Retinal origin of direction selectivity in the superior colliculus

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Detecting visual features in the environment, such as motion direction, is crucial for survival. The circuit mechanisms that give rise to direction selectivity in a major visual center, the superior colliculus (SC), are entirely unknown. We optogenetically isolate the retinal inputs that individual direction-selective SC neurons receive and find that they are already selective as a result of precisely converging inputs from similarly tuned retinal ganglion cells. The direction-selective retinal input is linearly amplified by intracollicular circuits without changing its preferred direction or level of selectivity. Finally, using two-photon calcium imaging, we show that SC direction selectivity is dramatically reduced in transgenic mice that have decreased retinal selectivity. Together, our studies demonstrate a retinal origin of direction selectivity in the SC and reveal a central visual deficit as a consequence of altered feature selectivity in the retina.

Neurons in the brain are specialized in detecting unique features in the environment. In the visual system, many neurons at various stages of processing respond selectively to stimuli moving along specific directions or having particular orientations1–3. Such direction and orientation selectivity are critical for motion sensing and image processing, leading to visually guided behaviors that are important for survival. Not surprisingly, the circuit mechanisms of direction and orientation selectivity have been extensively studied. However, these studies have mostly focused on the retina and primary visual cortex (V1)2,3 while neglecting the SC, a major retinal target and vision center.

The SC, or optic tectum, is an evolutionarily conserved structure that receives direct retinal input in all vertebrates4–6. It was the most sophisticated visual center until the neocortex recently evolved in mammals. Even in mice, a mammalian species that has become a useful model in vision research7, 85–90% of retinal ganglion cells (RGCs) project to the SC8, making it the most prominent visual structure in this species. Although the SC is mostly known for its functions in initiating rapid gaze shift toward salient stimuli, neurons in its superficial layers (i.e., the visual layers), including the stratum griseum superficiale (SGS) and stratum opticum (SO), display diverse visual response properties. In particular, selectivity for motion direction has been observed in the superficial SC of all mammalian species, and most of the DSGCs project to the superficial SC18. In mice, several subtypes of DSGCs preferentially terminate their axons in the upper half of the SGS, while most of the non-DSGCs tend to project to the lower half19–22. Notably, DS collicular neurons are also organized in a depth-specific manner, where they are most concentrated in the topmost lamina of the SGS and become less prevalent with depth23. This correspondence between the anatomical and functional organization supports the idea that similarly tuned DSGCs could project to common targets, thereby providing SC neurons with a synaptic drive that is biased toward certain directions.

Second, direction selectivity in the SC could arise from a specific arrangement of retinal inputs that are not individually tuned. In his motion-detector model, Reichardt24 proposed that direction selectivity could be generated by nonselective inputs that have different temporal delays. In such a model, these inputs are arranged such that stimuli moving in the preferred direction would result in the synchronous arrival of synaptic inputs and consequently a large depolarization in the postsynaptic cell onto which they converge. On the other hand, stimuli moving in the opposite direction would cause only a small depolarization because the inputs would arrive asynchronously. This scenario was indeed shown to underlie direction selectivity in cat V1, as revealed by both extracellular and intracellular recordings25,26.

Received 29 August 2016; accepted 11 January 2017; published online 13 February 2017; doi:10.1038/nn.4498
posed by Barlow and Levick for retinal direction selectivity 27. circuits, such as tuned or spatially offset inhibition, as originally pro-
selectivity could then arise via dynamic interactions within the local In this scenario, the combined retinal input that a DS SC neuron
between DSI-spike and DSI-
was as steep as seen in the DS cells in cat or mouse visual cortex. The ratio
depolarization to bars moving along their nonpreferred directions Consistently, some SGS cells showed very weak, barely detectable
Notably, the
are a substantial population that was highly selective in its spiking responses (for example, Fig. 1b–c). Using the normalized vector sum as an index (see Online Methods for details), which we refer to as the global direction selectivity index (gDSI), we found that 27% of the SGS neurons were highly DS in their spiking (gDSI of spikes ≥ 0.25; n = 14 of 52 cells), consistent with our previous extracellular studies across the entire depth of the SGS28. The gDSIs of spikes (gDSI-spike) and of Vm (gDSI-Vm) were overall correlated for individual cells, but gDSI-spike was greater in most cases (Fig. 1f), as expected from the nonlinear effect of the spike threshold13. As the vast majority of selective SGS neurons (gDSI-spike ≥ 0.25) had gDSI-Vm ≥ 0.1 (n = 11 of 14 cells), we subsequently used 0.1 as a cutoff for classifying Vm and excitatory postsynaptic current (EPSC) responses as highly DS. Notably, the Vm-to-spike transformation in the SGS neurons was not as steep as seen in the DS cells in cat or mouse visual cortex. The ratio between DSI-spike and DSI-Vm was ~3 in cat V126 and ~6 in mouse V129, but only ~1.2 in mouse SGS. In fact, a number of the recorded cells showed nearly identical tuning curves and gDSI values between their spiking and Vm responses (Fig. 1f and Supplementary Fig. 1). Consistently, some SGS cells showed very weak, barely detectable depolarization to bars moving along their nonpreferred directions (for example, Supplementary Fig. 1), a phenomenon rarely seen in cortical DS cells56,29. Furthermore, the preferred directions of SGS cells were similar for spike and Vm responses, especially for highly DS cells (Fig. 1g). These observations thus suggest that synaptic inputs likely play a more important role in determining direction selectivity in the mouse SGS.

SGS direction selectivity originates from individually tuned retinal input SGS neurons receive several sources of synaptic inputs, including both excitation and inhibition. To isolate the excitatory inputs, we next performed whole-cell voltage-clamp recording. These experiments were done in transgenic mice that expressed channelrhodopsin-2 (ChR2) in GABAergic inhibitory neurons. By illuminating the exposed SC with a blue LED (light-emitting diode), we were able to photoactivate local inhibitory neurons and suppress excitatory neurons in the SGS (Supplementary Fig. 2). This allowed us to achieve three goals. First, we could identify whether the recorded cells were excitatory or inhibitory by their responses to LED photoactivation (Supplementary Fig. 3). Second, we were able to determine the reversal potential of inhibitory currents in the recorded excitatory neurons individually (Supplementary Fig. 3). As expected from the fact that the same internal solution was used in all recordings, the inhibitory reversal potential was very consistent across cells (~64.92 ± 0.21 mV; n = 43 cells; Supplementary Fig. 3). We thus used the same holding potential (~65 mV) for voltage clamp of inhibitory neurons, even though the reversal potential could not be determined directly in these cells because of ChR2 activation. Finally, optogenetic activation of local inhibitory neurons in these mice could silence excitatory neurons in the SGS and remove local excitatory interactions, thereby exposing the retinal input to the recorded cells (Supplementary Fig. 2). This allowed us to analyze and compare the selectivity of retinal and total excitatory inputs to each SGS neuron individually.

We first analyzed the total EPSCs in the absence of LED photoactivation. Consistent with the above observation of highly tuned Vm responses, the visually evoked total EPSCs in many SGS neurons, including both excitatory and inhibitory neurons, were DS (for example, Fig. 2a,b and Supplementary Fig. 4). In fact, the gDSI distributions of visually evoked peak excitation (gDSI of EPSC (gDSI-EPSC) = 0.13 ± 0.01, n = 87) and Vm responses (gDSI-Vm = 0.12 ± 0.02, n = 52) were very similar (Fig. 2b and see Online Methods for details of determining peak EPSCs and Vm responses). Furthermore, we recorded both Vm and EPSC responses in a small number of cells (n = 23) and found that their gDSI values were nearly identical (Fig. 2c). Specifically, for cells that were highly selective in their Vm (gDSI-Vm ≥ 0.1; Fig. 2e), all of them (n = 7 of 7) received selective EPSCs (gDSI-EPSC ≥ 0.1). Conversely, all of the cells whose gDSI-Vm < 0.1 had gDSI-EPSC < 0.1 (n = 16 of 16). In other words, the excitatory input that individual SGS neurons received was a deciding factor in their degree of direction selectivity. Similarly, the preferred direction of the Vm was also determined by the EPSC (Fig. 2f). These results thus suggest that local inhibition in the SGS did not generate direction selectivity de novo from nonselective excitatory input.

Most SGS neurons still showed visually evoked excitation upon optogenetic silencing of local excitatory neurons (n = 41 of 48, 85.4%), including both excitatory and inhibitory neurons (Supplementary Fig. 5), indicating that they received direct retinal inputs. In particular, a slightly larger portion of the DS cells (n = 15 of 16, 93.8%) were directly innervated by the retina than non-DS cells (n = 26 of 32, 81.3%; Fig. 2g). When comparing the peak amplitudes of total and retinal EPSCs in the same cells (tEPSCs and rEPSCs, respectively), we found that the retinal inputs were amplified by intracollicular circuits in a largely linear fashion (for example, Fig. 2c), similarly to the transformation of inputs from thalamus to visual cortex30,31. The amplification ratio ranged from 1.11 to 7.39, with a mean of 3.09 ± 0.24 (n = 41; Fig. 2h–i). Consistent with the largely linear amplification (Supplementary Fig. 6), rEPSCs and tEPSCs of the same cells were similarly tuned in their peak response amplitudes (for example, Fig. 2a–b). Indeed, the gDSI values and preferred directions were well correlated between rEPSCs and tEPSCs, and this was the case for both excitatory and inhibitory neurons (Fig. 3a–c). In other words, the DS SGS cells, i.e., cells that have high gDSI values for their tEPSC and
resulting $V_{m}$, receive similarly tuned retinal excitation. Furthermore, the intracollicular excitatory inputs, determined as the difference between tEPSC and rEPSC (Supplementary Fig. 7), displayed selectivities largely similar to those of the retinal inputs (Fig. 3d–f). These analyses thus indicate that the direction selectivity of SGS neurons was determined by their retinal inputs, which were further amplified by similarly tuned intracollicular inputs to maintain the directional preference and level of selectivity.

The selective retinal inputs could result from summing inputs that are individually tuned, such as from DSGCs (Fig. 4a,b). Alternatively, individual retinal inputs may not be DS, but their integration in the postsynaptic neuron could take place in a precise spatiotemporal manner to generate larger EPSC peaks at the preferred direction than at the opposite direction. For example, a difference in response latency between the non-DS retinal inputs could cause them to arrive at the postsynaptic cell synchronously in response to the preferred direction but asynchronously to the opposite direction, thus resulting in different peak amplitudes (Fig. 4c), as proposed originally by Reichardt for motion detection. 24. In this latter scenario, the total charge of the retinal EPSCs would be much less selective than their peaks or even entirely nontuned, which would be analogous to the emergence of orientation selectivity in the visual cortex. 30. To determine which of the two scenarios is true for the retinocollicular transformation of direction selectivity, we calculated the integral of rEPSCs during responses evoked by the moving bars (see Online Methods for details) and compared its direction selectivity to that of the peak
Figure 2  Voltage-clamp recording and optogenetic silencing to isolate retinal excitation to SGS neurons. (a) Trial-averaged EPSC traces of an example SGS neuron to bars moving along 12 directions in the absence (left panel) or presence (right) of LED illumination (as indicated by the blue bar). The red dotted lines indicate the mean current level in the absence of visual stimulus. (b) Direction tuning curves of total EPSC (tEPSC, top) and retinal input (rEPSC, bottom) for the cell in a. The red dotted lines indicate 3 standard deviations (s.d.) above the mean current level as determined in the absence of visual stimulus. Data are presented as mean ± s.e.m.; n = 8, 7, 7, 6, 7, 7, 7, 7, 6 and 6 trials in the top panel and n = 3 for all directions in the bottom panel. (c) Scatter plot of tEPSC versus rEPSC for the cell in a. Peak rEPSC and tEPSC amplitudes are plotted for the responses to 12 directions of bars. The dashed line is the linear regression of the data points (R² = 0.95, F1,10 = 192.7, P < 0.001, linear regression; r = 0.98, P < 0.001, Pearson correlation). (d) Cumulative distributions of gDSI for tEPSC (n = 87 cells from 58 mice, red) and for Vm (n = 52 cells from 41 mice, black), indicating that tEPSC and Vm have nearly identical gDSI distributions (P = 0.30, K-S statistic = 0.17, Kolmogorov-Smirnov test). (e) Scatter plot of gDSI values for tEPSC and Vm in the same cells (n = 23 cells from 21 mice). They are well correlated (r = 0.99, P < 0.001, Pearson correlation) and similar in values (P = 0.18, W = 90, Wilcoxon signed-rank test). Note that the plot is shown in log–log axis to better illustrate the cells with low gDSI. The dotted lines indicate gDSI levels of 0.1. (f) Scatter plot of the preferred direction (prefD) for Vm versus tEPSC. The diameter of each dot is scaled to the gDSI-Vm of that cell. Note that the preferred directions are similar for tEPSC and Vm responses, especially for highly selective cells. The solid lines in both e and f are the lines of identity. (g) Percentage of cells that receive direct retinal input in DS (n = 15 of 16) and non-DS (n = 26 of 32) SGS neurons. (h) Distribution of amplification ratios of all cells that receive direct retinal inputs (n = 41). The red line indicates the mean of the distribution. (i) Similar amplification ratio between DS (3.37 ± 0.46, n = 15 cells from 13 mice) and non-DS (2.94 ± 0.26, n = 26 cells from 20 mice) SGS neurons (P = 0.41, U = 164, Mann-Whitney U-test). Data are presented as mean ± s.e.m.
The integral of retinal input was in fact similarly selective or even more selective in a few cells, compared to the peak rEPSC (Fig. 4d–e and Supplementary Fig. 8), ruling out the aforementioned second scenario. The averaged tuning curves of peak rEPSC and integral rEPSC were very similar, nearly identical to that of total EPSC (Fig. 4f), indicating that SC direction selectivity originates from individually tuned retinal inputs. Finally, the same integral-versus-peak analysis showed that the intracollicular excitatory inputs also followed the first scenario (Supplementary Fig. 7), indicating that DS SGS neurons were preferentially connected with other collicular neurons that prefer similar directions.

Genetic disruption of retinal direction selectivity reduces selectivity in the SGS

Our in vivo whole-cell experiments support the conclusion that the direction selectivity of SGS neurons originates from converging inputs of similarly tuned DSGCs. If this is indeed the case, a reduction of retinal direction selectivity would compromise the selectivity in the SGS. We next tested this prediction using genetic manipulation. GABAergic inhibition provided by starburst amacrine cells is a critical factor in generating direction selectivity in the retina1,2, and it can be eliminated by knocking out (KO) the vesicular GABA transporter (Vgat) gene Slc32a1 from these cells by crossing loxP-flanked Slc32a1 (Slc32a1Vlox/lox or VgatVlox/lox) with choline acetyltransferase (ChAT)-IRES-Cre mice32. We then performed two-photon calcium imaging in the ganglion cell layer of these KO mice using the genetically encoded indicator GCaMP6s33. In particular, we focused on the ON-OFF DSGCs that are the ones that primarily project to the SGS18. In wild-type (WT) littermate controls, 9.3% of cells in the ganglion cell layer were ON-OFF DSGCs (Fig. 5a–c; n = 60 of 648 cells from 9 mice), consistent with previous studies34,35. In contrast, in KO mice, the percentage of cells that displayed ON-OFF DS responses was significantly reduced (Fig. 5c; n = 19 of 566 cells, 3.4%, from 14 mice; P < 0.001, χ2 test). Because cholineric inputs to the SC terminate in the intermediate and deep layers and do not co-release GABA, these ChAT-Vgat KO mice provided us with a unique opportunity to study the effect of altered retinal direction selectivity on the visual response properties of superficial SC neurons.

We first performed intrinsic imaging and found normal SC retinotopic maps in the KO mice (Supplementary Fig. 9). Next, we performed two-photon calcium imaging of the topmost SGS lamina, which we previously showed is enriched with DS neurons that have overlapping ON-OFF receptive fields23. The receptive field structures of the imaged cells were largely normal in the KO mice, with subtle increases in subfield size and completely normal ON-OFF overlaps (Supplementary Fig. 10). The small increase in subfield size was consistent with the reduced inhibition in their retina of the KO mice. Also consistent with this was the finding that slightly more cells were responsive in the KO mice. When stimulated with drifting gratings or sweeping bars, 46.5% (310 of 667 cells to gratings) or 47.2% (315 of 677 cells to bars) were responsive in the KO mice. As expected, the vast majority of the responsive cells in this lamina were DS in WT (Fig. 5d–f; gDSI ≥ 0.25; n = 235 of 310 cells to gratings, 76%; and 146 of 315 cells to bars, 46%). Preferred motion directions were more widely represented than the four cardinal directions in the retina (Supplementary Fig. 11), presumably due to specifically combining inputs of DSGCs that prefer neighboring cardinal directions. Notably, in the KO mice, many fewer cells were DS in this lamina (n = 84 of 407 to gratings, 21%; and 35 of 505...
The solid lines in Figure 4 show that retinal inputs exhibit similar amplitudes to all directions. These non-DS retinal inputs arrive at the postsynaptic cell synchronously in response to the preferred direction (top) and asynchronously to the opposite direction (bottom), thus resulting in different peak amplitudes. In this scenario, the total charge of rEPSC would be much less selective than their peaks. (c) The integral and peak of retinal EPSCs prefer similar directions in DS SGS neurons. The solid lines in d and e are the lines of identity. (f) Averaged tuning curves for rEPSC peaks (black), rEPSC peaks (blue) and rEPSC integrals (red) in DS SGS neurons (n = 15 cells from 13 mice). Individual curves were normalized by their maximum responses and aligned to their preferred directions. They were then averaged for plotting. Error bars represent s.e.m.

**DISCUSSION**

In this study, we both isolated and manipulated the retinal input in order to study its role in generating SGS direction selectivity. The whole-cell recording and optogenetic silencing experiments demonstrate that DS SGS neurons received retinal input that was already selective, generated by precisely converging inputs from similarly tuned DSGCs. The selective retinal input was amplified by intracollicular circuits without changing its preferred direction or level of selectivity. The resulting membrane potential depolarization in the SGS neuron then led to a slightly more selective spiking response due to the nonlinear effect of thresholding. Consistent with these results, we also found that SGS direction selectivity was reduced in mice with altered retinal selectivity. Our studies thus demonstrate that SGS neurons inherited their direction selectivity from DSGCs in the retina, a finding that has important implications for understanding signal processing in the early visual system.

It is known that individual SGS neurons are innervated by several RGCs. Consequently, in order to provide DS excitation to the postsynaptic neuron, the converging DSGCs must prefer similar directions. In addition, a new directional preference would emerge when the DSGCs that prefer neighboring cardinal directions precisely converge. Our findings therefore indicate that well-controlled developmental mechanisms must exist to ensure the precise and selective targeting of DSGCs in the SGS. Consistent with this idea, several subtypes of DSGCs have been found to project primarily to the upper SGS, which contains more DS cells than the lower SGS. How such depth-specific targeting is established during development and how even more precise patterns of connectivity are generated at the level of individual cells have not yet been studied.

Our data further show that the intracollicular excitation that DS SGS neurons received was also tuned to similar directions, thereby amplifying the retinal inputs without changing their preferred direction or levels of selectivity. This result thus indicates that, within the SGS, excitatory neurons that prefer similar directions are preferentially connected. A similar nonrandom connectivity has been revealed for orientation-selective cells in the mouse visual cortex, and its emergence requires visual experience. The exact wiring diagram of the SGS circuity is not yet known, but given that the mouse retinocollicular pathway is already a productive model for studying cell types and neural development, future research may provide a more detailed understanding of these circuits.
Figure 5 Genetic disruption of retinal direction selectivity reduces selectivity in the SGS. (a) A schematic of two-photon calcium imaging of retina (top). The bottom panel shows a max-intensity projection of GCaMP6 fluorescence in an example field of view. Scale bar, 25 μm. (b) Top (trace 1): Ca²⁺ signals of the RGC circled in a to the presentation of moving bars in eight directions (different colors represent separate trials). The gray shade corresponds to the time interval in which the bar stimulus sweeps across the field of view and the arrows represent the directions of movement in relation to the polar plots on the right. This cell showed DS responses to both leading and trailing edges of the moving bars, indicating that it was an ON-OFF DSGC. Bottom (trace 2): an ON-OFF cell from a conditional KO mouse. Corresponding polar plots are shown to the right. (c) Summary plot showing the percentages of ON-OFF DSGCs in WT (black, n = 60 of 648 cells from 9 mice) and KO (red, n = 19 of 566 cells from 14 mice) retinas (P < 0.001, χ² = 17.3, χ² test). Data points represent percentages of ON-OFF DSGCs in individual mice. (d) A schematic of two-photon imaging of the SGS (top) and an example field of view from a WT (bottom). Scale bar, 20 μm. (e) Ca²⁺ signals of the two neurons (1 and 2) circled in d and of two neurons from a Vgat KO (3 and 4), in response to drifting gratings. The gray boxes mark the duration of stimulation. The movement directions of the bar are represented by arrows on top. Corresponding polar plots are shown to the right. (f) gDSI distribution of WT (top, black) and KO (bottom, red) cells to drifting gratings. The solid green lines indicate the medians of distributions. (g) Average WT (black, n = 310 cells from 5 mice) and KO (red, n = 407 from 8 mice) tuning curves to gratings after aligning each cell’s preferred direction at 0. Data are presented as mean ± s.e.m. *P < 0.001, Mann-Whitney U-test. (h) Cumulative distribution of the data shown in f (P < 0.001, K-S statistic = 0.61, Kolmogorov-Smirnov test). (i) Cumulative distribution of gDSI to sweeping bars (P < 0.001, K-S statistic = 0.43, Kolmogorov-Smirnov test). Scale bars for the Ca²⁺ signals and polar plots in b and e represent the change in fluorescence from baseline (∆F/F₀).

studies of this pathway will likely reveal the molecular and cellular mechanisms that establish the precise connections from the retina to the SGS and within the SGS, both of which are necessary to generate and maintain feature selectivity in the superior colliculus.

Direction-selectivity is an evolutionarily conserved property seen in many visual structures and in various species. In zebrafish, for example, RGC subtypes that prefer different directions project to segregated layers in the optic tectum, and the tectal neurons with matching preferred directions arborize their dendrites in the corresponding layers. This suggests that the DS retinal inputs could determine the direction preference of tectal neurons, consistent with our findings here in the mouse SC. In monkeys, a very small population of DS neurons was found in the SC, largely consistent with the fact that DSGCs have so far remained elusive in primates. It is certainly possible that DSGCs may be discovered with new genetic and imaging techniques in the future and that they may give rise to the observed SC direction selectivity. Alternatively, the weak DS responses in the primate SC could result from the excitatory input from visual cortex, which includes DS cells. This possibility has in fact been addressed in cats by lesioning or cooling the cortex, but unfortunately these studies yielded conflicting results. On the other hand, cortical input does not appear to affect SC selectivity in rodents. For example, in ground squirrels, SC direction selectivity remain unchanged when visual cortex is removed, leading to the proposal that the DS
cells receive their inputs from the retina. We have recently shown that in mice cortical inputs do not affect the magnitude or looming speed tuning of SC responses under anesthesia and only increase the response magnitude in the awake condition. Our current study, in which the animals were anesthetized and their V1 removed, demonstrates that retinal input was the origin of the direction selectivity in the mouse SC. Future studies will be needed to determine whether cortical input could modulate SC direction-selective responses under certain behaviors in mice or even give rise to SC direction selectivity in primates.

In addition to direction selectivity, SGS neurons also display a number of other response properties, such as size preference, motion selectivity and speed tuning. These properties could be generated by integrating inputs from DSGCs, other RGC subtypes, local intracollicular circuits and afferent inputs from visual cortex. In terms of local circuits, the SGS contains a large population of inhibitory neurons. Inhibition could sharpen direction selectivity if it is tuned to the opposite direction or offset spatiotemporally, as shown for DS neurons in zebrafish tectum and mouse visual cortex. Although we did not directly address the role of synaptic inhibition in this study, our data indicate that it is not required to generate direction selectivity in SGS neurons or to sharpen its tuning. This is because the excitatory inputs that individual DS neurons receive are already selective and tuned to the same level as $V_{1/2}$. On the other hand, inhibitory neurons in the topmost SGS lamina are known to be DS. It is thus conceivable that these inhibitory neurons may provide direction-specific interactions between stimulus center and stimulus surroundings in response to complicated visual scenes. Future studies will be needed to determine the spatial and direction tuning of inhibition in SGS neurons in order to reveal the functions of the SGS in visual processing.

In conclusion, the diverse response properties in the SC are generated by selective and precise connections in the retinocollicular and intracollicular circuits. Using the genetic and optogenetic tools available in mice, we discovered the neuronal mechanism underlying direction selectivity, one of the most important properties. Given the fundamental importance of the SC in visually guided behaviors, we are confident that our findings will motivate exciting future studies of visual system organization, function and development.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank X. Zhao and H. Chen for their help with data analysis. For the use of GCaMP6s, we gratefully acknowledge V. Jayaraman, R.A. Kerr, D.S. Kim, L.L. Looger and K. Svoboda from the GENIE Project, Janelia Farm Research Campus, Howard Hughes Medical Institute. This research was supported by US National Institutes of Health (NIH) grants (EY026286 to J.C. and X.L., and EY024016 to W.W.), National Natural Science Foundation of China (NSFC) grant (81371049 to X.S.), China Scholarship Council (CSC) scholarship (201309120003 to X.S.) and Tianjin 131 Innovative Talent Project first-level talent scholarship (to X.S.).

AUTHOR CONTRIBUTIONS

X.S., J.B., X.L., W.W. and J.C. designed the experiments. X.S. performed in vivo whole-cell recording experiments and analyzed the data. J.B performed in vivo two-photon imaging experiments and analyzed the data. H.A.L. and D.K. performed retinal imaging experiments and analyzed the data. J.C. performed intrinsic imaging. Y.J. performed histology. W.W. and J.C. guided data analysis and oversaw the project. All authors discussed the results and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animal preparation. C57BL/6 wild-type (WT) and transgenic mice of both sexes were used in this study: Gad2-IRES-cre (Stock no. 010802) and Aii32 (RCL-ChR2(H134R)/EYFP, Stock no. 012569) mice were acquired from Jackson Laboratory and crossed to generate heterozygous offspring that express ChR2 in glutamate decarboxylase 2–positive (GAD2+2) cells for in vivo optogenetic experiments (n = 100). Additional WT mice were also used for in vivo whole-cell recording (n = 25). For two-photon calcium imaging in the retina and SC, low-flanked Slc32a1 (Slc32a1fluosc/ or Vgatfluosc) mice (Stock no. 012897) and ChAT-IRES-Cre mice (Stock no. 006410) were originally acquired from Jackson Laboratory and crossed to knock out (KO) the vesicular GABA transporter (Vgat) gene (Slc32a1) from choline acetyltransferase (ChAT+) cells. These strains were backcrossed to the C57BL/6 background32. Both KO mice (n = 22) and littermate controls (n = 14) were used. All mice were kept on a 12-h light:12-h dark cycle, with one to five mice per cage. All experimental procedures were approved by the Northwestern University and the University of Chicago Institutional Animal Care and Use Committees.

For in vivo experiments, mice between postnatal day 45 and 90 were anesthetized with urethane (1.2 g/kg in 10% saline solution, i.p.) and then sedated by chlorpromazine (10 mg/kg in water, i.m.) as described before23,48. Atropine (0.5 mg/kg in 10% saline) and dexamethasone (2 mg/kg in 10% saline) were administered subcutaneously. The animal was then transferred onto a heating pad for recording or imaging. The animal’s body temperature was monitored through a rectal thermoprobe and maintained at 37°C through a feedback heater control module (Frederick Haer Company, Bowdoinham, Maine). The toe–pinch reflex was monitored during experiments to test for depth of anesthesia. Additional urethane (0.2–0.3 g/kg) was administered when necessary. After the mouse was anesthetized, the scalp was shaved and skin removed to expose the skull. For whole-cell and single-unit recording, a metal plate was mounted on top of the skull with Metabond (Parkell, Edgewood, NY) mixed with black ink. The plate was then mounted to a steel stand on the vibration isolation table. A thin layer of silicon oil was applied on both eyes to prevent drying. A craniontomy (~4.0 × 2.0 mm²) was performed on the left hemisphere, and the tissues, including the entire V1, overlaying the SC were removed by aspiration to expose the SC. The procedures for imaging are described below.

In vivo whole-cell recording. Blind whole-cell patch-clamp recording was performed to record SGS neurons intracellularly. Glass pipettes had tip openings of 1.5–2.0 μm (5–7 MΩ). For current-clamp recordings, the K+-based internal solution contained 135 mM potassium-glucuronate, 7 mM KCl, 0.5 mM EGTA, 10 mM HEPES, 10 mM sodium-phosphocreatine, 4 mM Mg-ATP, 0.4 mM Na-GTP and 0.5% biocyctein. The pH was adjusted to 7.25 with KOH. For voltage-clamp recordings, both K+-based (n = 66) and Ca2+-based (n = 21) internal solutions were used. The Cs+-based internal solution contained 125 mM cesium-glucuronate, 2 mM CsCl, 0.5 mM EGTA, 10 mM HEPES, 1 mM QX-314, 5 mM TEA-Cl, 10 mM sodium-phosphocreatine, 4 mM Mg-ATP, 0.4 mM Na-GTP and 0.5% biocytin. The pH was adjusted to 7.25 with CsOH. The K+-based internal solution was used to record EPSCs and Vm from the same cells, and the Ca2+-based internal solution was used only for recording EPSCs. No difference was found between using K+-based and Ca2+-based internal solutions for recording EPSCs. Glass pipettes were advanced perpendicularly to the horizontal plane of the mouse’s head until just touching the SC surface. We then added 2% agarose in artificial cerebrospinal fluid solution (ACSF, containing 140 mM NaCl, 2.5 mM KCl, 11 mM glucose, 20 mM HEPES, 2.5 mM CaCl2, 3 mM MgSO4 and 1 mM Na2HPO4) onto the exposed SC to stabilize the brain. A small piece of wet gauze was placed onto the agarose to prevent drying. Pipettes were then inserted into the SC.

Electrical signals were amplified using a MultiClamp 700B (Axon Instruments, CA) and acquired with a System 3 workstation (Tucker Davis Technologies, FL) at 10 kHz. Pipette capacitance and the electrode resistance were compensated initially. For current–clamp recordings, only responsive cells with stable resting membrane potentials and series resistances lower than 80 MΩ across the duration of the recordings were included in our analysis. No current injection was applied during recordings except to measure series resistance40. For voltage-clamp recordings, neurons were clamped at the reversal potential of ionization, which was determined by adjusting the holding potential to minimize the amplitude of the inhibitory postynaptic current evoked by photostimulation of GAD2+ neurons (Supplementary Fig. 3). The mean reversal potential for inhibitory currents in excitatory cells was −64.92 ± 0.21 mV (n = 43). For recordings of inhibitory cells, −65 mV was used as the holding potential to isolate excitatory currents. Only responsive cells with stable current baselines and series resistance < 50 MΩ across the duration of the recordings were included in our analysis. Note that the reported values were not corrected for the junction potential. The depths of recorded cells were between 0 and 300 μm (reading from the micromanipulator) from the point where the pipette broke into the thin membrane on the SC surface as indicated by a large sudden and quickly recovered electrical signal change. The morphology of nine biocytin-stained cells further confirmed the accuracy of the manipulator readings and confirmed that all the cells were in the SGS.

Histology. After in vivo whole-cell recordings, mice were overdosed with euthanasia solution (150 mg/kg pentobarbital) and perfused with PBS and then 4% paraformaldehyde (PFA). The brain was immersed in 4% PFA overnight. Coronal slices 150 μm thick were cut from the fixed brain using a vibrating blade microtome (VT1000S, Leica Microsystems). The labeled cells were revealed by visualizing biocytin with streptavidin–Alexa Fluor 488 conjugate (Invitrogen). Images were captured using a Zeiss LSM5 Pascal confocal microscope (Carl Zeiss, Jena, Germany) in z-series scanning and reconstructed in Filament Tracer of Imarisl (Bitplane, Zurich, Switzerland).

In vivo extracellular recording. Tungsten electrodes (5–10 MΩ, FHC) were inserted perpendicularly into the SC. The SC surface was estimated visually under the microscope. We then applied 2% agarose in ACSF onto the exposed SC. SGS neurons were recorded between 0 and 300 μm below the surface. The electrical signals were filtered between 0.3 and 5 kHz for spikes and between 10 and 300 Hz for local field potentials, and sampled at 25 kHz using a System 3 workstation (Tucker Davis Technologies, FL). The spike waveforms were further sorted offline in OpenSorter (Tucker Davis Technologies, FL) to isolate single units as described before24,48.

Two-photon calcium imaging of SGS neurons. We followed our recently published procedures for two-photon imaging of the superficial SGS neurons23. Briefly, a craniontomy was performed on the left hemisphere, starting at the lambda point and extending ~3 mm both laterally and rostrally. Tissues overlaying the SC were removed by aspiration. Once the SC was exposed, a glass pipette (inner diameter of 10–20 μm) filled with freshly made solution containing the fluorogenic calcium-sensitive dye Cal-520AM51 (1.13 mM, ATT Bioquest) and 4% paraformaldehyde (PFA) was lowered into the tissue. Twenty pulses of 2.5 μl each (45 ml total volume), at 20-s intervals, were delivered to inject the Cal-520 solution at a depth of 500 μm below the surface. The same procedure was repeated after retracting the pipette to a depth of 250 μm. The pipette was left in the tissue for 1–2 min before being slowly retracted. The SC was then covered by ACSF. Imaging was performed 1–2 h after loading.

Once the injection procedure was complete, a small metal plate was mounted on the mouse’s head with Metabond (Parkell, Edgewood, NY), which, when clamped under the microscope, resulted in the imaged SC surface being largely flat and perpendicular to the optical axis of the objective. A shield was placed around the craniontomy to block light from the visual stimulus during imaging. The SC was covered by 3% agarose in ACSF for stability. Imaging was performed with a two-photon microscope (2P-SC, Bruker Nano Surface Division) and a Ti:sapphire laser (Coherent Chameleon Ultra II) at an excitation wavelength of 800 nm and using a 40×, 0.8-NA objective (Leica). Data were acquired using PrairieView software with a spiral scan at 2× optical zoom, resulting in a circular field of view with a diameter of 135 μm. Image resolution was 256 × 256 pixels and the acquisition rate was 8.079 Hz.

Optogenetic silencing of SGS excitatory neurons. To photostimulate ChR2-expressing cells, we used an optic fiber (0.2-mm core diameter) driven by a blue LED (470 nm, Doric Lenses) placed ~0.5 mm above the exposed SC. The tip of the LED fiber was placed at a similar position in all mice. During recordings, it was buried in the agarose that was applied to reduce the pulsation of the brain and protect the tissue. To prevent direct photostimulation of the eyes by the LED light, the Metabond used for mounting the head plate was prepared with black ink. The agarose surface was painted with black ink, and a piece of thick black paper was carefully placed around the fiber to ensure that light could not be seen from the front and sides, as described before48. The LED was driven by a
square wave starting 500 ms before the onset of each visual stimulus and ending 100 ms after the offset of each visual stimulus (3,600 ms total for sweeping bars, as described below). The intensity of LED light was ~160 mW/mm² at the tip of the optic fiber in all recordings, which was confirmed to be reliably effective in silencing SGS excitatory neurons (Supplementary Fig. 2).

Visual stimulation. For in vivo experiments, visual stimuli were generated with Matlab Psychophysics toolbox25,26 on an LCD (37.5 cm x 30 cm, 60 Hz refresh rate, ~50 cd/m² mean luminance) or CRT monitor (40 cm x 30 cm, 60 Hz, ~35 cd/m² luminance). The monitor was placed 25 cm away from the eye contralateral to the recording and imaging site (the right eye), and the screen was slightly adjusted for each cell so that its receptive field was completely covered. For two-photon imaging, the screen was also tilted at an angle matching that of the mouse's head, given that the mouse's nose was slightly elevated to correct for the curvature of SC and allow imaging from a relatively flat surface. The screen was adjusted so that the imaged cells' receptive fields were near the center of the screen. The ipsilateral eye was covered throughout the experiments.

Two types of visual stimuli were used to determine the DS of SGS neurons. First, sweeping bars, 5° wide and drifting at a speed of 30° per s, were used in both physiology and imaging experiments. The drifting directions were varied between 0° and 330° (12 steps, 30° spacing), which were presented in a pseudorandom sequence together with a 'blank stimulus' (grey screen at the mean luminance). In whole-cell recording, the interstimulus interval was 0.5 s or 1 s when there was no LED illumination and was 10 s when LED was used to allow the stimulated inhibitory cells to recover. In two-photon imaging, the interstimulus interval was 3 s. Second, drifting sinusoidal gratings were also used in the imaging experiments, at 0.08 c/°, 2 Hz, 100% contrast23. They were presented at 12 movement directions (0°–330°, with 30° increments) in a pseudorandom order within a circular window (32° in diameter and surrounded by a gray background) near the center of the imaged cells' receptive fields (which was determined by flashing white or black squares as described in reference23). The stimulus duration was 3 s and the interstimulus interval was 5 s. Each stimulus was repeated 4–6 times for imaging and 3–8 times for recording.

To examine whether optogenetic activation of GABAergic neurons could affect retinal transmission by potentially acting presynaptically through GABAB receptors, we recorded visually evoked local field potentials (LFP) in the SGS, before and 15–45 min after administration of the GABAB receptor antagonist CGP54626 (10 μM) to the SC surface. The visual stimulus was a 20° diameter circle flashing on and off, centered at the receptive field of the recorded site. We recorded 40–60 trials to calculate the average response for quantification.

Data analysis. Whole-cell recording data were first analyzed using a custom Matlab program (originally written by a former lab member Dr. Xinyu Zhao). For whole-cell recording, data collection and analysis were not performed blind to the conditions of the experiments because it does not apply. For current-clamp data, spikes were detected by calculating the first derivative of raw voltage traces (dV/dt), and the start of a spike was the time point when dV/dt reached a manually set positive threshold. Individual traces were carefully inspected to ensure proper spike detection. Peristimulus spike time histograms (PSTHs) were calculated by trial-averaging the spike counts in each 50-ms time bin. Subthreshold \( V_m \) traces were extracted by removing spikes from the raw voltage traces using a 6-ms median filter. The subthreshold \( V_m \) traces were trial-averaged for each stimulus condition. The trial-averaged \( V_m \) trace for the blank stimulus (i.e., gray screen) was used to calculate the mean \( \langle V_m \rangle \) baseline and s.d. of spontaneous \( V_m \) fluctuations. The \( V_m \) baseline was then subtracted from the trial-averaged \( V_m \) trace for each visual stimulus condition, i.e., bars of certain direction.

For voltage-clamp data, the current traces \( I_m \) were first smoothed by a 40-ms mean filter17,18 and then trial-averaged for each stimulus condition. For visual stimulus conditions in the absence of LED photoactivation (i.e., LED-off), the EPSC baseline was calculated as the mean of the trial-averaged \( I_m \) trace to the blank stimulus and subtracted from the trial-averaged trace of each condition. For LED-on conditions, \( I_m \) traces were similarly trial-averaged for each stimulus condition. For inhibitory cells, all traces showed LED-activated ChR2 currents in addition to visually evoked EPSCs (Supplementary Fig. 5). The trace for the blank condition was flat in most excitatory cells (Supplementary Fig. 5), but in some cells, a slowly increasing inward current was seen during LED stimulation; this was possibly the 'bystander currents' mediated by acid-sensing ion channels44. The dynamic of this slow current, when present, was always the same for all stimulus conditions of the same cell, including the blank stimulus. We therefore did a point-by-point subtraction of a further-smoothed trace of the blank condition from the trace of each condition. This was done for both excitatory and inhibitory cells to remove the LED-evoked currents, while keeping the fluctuations in each trace (including the blank condition, which was used to determine baseline fluctuation). Finally, the intracollicular EPSC traces were generated by a point-by-point subtraction of retinal EPSCs (LED-on) from the total EPSCs (LED-off) traces (Supplementary Fig. 7).

For analyzing \( V_m \) and EPSCs, we determined time windows of responses to the sweeping bars. This was necessary to find 'response' magnitude for directions that evoked weak or no responses. To do this, we first calculated a cutoff threshold, which was the \( V_m \) or EPSC value 2 s.d. away from the mean of the baseline fluctuation (determined from the blank condition as described above). The widest segment of the traces that were above (for \( V_m \)) or below (for EPSCs) the threshold was determined as the response time window for each stimulus condition. This time window was expanded if there were any short above-threshold segments within 150 ms of the two sides. Next, the conditions that evoked wider time windows were used to guide the analysis of other conditions, to ensure that the estimation of response window was not too conservative or inaccurate for nonpreferred directions. Specifically, for conditions where the window was narrower than 1/3 of the widest window of this cell (or 333 ms if the widest window was longer than 1 s), the response-time window determined from the opposite direction, reversed in timing, was used. All traces were checked visually to confirm that the time windows were determined properly. Peak \( V_m \) and spike rate were calculated for the response time window of each stimulus condition, subtracting the mean values of blank condition. For EPSCs, they could spontaneously fluctuate across the threshold, thus leading to an overestimation of the weak responses. Therefore, for data shown in the main text, if the peak EPSCs were within 3 s.d. of the baseline, they were manually set to 0. We compared this with other methods of calculation (Supplementary Fig. 8) and found no difference in our conclusions. Finally, the EPSC charge integral for each stimulus condition was quantified as the time integral of the data points in the response time window as determined above.

For SC two-photon imaging and data analysis, the experimenter was blind to the genotype of the mice. Animals that had visible tissue damage to their SC after dye loading or for which the dye failed to be incorporated into the cells were not subject to imaging. Data analysis was performed on all animals that were subject to imaging, and no data points were excluded from the resulting data sets. We followed our published analysis procedures25,29. Briefly, regions of interest (ROIs) were drawn manually on the average images, and the intensity values for all pixels in each ROI were averaged for each frame to obtain the raw Ca²⁺ signal of each cell. From the raw signal, for each stimulus presentation, \( \Delta F/F_0 = (F - F_0)/F_0 \) was calculated, where \( F_0 \) was the mean of the baseline signal over a fixed interval of 1.25 s (for gratings) or 0.75 s (for bars) before stimulus onset; and \( F \) was the fluorescence signal from 250 ms after stimulus onset to 500 ms after stimulus offset. A cell was considered responsive if its mean \( F \) (for gratings) or peak \( F \) (for bars) was more than two s.d. above its \( F_0 \) for at least one of the stimulus conditions. The mean (for gratings) or peak (for bars) value of \( \Delta F/F_0 \) for each of the stimulus conditions was then used to determine the direction tuning curves for every responsive cell.

To quantify the degree of direction selectivity, we calculated a global direction selectivity index (gDSI), which is the vector sum of responses normalized by the scalar sum of responses23,49:

\[
gDSI = \frac{\sum R_{\text{pref}, \theta} \theta}{\sum R_{\text{opp}}},
\]

where \( R_{\text{pref}} \) is the response magnitude of spikes, \( V_m \), EPSC or \( \Delta F/F_0 \) at 0 direction of bars or gratings. The preferred direction is quantified as the angle of the vector sum of responses. Previous studies of direction selectivity mostly used

\[
\text{DSI} = \frac{R_{\text{pref}} - R_{\text{opp}}}{R_{\text{pref}} + R_{\text{opp}}},
\]

where \( R_{\text{pref}} \) is the cell's maximal response and \( R_{\text{opp}} \) is the cell's response to the opposite direction. To facilitate comparisons with such studies, we plotted the relationship between the gDSI and DSI of individual neurons for both whole-cell and imaging data (Supplementary Fig. 12).
To classify whether a cell was direction selective in Figure 4, we used a criterion of gDSI-EPSC ≥ 0.1. Of the 19 cells that met this criterion and received direct retinal inputs, four cells showed a small but obvious second peak in their tuning curves at the direction opposite to the preferred direction, thus leading to a global orientation selectivity index

\[
gOSI = \frac{\sum_R g_{R,2} \cdot \frac{\Delta F}{F_0}}{\sum_R g_{R,1}}
\]

greater than their gDSI. The other 15 cells had gDSI values greater than their gOSI. We therefore did not include these four cells in the DS group, but none of our conclusions would change if these four cells were included.

**Retinal calcium imaging and data analysis.** WT littermate controls and Vgat KO mice were given intravitreal injections after eye opening at P18 with an AAV2 viral vector carrying GCaMP6s (University of Pennsylvania Vector Core). After 21 d, the injected mice were dark-adapted for 1 h and their retinas were dissected in the dark under infrared (IR) light. During dissection, the retina was cut into dorsal and ventral pieces following the procedure described by Wei et al.52 and the nasal direction was noted for each piece. The dissected retinas were kept in darkness at room temperature (21–22 °C) in Ames’ medium bubbled with 95% O2/5% CO2 until use (0–7 h), and then imaged at 33 °C. Cells were imaged at 33 °C and IR light was used to visualize GCaMP6s+ cells. GCaMP6s expression was imaged at 21–22 °C with a white organic light-emitting display (OLEDXL, eMagin; 800 × 600 pixel resolution). To classify whether a cell was direction selective in this study, we used a criterion of gDSI-EPSC ≥ 0.1. Of the 19 cells that met this criterion and received direct retinal inputs, four cells showed a small but obvious second peak in their tuning curves at the direction opposite to the preferred direction, thus leading to a global orientation selectivity index

\[
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Statistics. All pooled data were presented as mean ± s.e.m. Statistical significance was calculated using nonparametric, two-sided, Mann-Whitney U-tests, Wilcoxon signed-rank tests, Kolmogorov-Smirnov (K-S) tests or χ2 tests, as mentioned in the text. All analyses and graph plotting were performed in Matlab (MathWorks) or Prism (GraphPad Software, Inc.). No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in the field. We did not randomly assign animals to groups for whole-cell recording because it does not apply to these experiments. For two-photon imaging experiments, KO mice and littermate controls were assigned according to their genotypes. A Supplementary Methods Checklist is available.

Data and code availability. The data that support the findings of this study and the custom Matlab code are available from the corresponding author upon reasonable request.

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