“Silent” NMDA Synapses Enhance Motion Sensitivity in a Mature Retinal Circuit

Highlights

- NMDA/non-NMDA/GABA signals driving DSGCs do not scale proportionately
- A high-sensitivity NMDA pathway to DSGCs is shared with starbursts
- SBEM reveals common glutamate input to starbursts and DSGCs
- Contextual “multiplicative” and “additive” scaling by NMDA receptors

Authors

Santhosh Sethuramanujam,
Xiaoyang Yao, Geoff deRosenroll,
Kevin L. Briggman, Greg D. Field,
Gautam B. Awatramani

Correspondence
gautam@uvic.ca

In Brief

Sethuramanujam et al. demonstrate that common bipolar input to DSGCs and GABAergic/cholinergic starburst amacrine cells is differentially processed using NMDA and AMPA receptors, respectively. Results further indicate how this synaptic arrangement enhances the DSGC’s ability to code direction at threshold contrast.
“Silent” NMDA Synapses Enhance Motion Sensitivity in a Mature Retinal Circuit

Santhosh Sethuramanujam,1 Xiaoyang Yao,2 Geoff deRosenroll,1 Kevin L. Briggman,3 Greg D. Field,2 and Gautam B. Awatramani1,4,*

1Department of Biology, University of Victoria, Victoria, BC V8W 3N5, Canada
2Department of Neurobiology, Duke University School of Medicine, Durham, NC, USA
3Department of Computational Neuroethology, Center of Advanced European Studies and Research (caesar), 53175 Bonn, Germany
4Lead Contact
*Correspondence: gautam@uvic.ca
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SUMMARY

Retinal direction-selective ganglion cells (DSGCs) have the remarkable ability to encode motion over a wide range of contrasts, relying on well-coordinated excitation and inhibition (E/I). E/I is orchestrated by a diverse set of glutamatergic bipolar cells that drive DSGCs directly, as well as indirectly through feedforward GABAergic/cholinergic signals mediated by starburst amacrine cells. Determining how direction-selective responses are generated across varied stimulus conditions requires understanding how glutamate, acetylcholine, and GABA signals are precisely coordinated. Here, we use a combination of paired patch-clamp recordings, serial EM, and large-scale multi-electrode array recordings to show that a single high-sensitivity source of glutamate is processed differentially by starbursts via AMPA receptors and DSGCs via NMDA receptors. We further demonstrate how this novel synaptic arrangement enables DSGCs to encode direction robustly near threshold contrasts. Together, these results reveal a space-efficient synaptic circuit model for direction computations, in which “silent” NMDA receptors play critical roles.

INTRODUCTION

Synapses lacking the functional expression of AMPA receptors are often considered “silent” (Kerchner and Nicoll, 2008), as glutamate binding to NMDA receptors alone does not substantially activate them (due to the voltage-dependent block of NMDA receptors by external Mg2+ ions). Studies over the past two decades have established silent synapses as prominent cellular substrates for synaptic plasticity in the developing brain (reviewed by Kerchner and Nicoll, 2008). However, their unique dependency of two simultaneous conditions—presynaptic glutamate release and postsynaptic depolarization—also make NMDA-dominated synaptic pathways attractive candidates for mediating silent modulatory signals observed in mature brain (Herrero et al., 2013; Rivadulla et al., 2001; Self et al., 2012; Shima and Tanji, 1998; Wang, 2001). For example, elegant in vivo pharmacological studies demonstrate that the modulatory effects of covert attention signals on the responsiveness of neurons in the visual cortex are abolished when NMDA receptors are antagonized (Herrero et al., 2013; Self et al., 2012). However, there is little direct evidence for silent synapse expression in the mature CNS (reviewed by Hanse et al., 2013; Kerchner and Nicoll, 2008), and the synaptic mechanisms by which they enhance neural computations remain unexplored.

Assessing the contributions of silent NMDA receptor-dominated pathways to neural computation is difficult because these pathways are poorly defined and intermingled with other excitatory pathways, such as those driven by AMPA receptors and/or non-glutamatergic receptors. Interestingly, in the direction-selective (DS) retinal circuit that we examine here, we recently observed that under cholinergic receptor blockade, bipolar cell glutamate inputs to ON-OFF DS ganglion cells (DSGCs; output neurons that relay directional information from retina to higher visual centers) evoked by low-contrast stimuli were mediated by silent NMDA synapses (Sethuramanujam et al., 2016). The AMPA receptor-mediated component of the DSGC’s synaptic response only appeared at higher stimulus contrasts. In contrast, however, a number of other studies indicate that AMPA/NMDA receptor-mediated inputs to DSGCs scale together as a function of contrast, consistent with observations in other types of ganglion cells, suggesting that AMPA and NMDA receptors are driven by a common source of glutamate (Buldyrev et al., 2012; Diamond and Copenhagen, 1995; Manookin et al., 2010; Poleg-Polsky and Diamond, 2016b; Stafford et al., 2014). These diverging results have given rise to two distinct models that explain how the DS circuit coordinates excitation and inhibition (E/I) over a range of stimulus contrasts (Figures 1A and 1D), which underlies the DSGC’s robust ability to compute direction (Grzywacz and Amthor, 2007; Nowak et al., 2011; Poleg-Polsky and Diamond, 2016b).

In the conventional model, DSGCs compute direction by comparing the relative strength of non-directional bipolar cell inputs mediated by AMPA/NMDA receptors with highly directional starburst inputs mediated by GABA_B receptors (reviewed by Mauss et al., 2017; Vaney et al., 2012). In this model, changing the strength of bipolar cell input relative to inhibition strongly affects the tuning properties of DSGCs. Thus, maintaining DS tuning properties over a range of contrasts requires glutamate,
GABA, and acetylcholine (ACh) signals to DSGCs to scale proportionally as a function of contrast (Figure 1B). To achieve such presynaptic balance, glutamate release from bipolar cells onto DSGCs must be curbed until stimuli are sufficiently strong to evoke GABA release from starbursts. This is thought to be accomplished by using high- and low-sensitivity bipolar cells (Ichinose et al., 2014; Odermatt et al., 2012; Poleg-Polsky and Diamond, 2016b) to independently drive starbursts and DSGCs, in a way that compensates for the starburst’s threshold non-linearity (Figures 1A and 1B) (Poleg-Polsky and Diamond, 2016b).

As this model requires the matching of presynaptic GABA/glutamate/ACh signals, we refer to it as the “matched” model for direction selectivity in ganglion cells.

In the alternate “silent synapse” model, glutamate signals produced by low-contrast stimuli (e.g., that fail to evoke starburst release) are “unmatched” with GABA and ACh. In this model, unbalanced glutamate signals are rendered silent at DSGCs because they are processed predominantly by NMDA receptors (Figures 1D and 1E), which alone do not drive spiking (Sethuramanujam et al., 2016). NMDA receptors serve to amplify coincident ACh/GABA signals from starbursts that contain accurate directional information (Lee et al., 2010; Sethuramanujam et al., 2016). Thus, the requirement for high/low-sensitivity bipolar cells to drive balanced E/I is obviated. Importantly, in this model, the bipolar inputs in the low-contrast regime are strictly modulatory and their strength is expected to alter response amplitude, but not the direction tuning properties of DSGCs, contrasting with their role in the matched model.

The types of bipolar cells in the DS circuit have been described in great anatomical and physiological detail (Ding et al., 2016; Duan et al., 2014; Greene et al., 2016; Helmstaedter et al., 2013; Kim et al., 2014). Together, these studies suggest that overlapping types of bipolar cells drive DSGCs and starbursts. Furthermore, previous anatomical studies relied on contact area analysis as a proxy for synaptic connectivity, which in some cases can be ambiguous (Ding et al., 2016). Therefore, whether starbursts and DSGCs are driven independently, or by shared input, remains an open question. Conclusive resolution of this issue would enable matched and silent synapse models for direction selectivity to be clearly distinguished.

At an operational level, both models ensure direction selectivity is maintained through the DSGC’s dynamic range. However, one potentially important difference could manifest in the way NMDA receptors scale the DSGC’s contrast response function. In the matched model, NMDA receptors scale the DSGC’s response in a multiplicative manner, simply because they scale together with non-NMDA receptor-mediated inputs (Murphy and Miller, 2003; Poleg-Polsky and Diamond, 2016a, 2016b). Multiplicative scaling increases the gain of the DSGC input-output function (increase in $R_{\text{max}}$; Figure 1C). Conversely, in the silent synapse model, the NMDA/non-NMDA ratio changes with stimulus contrast (Figure 1E). Thus, NMDA receptors should amplify responses more strongly at low contrasts and possibly increase the overall sensitivity of the DSGC’s response to motion. A shift of the DSGC’s input-output function along the contrast axis is considered an additive operation (Figure 1F) (Silver, 2010). This would be greatly advantageous, provided the strong amplification supplied by NMDA receptors does not disrupt DS coding in this regime.
To reconcile these conflicting views and further our understanding of the workings of the DS circuit, we monitored the sensitivity of NMDA and non-NMDA pathways simultaneously in neighboring pairs of starbursts and DSGCs. This analysis indicated the presence of a high-sensitivity silent NMDA receptor-mediated pathway to DSGCs that was shared with starbursts (although starburst inputs were processed by AMPA receptors). Direct visualization of synapses with serial block-face electron microscopy (SBEM) (Denk and Horstmann, 2004) indicated an abundance of common input to DSGCs and starbursts from individual bipolar cells. Finally, pharmacological analysis of DSGC spiking responses recorded on a large-scale multi-electrode array (MEA) indicated that NMDA receptors enhance the contrast sensitivity of DSGC output while preserving their directional tuning properties, down to threshold contrast levels. Together, these findings lead us to propose a unifying “silent synapse” model for direction selectivity in which NMDA receptors help coordinate E/I in a way that maximizes output sensitivity (Figure 1F).

RESULTS

In this study, our experiments address three specific questions. (1) What are the relative contrast sensitivities of NMDA versus non-NMDA receptor-mediated inputs to DSGCs? (2) Do starbursts and DSGCs share common bipolar cell input? (3) How do NMDA receptors modulate the DSGC’s output as a function of stimulus direction versus stimulus contrast?

A High-Sensitivity Glutamatergic Pathway to DSGCs Mediated by NMDA Receptors

In whole-mount retinal preparations, genetically labeled starbursts and DSGCs were targeted for patch-clamp analysis using two-photon microscopy (Rivlin-Etzion et al., 2011; Trenholm et al., 2011). To accurately estimate the relative contrast sensitivities of NMDA and non-NMDA receptor-mediated inputs to DSGCs, we simultaneously measured responses to a series of moving spots of increasing contrasts (250 µm diameter, velocity ~1 mm/s, Weber contrast 3%–300%) in neighboring pairs of starbursts and DSGCs (Figure 2A). Peak synaptic responses were fit to the Naka-Rushton function to estimate the contrast sensitivity of the input pathways (listed in Table S1). The starburst excitatory response (measured in whole-cell voltage-clamp mode, \( V_{\text{HOLD}} = -60 \, \text{mV} \)) provided an internal control. This was particularly important because it helped overcome the alterations in the contrast response functions that arose from variability in the absolute sensitivity of individual cells estimated in different retinal preparations (Figure S1).

We found that excitatory inputs to starbursts were significantly more sensitive to stimulus contrast compared to both non-NMDA (nACh+AMPA) and GABA receptor-mediated inputs to DSGCs, measured at –60 mV (~\( E_{\text{CI}} \)) and 0 mV (~\( E_{\text{EXCITATION}} \)), respectively (Figures 2B and 2C; \( p < 0.005 \)). The non-NMDA and GABA inputs scaled almost perfectly together (Figures 2B and 2C; Table S1; \( p = 0.41 \)). These measurements were consistent with the notion that bipolar cells with different sensitivities drive starbursts and DSGCs, and that E/I inputs to DSGCs scale together as a function of contrast (Poleg-Polsky and Diamond, 2016b). However, when inputs to DSGCs were measured at +40 mV, a more complex circuit arrangement was revealed.

At depolarized potentials, the sensitivity of synaptic inputs measured in DSGCs was well matched to that of starburst inputs (Figures 2B and 2D; Table S1; \( p = 0.14 \)). The simplest interpretation of this finding is that depolarization reveals an additional contribution of NMDA receptors (by removing Mg²⁺ block) driven by a high-sensitivity pathway. Confirming this notion, the application of a selective NMDA receptor antagonist (50 µM D-AP5) shifted the DSGC’s contrast response function to the right (Figure 2E; Table S1; \( p < 0.05 \)). Similar results were obtained when NMDA and AMPA pathways were isolated pharmacologically (in the presence of GABA_A/ACH/kainate receptor antagonists; Figure 2F). However, the data under pharmacological isolation should be interpreted with caution, as these conditions might exacerbate glutamate “spillover,” leading to an overestimate of NMDA receptor contributions (Sagdullaev et al., 2006; Zhang and Diamond, 2009).

Two lines of evidence suggest that the effects of AP5 are largely postsynaptic in origin. First, the sensitivity of the residual synaptic responses measured in D-AP5 was similar to the non-NMDA and inhibitory inputs measured in control conditions (compare Figures 2C and 2E; Table S1). Second, the NMDA antagonist did not affect the sensitivity of the starbursts (Figure S3) (Poleg-Polsky and Diamond, 2016a). Taken together, these results suggest that multiple bipolar cells with different sensitivities drive starbursts and DSGCs in parallel, but do so using distinct complements of glutamate receptors.

The relative high sensitivity of the NMDA pathway to DSGCs was also apparent upon examining threshold responses. Responses to weak stimuli (<20% Weber contrast) were detected in DSGCs only at the most depolarized potentials (+40 mV; Figure 2B), a signature of silent NMDA synapses (Kerchner and Nicoll, 2008). Indeed, these responses were completely blocked by D-AP5, confirming that they were mediated by NMDA receptors (Figure 2E; \( n = 5 \)). In addition, the peak amplitude of the threshold responses evoked by preferred- and null-direction motion was similar (Figure S2; preferred = 132 ± 36 pA; null = 143 ± 45 pA; \( p = 0.41 \); responses measured at 12% ± 2% Weber contrast). This not only suggests that glutamate release from bipolar cells is non-directional at its threshold (Park et al., 2014; Yonehara et al., 2013), but also confirmed that responses did not contain inhibitory currents, since inhibition measured at 0 mV was strongly DS at its threshold (note that the threshold for inhibition was relatively higher compared with threshold for NMDA excitation; Figure S2). Importantly, the minimal contrast level for evoking NMDA inputs to DSGCs matched the threshold of inputs to starbursts (Figure 2B, left panel; Table S1). Together, these results support the notion that a high-sensitivity glutamate pathway drives DSGCs and starbursts in parallel, and establishes NMDA as the dominant synaptic conductance in DSGCs near threshold contrasts.

Previous studies provided mixed views on whether presynaptic pathways to DSGCs, including those mediated by AMPA and NMDA receptors, scale proportionately as a function of contrast (Lipin et al., 2015; Poleg-Polsky and Diamond, 2016b;
Here, the paired recordings directly demonstrate that high-sensitivity inputs to starbursts scale with NMDA receptor-mediated inputs, but not with GABA/AMPA/ACh inputs to DSGCs (Figure 2). These differences were not easily apparent if DSGC responses alone were averaged over a small population without accounting for the contrast sensitivity of a nearby starburst (Figure S1). This is likely one source for the inconsistencies in previous studies. It is also possible that different experimental conditions contribute to some of the observed differences.
A Shared Glutamatergic Pathway to Starbursts and DSGCs

The finding that glutamate receptor-mediated synaptic responses in starbursts and DSGCs could share the same sensitivity and threshold suggests that they arise either from the same presynaptic bipolar cells, or from different bipolar cells with similar sensitivities. To examine the functional connectivity patterns, we next examined the degree to which spontaneous NMDA and AMPA receptor-mediated inputs were temporally correlated in neighboring starbursts and DSGCs with overlapping dendritic fields (inter-somatic distance < 50 μm; Figure 3).

In these experiments, AMPA and NMDA receptor-mediated activity was isolated pharmacologically using the cocktail of GABA_A/nACh/kainate receptor antagonists. The presence of the kainate receptor antagonist (10 μM UBP310), which selectively hyperpolarizes OFF bipolar cells (Borghuis et al., 2014), was used to limit activity from the OFF pathway. Under these conditions, spontaneous excitatory postsynaptic currents (EPSCs) occurred in bursts of varying amplitudes (10–300 pA) that could last for hundreds of milliseconds (Figures 3A and 3D), reflecting the coordinated release of multiple vesicles and possibly glutamate “spillover” (Sagdullaev et al., 2006; Zhang and Diamond, 2009). While this made it difficult to carry out a quantitative analysis to estimate the precise number of shared inputs to starbursts and DSGCs (Grimes et al., 2014), it provided a strong indication of the pharmacological properties of shared and unshared input occurring on a coarser scale, as described below.

Spontaneous activity in starbursts and DSGCs was strongly correlated (CC = 0.57 ± 0.08; n = 6) on a millisecond timescale with a half-width at half-maximum of 70 ± 11 ms (Figure 3C). This strong and temporally precise correlation observed between starburst and DSGC inputs indicates a common presynaptic source (Grimes et al., 2014; Trong and Rieke, 2008). Application of D-AP5 strongly reduced correlated activity (Figures 3B and 3C; CC = 0.19 ± 0.08; n = 6; p < 0.001). Similar results were obtained when NMDA receptors were blocked postsynaptically either by measuring AMPA EPSCs at −60 mV (CC_{60 mV} = 0.16 ± 0.03; n = 3; data not shown) or by including MK-801 in the electrode solution (Figures 3D–3F; CC_{initial} = 0.6 ± 0.09; CC_{late} = 0.2 ± 0.04; n = 6; p < 0.01). Together, these results indicate that the effect of blocking NMDA on correlated activity was largely postsynaptic in origin. It is important to note that the weak correlations observed during NMDA receptor blockade are not a result of reducing the amplitude of spontaneous events in DSGCs. The example DSGCs shown in Figure 3 were chosen to illustrate this point. In these DSGCs, the extent by which NMDA blockade reduced responses was different (~70%, Figures 3A and 3B versus ~25% reduction, Figures 3D and 3E), yet the residual non-NMDA events were correlated with starburst activity to a similar extent (Figures 3C and 3F).

In addition, the weak correlations in the AMPA receptor-mediated inputs, in conjunction with the distinct contrast sensitivities of AMPA and NMDA inputs (Figure 2F), argue against the idea that inhibitory receptor blockade induced widespread synchrony. These observations suggest that AMPA receptor-driven synapses in DSGCs are not shared with starbursts to the same extent as NMDA receptor-driven synapses. While it is not possible to directly determine whether spontaneous correlated activity derives from the high- or low-sensitivity glutamate pathway in the DS circuit, the finding that NMDA receptors alone mediate the threshold response in DSGCs (Figure 2B) leads us to posit that it is the high-sensitivity pathway that is shared.

Figure 3. The NMDA Receptor-Mediated Pathway to DSGCs Is Strongly Shared with Starbursts

(A and B) Spontaneous AMPA/NMDA inputs measured simultaneously in a starburst and a neighboring DSGC in control Ringer’s (A) or in the added presence of D-AP5 (B). Starbursts were held at −60 mV and DSGCs at +40 mV. AMPA/NMDA inputs were isolated in the cocktail of antagonists (SR-95531, hexamethonium, and UBP310).

(C) A comparison of the cross-correlation function for activity in the pair shown in (A) and (B) (left), or population average (n = 6 pairs; right; mean ± SEM).

(D and E) Spontaneous AMPA/NMDA inputs recorded in a starburst-DSGC pair with MK801 in the DSGC internal. (D) and (E) show the recording at 4 and 16 min after break-in, respectively.

(F) A comparison of the cross-correlation function for activity in the pair shown in (D) and (E) (left). The average cross-correlation function computed across six pairs measured during the early or late phases of the recording is illustrated in the right panel (mean ± SEM).

Anatomical Evidence for Common Synaptic Input to Starbursts and DSGCs

While previous studies have identified overlapping types of bipolar cells driving starbursts and DSGCs, whether an individual bipolar cell can contact both a starburst and a DSGC is not clear (Ding et al., 2016; Duan et al., 2014; Greene et al., 2016; Helmsstaedter et al., 2013; Kim et al., 2014). To determine whether the underlying anatomical connectivity supports the functional
evidence for common input, we next examined connectivity in an SBEM dataset in which intracellular structures were preserved (Ding et al., 2016). Bipolar cell ribbon synapses could be identified by the ribbon-containing terminal apposed to two postsynaptic structures in the form of a dyad (Figures 4A and 4B). The ribbons themselves could be unequivocally identified in 3D as faint gray sheets decorated by a halo of vesicles (Ding et al., 2016), rather than as intense, electron-dense structures that are typically observed in post-stained serial sections. A 3D reconstruction of the same dyad (right; color-coded as in left); the 3D ribbon reconstruction itself is shown in black. Scale bar, 1 μm.

To investigate the possibility of common input, we reconstructed the entire axonal terminal of a bipolar cell contacting the ON dendrites of a DSGC. In this case, the bipolar cell was identified as type BC5t based on its characteristic “thick” stratification pattern (Ding et al., 2016; Greene et al., 2016). In this terminal, we identified 22 ribbon synapses distributed across the axonal tree (Figure 4C). To determine which cells this bipolar cell activated, we partially reconstructed the two processes in these dyads were reconstructed to identify postsynaptic partners, including DSGCs, starbursts, WACs, and narrow-field amacrine cells (NACs). Scale bar, 10 μm. (D) The frequency of the bipolar cell contacts with four cell types (DSGCs, starbursts, WACs, and NACs) is shown in the matrix. The combinations are color-coded as indicated in (C).

(E) Alternatively, dyadic connections (n = 64; ~20–30 bipolar cells) were identified on the ON dendritic tree of a DSGC (gray), from which the second postsynaptic process was traced. Dyads on the reconstructed DSGC dendritic tree (gray) are color-coded according to the second dyadic partner: another DSGC (red), starburst (cyan), or wide-field amacrine cell (blue). Scale bar, 25 μm. (F) Fraction of DSGC, starburst, or wide-field processes that are shared with dyads contacting a single DSGC (gray cell in E).
approach to confirm the prevalence of common input to DSGCs and starbursts. This entailed viewing shared connections from the perspective of the DSGC. We annotated 64 ribbon synapses (arising from ~20–30 bipolar cells, assuming each bipolar cell makes, on average, 2–3 synapses/DSGC, as they do for starbursts) (Ding et al., 2016), contacting a single DSGC in the ON sublamina of the inner plexiform layer and then traced the second postsynaptic partner present in the dyad (Figure 4E). This analysis revealed that dyads in a DSGC were shared with another DSGC (53%), with an ON starburst (41%; Figures 4E and 4F), or in the rare case with a wide-field amacrine cell (6%). While these analyses preclude the identification of the types of bipolar cells contacting starbursts and DSGCs, they strongly indicate that these cells share bipolar cell input, both on the fine spatial scale of dyads (Dacheux et al., 2003) and on the coarser scale of whole bipolar cell axon terminals, providing strong support for our physiological measurements indicating common input.

**“Arithmetical” Scaling Operations Mediated by NMDA Receptors**

Having gained functional and anatomical evidence for the silent synapse model, we next sought to understand the rationale for such a design. We envisioned that the high-sensitivity pathway would have the largest impact on responses evoked by low-contrast stimuli, but how it affected the DSGC’s input-output function (response gain and/or $C_{50}$) and DS coding at lower contrasts was harder to predict based on the conductance measurements alone. We employed a simple two-compartmental computational model (Figure 5; see STAR Methods for details) to build an intuition of how NMDA receptors may behave differently across stimulus contrasts and directions. Responses were driven by inhibitory and excitatory synaptic inputs that grew in contrast, similar to our experimental measurements. To simulate stimulus direction, excitation was set to be non-directional while GABA inhibition was highly directional (Poleg-Polsky and Diamond, 2016a). Interestingly, although the DSGC spiking response was modulated by stimulus contrast and stimulus direction to a similar extent, NMDA receptors had a different effect in each context (Figures 5).

In the matched model configuration, in which synaptic inputs scale proportionately as a function of contrast, we found that NMDA-mediated inputs amplified responses in a multiplicative manner (Murphy and Miller, 2003; Poleg-Polsky and Diamond, 2016a). This is indicated by the stable fractional contribution of NMDA receptors across the entire contrast range (Figure 5A, right axis). However, in a similar model in which the contrast sensitivity of the NMDA inputs was increased according to our experimental measurements (Figures 2C–2E), the NMDA receptor contribution to the DSGC’s spiking response became highly dependent on stimulus contrast (Figure 5D, right axis), being...
maximal at the lowest contrasts. As a result, the contrast response function in the absence of NMDA receptors was rightward shifted (C_{50}, 26.5% Weber contrast in control; 32.5% without NMDA receptors) (Figure 5D). However, as this increase in sensitivity was an effect of a disproportionate contrast-dependent scaling of the DSGC’s spiking response amplitude (y-scaling), this operation can be considered “pseudo-additive,” to distinguish it from real x-scaling operations (Silver, 2010). Interestingly, even at low contrasts (30%) where NMDA receptors strongly amplify responses, NMDA receptors scaled responses in a way that preserved DS tuning properties of the model DSGC (Figure 5G), similar to their effects observed at higher contrasts (Poleg-Polsky and Diamond, 2016a). Thus, a simple model that captures the multiplicative effects of NMDA receptors predicts that the high-sensitivity NMDA pathway produces an additive scaling of the DSGC output as a function of contrast.

To understand the synaptic mechanisms underlying the context-dependent modulation by NMDA receptors, we examined the synaptic currents/conductances underlying the DSGC’s responses under different conditions (Figures 5B, 5E, and 5H). In the case of the silent synapse model, at low contrasts, the total current through the NMDA receptors was large relative to the non-NMDA and GABA receptor-mediated current (Figure 5E). The NMDA/non-NMDA ratio subsequently falls with increasing contrast because the non-NMDA conductance sharply increases (Figure 5F). This occurs despite the voltage-dependent increase of the absolute NMDA conductance observed with increasing contrasts. Consequently, the relative contribution of NMDA to spiking decreases over the contrast range, resulting in the pseudo-additive scaling operation observed in the silent synapse model (Figure 5D). However, in other cases (direction tuning or in the matched model), the NMDA/non-NMDA conductance ratio increases with response magnitude (Figures 5C and 5I), giving rise to multiplicative scaling (Figures 5A and 5G). Note, if NMDA receptors were made to be voltage-independent in the model, the multiplicative scaling properties on the directional responses are lost (Figure S4) (Poleg-Polsky and Diamond, 2016a). Thus, these simple models simulating the DSGC’s responses over direction and contrast indicate that the relative NMDA conductance (rather than absolute NMDA conductance/current) is a key factor that determines how NMDA receptors shape responses.

To test these model predictions, we examined the effects of blocking NMDA receptors on the spiking behavior of DSGCs. An MEA was used to record activity from a population of DSGCs across a range of directions and contrast levels (eight directions; 5%-300% Weber contrast). ON-OFF DSGCs were distinguished from other ganglion cells based on their direction and speed tuning (Figures 6A and SS; STAR Methods). A heatmap of the average number of spikes/trial measured across the population of DSGCs plotted as a function of direction and contrast (Figure 6C), depicts for the first time the ability of mouse DSGCs to maintain their directional tuning properties across a large contrast range, consistent with the behavior of their counterparts in the rabbit retina (Grzywacz and Amthor, 2007; Nowak et al., 2011). However, small but statistically significant contrast-dependent changes in tuning reflected in direction selectivity index (DSI; STAR Methods) were observed in the low- (DSI_{10%} = 0.46 ± 0.04; DSI_{20%} = 0.61 ± 0.02; p < 0.005, Wilcoxon signed-rank test) and high-contrast ranges (DSI_{110%} = 0.57 ± 0.02; DSI_{130%} = 0.47 ± 0.02; p < 0.05, Wilcoxon signed-rank test).

We found blocking NMDA receptors with D-AP5 reversibly reduced the response amplitude for all four populations of ON-OFF DSGCs (Figure 6B) and thus the data from all types were combined. Consistent with previous studies, the NMDA receptor antagonist did not strongly affect the directional tuning properties of DSGCs (control DSI_{40%} = 0.65 ± 0.01; D-AP5 DSI_{40%} = 0.63 ± 0.02; p = 0.39; Wilcoxon signed-rank test; Figures 6C–6E). Moreover, the fractional response blocked by NMDA antagonists did not change with direction across a range of stimulus contrasts (Figures 6G and 6H), indicating the robustness of the multiplicative scaling properties of NMDA receptors (Poleg-Polsky and Diamond, 2016a).

However, when viewed as a function of stimulus contrast, we found NMDA receptors did not scale responses in a multiplicative manner, consistent with the prediction of our silent synapse model neuron. D-AP5 reduced responses evoked by low-contrast stimuli (<20% contrast) more strongly than it reduced responses to high-contrast stimuli (>20% contrast; Figures 6F and 6H; p < 0.001, Wilcoxon signed-rank test). This resulted in an increase in both the semi-saturation constant (control, 55.5% ± 5% Weber contrast; AP5, 72.6% ± 4%; p < 0.0001, Wilcoxon signed-rank test) and the absolute threshold (control, 30% ± 3%; AP5, 51% ± 5%; p < 0.0001; Wilcoxon signed-rank test) of the DSGC’s contrast response function, which are hallmarks of additive operations (Figure 6F) (Silver, 2010). The NMDA transformation across the majority of the contrast range is explained by arithmetically scaling the responses to increasing contrasts in the y-dimension, using additive (77%) and multiplicative (23%) scaling factors (Figure 6G). Importantly, the additive shifts occurred for all stimulus directions, as indicated by the percent block plots (Figures 6G and 6H). In contrast, simultaneously recorded ON ganglion cells with brisk transient responses exhibited only a minor change in their contrast sensitivity upon D-AP5 application (C_{50} in control, 27.1% ± 1%; C_{50} in AP5, 30.0% ± 1%; p < 0.0001, Wilcoxon signed-rank test; Figure S5), indicating that time-dependent changes in overall retinal sensitivity did not confound the pharmacology. Thus, NMDA receptors instantiate multiple “arithmetical” operations that enhance the DSGC’s sensitivity, without compromising direction encoding.

Importantly, the large contribution of NMDA receptors to the low-contrast response is not due to a simple thresholding effect. This is directly indicated by the finding that responses of similar amplitudes to stimuli moving in non-preferred directions (high contrast) were significantly less affected by D-AP5 (Figure 6H). This can be seen more clearly when the percent block is plotted against the number of spikes (Figure S6C). The reason for the different amounts of blocks under these two conditions (low contrast in the preferred direction and high contrast in the null direction) is that at low contrasts, the NMDA synaptic conductance is the dominant conductance (Figure 2B), while in the non-preferred directions, it is dwarfed by the opposing inhibitory conductances that combine to
generate weak spiking responses (Taylor and Vaney, 2002). Therefore, the contribution of NMDA receptors in this context depends on their conductance relative to other synaptic conductances. Thus, the large contribution of NMDA receptors at low contrasts, imparted by the high-sensitivity pathway (Figure 5E), is paramount to creating the observed pseudo-aditive effect of NMDA on contrast-modulated DSGC spiking responses.

**Figure 6. Additive and Multiplicative Scaling Operations Mediated by NMDA Receptors**

(A) An example DSGC response recorded on an MEA. Spikes were evoked by drifting bars (240 µm wide on the retina, moving at 960 µm/s; 8 directions, 7 contrasts indicated by different colors; 10 trials for each contrast). ON-OFF DSGCs were distinguished from other ganglion cells based on their tuning properties (Figure S5). The bottom plot shows the tuning curves of this DSGC plotted for multiple contrasts (mean ± SEM). Responses at each contrast were fit with a cosine function.

(B) NMDA receptor antagonist, 50 µM D-AP5, reversibly reduced responses (preferred motion; 20%–40% contrast) to a similar extent in the four subtypes of ON-OFF DSGCs.

(C) A heatmap of the population DSGC response as a function of direction and contrast measured in control conditions (the color bar indicates the average number of spikes/trial on a logarithmic scale). Responses were averaged across the four types of ON-OFF DSGCs (21 superior, 12 inferior, 27 anterior, and 6 posterior-coding DSGCs).

(D) Same as (C), except responses were measured in the presence of D-AP5.

(E) Cross-sections of the heatmaps along the direction axis, in control and in D-AP5 (1a from C; 1b from D). The solid line indicates the Gaussian fit of the control data (black circles). The same Gaussian fit could be scaled to approximate the response measured in D-AP5 (red circles; purple shaded region indicates the D-AP5-sensitive component that could be accounted for by multiplicative scaling; Figure S6). Data are represented as mean ± SEM.

(F) Cross-sections of the heatmaps along the contrast axis, in control and in D-AP5 (2a from C; 2b from D) for preferred direction stimuli. The control response (black circles) was fit with the hyperbolic Naka-Rushton equation (solid line; STAR Methods). The response measured in D-AP5 (red circles) could be approximated by scaling the control response in the Y-dimension as depicted by the dashed line (purple and yellow shaded regions indicate the D-AP5-sensitive component that could be accounted for by purely multiplicative or additive scaling operations, respectively) (Figure S5 illustrates how these scaling factors were determined). D-AP5 did not affect the responses of ON transient ganglion cells (Figure S5), which was used as an indicator of the stability of contrast sensitivity of the retina for the duration of the experiment. Data are represented as mean ± SEM.

(G) A heatmap indicating the relative suppression of responses by D-AP5 across contrasts and direction (scale bar represents percent [%] suppression; [Control-AP5]/control*100).

(H) Cross-sections of the heatmaps along the direction (gray; 1c from G) and contrast axes (black; 2c from G). The fraction of the response blocked by D-AP5 did not systematically change with direction, but varied strongly with contrast. Data are represented as mean ± SEM.
DISCUSSION

Our results characterizing the input/output function of DSGCs and starburst amacrine cells shed new light on the precise arrangement of the feedforward circuitry, highlighting the computational advantages gained by the differential expression of AMPA/NMDA receptors at specific inner retinal synapses.

A Unified Model for Contrast Invariant DS Tuning Utilizing Silent Synapses

Whether starbursts and DSGCs receive common input from bipolar cells is an important aspect of the DS circuit that remains debated (Poleg-Polsky and Diamond, 2016b; Helmstaedter et al., 2013). Here, we unequivocally identify a high degree of shared input to starbursts and DSGCs in a recent SBEM dataset (Ding et al., 2016). These results effectively complement previous studies that identify the types of bipolar cells driving starbursts and DSGCs, and together suggest that the most likely candidates for common input are the bipolar cell types 5i, 5o, and 5t (BC5s) (Ding et al., 2016; Duan et al., 2014; Greene et al., 2016; Helmstaedter et al., 2013; Kim et al., 2014; H.S. Seung, personal communication). In addition, the likely sources of uncommon input are the type 7 bipolar cells, which make strong contact with starbursts (20%–45%; Helmstaedter et al., 2013; Greene et al., 2016; Ding et al., 2016), but not DSGCs, and an anomalous source of glutamate from a specialized subset of VGlut3+ amacrine cells, which appear to drive DSGCs, but not to starbursts (Lee et al., 2014).

The presence of profuse shared afferents reinforces the general question of how feedforward E/I can be balanced in the face of threshold circuit non-linearities. Other feedforward circuits appear to circumvent non-linear processing using several specializations, all of which lead to the preferential recruitment of inhibitory interneurons. For example, afferent inputs/projections to inhibitory neurons may have higher vesicular release probabilities (Acsády et al., 1998; Maccarelli et al., 1998; Toth et al., 2000), a larger number of synapses (Bruno and Simons, 2002; Stokes and Isaacson, 2010), and/or AMPA receptor subtypes with larger unitary conductances relative to synapses driving principal neurons (Cruikshank et al., 2007; Gabernet et al., 2005; Lawrence and McBain, 2003). At thalamocortical synapses, the AMPA/NMDA receptor ratio differs between principal cells and interneurons in a way that drives inhibition with higher fidelity (Angulo et al., 1999; Hull et al., 2009; Krukowski and Miller, 2001). However, the drawback of driving inhibitory pathways more effectively is that it decreases the overall sensitivity of the circuit (Poleg-Polsky and Diamond, 2016b).

Here we propose a novel scheme in which silent NMDA receptors play a dominant role in orchestrating E/I balance at low contrast, while maximizing circuit sensitivity (Figures 1D–1F). The rationale for NMDA receptor expression becomes clear when considered in relation with the starburst input/output. Starbursts only release their neurotransmitters when their inputs are stimulated to 38% ± 5% of their maximum (Figure 2C). Because DSGCs are driven by shared input, in this regime, presynaptic glutamate and GABA signals are not balanced. As these glutamate signals are non-directional (Figure S2) (Park et al., 2014; Yonehara et al., 2013), they would likely produce non-directional spiking responses in DSGCs if conventional AMPA synapses were utilized. By using silent synapses, DSGC responses become reliant on coincident starburst cholinergic inputs for their activation (Brombas et al., 2017; Sethuramanujam et al., 2016), and thus circumvent the potential deleterious effects of non-linear processing by starbursts.

We further propose that the high-sensitivity silent NMDA receptor-mediated inputs to DSGCs and the AMPA/KA receptor-mediated inputs to starbursts originate from the same bipolar presynaptic terminals. This is based on the findings that (1) ~40% of the dyadic input to DSGCs is shared with starbursts (Figure 4); (2) NMDA inputs to DSGCs and AMPA inputs to starbursts have similar threshold sensitivities (Figure 2); and (3) spontaneous NMDA, but not AMPA, receptor-mediated activity in DSGCs was strongly correlated with starburst input (Figure 3). It should be mentioned that the measurements of correlated activity were performed under artificial conditions to isolate AMPA and NMDA receptor components, which could exaggerate correlations through indirect network mechanisms. For example, NMDA receptors that have a higher affinity for glutamate compared to AMPA receptors could in theory sense glutamate “spillover” from synapses that drive starbursts, especially under conditions in which GABA receptors are blocked (Sadgulaev et al., 2006; Zhang and Diamond, 2009). However, we found spontaneous NMDA receptor-mediated EPSCs in DSGCs to be almost as large as AMPA EPSCs measured in starbursts, making it unlikely that NMDA inputs were mediated solely by spillover mechanisms, which tend to drive relatively weaker responses in other areas of the brain (Szapiro and Barbour, 2007). Nevertheless, future studies using immuno-EM are required to confirm that AMPA and NMDA receptors are differentially expressed postsynaptically at the level of single bipolar dyads. The age-old “rule” stating that only one of the two postsynaptic processes at a dyad expresses a given ionotropic glutamate receptor subtype (Boycott and Wässle, 1999) suggests this as an intriguing possibility.

In addition to silent synapses, four other important circuit features ensure that DSGCs are able to encode direction under low-contrast conditions. First, GABAergic inhibition to DSGCs was found to be DS at the lowest detectable contrast (Figure S2). This is consistent with the idea that starburst dendrites strongly rely on high-threshold voltage-gated Ca2+ channels for transmitter release as well as for generating robust direction responses (Hausseit et al., 2007; Tukker et al., 2004). Second, in contrast to earlier reports (Lee et al., 2010), we found GABA and ACh inputs to be activated at similar contrast thresholds (Figure 2C). The precise activation of cholinergic inputs could be inferred from measurements of excitation made at ~60 mV because they are known to dominate the non-NMDA excitatory inputs to DSGCs at low stimulus contrasts (Sethuramanujam et al., 2016). Although cholinergic signals are non-DS, the combination of E/I signals generated by the starburst network contains accurate direction information (Sethuramanujam et al., 2016). Third, even near contrast threshold, where NMDA conductances are large (1 nS Gmax estimated at +40 mV), ACh input never “kicks” NMDA receptors into a self-regenerating mode. This indicates that NMDA receptor activity is well balanced by opposing conductances, likely provided by the combination of...
GABA (Krukowitz and Miller, 2001; Poleg-Polsky and Diamond, 2016a; Rivadulla et al., 2001), voltage-dependent K⁺, and leak conductances (Ferster and Miller, 2000; London and Häusser, 2005; Schiller et al., 2000). Together, this ensures that NMDA receptors amplify starburst ACh/GABA signals without altering the direction coding of DSGCs. Fourth, the neighboring DSGCs receive common input from bipolar cells (~50% dyadic connections are shared with other DSGCs; Figure 4), regardless of the direction they code. This suggests that NMDA modulation would occur in a correlated way across the four types of DSGCs, helping to preserve the population code (Zylberberg et al., 2016). Thus, it appears that silent synapses engage with diverse mechanisms to produce a robust DS code, down to threshold levels.

In one respect, the silent synapse model appears inefficient compared to the matched model, as scaling glutamate and ACh in proportion would avoid the “silent” regime in which glutamate does not produce spiking responses. However, if ACh and NMDA inputs had similar contrast scaling, then their interactions at threshold contrasts would be weak (Figure 5), as there would only be a few glutamate-bound NMDA receptors. By sharing signals generated by the high-sensitivity pathway, many NMDA synapses are “primed” to amplify even weak coincident cholinergic inputs, requiring fewer starburst inputs to generate a spiking response. The shifting of the DSGC’s input-output function along the contrast axis by NMDA receptors highlights the functional advantage of the silent synapse over the matched model, in which responses are expected to scale purely multiplicatively. By sharing starburst input with DSGCs, the DS circuit ensures that information carried by high-sensitivity bipolar cells is fully utilized.

STAR★METHODS
Details provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION
Supplemental Information includes six figures and one table and can be found with this article online at https://doi.org/10.1016/j.neuron.2017.09.058.

AUTHOR CONTRIBUTIONS

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We thank Drs. Jeff Diamond, Alon Poleg-Polsky, Jon Cafaro, Court Hull, Kerry Delaney, and Ben Murphy-Baum for their useful discussions and comments on the manuscript; J. Boyd (University of British Columbia) for help with two-photon imaging software; and A. Sher, J. Leung, and Laura Hanson for providing helpful technical support. This work was supported by operating grants from the NIH/NEI EY024567 (G.D.F.) and Canadian Institutes of Health Research (CIHR-130268-2013) awarded to G.B.A.

REFERENCES


STAR METHODS

KEY RESOURCES TABLE

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Experimental Models: Organisms/Strains

Mouse: wild-type (C57BL/6J) The Jackson Laboratory RRID: IMSR_JAX:000664
Mouse: Hb9-EGFP Gift from R. Brownstone (University of Dalhousie) RRID: MGI_109160
Mouse: Trhr-EGFP Gift from M. B. Feller (University of California, Berkley) (Rivlin-Etzion et al., 2011) RRID: MMRRC_030036-UCD
Mouse: Chat-IRES-Cre The Jackson Laboratory RRID: MGI_5475195
Mouse: Ai9 The Jackson Laboratory RRID: MGI_3809523

Software and Algorithms

MATLAB MathWorks http://www.mathworks.com/; RRID: SCR_01622
LABVIEW National Instruments http://www.ni.com; RRID: SCR_014325
ITK-SNAP (Yushkevich et al., 2006) http://www.itksnap.org/pmwiki/pmwiki.php; RRID: SCR_002010
KNOSSOS N/A http://knossostool.org/; RRID: SCR_003582
NEURON (Hines and Carnevale, 1997) https://www.neuron.yale.edu/neuron/; RRID: SCR_005393

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Gautam B. Awatramani (gautam@uvic.ca).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
Experiments were performed using healthy adult (either sex) C57BL/6J (RRID: IMSR_JAX:000664), Hb9-EGFP (RRID: MGI_109160), Trhr-EGFP (RRID: MMRRC_030036-UCD) and Chat-IRES-Cre (RRID: MGI_5475195) crossed with reporter mice, Ai9 (RRID: MGI_3809523). Animals were housed in 12hr light-dark cycles, in groups up to 5 animals per cage. All procedures were performed in accordance with the Canadian Council on Animal Care and approved by the University of Victoria’s Animal Care Committee or Duke University’s Animal Care and Use Committee.

METHOD DETAILS

Dual Patch Clamp Recordings
Mice were dark-adapted for approximately 30–60 min before being briefly anesthetized and decapitated. The retina was dissected in Ringer’s solution under infrared light. The isolated retina was then mounted on a 0.22 mm membrane filter (Millipore) with a pre-cut window to allow light to reach the retina and enabling the preparation to be viewed with infrared light using a Spot RT3 CCD camera (Diagnostic Instruments) attached to an upright Olympus BX51 WI fluorescent microscope outfitted with a 40 x water-immersion lens (Olympus Canada). The isolated retina was then perfused with warmed Ringer’s solution (35–37°C) containing 110 mM NaCl, 2.5 mM KCl, 1 mM CaCl2, 1.6 mM MgCl2, 10 mM dextrose and 22 mM NaHCO3 that was bubbled with carbogen (95% O2:5% CO2). Unless otherwise noted, all reagents were purchased from Sigma-Aldrich Canada. NBQX, D-AP5, MK-801 and UBP310 were purchased from ABCAM Biochemicals.
DSGCs and starbursts were identified using two-photon laser-scanning microscopy techniques. Voltage-clamp whole-cell recordings were made using 4–7-MΩ electrodes containing 112.5 mM CH3CsO3S, 7.75 mM CsCl, 1 mM MgSO4, 10 mM EGTA, 10 mM HEPES, 5 mM OX-314-bromide (Tocris) and 100 μM spermine (ABCAM Biochemicals). The pH was adjusted to 7.4 with CsOH. The reversal potential for chloride was calculated to be −56 mV. The recordings were neither corrected for series resistance nor the junction potential. Recordings were made with a MultiClamp 700B amplifier (Molecular Devices). Signals were digitized at 10 kHz (PCI-6036E acquisition board, National Instruments) and acquired using custom software written in LabVIEW. The traces were viewed and analyzed offline with custom written software in MATLAB.

Visual stimuli were produced using a digital light projector (Hitachi Cpx1, refresh rate 75 Hz), focused onto the outer segments of the photoreceptors using the sub-stage condenser. The background luminance, measured with a calibrated spectrophotometer (Ocean Optics), was set to 10 photoisomerisations/s (R*/sec). Stimuli were created in the MATLAB environment (Psychtoolbox). Spots (250 μm diameter) with positive contrasts, ranging between 3% and 300% (Weber contrast) were moved across the retina at a velocity of 1mm/s, along the preferred or null axis of the DSGC, as indicated. The responses were fitted to the Naka Rushton equation $R_{\text{max}} = \frac{C}{(C+C_{50})^{n}}$), where $C$ indicates the contrast, $R_{\text{max}}$ is the maximum response, $C_{50}$ the semi-saturation constant and $n$ is a coefficient proportional to the slope of the contrast response function at $C_{50}$. Correlated activity in starbursts and DSGCs were estimated by comparing the spontaneous activity in dark over 5 s intervals.

Multi-Electrode Array Recordings
Dorsal peripheral retina was dissected and mounted with ganglion cell side down on an array of 519 electrodes with 30 μm spacing, covering a hexagonal region of ~0.5 μm² (Yu et al., 2017). The retina was perfused with Ames’ solution (30-31°C, 7-12 mL/min) bubbled with 95% O2 and 5% CO2, pH 7.4. Recordings were analyzed offline to identify and sort the spikes of different cells, as described previously (Field et al., 2007). Visual stimuli were presented on an OLED video display (emagin) using custom software written in MATLAB (MathWorks); stimuli were focused onto the photoreceptor layer. Light intensities were set using a calibrated photodiode (Gamma Scientific). Experiments were performed at a light intensity corresponding to ~7000 P*/cone/s (for the middle wavelength sensitive opsin).

Candidate spike events were detected using a threshold on each electrode, and voltage waveforms on the electrode and nearby electrodes around the time of the spike were extracted. Clusters of similar spike waveforms were identified as candidate neurons if they exhibited a refractory period and accounted for more than 100 spikes in recording over 30 min. Duplicate spike trains identified across different electrodes were identified by temporal cross-correlation and removed. DSGCs were identified from the magnitudes of vector summed response to square-wave drifting gratings moving in 8 directions (spatial and temporal periods 960 μm and 2 s, respectively), using a Gaussian Mixture model (Figure S5C). The cluster with larger average magnitudes identified DSGCs. ON and ON-OFF DSGCs were further segregated by their speed tuning. Square-wave gratings drifting at 8 speeds (ranging from 0.04 to 4.8 mm/s) were used to measure speed tuning curves (data not shown). Cells with broad speed tuning were identified as ON-OFF DSGCs, while cells that preferred lower speeds were identified as ON DSGCs. ON-OFF DSGCs exhibited 4 direction preferences while ON DSGCs exhibited 3 (data not shown). The relative direction preference was determined by using the electrophysiological image (EI), which is a movie of the average electrical activity produced across the electrode array by a neuron. Since axons of dorsal RGCs travel ventral, DSGCs with preferred direction matching the axon direction were identified as superior DSGCs, other directions were determined by whether the retina was dissected from the left or right eye. Direction tuning curves and contrast response functions (Figure 6) were measured with moving bar stimuli. The bar was 240 μm wide on the retina and moved at 960 μm/s. The direction-tuning curve was centered at the preferred direction and fit with a cosine or Gaussian equation, as indicated (Nowak et al., 2011).

Electron Microscopy Analysis
A previously published dataset acquired using scanning SBEM was analyzed (retina k0563; Ding et al., 2016). Voxel dimensions were 12 X 12 X 25 nanometer (nm) (x, y, and z, respectively). Potential DSGCs were first identified as ganglion cells with bifratified synapses in the IPL. Next, synapses from starbursts on the dendrites of these cells were identified; partial reconstruction of the ON and OFF starbursts confirmed their co-stratification with the ganglion cell dendrites indicating a DSGC. Bipolar cell ribbon synapses on the DSGC dendrites were identified and other post-synaptic elements were annotated by partial dendritic reconstruction (Figure 4). The whole axon terminal of a bipolar cell synapsing with the DSGC was reconstructed to annotate its ribbon synapses and identify dyadic partners. All analyses were performed by tracing skeletons and annotating synapses using the Knossos software package (https://knossostool.org/). Volumetric reconstructions of synapses were performed using ITK-SNAP (http://www.itksnap.org/pmwiki/pmwiki.php) (Yushkevich et al., 2006).

Simulations
A simple two-compartment model containing a thin passive compartment attached to an active soma was built in the NEURON simulation environment (Hines and Carnevale, 1997). The synaptic inputs (NMDA, non-NMDA and inhibition) were placed at the end of the thin passive compartment. Physical parameters were set such that electrotonic properties of the model neuron allowed the membrane voltage at the synaptic site to vary from that of the soma (see table below). The synaptic inputs were modeled with reversal potentials for non-NMDA and NMDA at 0mV and inhibition at −60mV. Inhibitory and non-NMDA conductances were

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implemented with the built-in Exp2Syn point process of the NEURON environment, and NMDA was implemented by modifying the
Exp2Syn process to include a voltage function \( g(v) = \frac{G_{\text{max}}}{1 + 0.213 * e^{\gamma v}} \), \( n = 0.25, \gamma = 0.08 \). To simulate voltage
independent NMDA activity, \( g \) was set to 20% of \( G_{\text{max}} \) (the conductance at \(-30\,mV\), using the equation above), at all voltages.

<table>
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<td>1 ( \mu )m</td>
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<td>Length</td>
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<td>cm</td>
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<td>( g_{\text{kmbar}} )</td>
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<td>HHst noise</td>
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</table>

Contrast responses were simulated by scaling the synaptic input conductances to the Naka-Rushton fits (see table below; Figure 2). Note that these values were slightly different from Table S1 as they are taken by fitting the average responses. For the ‘matched’ models, the NMDA conductances were scaled to the non-NMDA Naka-Rushton fit. For direction, the amplitude of non-NMDA and NMDA conductances were set as constant, while the amplitude and timing of inhibitory conductances were varied simulating 16 different directions (11.25° apart). These values were modulated such that the model output best approximated the directional tuning observed in the MEA experiments.

<table>
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<th>Naka-Rushton fits ( g = g_{\text{max}}*(C^n/(C^n+C_{C50}^n)) )</th>
<th>( C_{50} ) (SAC ( C_{50} = 17 ))</th>
<th>( n )</th>
<th>( g_{\text{max}} ) (nS)</th>
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<td>( E )</td>
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<td>2.853</td>
<td>35</td>
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In the spiking models, the soma was built with active properties (Nav, Kv, noise) implemented using a stochastic Hodgkin-Huxley (HHst) model (Linaro et al., 2011). Spike outputs were simulated over 20 trials with unique pseudo-random noise provided by HHst. The total number of spikes generated by the synaptic inputs was estimated as the response.

QUANTIFICATION AND STATISTICAL ANALYSIS

All population data has been expressed as mean ± SEM and are indicated, along with the number of samples, in the figure legend. Student’s t test or a Wilcoxon signed-rank test was used to compare values under different conditions (unless indicated in the main text, Student’s t test was used), and the differences were considered significant when \( p \leq 0.05 \).

DATA AND SOFTWARE AVAILABILITY

The data analysis is available on request from the Lead Contact.