey background (96% Michaelson’s contrast) at an intensity of
3.1 x 10⁶ R²/s/rod. Stimuli were individually presented in 100 block-shuffled positions, repeated
three times, with each stimulus lasting for 0.5 seconds followed by a 1.2 sec interstimulus
interval of grey background. Stimuli were presented within a 10x10 grid, onto a stimulus field of
500 x 500 µm, with the DSGC soma located in the center of the stimulus field (See Figure 2). A
20X water-immersion objective (Olympus LUMPlanFl/IR 360/1.0 NA) was used to target cells for
voltage clamp recordings, which were simultaneously acquired using methods described
below.

Two-photon targeted whole-cell voltage-clamp recordings

Oriented retinas were placed under the microscope in oxygenated Ames’ medium at 32–34˚C.
Identification and recordings from GFP+ cells were performed as described previously (Wei et
al., 2010). In brief, GFP+ cells were identified using a custom-modified two-photon microscope
(Fluoview 300; Olympus America) tuned to 920 nm to minimize bleaching of photoreceptors.
The inner limiting membrane above the targeted cell was dissected using a glass electrode. Cell
attachment voltage clamp recordings were performed with a new glass electrode (4-5 MΩ) filled
with internal solution containing the following (in mM): 110 CsMeSO₄, 2.8 NaCl, 20 HEPES, 4
EGTA, 5 TEA-Cl, 4 Mg-ATP, 0.3 Na₃GTP, 10 Na₂Phosphocreatine, QX-Cl (pH = 7.2 with CsOH,
osmolarity = 290, ECl⁻ = -60 mV). Whole cell recordings were performed with the same pipette after obtaining a giga ohm (1GΩ) seal and breaking into the cell membrane. Holding voltages for measuring excitation and inhibition after correction for the liquid junction potential (10 mV) were 0 mV and -70 mV, respectively. Signals were acquired using Clampex 10.4 recording software and a Multiclamp 700A amplifier (Molecular Devices), sampled at 10 kHz, and low pass filtered at 6 kHz.

Two-photon microscopy and morphological reconstruction

After physiological recordings of DSGCs were completed, Alexa-594-filled DSGCs were imaged using two photon excitation at 800 nm. At this wavelength, GFP is not efficiently excited, but Alexa 594 is brightly fluorescent. 480x 480 µm Image stacks were acquired at z intervals of 1.0 µm and resampled fifteen times for each stack using a 20X objective (Olympus LUMPlanFl/IR 2x digital zoom, 1.0 NA) 30kHz resonance scanning mirrors covering the entire dendritic fields of the DSGCs. Image stacks of DSGCs were then imported to FIJI (NIH) and a custom macro was used to segment ON and OFF dendrites based on their lamination depth in the inner plexiform layer (ON layer 10-30 µm, OFF layer 35-55 µm depth). Following ON and OFF dendritic segmentation, we used the Simple Neurite Tracer plugin on FIJI to skeletonize and then binarize the ON and OFF dendritic segments for morphological analyses.

Pharmacology

For experiments conducted in Hexamethonium (Millipore Sigma), we diluted 100 µM in AMES media, and allowed it to perfuse for 5-10 mins at a perfusion rate of 1 mL/min.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Tests

Mean ± standard deviations for all angles performed using circular mean and circular standard deviations. Details of statistical tests, number of replicates, and p values are indicated in the figures and figure captions. P values less than 0.05 were considered significant.

Data analysis

For voltage clamp recordings during moving stimuli, traces were first average across the 3 trials for each direction and inspected to ensure the consistency of the responses across trials. Average traces were baseline subtracted based on the last 500 msec of recording or a user defined interval after manual inspection. Peak currents were calculated from average baseline subtracted traces and were the maximal (IPSC) or minimal (EPSC) points during two separate 1.9 s windows in which the ON and OFF responses occurred. The peak currents in each direction were used to calculate the vector sum of the current responses. For timing analysis, PSC traces were low pass filtered using an 80 msec moving average, and peak time for excitation and inhibition was extracted for ON and OFF responses. Null directions for both ON and OFF responses used to calculate peak responses and were defined as the angle of the vector sum of ON and OFF peak IPSCs and the preferred directions were defined as 180 - null direction.
The directionally selective index (DSI) was calculated for the peak amplitude of the inhibitory post synaptic currents (IPSCs) as: \( \frac{ND-PD}{ND+PD} \) where ND is the amplitude of the peak current in the null direction, and PD is the amplitude of the peak current in the preferred direction. We also used the magnitude of the vector sum of the spike responses as another measurement of directional tuning (Vector Sum = 1-Circular Variance of the spike responses (Mazurek et al., 2014)).

**Quantification of receptive fields:** For voltage clamp recordings during static stimuli, we first divided each trace into the ON and OFF response based on the location where the stimulus was present. Next, we calculated the center of mass (COM) of the peak current amplitudes of the stimulus field using the following equations:

\[
\text{COM}_x = \frac{\sum_{i=1}^{N} m_i x_i}{M}, \quad \text{COM}_y = \frac{\sum_{i=1}^{N} m_i y_i}{M}
\]

Where \( x \) and \( y \) are the cartesian coordinates of the center of mass, \( N \) is the total number of stimulus squares (100), \( m \) is the peak current amplitude at each coordinate location and \( M \) is the sum of peak current amplitudes across the entire receptive field.

To measure the displacement and orientation of the receptive fields relative to the soma, we calculated the magnitude and angle, respectively, of vector from the soma to the center of mass of the receptive field using the following equations:

\[
\text{Vector Magnitude} = \sqrt{\vec{A}_x^2 + \vec{A}_y^2}, \quad \text{Vector Angle} = \tan^{-1}\left(\frac{\vec{A}_x}{\vec{A}_y}\right).
\]

To quantify spatially offset inhibition, we calculated the vector from the center of mass of the excitatory receptive field to the center of mass of the inhibitory receptive field.

**Quantification of dendrites:** To compare DSGC dendrites to the synaptic inputs evoked by static stimuli, we skeletonized dendrites using methods described above. Next, we calculated the vector from soma to the COM of the dendritic pixels; the magnitude of the vector indicates the magnitude of dendritic asymmetry relative to the soma, while the angle of the vector indicates the orientation dendrites relative to the soma. To directly compare the DSGC dendrites to the IPSC and EPSC receptive fields, we binned the dendritic skeleton into a 10 x 10 matrix by summing the binarized pixels in each bin, with the soma located in the center of the matrix. We calculated the vector from soma to the COM of the binned dendritic pixels; the magnitude of the vector indicates the magnitude of dendritic asymmetry relative to the soma, while the angle of the vector indicates the orientation dendrites relative to the soma. In Figure 2A, we...
show the responses of an example vDSGC and nDSGC recorded in control conditions as well as their binned dendrites.

**Quantification of receptive and dendritic field sizes:**
To quantify receptive field size, the locations in the excitatory and inhibitory receptive that had responses below and above a set noise threshold of 50 pA were assigned binary values based on whether the response at each location exceeded this threshold. Next, we calculated the area total area of the grid with responses that exceed the threshold. To quantify dendritic field size in a manner that is comparable to the receptive field size, we binned dendritic pixels into a 10 x 10 matrix and then used the same method to calculate dendritic area, without applying noise thresholds since dendrites were skeletonized prior to this analysis using the method described above.

**RESULTS**

**DSGCs with asymmetric dendrites exhibit greater directional tuning of inhibition than DSGCs with symmetric dendrites.**
Our goal was to determine whether the synaptic organization onto DSGCs is dependent on the dendritic morphology. We first quantified the difference in dendritic asymmetry in vDSGCs vs. nDSGCs, by calculating the magnitude of the vector from the soma to the center of mass of the dendritic pixels. We found that both ON and OFF dendrites of vDSGCs were significantly more asymmetric than nDSGCs (mean ± S.D.: vDSGCs: ON = 68.2 ± 25.0 µm, OFF = 65.2 ± 25.8 µm, n = 23; nDSGCs: ON = 44.3 ± 20.0 µm, OFF = 37.6 ± 17.5 µm, n = 16) (Figure 1A). As reported previously, the asymmetry in the dendrites of nDSGCs are not consistently aligned with their preferred direction (Rivlin-Etzion et al. 2011).

To assess the impact of dendrite morphology on the tuning of inhibition, we conducted voltage clamp recordings of both vDSGCs and nDSGCs and isolated inhibitory postsynaptic currents (IPSCs) in response to a bar of light moving in eight different directions (Figure 1B). Despite previous MEA studies showing that comparable spike tuning of both DSGC subtypes under our stimulus conditions (Yao et al. 2020), asymmetric vDSGCs had a significantly higher direction selectivity index (DSI), compared to nDSGCs (DSI vDSGCs: ON = 0.47 ± 0.18, OFF = 0.55 ± 0.12; nDSGCs: ON = 0.31 ± 0.13, OFF = 0.35 ± 0.10; Figure 1C). Hence, vDSGCs with asymmetric dendrites had greater tuning of inhibition.

Previous studies have reported differences in the relative timing of excitatory and inhibitory synaptic inputs for preferred and null direction stimulation, consistent with the presence of spatially offset inhibition (Fried et al., 2002; Taylor & Vaney, 2002). Here, we report similar differences in timing, with inhibitory inputs delayed relative to excitatory input for preferred direction stimulation in symmetric, nDSGCs (E-I timing diff: ON = 257 ± 134 msec, OFF = 173 ± 325 msec) and asymmetric vDSGCs (E-I timing diff mean ± S.D.: ON = 81 ± 262 msec, OFF = 292 ± 468 msec), though there was greater variability during preferred direction motion for asymmetric...
vDSGCs due to the small amplitude of the inhibitory currents (Figure 1E). For both nDSGCs and vDSGCs, null direction motion elicited a much smaller temporal difference between the excitatory and inhibitory responses (Table 1). We also represented these timing differences as spatial offsets by multiplying by the velocity of our stimulus (250 µm/sec = 8.1°/sec). These data suggest that, for both asymmetric vDSGCs and symmetric nDSGCs, spatially offset inhibition contributes to the DS computation.

**Receptive field mapping of DSGCs reveals similar spatially offset inhibition for DSGCs with symmetric or asymmetric dendrites.**

Previously, we showed that in asymmetric vDSGCs, the centers of mass of the spatial receptive fields for excitation and inhibition are both offset toward the preferred direction with inhibitory receptive fields further offset than the excitatory receptive fields (El-Quessny et al., 2020). However, for symmetric nDSGCs, the relative arrangement of excitatory and inhibitory receptive fields is unknown. Hence, we mapped the excitatory and inhibitory receptive fields by recording synaptic currents evoked by squares of light sequentially presented at 100 block-shuffled locations within a soma-centered grid (Figures 2A). We stimulated a 500x500 µm area spanned by a 10x10 grid. We presented a 30x30 µm light flash within the center of each grid to prevent any blooming artifacts of the visual stimulus.

To characterize the relative position of excitatory and inhibitory receptive fields, we computed the center of mass for dendrites, excitatory receptive fields, and inhibitory receptive fields (Figure 2B) and compared both the relative displacement and orientation of the inhibitory receptive field to the excitatory receptive field (Figures 2C). We found that the excitatory and inhibitory receptive fields of both vDSGCs and nDSGCs exhibited some spatial offset (Figure 2E). Though the relative magnitude of spatially offset inhibition (magnitude of the vector from excitation to inhibition) was slightly greater in nDSGCs ($\text{mean} \pm \text{S.D. ON}=38 \pm 25 \mu m$, $\text{OFF} = 33 \pm 26 \mu m$), compared to vDSGCs (Figure 2E) ($\text{mean} \pm \text{S.D. ON} = 21 \pm 15 \mu m$, $\text{OFF} = 20 \pm 14 \mu m$), we were surprised to find that they were comparable to each other despite their distinct dendritic morphologies. Moreover, we observed that the direction of the spatially offset inhibition on average clustered around the preferred direction but there was significant variance for both nDSGCs (Deviation from PD mean $\pm$ S.D., ON: $7.9 \pm 70^\circ$; OFF: $-4.9 \pm 70^\circ$), and vDSGCs (Deviation from PD mean $\pm$ S.D., ON: $-7.5 \pm 87^\circ$; OFF: $-40 \pm 72^\circ$) (Figure 2D and E).

Although we observed a shift in the position of inhibitory receptive fields relative to excitatory receptive fields in both cell types, there was also a striking correlation between them. First, we observed a strong positive correlation between the location of excitation and inhibition relative to the soma (Figure 2F). Note, this correlation was stronger in asymmetric vDSGCs (Table 2) consistent with previous findings (El-Quessny et al., 2020). Second, we observed a strong correlation between the strength of excitation and inhibition measured at each pixel (Figure 2G), where the amplitude of excitation explains on average 65% and 51% of the variance in the amplitude of inhibition in vDSGCs and nDSGCs, respectively (Figure 2H). This strong local correlation is consistent with the tight alignment of ACh-GABA co-transmission from SAC.
varicosities (Brombas et al., 2017; Jain et al., 2020; Lee et al., 2010; Sethuramanujam et al., 2016).

To assess the organization of the excitatory and inhibitory receptive fields along the preferred-null axis, we collapsed the synaptic currents recorded with the static stimulus along the axis orthogonal to their preferred direction and plotted the normalized distribution of excitation and inhibition (Figure 3A). We found both vDSGCs and nDSGCs exhibit a comparable skew in the spatial distribution of excitatory and inhibitory synapses towards their preferred directions (Figure 3B and C). Together, these data indicate that nDSGCs and vDSGCs exhibited similar spatially offset inhibition despite significant differences in their dendritic morphology.

**DSGC dendritic morphology does determine the organization of spatial receptive fields**

We next explored whether the small displacements for the EPSC and IPSC receptive field centers from the soma were correlated with variations in the spatial arrangement of the DSGC dendrites (Figure 4A). To do that, we compared the distance and orientation of the center of mass relative to the soma of the EPSC and the IPSC peak current amplitudes of the ON and OFF responses from the soma (Figure 2B) to those of the dendrites. Consistent with our previous study (El-Quessny et al., 2020), we found that the orientation of vDSGC dendrites (Angle mean ± SD, ON = 242 ± 42°, OFF = 230 ± 41°, n=20), excitatory receptive fields (Angle mean ± SD ON = 267 ± 45°, OFF= 260± 45°, n=17) and inhibitory receptive fields (Angle mean ± SD ON= 264 ± 41°; OFF= 260± 43°, n=20) were all ventrally pointing (ventral corresponds to 270°) (El-Quessny et al., 2020). In contrast, nDSGC dendrites (Angle mean ± S.D. ON= 147 ± 67°, OFF= 234 ± 76°, n=20), excitatory receptive fields (Angle mean ± S.D. ON: 196 ± 65°; OFF: 197± 61°,n=15) and inhibitory receptive fields (Angle mean ± S.D.: ON= 296 ± 75°; OFF = 278± 96°,n = 20) did not exhibit a biased orientation toward the nasal direction (nasal corresponds to 0/360°) (Figure 4B and C and Table 3). We also found that EPSC and IPSC receptive fields were significantly larger than the dendritic fields in both vDSGCs (EPSC/Dendrite Ratio mean ± SD: ON=1.9 ± 0.76, OFF = 1.9 ±0.93; IPSC/Dendrite: ON=1.6 ± 0.62, OFF = 1.9 ±0.91) and nDSGCs (EPSC/Dendrite Ratio mean ± SD: ON=2.1 ± 1.2, OFF = 2.6 ±1.4; IPSC/Dendrite: ON=2.1 ± 1.4, OFF = 3.1 ±1.6) (Figure 4D), contrary to previous studies in rabbit DSGCs (Brown et al., 2000; Yang & Masland, 1994).

In the above experiments, EPSCs are mediated by a combination of activation of nicotinic acetylcholine receptors (nAChRs) and glutamate receptors. In a subset of experiments, where we pharmacologically blocked cholinergic excitation, we found that the orientation of the glutamate receptive field in vDSGCs (Angle mean ± SD ON= 267 ± 47°; OFF= 273± 54°, n=6) was also ventrally oriented. In contrast, the orientation of the glutamate receptive field in nDSGCs was not oriented toward its preferred direction but rather, on average, was oriented towards the DSGCs’ null direction (180°) (mean ± SD ON= 200 ± 68°; OFF= 220± 72°, n=10) (Figures 5A and B) (Table 4). This is consistent with recent reports investigating another nDSGC subtype, where the glutamatergic receptive field was also skewed towards the DSGC’s null direction (Ding et al., 2021). Additionally, though glutamatergic receptive field were significantly larger than dendritic field size (Figure 5C), they were closer in area than mixed glutamatergic-cholinergic receptive field size (compare Figure 5C with 5D, left), indicating that cholinergic...
inputs from SACs contribute excitatory inputs outside of the DSGC dendrites. These data reveal that while asymmetric dendritic morphology of vDSGCs can predict the locations of the center of their receptive fields, dendritic field size does not dictate the size of the inhibitory or mixed excitatory receptive fields in either vDSGCs or nDSGCs.

DISCUSSION

Dendritic morphology is thought to influence synaptic organization. Here, we show that dendritic morphology impacts the amount of tuned inhibition whereby DSGCs with asymmetric dendrites exhibit more strongly tuned inhibitory inputs than DSGCs with symmetric dendrites but both cell types exhibit comparable spatially offset inhibition. Moreover, we found in both cell types, that the receptive fields for excitation and inhibition are similarly oriented to each other and are locally correlated in strength. Finally, our results indicate that spatial receptive fields are significantly larger than dendritic fields and are not strongly dictated by the dendritic structure. Here, we discuss the implications of these findings for direction selectivity in the mouse retina.

Asymmetric dendrites may lead to stronger tuning of inhibitory inputs

We found that vDSGCs had stronger inhibitory tuning than symmetric nDSGCs, driven primarily by a decrease in the amount of inhibition during preferred side stimulation (Figure 1). One interpretation of these findings is that the absence of preferred side dendrites reduces the likelihood of these preferred side SAC-DSGC synapses. Serial EM reconstructions indicate that the presence of SAC-DSGC synapses is correlated with an anti-parallel organization of SAC processes and the preferred direction of the DSGC, however, this wiring rule applies across the entire dendritic tree of a symmetric DSGC (Briggman et al., 2011). Our finding that a DSGC with an asymmetric dendritic tree exhibits a relative reduction in synapses with SAC processes oriented parallel to the DSGC’s preferred direction would imply that the orientation of the dendritic branch of the DSGC itself may play a role in instructing this wiring, potentially by increasing the proportion of antiparallel compared parallel SAC-DSGC connections. Though there is no evidence for this in the adult mouse DS circuit, this scenario has not been explicitly tested. For comparison, asymmetric dendritic organization is crucial for the wiring of inputs to DS neurons in the drosophila, where connectome analysis reveals dendritic asymmetry mediates the physical displacement of null and preferred side inputs (Shinomiya et al., 2019). Another example can be found in the mouse spinal cord, where the relative orientation of pre- and postsynaptic processes instructs circuit wiring (Balaskas et al., 2019).

Despite the different in tuning of inhibition, vDSGCs and nDSGCs have been shown to exhibit similar spike tuning properties under our stimulus conditions (Yao et al., 2018). We think that this is due to the fact that tuning is set by the inhibition generated by null direction; namely if there is sufficient inhibition, then cells will be similarly tuned (Koch et al., 1983; Taylor et al., 2000).
An alternative interpretation is that different subtypes of DSGCs receive different levels of non-DS inhibition from other sources (Pei et al., 2015; Morrie and Feller, 2018; Yang and Field, 2021) such as VIP amacrine cells (Park et al. 2015). For example, in another population of nasal preferring DSGCs, paired recordings with SACs show that asymmetric inhibition is impaired when the vesicular GABA transporter (VGAT) is knocked out from SACs, compared to wildtype animals (Pei et al. 2015). In these knockouts, the amplitude of inhibitory input was reduced in response to null but not preferred direction stimulation, pointing to a role for non-SAC sources of inhibition during preferred direction motion.

**DSGC dendrites and the spatial organization of their receptive fields**

Using receptive field mapping based on stationary stimuli, we find that there was an overall shift in the inhibitory receptive field relative to the excitatory receptive field for DSGCs with both symmetric and asymmetric dendrites. This is consistent with our previous work has shown that vDSGCs exhibit ventrally offset inhibitory receptive fields, regardless of their altered morphology following dark rearing (El-Quessny et al., 2020).

In our present study, the spatial offset between the excitatory and inhibitory receptive fields was on average less than 50 µm (Figure 2E), which is the resolution of our mapping. In a previous study, which uses a slightly larger stimulus to map the synaptic input receptive fields of vDSGCs, a slightly larger shift was observed in the excitatory and inhibitory receptive fields (Trenholm et al. 2013), indicating the importance of the mapping resolution in estimating spatial offset. Interestingly, the spatial offset between excitatory and inhibitory receptive fields scales with that observed in the rabbit retina, which predicted spatial offsets of 150 microns, or roughly half the dendritic tree of rabbit DSGCs (Fried et al., 2002). Given the larger dendritic field of rabbit ON-OFF DSGCs (~ 600 µm diameter) (Oesch et al., 2005; Yang & Masland, 1994) compared to mouse ON-OFF DSGCs (~200 µm diameter) (Rivlin-Etzion et al., 2011), we believe that the observed spatial offset scales with dendritic field size across both species. Additionally, the observed spatial offset is comparable but a bit smaller than predicted by the temporal offsets induced by drifting bar (Figure 1E) and the displacement of the inhibitory receptive field is smaller than that predicted by the temporal offsets previously reported for symmetric nDSGCs (270 msec at 500 µm/s corresponding to 135 µm, (Pei et al., 2015). This may be due to different stimulus sizes leading to differential recruitment of lateral inhibitory circuits. Another difference is that stationary stimuli may more strongly activate symmetric sources of inhibition onto DSGCs that arise from non-starburst amacrine cells (Morrie & Feller, 2018; Wei, 2018).

We also found that both excitatory and inhibitory receptive fields are much larger than dendritic fields (Figure 3). Blockade of nAChR signaling reduced the size of the excitatory receptive field to that of the dendrites (Figure 5), consistent with a larger excitatory receptive field due to cholinergic inputs from SACs (Lee et al., 2010; Sethuramanujam et al., 2016). This data is in line findings that glutamatergic receptive fields being closely aligned to the DSGC dendrite (Jain et al., 2020; Rasmussen et al., 2020; Sethuramanujam et al., 2018) (Yang &
Masland, 1994). Another possibility not explored here is the role of gap junctions in expanding receptive field size as recently described for F-mini ON RGCs (Cooler & Schwartz, 2021) and in vDSGCs (Trenholm et al., 2013).

It is important to note that the strength of synapses revealed by stationary receptive field mapping is different than what is activated during moving stimuli. Motion stimuli evoke directional release of GABA from SACs (Euler et al. 2002, Vlasits et al. 2016, Ding et al. 2016), and glutamate from bipolar cell terminals (Matsumoto et al. 2020). Paired recordings between SACs and DSGCs indicate that the strength of ACh synapses are symmetric, and likely mediated by diffuse release of ACh (Lee et al., 2010), while motion stimuli may lead to asymmetric release of ACh during low contrast stimuli (Poleg-Polsky & Diamond, 2016; Sethuramanujam et al., 2016). Furthermore, optogenetic stimulation of SACs expressing channelrhodopsin leads to cholinergic excitation preceding GABAergic inhibition and exhibiting faster receptor kinetics, during preferred direction motion, with all other mechanisms of synaptic inputs blocked (Hanson et al., 2019; Pottackal et al., 2020). However, receptive field mapping informs us of the overall synaptic distribution onto DSGC dendrites that could be activated by a variety of different visual stimuli. For example, a recent study has also implicated variations in the strength of excitatory receptive field, along the null-preferred axis, is critical for the ability to encode the location of moving stimuli and is revealed when the motion stimulus is interrupted by stationary occluder (Ding et al, 2021).

Here, we find that the while the glutamatergic receptive fields of nDSGCs are skewed towards the DSGC’s null direction, consistent with previous reports (Ding et al., 2021), the glutamatergic receptive fields of vDSGCs are skewed towards their preferred side. However, vDSGCs were previously reported to exhibit lag normalized synaptic responses due to gap junction coupling, enabling them to encode object location (Trenholm et al., 2013). Together, these data indicate that vDSGCs and nDSGCs may employ distinct mechanisms for encoding object location.

Local dendritic computations support direction selectivity in DSGCs

As noted above, the extent of direction selective tuning is set by the presence of a sufficient level of inhibition. Interestingly, there is strong evidence that the direction selective computation is made locally on DSGC dendrites, i.e. that motion stimuli confined to small segments of the DSGC dendritic tree still elicit a directional responses (Wei, 2018). First, we found that for both vDSGCs and nDSGCs, inhibitory and excitatory receptive fields exhibiting correlated synaptic strengths (Figure 3), indicating that regions of the receptive field with a higher number of excitatory synapses is countered by an increase in inhibitory synapses. Second, local asymmetric release of GABA are supported by the SAC plexus (Sun et al., 2013). Directional computations within the SAC dendrites are localized (Koren et al., 2017; Morrie & Feller, 2018; Poleg-Polsky et al., 2018). In fact, changes in this density of this plexus appears to be correlated with tuning: decreases in the coverage factor of SAC dendritic arbors (Morrie & Feller, 2018) diminishes DS tuning, while increases in the coverage factor of SAC dendritic
arbors increases DS tuning (Soto et al., 2019). In the mouse retina, indicating that the density of the SAC dendritic plexus determines asymmetric inhibition of all DSGCs.

Computational modeling showed that nonlinear conductance within the dendritic tree promotes a multi-compartmental model, allowing local interactions between excitation and inhibition to shape dendritic DS, while SAC ablation abolished DS (Jain et al., 2020; Sivyer & Williams, 2013). A multi-compartmental model is specifically relevant for vDSGCs, whose form-function correlation enables them to nonlinearily integrate synaptic inputs along their dendrites (El-Quessny et al., 2020; Trenholm et al., 2011, 2013). This may explain how vDSGCs rely more heavily on asymmetric versus spatially offset inhibition, relative to nDSGCs which do not have a form-function relationship.

In conclusion, we show that DSGCs exhibit two parallel mechanisms for computing motion direction. The first is based on tuned inhibition, which we find is influenced by the morphology of the DSGC, and the second is based on spatially offset inhibition which is not influenced by the DSGCs’ dendritic orientation, size or asymmetry.
### TABLE 1
(Related to figure 1)

<table>
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<tr>
<td></td>
<td>vDSGCs</td>
<td>nDSGCs</td>
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<tr>
<td>Mean (µm)</td>
<td>Mean</td>
<td>SD</td>
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<tr>
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<tr>
<td>Dendrite Angle ('')</td>
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<tr>
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<td>IPSC Amplitude (PD) (µA)</td>
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<td>E - I Timing difference (PD) (msec)</td>
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<tr>
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<td>E - I Spatial Offset (PD) (µm)</td>
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### TABLE 2
(Related to Figure 2)

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<td>Mean (µm)</td>
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<td>SD</td>
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<td>RF Spatial offset magnitude (E-I) (µm)</td>
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<td>RF Spatial offset deviation from PD ('')</td>
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<td>R</td>
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<td>R</td>
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<td>EPSC angle Vs. IPSC angle</td>
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R: Pearson's correlation
p: p-value
### TABLE 3
(Related to Figure 4)

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<td></td>
<td>SD</td>
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<td>EPSC area/Dendrite area</td>
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<td>IPSC area Vs. Dendrite area Pearson’s Correlation</td>
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R: Correlation coefficient
p: p-value

### TABLE 4
(Related to Figure 5)

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<th>ON Responses</th>
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<td>vDSGCs Mean</td>
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<tr>
<td></td>
<td>SD</td>
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<tr>
<td>Soma to EPSCGlu COM vector magnitude (µm)</td>
<td>58.28</td>
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<tr>
<td>Soma to EPSCGlu COM vector Angle (°)</td>
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<td>EPSCGlu area Vs. Dendrite area Pearson’s Correlation</td>
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<tr>
<td>IPSC area Vs. Dendrite area Pearson’s Correlation</td>
<td>0.13</td>
<td>0.60</td>
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</table>

R: Correlation coefficient
p: p-value
FIGURE CAPTIONS

Figure 1: DSGCs with asymmetric dendrites exhibit more asymmetric inhibition but similar temporal offset to DSGCs with symmetric dendrites

A) Left: Skeletonized vDSGC (orange) and nDSGC (blue) showing asymmetry and ventral orientation of vDSGCs, in contrast to the symmetry of nDSGCs. Right: Summary data comparing dendritic asymmetry of vDSGCs (n=23) and nDSGCs (n=16) as measured by the magnitude of the vector from the soma to center of mass of the ON (filled) and OFF (open) dendrites. Red data points indicate the measurements for example cells on the left. Statistical significance assessed by one-way ANOVA (p=4x10^-4) and Dunn-Sidak post-hoc test (**p<0.01).

B) Example tuning curve and mean traces of the inhibitory postsynaptic currents in vDSGCs (orange, left) and nDSGCs (blue, right) in response to a moving bar stimulus. ON (solid lines) and OFF (dashed lines) tuning curves and vector sums are based on peak current amplitudes in each direction.

C) Left: Scatter plot of the peak amplitude of IPSCs in response to preferred versus null direction motion in vDSGCs (orange) and nDSGCs (blue). SEM for ON (dark shade) and OFF (light shade) responses indicated on the plot. Right: Box plot summarizing the tuning of vDSGCs (orange) and nDSGCs (blue) as measured by the direction selectivity index. ON (filled) and OFF (open) responses separately. Unity line (grey dashed) indicating where preferred (PD) = null (ND) IPSC peak amplitude. Statistical significance assessed by Wilcoxon Rank-Sum test (** p<0.01, *** p<0.001).

D) IPSC and EPSC traces in response to the preferred direction (PD, top) and null direction (ND, bottom) for the example vDSGCs (orange) and nDSGCs (blue). Arrows indicating peak timing for IPSCs (magenta) and EPSCs (green) after applying an 80 ms moving average. Note, the small amplitude and complex kinetics of preferred direction IPSCs in vDSGC prevents a reliable calculation of the timing of the peak current. E) Summary data representing spatial offset based on the timing differences of the peak excitatory (E) and inhibitory (I) currents in response to preferred direction (PD) and null direction (ND) stimulation for ON (filled) and OFF (open) responses in vDSGCs (orange) and nDSGCs (blue). Statistical significance for nDSGCs assessed by paired t-test (p>0.05).

Figure 2: vDSGCs and nDSGCs have similar spatially offset inhibition and exhibit strong local correlations in excitation and inhibition.

A) Example vDSGC (top) and nDSGCs (bottom) receptive field displaying mean inhibitory and excitatory postsynaptic responses for each stimulus presentation. Asterisk in the center of the stimulus field denotes soma location. ON (cyan) and OFF (purple) dendritic skeletons are overlaid. Inset shows stimulus presentation of 30 x 30 µm light within a 50 x 50 µm area to evade scattering effects.

B) Heat map of dendritic density (left), the EPSC peak current amplitude (middle) and IPSC peak current amplitude (right) for ON (top) and OFF (bottom) responses of the example vDSGC (top row) and nDSGC (bottom row) to the left. Scale bar = 100 µm.

C) Summary data plotting the average IPSC (I) receptive field (vDSGCs, n=17; nDSGCs, n=15 cells), centered on the EPSC (E) receptive field center of mass (ECOM). ON (top) and OFF (bottom) responses are analyzed separately. Scale bar = 100 µm.
D) Summary data represented as polar plots of the vectors from the excitatory (center) to the inhibitory receptive fields in vDSGCs (orange, left) and nDSGCs (blue, right) for ON (solid) and OFF (dashed) responses.

E) Left: Summary data showing magnitude (left) of spatially offset inhibition (Vector from E to I) for vDSGCs (orange) and nDSGCs (blue). Spatial offset predicted from the temporal offset of excitation and inhibition during preferred direction motion of a moving bar stimulus (Figure 1) indicated in grey. Statistical Significance across cell types assessed with Wilcoxon Rank-Sum test (*p<0.05). Statistical significance between measured and predicted spatial offset determined by one-sided t-test (all p values< 0.001). Right: Summary data showing the angular deviation of spatially offset inhibition from the preferred direction of vDSGCs (orange) and nDSGCs (blue).

F) Summary data representing the orientation of the EPSC’s receptive field relative to the orientation of the IPSC’s receptive field in vDSGCs (orange, top) and nDSGCs (blue, bottom) for ON (filled) and OFF (open) responses. Pearson’s correlation coefficients presented in Table 2.

G) Example scatter plots of EPSC vs. IPSC amplitude per pixel in vDSGCs (orange, left) and nDSGCs (blue, right) for ON (filled) and OFF (open) responses. Trend lines computed using least squares regression. Pixels with current amplitude below 5% of the maximum were excluded. Inset: Coefficient of determination (R2) for each example cell.

H) Summary data of R2 values for each vDSGC (orange) and nDSGC (blue).

Figure 3: vDSGCs and nDSGCs display comparable distribution of synaptic inputs along their preferred-null axis.

A) Summary data displaying the normalized amplitude of the inhibitory (magenta) and excitatory (green) inputs along the null-preferred axis of vDSGCs (top, n=17 cells) and nDSGCs (bottom, n=15 cells). ON (left) and OFF (right) responses plotted separately.

B) Summary data representing the distribution of the locations of the peak inhibitory (magenta) and excitatory (green) inputs along the null-preferred axis of vDSGCs (top) and nDSGCs (bottom).

C) Summary data representing the locations of the peak excitatory (E) and inhibitory (I) inputs along the null-preferred axis of vDSGCs (orange, top) and nDSGCs (blue, bottom). Statistical Significance determined with a paired t-test (p>0.05).

Figure 4: Spatial organization of receptive fields differs from dendritic morphology.

A) Example vDSGC (left) and nDSGC (right) dendritic skeletons. Orientation on the retina indicated by arrows, with preferred direction in bold. Scale bar = 100 µm.

B) Summary data represented in polar plots of the vectors from the soma to the dendrites (left), the excitatory (middle) and the inhibitory (right) receptive field center of mass in vDSGCs (top, orange) and nDSGCs (bottom, blue). Data for ON (solid) and OFF (dashed) plotted separately.

C) Summary data displaying the relationship between the orientation of dendritic morphology and the orientation of the vector from the soma to the excitatory receptive field (EPSC) center of mass (left) and to the inhibitory receptive field (IPSC)(middle)in vDSGCs (orange) and nDSGCs (blue). Data for ON (filled circle) and OFF (open circle) plotted separately. Pearson’s correlation coefficients determined no significant correlations between dendrite angle and EPSC or IPSC locations (p> 0.05).
D) Summary data comparing the relationship between dendritic area and EPSC (left) and IPSC (right) response areas within the receptive field, and the area of the dendrites for each vDSGC (orange) and nDSGC (blue). Data for ON (filled circle) and OFF (open circle) plotted separately. Statistical significance of the EPSC/Dendrite and IPSC/Dendrite ratio determined with one-sided t-test and compared to a ratio of 1 (PSC = Dendrite area) - All p values <0.001.

**Figure 5: DSGC glutamatergic receptive field is more restricted to the dendritic field.**

A) Left: Summary data represented as polar plots of the vectors from the soma to the excitatory glutamate receptive field center of mass in the presence of 100 µM Hexamethonium in vDSGCs (orange, top) and nDSGCs (blue, bottom) for ON (solid) and OFF (dashed) responses. Right: Summary data representing the deviation of the vector angle (right) from the vDSGC (orange, top) and nDSGC (blue, bottom) preferred direction. Data for ON (filled circle) and OFF (open circle) plotted separately.

B) Summary data displaying the relationship between the orientation of the vector from the soma to the glutamatergic excitatory receptive field (EPSCGlu) center of mass, relative to the orientation of the dendritic center of mass in vDSGCs (orange, n=5 cells) and nDSGCs (blue, n=9 cells).

C) Summary data comparing the relationship between dendritic area and the glutamatergic excitatory (EPSCGlu) response areas within the receptive field, and the area of the dendrites for each vDSGC (orange) and nDSGC (blue). Data for ON (filled circle) and OFF (open circle) plotted separately. Statistical significance of the EPSCGlu/Dendrite ratio determined with one-sided t-test and compared to a ratio of 1 (EPSCGlu=Dendrite area) - All p values <0.001.
REFERENCES


underlying direction selectivity in the retina. *Nature*. https://doi.org/10.1038/nature09600


FIGURE 1

A. Dendrite asymmetry (µm)

B. DSI

C. DSI

D. PD

E. E and I offset
FIGURE 2

A. vDSGC

B. Dendrites Excitation Inhibition

C. I relative to E

D. nDSGC

E. Spatial offset

F. vDSGC nDSGC

G. Deviation from PD

H. Excitation Vs. Inhibition Amplitude (R^2)
FIGURE 3

A. Normalized Peak Amplitudes

B. E and I peak locations

C. E and I peak locations
FIGURE 4

A. vDSGCs and nDSGCs with dendrites showing ON and OFF states.

B. Dendrites with Excitatory RF and Inhibitory RF diagrams.

C. Scatter plots showing EPSC Angle and Dendrite Angle, and IPSC Angle and Dendrite Angle.

D. Scatter plots showing EPSC area and IPSC area against Dendrite area.

Legend: ON - ON state, OFF - OFF state, Dendrite Angle, EPSC Angle, IPSC Angle.
Figure 5

A. Glutamate RF

B. EPSC$_{Glu}$ Angle

C. vDSGC

Glutamate RF = Dendrite

EPSC$_{Glu}$ area (µm$^2$)

Dendrite area (µm$^2$)