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The First Stage of Cardinal Direction Selectivity Is Localized to the Dendrites of Retinal Ganglion Cells

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SUMMARY

Inferring the direction of image motion is a fundamental component of visual computation and essential for visually guided behavior. In the retina, the direction of image motion is computed in four cardinal directions, but it is not known at which circuit location along the flow of visual information the cardinal direction selectivity first appears. We recorded the concerted activity of the neuronal circuit elements of single direction-selective (DS) retinal ganglion cells at subcellular resolution by combining GCaMP3-functionalized transsynaptic viral tracing and two-photon imaging. While the visually evoked activity of the dendritic segments of the DS cells were direction selective, direction-selective activity was absent in the axon terminals of bipolar cells. Furthermore, the glutamate input to DS cells, recorded using a genetically encoded glutamate sensor, also lacked direction selectivity. Therefore, the first stage in which extraction of a cardinal motion direction occurs is the dendrites of DS cells.

INTRODUCTION

The visual system analyzes different categories of motion from the image flow that is projected onto the photoreceptors. Even at the front of the visual stream, in the retina, a number of parallel circuits extract information about motion. Within the different motion categories, most retinal hardware is dedicated to the analysis of the direction of motion (Barlow and Hill, 1963; Barlow et al., 1964; Vaney et al., 2012; Wei and Feller, 2011). Three different groups of ganglion cell types are dedicated to this task in mice: ON-OFF (Huberman et al., 2009; Kay et al., 2011; Trenholm et al., 2011; Weng et al., 2005), ON (Sun et al., 2006; Yonehara et al., 2008, 2009), and OFF (Kim et al., 2008) DS cells. Individual cell types within these three groups respond preferentially to one of the four cardinal directions—backward, upward, forward, or downward—and project their axons to various target brain regions, including the lateral geniculate nucleus, the superior colliculus, and the medial or dorsal terminal nuclei.

Both ON-OFF and ON DS cells are remarkably selective for motion direction along the axis of their preferred direction, producing no spikes, or only a few, when an image is moving opposite to the preferred, the so-called null, direction. This high degree of selectivity along the cardinal directions may be achieved by incrementally increasing direction selectivity along the photoreceptor-bipolar cell-ganglion cell route of visual information (Fried et al., 2002, 2005) or, alternatively, the first stage of cardinal direction selectivity is localized to retinal ganglion cells (Borst, 2001; Taylor et al., 2000).

Supporting evidence for the incremental computation of direction selectivity (Figure 1A) has come from electrophysiological studies that have shown that both the excitatory and the inhibitory input currents recorded at the cell body of DS cells were direction selective (Fried et al., 2002, 2005; Sun et al., 2006). ON-OFF and ON DS cells receive glutamatergic excitatory input from specific types of bipolar cells and inhibitory input from starburst amacrine cells. Therefore, it has been postulated that the computation of cardinal direction selectivity is first achieved at the axon terminals of bipolar cells (Borg-Graham, 2001; Fried et al., 2002) and a further increase in selectivity occurs at the dendrites of DS cells and that this pre- and postsynaptically distributed processing ensures robustness (Fried et al., 2002). It has been shown that starburst cells are necessary for the computation of direction selectivity (Yoshida et al., 2001) and it has been proposed that the spatially asymmetric connectivity from starburst cells, as well as dendritic computations within starburst cells (Euler et al., 2002; Hausselt et al., 2007; Lee and Zhou, 2006), provide the basis for the computation of direction selectivity. Experimental evidence for asymmetric connectivity from starburst cells to DS cells has been obtained for both ON-OFF (Briggman et al., 2011; Fried et al., 2002; Lee et al., 2010; Wei et al., 2011) and ON (Yonehara et al., 2011) DS cells. Recordings of direction-selective activity at subcellular

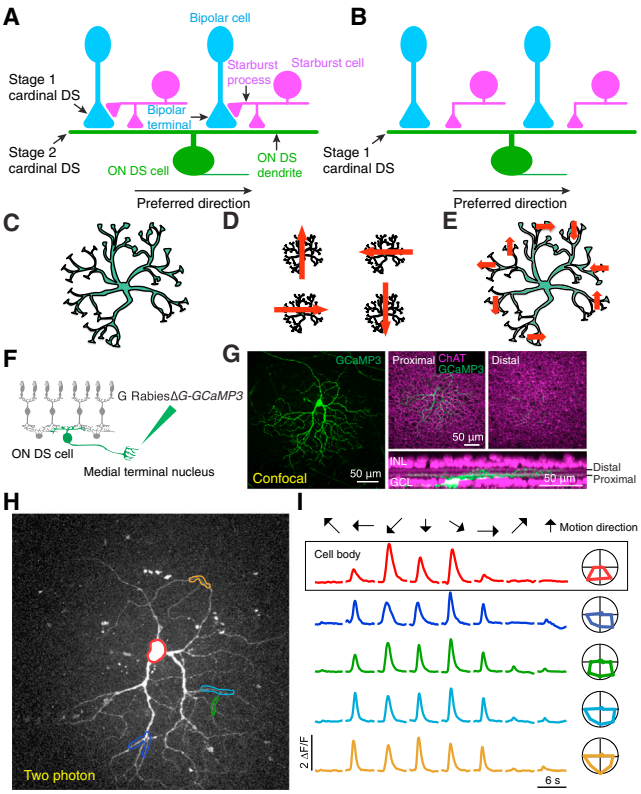

Figure 1. Dendritic Segments of ON DS Cells Are Direction Selective

(A and B) Two circuit models of cardinal direction selectivity in ON DS cells. (A) Cardinal direction selectivity is set up first at bipolar cell terminals and further enhanced at ganglion cell dendrites. (B) Cardinal direction selectivity is set up first at ganglion cell dendrites. (C–E) Schematics of three models for the functional units of direction selectivity in the bipolar cells: single bipolar cell type with axon terminals that are not selective for direction of motion (C); four different bipolar cell types having axon terminals at the same retinal layer, each bipolar cell type selective for a different direction (D); single bipolar cell type with four types of terminals, each selective for a different direction (E). (F) ON DS cells were targeted by GCaMP3-expressing, G-coated G-deleted rabies virus, which was injected into the medial terminal nucleus. (G) Confocal images of a retina in which an ON DS cell is labeled with GCaMP3 (green). Most dendritic segments of the labeled cell are stratified at the proximal ChAT-labeled layer (magenta). (H) Maximum image projection of two-photon image of the same ON DS cell shown in (G). Regions of interest are marked by colored lines. (I) Two-photon imaging of calcium transients in the cell body (top row) as well as in four dendritic segments of the ON DS cell shown in (H), in response to stimuli moving in eight different directions. Colors of the traces indicate locations of the recorded compartments in (H). Polar plots of the peak responses are shown on the right. See also Figure S1.

resolution has been shown at the dendrites of ON-OFF DS cells (Oesch et al., 2005), but not yet at the dendrites of ON DS cells. Direction selectivity has not yet been demonstrated directly at the axon terminals of bipolar cells that provide input to any of the DS cell groups.

The alternative model is that direction selectivity for cardinal directions appears first at the dendrites of the direction-selective ganglion cells (Figure 1B) (Taylor et al., 2000; Vaney et al., 2012). According to this view, activity at the bipolar terminals is not selective for motion direction (Figure 1C), and the direction-selective excitatory input measured at the cell bodies of DS cells reflects the technical limitations of patch-clamp recording: the inability of an electrode positioned at the cell body to voltage clamp at the location of synapses (Poleg-Polsky and Diamond, 2011; Vaney et al., 2012). This model is attractive, since the spatially asymmetric connectivity at the axon terminals of bipolar cells raises conceptual problems (Vaney et al., 2012). Since direction selectivity has been described for motion in three (ON DS cells) or all four (ON-OFF DS cells) cardinal directions, there should be either four types of bipolar cells, each being selective for one of the directions (Figure 1D), or each bipolar cell should perform parallel processing (Asari and Meister, 2012) so that the different axon terminals of the same bipolar cell have different preferred directions (Figure 1E). The first scenario would require many physiologically different types of bipolar cells; the second would require a sophisticated wiring between starburst cells and individual bipolar terminals.

To differentiate between these two alternative models for computing direction selectivity, we used monosynaptically restricted retrograde viral circuit tracing (Callaway, 2008; Osakada et al., 2011; Ugolini, 2011) initiated from individual upward or downward motion-selective ON DS cells (Yonehara et al., 2011). First, we determined the types of bipolar cells that are connected to these ON DS cells. Next, using GCaMP3 (Tian et al., 2009), a genetically encoded calcium indicator that we expressed from the tracer virus, we performed simultaneous two-photon imaging of activity from the dendrites of ON DS cells, the axon terminals of ON DS cell-connected bipolar cells, and the processes of starburst amacrine cells during visual motion stimulation (Reiff et al., 2010). The individual dendritic segments of ON DS cells were highly direction selective, with the same preferred cardinal direction. The processes of starburst cells were also direction selective along the centrifugal axis, from the cell body to the process tip (Euler et al., 2002). In striking contrast, the activity at the axon terminals of bipolar cells that were connected to ON DS cells were not direction selective. Finally, we monitored glutamate concentration around ON DS cell dendrites during motion stimulation using iGluSnFR, a genetically encoded glutamate sensor (Marvin et al., 2013). iGluSnFR signals were also not direction selective. Therefore, our results imply that cardinal direction selectivity appears first at the dendrites of DS cells.

RESULTS

Dendritic Segments of ON DS Cells Are Direction Selective

To investigate whether individual dendritic segments of ON DS cells are direction selective, we labeled ON DS cells with GCaMP3 using the retrogradely transported G-deleted rabies virus (Wickersham et al., 2007a) injected into the medial terminal nucleus where the axons of upward or downward motion-selective ON DS cells terminate (Figure 1F). Immunohistochemistry with the ChAT antibody, a marker of starburst cells and the retinal layer where ON DS cells extend their dendrites, revealed that most dendritic segments of GCaMP3-marked ganglion cells were stratified at the proximal ChAT-labeled layer (Yonehara

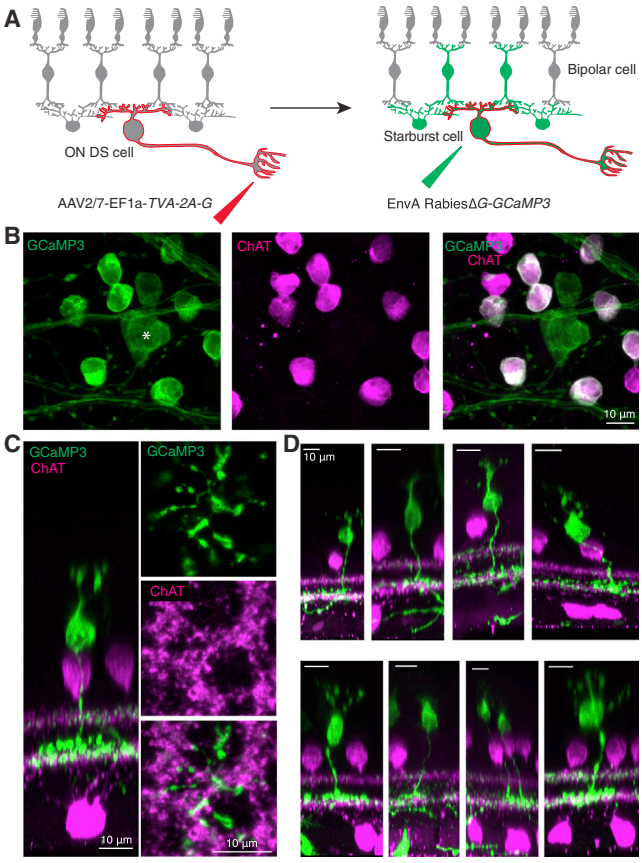

Figure 2. Type-5 Bipolar Cells Are Connected to ON DS Cells

(A) Schematics of the viral tracing approach used to label ON DS cells as well as their presynaptic bipolar cells and starburst amacrine cells. (B) Confocal images of a retina in which an ON DS cell (*) and starburst amacrine cells (magenta) are infected with G-deleted rabies virus expressing GCaMP3 (green). (C) Side view (left) and top view (right) of an example of a labeled type-5 bipolar cell. The image for the top view was taken at the depth of the proximal ChAT-labeled layer. (D) Nine more examples of labeled type-5 bipolar cells. See also Figure S2.

et al., 2008, 2009), suggesting successful targeting of ON DS cells (Figure 1G). We stimulated isolated retinas with a positive contrast spot moving in eight different directions, and performed two-photon imaging of GCaMP3-labeled ganglion cells (Figures 1H and 1I). Calcium responses in the cell body were highly direction selective. Next, we recorded calcium responses along dendrites of ON DS cells. We found that most dendritic segments examined were direction selective and showed the same preferred direction as the cell body. These experiments showed that the dendritic segments of ON DS cells, in a similar way to ON-OFF DS cells (Oesch et al., 2005), are direction selective.

To confirm that electrophysiological recordings from the cell bodies of ON DS cells display direction-selective excitatory and inhibitory input currents (Sun et al., 2006), we made two-photon targeted patch-clamp recordings from ON DS cells in Spig1-GFP mice (Yonehara et al., 2008, 2009), where upward-direction-selective ON DS cells are fluorescently labeled. We found both the excitatory and the inhibitory currents to be direction selective (Figure S1 available online).

Type-5 Bipolar Cells Are Connected to ON DS Cells

We sought to investigate the types of bipolar cells and amacrine cells that are connected to ON DS cells. We injected the retrograde tracer AAVs or herpes viruses expressing TVA receptor and rabies-G protein into the medial terminal nucleus. As a result, ON DS cells expressed the TVA receptor and the rabies-G protein (Figure 2A). We then injected GCaMP3-expressing, EnvA-coated G-deleted rabies viruses into the eye. Since EnvA specifically binds to TVA, rabies virus infected only ON DS cells. Due to the presence of rabies-G expressed from AAV/herpes viruses in ON DS cells, the G-deleted rabies viruses were complemented with rabies-G and crossed one synapse retrogradely to mark the monosynaptically connected cells (Wickersham et al., 2007b; Yonehara et al., 2011) (Figure 2A). The transsynaptic spread of rabies virus has been observed to be specific to synaptically connected neurons and not to adjoining neurons that are either not connected or gap-junction connected (Ugolini, 2011). Injection of EnvA-coated rabies virus into the eye without supplying the TVA receptor did not result in any labeling of retinal cells in 15 independent eye injections (Figure S2). Immunohistochemistry, together with three-dimensional (3D) confocal image reconstruction, showed that bipolar and amacrine cells were labeled together with ON DS cells. Most of the labeled amacrine cells were ChAT-positive starburst amacrine cells located in the ganglion cell layer (Figure 2B), confirming previous results that starburst cells are presynaptic to ON DS cells (Yonehara et al., 2011). Based on the confocal image stacks, we identified a morphological type of bipolar cell as presynaptic to ON DS cells. The axon terminals of all (21/21) bipolar cells were positioned slightly above the proximal ChAT-labeled layer and were therefore categorized as type-5 bipolar cells (Ghosh et al., 2004) (Figures 2C, 2D, and S2). We did not find any labeled type-6 or type-7 bipolar cells, even though the axon terminals of these bipolar cells are physically close to the dendrites of ON DS cells at the proximal ChAT-labeled layer (Ghosh et al., 2004) and have therefore had opportunities to contact ON DS cell dendrites.

Activity at the Axon Terminal of ON DS Cell-Connected Bipolar Cells Is Not Selective for Motion Direction

We used the same combination of rabies and AAV/herpes viruses that we had used for circuit labeling to record calcium responses via GCaMP3 from the axon terminals of labeled bipolar cells. We stimulated retinas with a positive contrast spot moving in eight different directions. We first imaged the cell body of an ON DS cell and made sure that it was direction selective. Next, we imaged calcium responses in the axon terminal endings of connected bipolar cells. Each axon terminal button of a connected bipolar cell was visible under the two-photon microscope (Figure 3A). Based on their appearance (large buttons), we could differentiate them from ON DS dendrites and starburst processes. To further ensure the subcellular and cellular identity of each recorded compartment, we obtained a two-photon image stack at the end of each recording session, fixed the retina, performed immunohistochemistry to label GCaMP3 and starburst cells, and reconstructed the labeled ON DS circuit using 3D confocal imaging. We then aligned the

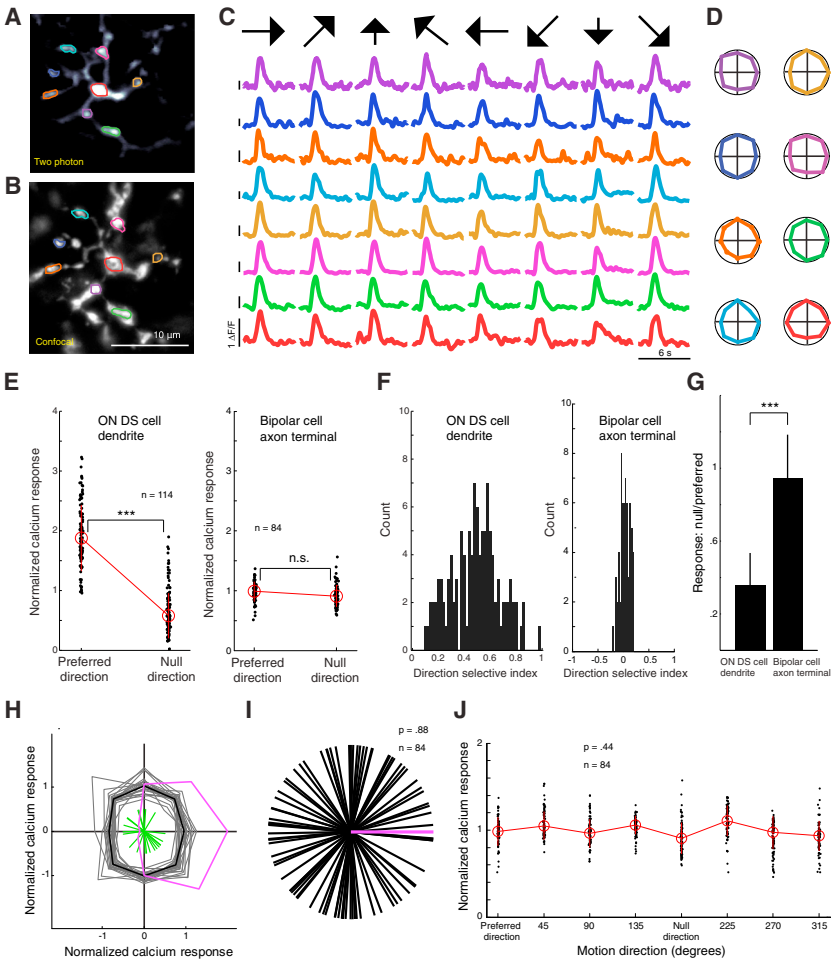

Figure 3. Activity at the Axon Terminal of ON DS Cell-Connected Bipolar Cells Is Not Selective for Motion Direction

(A) Top view of two-photon image of bipolar cell axon terminal labeled with GCaMP3 expressed from transsynaptic rabies virus initiated from an ON DS cell. Regions of interest are marked by colored lines. (B) Confocal picture of the same region as in (A). (C) Calcium transients recorded by two-photon imaging in response to stimuli moving in eight different directions. Colors of the traces indicate locations of the recorded compartments in (A) and (B). (D) Polar plots of peak responses to each direction of motion. Colors of the plots indicate the recorded compartments marked in (A) and (B). (E) Normalized ([ΔF/F]/mean[ΔF/F]) calcium responses of ON DS cell dendritic or bipolar cell axon terminal compartments in the preferred and null directions. The response of each recorded compartment is shown as a small dot. Large open dot and red vertical line represent the mean and ±SD, respectively. The preferred/null directions for the bipolar terminals refer to the preferred/null directions of the monosynaptically connected ON DS cell. (F) Histograms of the direction-selective index of ON DS cell dendritic (left) and bipolar cell axon terminal (right) compartments. (G) The ratio of the null direction response to the preferred direction response for ON DS cell dendrites and bipolar cell axon terminals. Data are represented as mean ± SD. (H) Polar plot of normalized calcium responses ([ΔF/F]/mean[ΔF/F] of each compartment) of an example ON DS cell body (magenta) and axon terminal compartments of the connected bipolar cells (gray and black) to each direction of motion. Gray plots and a black plot are the responses of each recorded compartment and the mean, respectively. Green bars indicate the direction of the vector sum of the activity of each bipolar terminal compartment within this single circuit. (I) The direction of the vector sum of the activity of each bipolar terminal compartment (black) across all recorded bipolar terminals relative to the preferred direction of the monosynaptically connected ON DS cell body (magenta). All preferred directions of ON DS cell bodies were chosen to point in the same direction. (J) Normalized ([ΔF/F]/mean[ΔF/F] of each compartment) calcium responses of bipolar cell axon terminal compartments in each direction of motion. The response of each recorded compartment is shown as a small dot. Large open dot and red line represent the mean and ±SD, respectively. See also Figure S3.

confocal and two-photon image stacks and determined whether the recorded compartments were axon terminals of bipolar cells, processes of starburst cells, or dendrites of ON DS cells (Figures 3A, 3B, and S3). Starburst processes could be identified because they were double-positive for GFP and ChAT. Strikingly, we found that none of the axon terminals of the 17 type-5 bipolar cells that belonged to the local circuit of nine ON DS cells that were recorded were direction selective: they responded in a similar way to all directions (Figures 3C–3J). The lack of direction selectivity was not only observed in averaged signals (three repetitions) but also in individual responses and we did not find response failures (Figure S3). The response vector of bipolar terminals pointed in random directions, neither aligned with the preferred direction of the connected ON DS cell nor with any other cardinal directions (Figures 3H and 3I). Some type-5 bipolar terminals provide synaptic input to ON DS cells, while others may drive different ganglion cell types; however, we found no direction-selective activity even when the analysis was restricted to those bipolar terminals that were positioned next to ON DS cell dendrites (Figures 4E–4G), suggesting that activity in all identified type-5 bipolar cell terminals are direction nonselective.

Concerted Activity at Subcellular Resolution within the Circuit of a Single ON DS Cell

To simultaneously observe the concerted activity of ON DS-connected bipolar terminals, starburst cell processes, and ON DS dendrites during motion stimulation, we imaged retinal regions around an ON DS cell where all of these three elements were labeled. It was possible to visualize the synaptic compartments of the circuit simultaneously, since these compartments are restricted to one two-photon image plane in the inner plexiform layer of the retina (Figures 4A and 4B). The simultaneous imaging of subcellular compartments clearly showed the different behavior of the three circuit elements: the axon terminals of bipolar cells were not direction selective, the processes of starburst cells showed ''local'' direction selectivity along the centrifugal axis (Euler et al., 2002), and the dendrites of ON DS cells

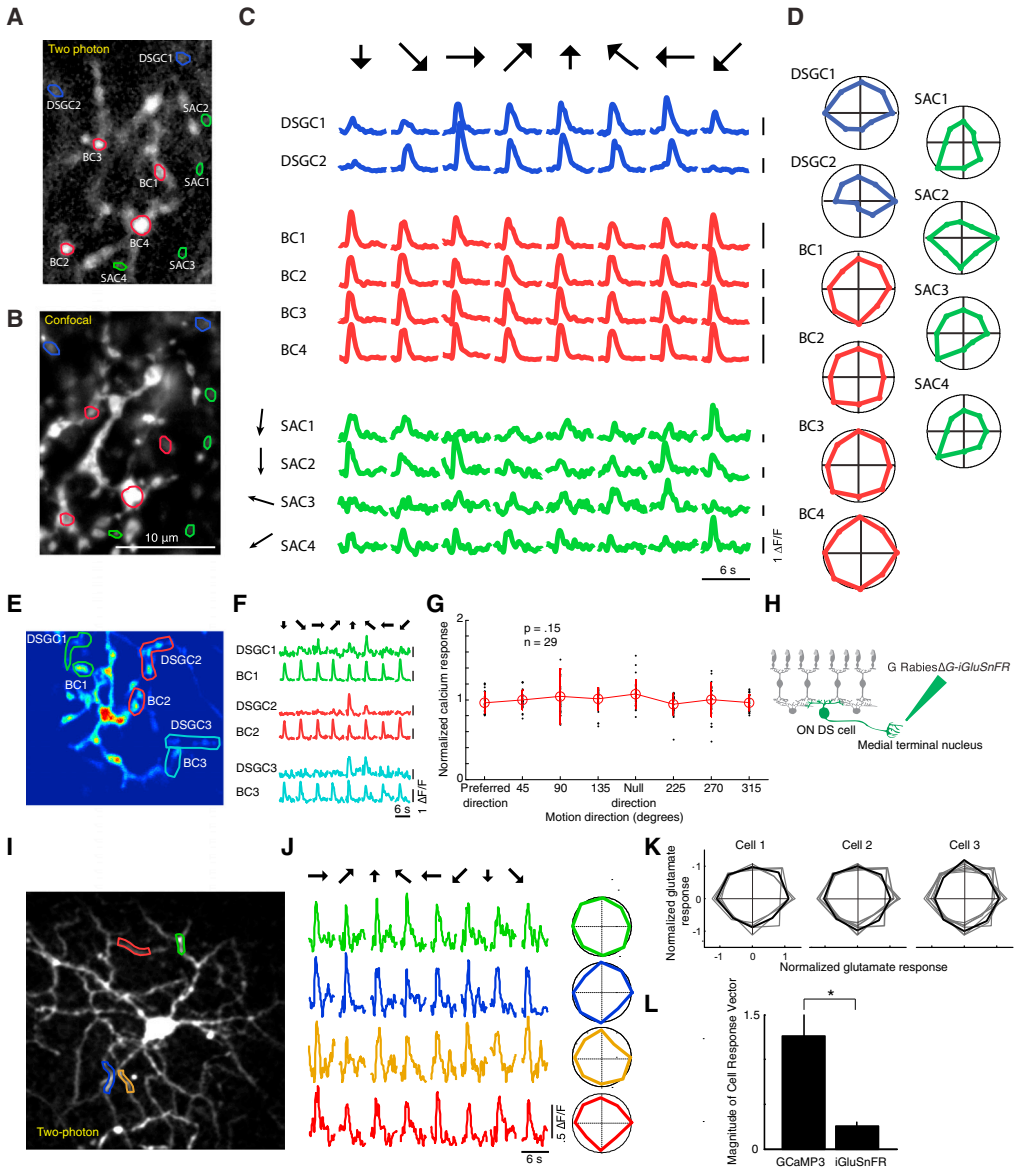

Figure 4. Concerted Activity at Subcellular Resolution within the Circuit of a Single ON DS Cell and Glutamate Input onto Dendrites of ON DS Cells

(A) Top view of two-photon image of bipolar cell axon terminal, starburst cell process, and ON DS cell dendrite labeled with GCaMP3 expressed from trans-synaptic rabies virus initiated from an ON DS cell. Regions of interest are marked by colored lines. BC, bipolar cell; SAC, starburst amacrine cell; DSGC, ON DS cell. (B) Confocal picture of the same region as in (A). (C) Calcium transients in ON DS cell dendrites (blue), bipolar cell axon terminals (red), and starburst cell processes (green) recorded by two-photon imaging in response to stimuli moving in eight different directions. Labels to the left of traces indicate locations of the recorded compartments in (A). The centrifugal directions of starburst cell processes are indicated by arrows at the left side. (D) Polar plots of peak responses to each direction of motion. (E) Two-photon image (top view) of GCaMP3-expressing bipolar cell axon terminals and ON DS cell dendrites shown as a heat map. Regions of interest are marked by colored lines. BC, bipolar cell; DSGC, ON DS cell. (F) Calcium transients recorded by two-photon imaging in response to stimuli moving in eight different directions. Colors of the traces indicate locations of the recorded compartments in (E). (G) Normalized ([ΔF/F]/mean[ΔF/F] of each compartment) calcium responses of those bipolar cell axon terminals that were juxtapositioned to monosynaptically connected ON DS cell dendrites. The response of each recorded compartment is shown as a small dot. Large open dot and red line represent the mean and ±SD, respectively. (H) ON DS cells were targeted by iGluSnFR-expressing, G-coated G-deleted rabies virus, which was injected to the medial terminal nucleus. (I) Maximum image projection of two-photon image of the same ON DS cell shown in Figure S4. Regions of interest are marked by colored lines. (J) Two-photon imaging of glutamate input signals onto the four color-marked dendritic segments of the ON DS cell shown in (I), in response to stimuli moving in eight different directions. Colors of the traces indicate locations of the recorded compartments in (I). Polar plots of the peak responses are shown on the right. (K) Polar plot of normalized glutamate signals ([ΔF/F]/mean[ΔF/F] of each compartment) in the dendrites of three different ON DS cells to each direction of motion. Gray plots and a black plot are the signals of each recorded dendritic compartment and the mean, respectively. (L) Comparing the magnitude of cell response vectors in calcium and glutamate recordings in ON DS cells (see Supplemental Experimental Procedures). Data are represented as mean ± SD.

were all "globally" direction selective along the same axis (Figures 4C and 4D).

Glutamate Signaling at ON DS Cell Dendrites Is Not Direction Selective

Neurotransmission from bipolar cells to ganglion cells is mediated by glutamate. To directly test whether the glutamate input signal to ON DS cells is direction selective or not, we developed a G-deleted rabies virus expressing a glutamate sensor iGluSnFR (Borghuis et al., 2013; Marvin et al., 2013) and injected it into the medial terminal nucleus (Figure 4H). Similar to GCaMP3-marked ganglion cells, most dendritic segments of iGluSnFR-marked ganglion cells were stratified at the proximal ChAT-labeled layer (Yonehara et al., 2008, 2009), suggesting successful targeting of ON DS cells (Figure S4). We stimulated isolated retinas with a positive contrast spot moving in eight different directions and performed two-photon imaging of iGluSnFR-labeled ganglion cells. The dendritic segments of five recorded ON DS cells did not have direction-selective iGluSnFR signals (Figures 4I–4L). These experiments suggested that the glutamate input signal to the dendrites of ON DS cells is not direction selective.

DISCUSSION

The key finding of this work is that direction-selective activity is absent both in the Ca signals measured at axon terminals of those specific bipolar cells, type-5, that connect to ON DS cells, as well as in the glutamate signals around the dendrites of ON DS cells. In contrast, the visually evoked Ca signals of the dendritic segments of ON DS cells are direction selective.

Mechanism of Direction Selectivity

Direction-selective computation in the retina, discovered in 1963 (Barlow and Hill, 1963), served for a long time as a model circuit to explain how a specific neuronal computation is implemented by neuronal hardware. It has been proposed that the key components of this computation are the centrifugal direction selectivity in starburst cells and the asymmetric connectivity between starburst and DS cells, as well as those bipolar cell axon terminals that provide input to DS cells. An increase in direction selectivity also occurs within ganglion cells, after the combination of inhibition and excitation, by the action of active conductances (Oesch et al., 2005; Schachter et al., 2010). In addition, in the case of the upward-motion-sensitive ON-OFF DS cell, the shape of the cell plays an important role in contributing to direction selectivity at slower speeds (Trenholm et al., 2011). There is direct proof of the centrifugal direction selectivity of starburst cells (Euler et al., 2002; Hausselt et al., 2007; Lee and Zhou, 2006) and the asymmetric connectivity between starburst and ON-OFF DS cells (Briggman et al., 2011; Fried et al., 2002; Lee et al., 2010; Wei et al., 2011), as well as ON DS cells (Yonehara et al., 2011). Asymmetric connectivity between starburst cells and bipolar cell axon terminals was inferred from indirect evidence, electrophysiological recordings from ganglion cell bodies (Fried et al., 2002, 2005). Our results, demonstrating the lack of direction selectivity in the Ca signals at bipolar cell axon terminals as well as the glutamate signals around ON DS cell dendrites, suggest that there is no spatially asymmetric connectivity to bipolar terminals and that the electrophysiological results probably reflect space-clamp problems at the synaptic sites (Poleg-Polsky and Diamond, 2011; Vaney et al., 2012). We found that direction selectivity in the cardinal directions is first achieved at the third (and last) neuron of the retina's excitatory neuronal chain.

In the rabbit retina, some of the dendritic segments of ON DS cells run together in tight fascicles (He and Masland, 1998). If the dendrites of ON DS cells are arranged similarly in mice, an alternative explanation for the lack of direction-selective glutamate signals in ON DS ganglion cells could be that glutamate spills over from the bipolar-to-ganglion cell synapse (Chen and Diamond, 2002; Sagdullaev et al., 2006), activating extrasynaptically expressed iGluSnFR in the cofasciculating dendrites. If these dendrites had different preferred directions, despite a potentially direction-selective glutamate signal in the synapse, motion in various directions would activate iGluSnFR on the same dendrite. Therefore, the recorded signal would appear direction nonselective. This explanation is, however, unlikely for the following two reasons. First, it was shown previously that glutamate transmission from mouse ON bipolar cells is mostly confined to the synapse. This is achieved by presynaptic inhibition to bipolar cell axon terminals through GABA C receptors, which limits the spillover of glutamate from the synapse to perisynaptic regions (Sagdullaev et al., 2006). In addition, evidence supporting the lack of extrasynaptic glutamate contributing to iGluSnFR signals in ganglion cells during light stimulation has also been recently provided, by showing that at the border between the termination of ON and OFF bipolar axon terminals, iGluSnFR-expressing ganglion cell dendrites do not report mixed ON and OFF signals (Borghuis et al., 2013). Second, only a fraction of the dendrites of rabbit ON DS cells run together (He and Masland, 1998). Therefore, even if the glutamate signal in the synapse were direction selective and if glutamate spill over would blur the iGluSnFR signals across cofasciculating dendrites, some dendritic segments would still show direction-selective responses. We found iGluSnFR signals to be direction nonselective in every dendritic segment analyzed.

The Concerted Activity of Circuit Elements at Subcellular Resolution

Recent advances in monosynaptic viral tracing have opened up the possibility to follow the activity of many neurons belonging to the same circuit (Osakada et al., 2011). Here, we used GCaMP3-functionalized viral tracing and two-photon imaging to record the activity of the circuit elements of single DS cells at subcellular resolution while these circuit elements were computing a specific task. We were able to image the different synaptic compartments of the circuit simultaneously because these compartments are arranged on a horizontal plane, within a layer in the inner plexiform layer of the retina. We then combined information from recordings with the Ca sensor GCaMP3 in both the presynaptic axon terminals and in the postsynaptic dendrites together with recordings using the glutamate sensor iGluSnFR in the postsynaptic dendrites to define the specific subcellular compartment, in this case the postsynaptic dendrite, at which a particular computation occurs. The recording of synaptic activity at three consecutive levels, Ca signals in axon terminals,

glutamate input signals at dendrites, and Ca signals in dendrites, within an identified circuit can not only reveal the computation logic of a neuronal circuit but could also be used to study the molecular logic of circuit assembly from specific cell-type components or to investigate genetic diseases that lead to synaptic dysfunction.

Unanswered Questions

A number of unanswered questions regarding computations by DS cell circuits in the retina remain. First, why are ON-OFF DS cells optimally stimulated with motion at higher speeds than ON DS cells (Sivyer et al., 2010)? Our finding that ON DS cells are specifically connected by type-5 bipolar cells, one of the three bipolar cell types that could provide synaptic input based on proximity, and another finding that type-7 cells connect to ON-OFF DS cells (Shi et al., 2011) either inclusively or exclusively, point to the bipolar cell input as one potential component of speed selectivity. Second, what are the roles of those amacrine cells (Chiao and Masland, 2003; Wright et al., 1997) that influence the DS circuit but are not necessary for direction selectivity? Third, so far most retinal studies, including this study, have focused on spatial asymmetries between inhibitory and excitatory circuit elements or on the centrifugal asymmetry within starburst cells as the explanation for direction selectivity. However, time delays among excitatory circuit elements, such as bipolar cells, could also contribute to direction selectivity (Reichardt-model), as it is predicted in insects (Borst and Euler, 2011). Future experiments with faster Ca sensors could address the question of whether type-5 cells are engaged in a Reichardt-model-like activation pattern and, thereby, enhance direction selectivity in DS cells. Time delays between the bipolar cells that provide input to starburst cells could also confer centrifugal direction selectivity upon the processes of starburst cells.

There are also unanswered questions regarding the message that DS cells send to the higher visual centers. How do the higher centers interpret the spiking pattern of DS cells? DS cells vary in their activity depending on the direction, speed, and contrast of the motion stimuli. How does the brain sort out these stimulus parameters, especially during natural vision, based on the spikes it receives from a single or multiple DS cells? New technologies combining genetic and transsynaptic labeling, together with optical or electrical readout of activity from the different circuit elements, will probably allow researchers in the field to approach these questions.

EXPERIMENTAL PROCEDURES

Monosynaptic Viral Tracing

For monosynaptic tracing, AAV or herpes virus was injected into the medial terminal nucleus at postnatal day 3 (P3). EnvA-coated rabies virus was injected intravitreously to the eye at P8–P9 using pulled-glass pipettes and a microinjector. Retinas were isolated for imaging and immunohistochemistry at P18–P21.

Two-Photon Targeted Patch-Clamp Recording

The two-photon microscope system and light pathways for light stimulation and targeted patch-clamp recordings from GFP-expressing cells have been described before (Farrow et al., 2013).

Two-Photon Calcium and Glutamate Imaging

The two-photon microscope system was equipped with a Mai Tai HP two-photon laser tuned to 920 nm and a 60× objective. Image data were acquired using custom software developed by Z. Raics. A TTL signal generated at the end of each line scan of the horizontal scanning mirror was used to trigger a UV LED projector (Reiff et al., 2010). Stimuli were presented during the fly-back period of the horizontal scanning mirror. The temporal switching between fluorescence recording and stimulus presentation was performed at a minimum frequency of 500 Hz, which is well above the flicker-fusion frequency of the mouse retina.

See Supplemental Experimental Procedures for detailed description of experimental procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.08.005.

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