Direction selectivity in the retina: symmetry and asymmetry in structure and function

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Abstract | Visual information is processed in the retina to a remarkable degree before it is transmitted to higher visual centres. Several types of retinal ganglion cells (the output neurons of the retina) respond preferentially to image motion in a particular direction, and each type of direction-selective ganglion cell (DSGC) is comprised of multiple subtypes with different preferred directions. The direction selectivity of the cells is generated by diverse mechanisms operating within microcircuits that rely on independent neuronal processing in individual dendrites of both the DSGCs and the presynaptic neurons that innervate them.

Microcircuit
An assembly of neural elements that are smaller than whole neurons but can independently perform computations.

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The visual system extracts many aspects of the visual scene from the image projected on the retina, including information about image motion and its direction. Direction-selective visual neurons not only provide information about the movement of objects such as predators and prey (local image motion) but also about the movement of the animal's head and eyes (global image motion). Almost 50 years ago, Barlow and Hill[1,2] discovered that direction selectivity could be generated in output neurons of rabbit retina, within two synapses of the photoreceptors, a finding that challenged the contemporary idea that such complex visual processing is an emergent property of higher visual centres[3].

Although the prevailing view at the time of this finding was that the retina is a relatively simple structure consisting of only five main classes of neurons, we now know that retinal neurons are comprised of as many as 100 distinct types, each with a characteristic morphology and specific response properties[4–6]. Nevertheless, the location of the retina and its highly organized laminar structure make it a particularly accessible part of the CNS for experimental investigation. Retinal ganglion cells (RGCs), the axons of which form the optic nerves, have relatively large somata located near the inner surface of the retina and can be readily studied both *in vivo* and *in vitro*. There are about 20 distinct types of RGCs, each of which extracts different information from the visual image for transmission to the visual centres in the brain. Because the visual field is mapped topographically on the retina, the receptive-field organization of the RGCs reflects the spatial arrangement of the interneurons that provide excitatory and inhibitory synaptic inputs to RGCs (FIG. 1).

The newly discovered direction-selective ganglion cells (DSGCs) had two receptive-field properties that were particularly striking[1,2]. First, moving visual stimuli that crossed the cell's receptive field elicited strong spiking when moving in a particular 'preferred' direction but little or no response when moving in the opposite 'null' direction (FIG. 2a–c); by contrast, the more common concentric RGCs were isotropic in their responses. Second, when a spot of light was flashed in the centre of the receptive field, the DSGC fired spikes both when the light was turned on (light On) and when it was turned off (light Off), whereas the concentric RGCs fired at either light On or light Off[7]. In the 50 years following the discovery of DSGCs, many studies on the cells and their input neurons have been conducted with the goal of understanding the cellular mechanisms that generate direction selectivity in the retina[8].

In this Review, we summarize the innovative morphological, physiological and developmental studies of the past decade that now provide a cohesive picture of the microcircuit that underlies the generation of direction-selective responses. In addition, we outline some of the perplexing issues that are central to the synaptic mechanisms of direction selectivity that remain to be resolved.

Receptive-field properties of DSGCs

An early influential study laid the foundation for subsequent research by carefully dissecting the receptive-field properties of DSGCs using basic visual stimuli[9]. Apparent-motion stimuli, consisting of two stationary light spots (A and B) or bars flashed in sequence to simulate movement in either the preferred or null direction,

provided insights into spatiotemporal interactions in the direction-selective circuitry[9]. When one light spot (either A or B) was flashed on its own, the DSGC fired at light On and light Off. When the spots were flashed successively in the null sequence (B then A), the response to spot A was largely abolished, indicating that inhibition of responses by null-direction motion is a key mechanism in the generation of direction selectivity. When the spots were flashed successively in the preferred sequence (A then B), the response to spot B was slightly greater than when flashed on its own, suggesting that the facilitation of responses by preferred-direction motion also plays a part. Although the DSGCs were shown to have receptive fields spanning about 3° of visual space (500 µm on rabbit retina), direction-selective spike responses were observed for image motions of less than 0.25° (40 µm), suggesting that the presynaptic circuitry that mediates direction selectivity is composed of multiple direction-selective 'subunits' that are replicated at small intervals across the receptive field of the DSGC[9]. In addition, null-direction inhibition could be elicited by apparent-motion stimuli separated by more than 1° of visual space, indicating that the subunits are large relative to their spacing, with adjacent subunits overlapping considerably.

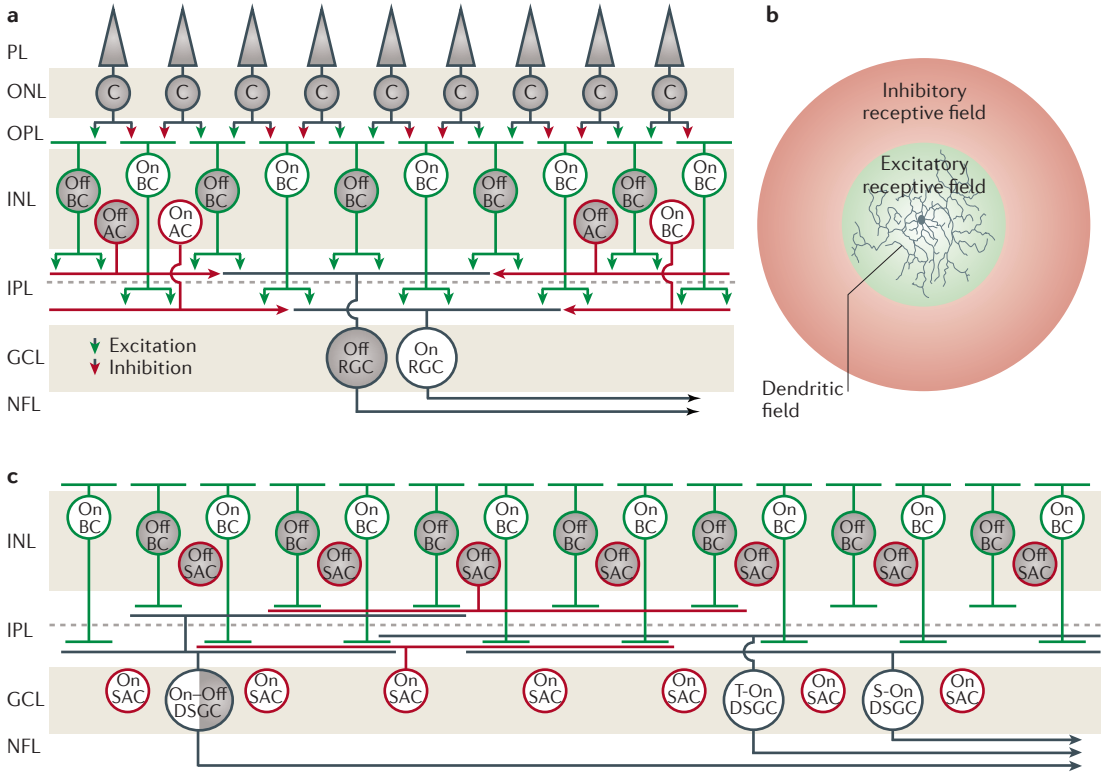

Figure 1 | **Neuronal organization of the retina. a** | A wiring diagram showing the laminar organization of the retina, the major classes of retinal neurons and their synaptic interactions. The cell bodies of the neurons are located in three somatic layers (the outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL)), and their processes interact in two intermediate layers (the outer plexiform layer (OPL) and inner plexiform layer (IPL)). Neurons that are depolarized when a light is turned on (white somata) and those that are depolarized when a light is turned off (grey somata) are also shown. Direct vertical pathways connect the cones (Cs), bipolar cells (BCs) and retinal ganglion cells (RGCs). The cones depolarize when a light is turned off and excite Off BCs (shown by green arrowheads) and inhibit On BCs (shown by red arrowheads). The Off BCs excite Off RGCs in the distal sublamina of the IPL, whereas the On BCs excite On RGCs in the proximal sublamina. The boundary between the Off and On sublaminae is shown by a dashed line. Lateral inhibitory pathways are provided by horizontal cells in the outer retina (not shown) and amacrine cells (ACs) in the inner retina; the dendrites of ACs both receive and make synapses. **b** | The visual receptive field of an RGC is composed of an excitatory receptive field (shown in green) derived from the BCs and an inhibitory receptive field (shown in red) derived from horizontal cells and ACs. The excitatory receptive field is not much wider than the dendritic field of the RGC, whereas the inhibitory receptive field, which overlaps the excitatory field, is much more extensive, reflecting the inhibitory input from large-field ACs. **c** | Major neuronal components of the direction-selective circuits in the retina. The On–Off direction-selective ganglion cells (DSGCs) are bistratified neurons that branch in both sublaminae of the IPL. They receive inputs from Off BCs and Off starburst amacrine cells (SACs) in the distal sublamina of the IPL and from On BCs and On SACs in the proximal sublamina; the somata of the On SACs are located in the GCL, unlike those of other types of ACs. The SACs have widely overlapping dendritic fields (for clarity, the dendrites of only one SAC of each type are shown), and the Off SACs co-stratify with the Off arbor of the On–Off DSGC, whereas the On SACs co-stratify with the On arbor. The transient On (T-On) and sustained On (S-On) DSGCs have much larger dendritic fields than the On–Off DSGCs; the S-On DSGCs partly co-stratify with the On SACs, whereas the T-On DSGCs stratify more distally in the On sublamina. NFL, nerve fibre layer; PL, photoreceptor layer.

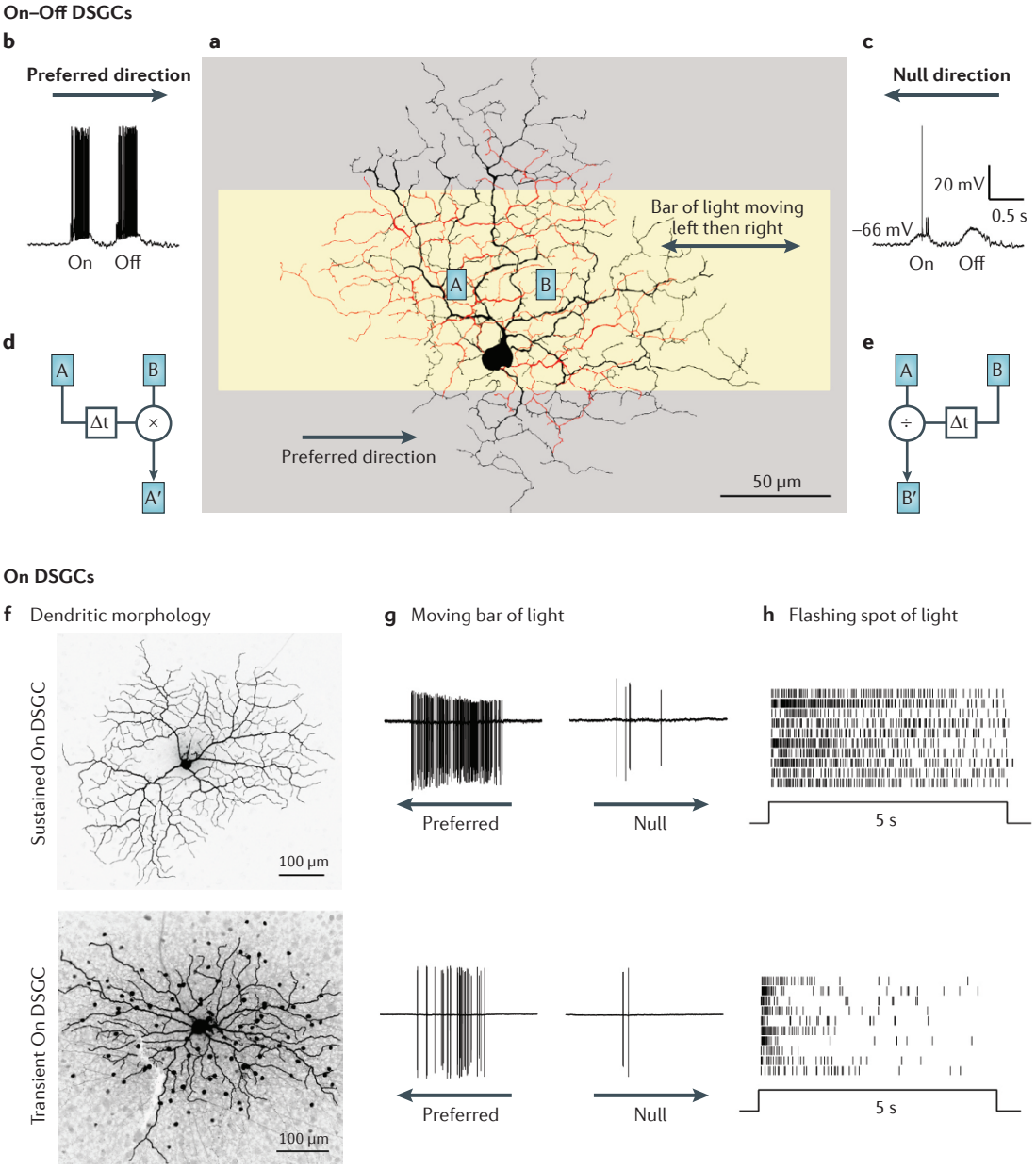

Figure 2 | **Dendritic morphology and receptive-field properties of direction-selective ganglion cells.** **a**–**e** | Properties of On–Off direction-selective ganglion cells (DSGCs) in rabbit retina. Dye filling of a physiologically characterized On–Off DSGC reveals its distinctive bistratified morphology (**a**), with many terminal dendrites distributed throughout the proximal On stratum (shown by black dendrites) and the distal Off stratum (shown by red dendrites). When an extended bar of light (yellow box in part **a**) is moved through the receptive field of an On–Off DSGC in the preferred direction, a somatic current-clamp recording shows that an On spike response is elicited by the leading edge of the bar and an Off spike response by the trailing edge (**b**). Image motion in the opposite null direction elicits little or no spike response (**c**). The generation of direction selectivity depends on nonlinear interactions between inputs (A and B) along the null-preferred axis. Facilitation in the preferred direction (**d**) acts multiplicatively to enhance spiking (producing output A′), whereas asymmetric inhibition in the null direction (**e**) acts divisively to suppress spiking (producing output B′). The facilitatory effect of A on the response to B in the preferred direction and the inhibitory effect of B on the response to A in the null direction need to be prolonged or act after a time delay (Δt) for the direction-selective circuitry to be responsive to moving stimuli. **f**–**h** | Properties of On DSGCs in rabbit retina. Injection of Neurobiotin into physiologically characterized On DSGCs reveals two distinct dendritic morphologies (**f**). The sustained On DSGC has many fine terminal dendrites within its dendritic field, whereas the transient On DSGC has a more sparse morphology; the transient On DSGC shows tracer coupling to overlapping populations of amacrine cells, whereas the sustained On DSGC does not show tracer coupling. Both types of On DSGCs show strong direction-selective spike responses to moving bars of light (**g**), but the sustained On DSGCs show more sustained firing than the transient On DSGCs to a stationary spot of light flashed in the receptive field. Raster plots show the responses of ten cells of each type (**h**). The dye-filled DSGC in part **a** is modified, with permission, from REF. 134 © (1989) John Wiley & Sons. Parts **g**–**h** are modified, with permission, from REF. 51 © (2010) Elsevier.

Box 1 | **Direction-selective ganglion cells with asymmetric morphology**

Several subtypes of On–Off direction-selective ganglion cells (DSGCs) and a novel type of Off DSGC in mouse retina have a strongly asymmetric morphology: the dendritic field is not concentric with the soma but systematically offset in a direction that approximately corresponds to the preferred direction of the subtype.

A newly characterized type of retinal ganglion cell (RGC) in mouse retina transgenically expresses green fluorescent protein (GFP) under the junctional adhesion molecule B (JAMB) promoter. These cells, named J-RGCs, have an asymmetric dendritic tree that extends from the soma towards the ventral retina and are strongly stimulated by visual stimuli moving ventrally on the retina (corresponding to superior object movement in visual space)[22]. Although the J-RGCs branch in the Off sublamina of the inner plexiform layer (IPL), distal to Off starburst amacrine cells (SACs), and give Off responses to small flashing spots centred on the soma of the J-RGC, their responses to moving dark spots seemed to be less direction selective than their responses to moving light spots. Moreover, unlike other types of DSGCs, the direction selectivity of the J-RGCs can be explained to a large extent by the asymmetric morphology and the substructure of the receptive field, with an Off region close to the soma and an On region located ventrally over the terminal dendrites. Correspondingly, the few J-RGCs with a symmetric morphology tree did not show direction-selective responses[22].

The superior-preferring On–Off DSGCs (BD-DSGCs) also have a dendritic tree that is usually offset from the soma in the preferred direction; however, in contrast to the J-RGCs, the few BD-DSGCs with a symmetric morphology showed direction-selective responses[41]. The anterior-preferring On-Off DSGCs (HB9-DSGCs) also have an asymmetric morphology, with the dendritic tree offset from the soma towards the temporal retina in all cells[42]. Surprisingly, the HB9-DSGCs still produce direction-selective spike responses when the GABAergic and cholinergic inputs are blocked, unlike rabbit DSGCs, although the directionality is reduced and only effective for slow-moving targets[42]. This directionality seemed to be generated postsynaptically because the remaining excitatory inputs from bipolar cells were not direction selective. The authors proposed that the asymmetric morphology of the HB9-DSGCs underlies this residual direction selectivity, and that the sites of dendritic spike initiation in the terminal dendrites are primed by a wave of excitation that is generated by centrifugal image motion from the soma to the distal dendrites[42], perhaps analogous to the intrinsic generation of direction selectivity in SAC dendrites[77,89,96]. It would be expected that such a postsynaptic mechanism would also contribute to the direction selectivity of the asymmetric BD-DSGCs and perhaps the J-RGCs in mouse retina. By contrast, there is no correlation between morphological asymmetry and preferred direction for rabbit DSGCs[28,29], perhaps because the inhibitory subunit mechanism is more refined than in mouse DSGCs, which require much larger displacements of the image to produce direction-selective responses[40].

From these types of experiments, it was deduced that each direction-selective subunit requires three general elements[9,10] (FIG. 2d,e). First, there needs to be a spatial asymmetry so that, for the null-direction inhibition, the response to B affects the response to A but the response to A does not affect the response to B, and vice versa for the preferred-direction facilitation. Second, the interaction between the responses needs to be nonlinear, otherwise the sum of inputs across the receptive field would be equal in the preferred and null directions. Third, because the stimulation by A and B occurs sequentially, the response to B needs to be prolonged enough to affect the response to A for the inhibitory interaction, and vice versa for the facilitatory interaction. The challenge for subsequent retinal studies has been to identify the neuronal substrate of the subunits and the cellular mechanisms that underlie the spatial asymmetry, the nonlinearity and the time delay.

Spike recordings from DSGCs in rabbit retina established that both the inhibitory transmitter GABA and the excitatory transmitter acetylcholine (ACh) have important roles in generating the receptive-field properties of DSGCs. The application of GABA type A ($GABA_A$)-receptor antagonists to the retina abolishes the direction selectivity of the spike responses, whereas the application of nicotinic cholinergic antagonists reduces the responses by a half but leaves the direction selectivity intact[11–16].

Although most DSGCs that are encountered in the retina respond at both light On and light Off, a second population of DSGCs responds only at light On[2]. These On DSGCs have much larger receptive fields than the On–Off DSGCs and only respond to slow image motion, whereas On–Off DSGCs respond over a wide range of image velocities[17,18]. As the excitatory receptive field of the On–Off DSGCs is surrounded by a strong inhibitory field, the cells respond poorly to visual stimuli that extend beyond the centre of the receptive field; they therefore signal local motion arising from objects moving within the visual field[19], such as conspecifics, prey or predators. By contrast, the On DSGCs have only a weak inhibitory surround and are responsive to global image motion resulting from self-movement of the animal; in particular, the slow velocity sensitivity of the On DSGCs is adapted to detecting retinal slip, the small displacement of an image on the retina that occurs when an eye movement does not precisely track motion in the visual field[20,21]. In addition to the On-Off DSGCs and the On DSGCs, a type of Off DSGC has recently been characterized in mouse retina[22] (BOX 1). Throughout the remainder of the article, where we use the term 'DSGCs', we are referring to On-Off DSGCs.

Subtypes of direction-selective RGCs

There are multiple subtypes of On–Off DSGCs and On DSGCs[23], but the numbers of different subtypes are uncertain. The identification of corresponding subtypes in different species, or even between studies in the same species, is not always straightforward.

On–Off DSGCs. In rabbit, mouse and rat retinae, there are four subtypes of DSGCs that respond preferentially to object movement in one of the four cardinal ocular directions in the visual field (anterior, posterior, superior and inferior directions)[23–26]. DSGCs have a distinctive bistratified morphology with many short terminal dendrites distributed throughout the dendritic field[27–30] (FIG. 2a). One dendritic arbor stratifies in the On sublamina of the inner plexiform layer (IPL), adjacent to the ganglion cell layer (GCL), whereas the other arbor stratifies in the Off sublamina, adjacent to the inner nuclear layer (INL)[31]. The different subtypes of DSGCs cannot be distinguished by the structure or size of their dendritic trees[28,29], at least in rabbit retina if not generally in mouse retina (BOX 1).

However, when DSGCs are injected with a gap-junction permeable tracer, a minority of cells in both rabbit and mouse retinae show tracer-coupling to surrounding DSGCs that have somata that form a regular array[30,32–34]. In rabbit retina, only the superior subtype shows tracer coupling[35], and this subtype accounts for approximately 3% of all RGCs, suggesting that the four subtypes of DSGCs together account for 12% of all

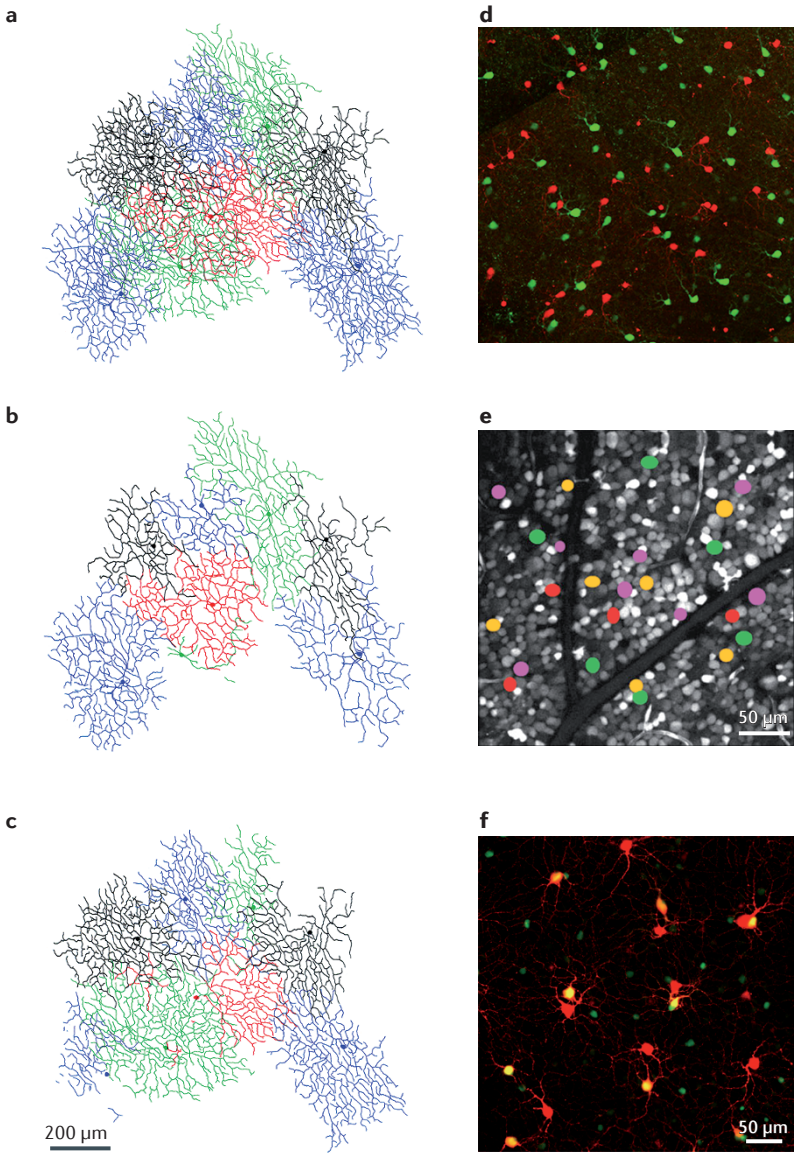

Figure 3 | **Cellular mosaics of direction-selective ganglion cells. a** | A Neurobiotin-filled On–Off direction-selective ganglion cell (DSGC; shown in red) in rabbit retina shows tracer-coupling to seven surrounding DSGCs of the same superior subtype (shown in black, blue and green) **b**,**c** | The strong territorial organization of the dendritic fields is apparent when the labelled dendrites are separated into the proximal On plexus (**b**) and the distal Off plexus (**c**). Although either of the dendritic fields may be located asymmetrically to the soma, there is no correlation between the offset direction and the preferred direction of the DSGC in rabbit retina. **d** | A mosaic of two subtypes of transgenically labelled On–Off DSGCs in mouse retina; one subtype responds to posterior movement (DRD4-DSGCs; green somata), whereas the other subtype responds to superior movement (BD-DSGCs; red somata). **e** | A mosaic of four subtypes of On–Off DSGCs with orthogonal preferred directions in mouse retina (coloured somata), as functionally mapped by population calcium imaging of all neurons in a small patch of the ganglion cell layer. The four colours correspond to the four cardinal directions of the colour-coded compass rose (FIG. 4a). **f** | A mosaic of two subtypes of On DSGCs in mouse retina projecting to the medial terminal nucleus (MTN). Both the superior and inferior subtypes are retrogradely labelled from the MTN with cholera toxin B (shown by red cells), but only the superior subtype expresses SPIG1–green fluorescent protein (GFP) (green cells) and therefore appears yellow in the double-labelled preparation. Parts **a**–**c** are modified, with permission, from REF. 33 © (1994) Society for Neuroscience. Part **d** is reproduced, with permission, from REF. 41 © (2011) Society for Neuroscience. Part **e** is modified, with permission, from REF. 25 © (2011) Macmillan Publishers Ltd. All rights reserved. Part **f** is reproduced from REF. 38.

RGCs[33]. The tracer-coupled DSGCs tile rabbit retina with little overlap of their dendritic fields, particularly in the On sublamina[33] (FIG. 3a–c). Dye filling of pairs of physiologically identified DSGCs showed that all four subtypes of DSGCs in rabbit retina have a similar territorial organization[36]: cells of the same subtype tile the retina, whereas cells of different subtypes may have widely overlapping dendritic fields.

Recent studies on mouse retina have identified specific molecular markers that are expressed by particular subtypes of DSGC[22,37–42], either endogenously or transgenically owing to positional effects near the site of transgene integration[41]. This has enabled DSGCs with a known preferred direction to be efficiently targeted for physiological recording, even before they are visually responsive. In mouse retina, only the posterior subtype of DSGC transgenically expresses green fluorescent protein (GFP) under the dopamine D4 receptor (DRD4) promoter[39]. DRD4–GFP is expressed in 3,000 cells in the developing retina and in 2,000 cells in the mature retina, and the labelled cells form a mostly regular array across the retina[40]; the DRD4-DSGCs would therefore account for 4% of all RGCs in the adult mouse retina, assuming a total population of 50,000 RGCs[43–45] (FIG. 3d). Dye injection of DRD4-DSGCs revealed that their dendritic trees extend as far as the soma of neighbouring cells[39,40]; subsequent studies have shown similar dendritic-field overlap in other subtypes of DSGCs in mouse retina[25,41,42]. This contrasts with the stronger territorial organization of DSGCs in rabbit retina[33,36].

It was subsequently reported that mouse retina contains a second posterior subtype of DSGC that transgenically expresses GFP under the thyrotropin-releasing hormone receptor (TRHR) promoter[40]; the 1,000 TRHR-DSGCs would account for around 2% of all RGCs in mouse retina. Both the DRD4- and TRHR-DSGCs have similar bistratified morphologies and co-stratify within the IPL, but the TRHR-DSGCs showed broader directional tuning on average. Although both subtypes project to the lateral geniculate nucleus of the thalamus and the superior colliculus of the midbrain (in the same way as rabbit On–Off DSGCs[46–48]), the detailed patterns of their projections differ, and the TRHR-DSGCs also project to the zona incerta in the subthalamus[39,40]. The functional significance of the differences between the two posterior subtypes is not known.

Another transgenic study on mouse retina has expanded the molecular analysis to all four subtypes of DSGCs[41]. One transgenic subtype, named W9-DSGCs, responded to posterior movement and seemed to be identical to DRD4-DSGCs, although co-expression of both markers was not tested. A second transgenic subtype, named BD-DSGCs[49], typically responded to superior movement (FIG. 3d). Unlike the DRD4-DSGCs, BD-DSGCs also project to the nucleus of the optic tract and weakly to the medial terminal nucleus (MTN) of the accessory optic system. Interestingly, the dendritic field of most BD-DSGCs was not concentric to the soma. Instead, it was offset in a direction that corresponded to the preferred direction, and this was also the case for a few BD-DSGCs near the dorsal pole of the retina that

responded preferentially to inferior movement. By contrast, such morphological asymmetry is not indicative of preferred direction for either the DRD4-DSGCs in mouse retina or the four subtypes of DSGCs in rabbit retina[28,29,39].

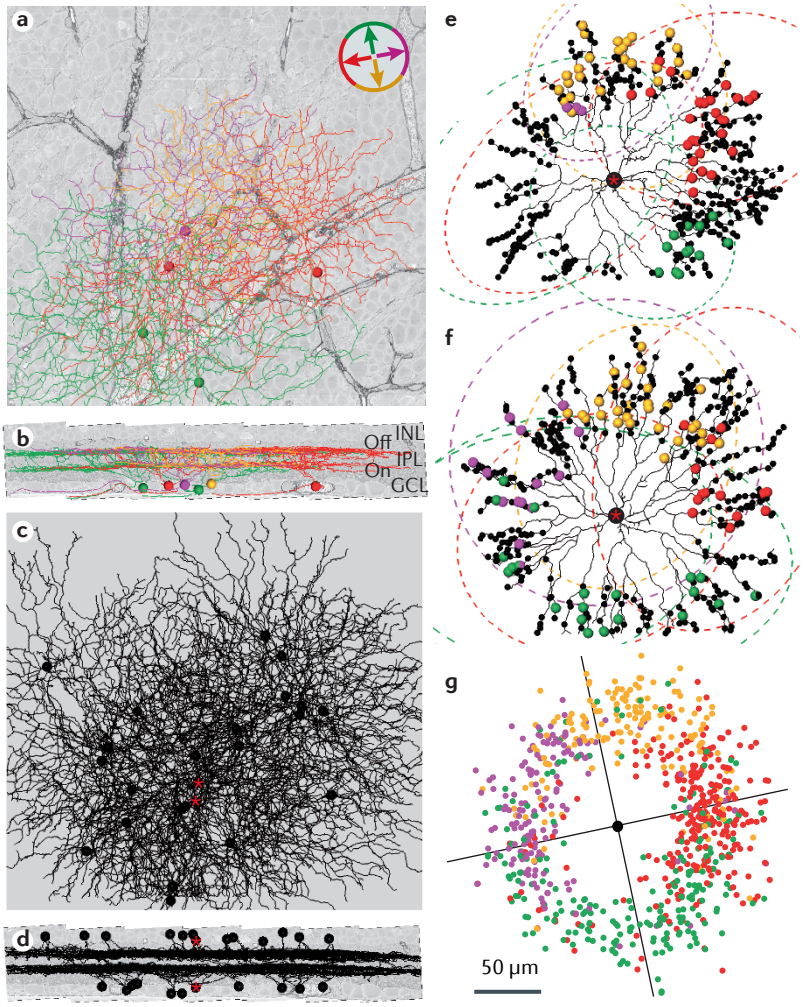

Figure 4 | **Synaptic connectivity between starburst amacrine cells and On–Off direction-selective ganglion cells. a–d** | After the functional identification of 25 direction-selective ganglion cells (DSGCs) in a small patch of mouse retina by population calcium imaging (FIG. 3d), six of the cells were reconstructed from serial block-face scanning electron micrographs (**a**,**b**), together with 11 of the On starburst amacrine cells (SACs) and 13 of the Off SACs that overlapped the DSGCs (**c**,**d**). The reconstructed DSGCs and SACs are shown in horizontal view in parts **a** and **c** and in vertical view in parts **b** and **d**. The two SACs marked with red asterisks in parts **c** and **d** correspond to those shown in parts **e** and **f**. The colours of the DSGCs indicate their preferred directions and correspond to the compass rose in part **a**. **e**–**g** | Mapping of the putative synapses made by the reconstructed SACs on the six reconstructed DSGCs (the dendritic fields of the reconstructed DSGCs are outlined with dashed ellipses) revealed that each DSGC received many more synapses from SAC dendrites pointing towards the null direction of the DSGC than towards the preferred direction; this was clear for both single On SACs (**e**), single Off SACs (**f**) and the aggregate data from all 24 reconstructed SACs aligned to the somata (**g**). For example, SAC dendrites pointing northwards selectively make contact (shown by yellow dots) with the DSGC that has a southwards preferred direction. The colours of the DSGCs indicate their preferred directions and correspond to the compass rose in part **a**. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer. Figure is modified, with permission, from REF. 25 © (2011) Macmillan Publishers Ltd. All rights reserved.

Microarray analysis and *in situ* hybridization were used to identify genes that are expressed at high levels in BD-DSGCs and to test whether these genes are expressed in other subtypes of DSGCs[41]. BD-DSGCs, and another subtype of DSGC that responded to inferior movement, endogenously expressed both cadherin 6 (*Cdh6*) and collagen 25A1 (*Col25a1*). These genes, which were not expressed by other retinal neurons, therefore provide a signature for DSGCs that respond to vertical movements (superior or inferior). Matrix metalloprotease 17 (*Mmp17*) was endogenously expressed by a small population of RGCs, 70% of which were DRD4-DSGCs, and it would be interesting to determine whether the other 30% of these cells corresponded to the TRHR-DSGCs. Cocaine- and amphetamine-regulated transcript (CART, encoded by *Cartpt*) was endogenously expressed by BD-DSGCs, and antibodies to CART labelled about 15% of all RGCs, including all BD-, W9- and DRD4-DSGCs, and nearly all RGCs that expressed *Col25a1* or *Mmp17* (REF. 41). The use of a transgenic line that randomly labels isolated RGCs showed that all presumptive DSGCs — and only presumptive DSGCs — were immunopositive for CART. This finding suggests that the DSGCs that respond to anterior movement also have a characteristic molecular signature, probably expressing *Cartpt* but not *Col25a1* or *Mmp17*.

It has recently been shown that the anterior subtype of DSGCs is selectively labelled in a mouse line that expresses GFP under the control of the promoter for motor neuron and pancreas homeobox 1 (*Mnx1*; also known as *Hb9*)[42]. The HB9-DSGCs form a regular somatic array and have a density of about 80 cells per mm^2, which the authors note is less than one-third of the density of 275 cells per mm^2 that was initially reported[39] for the posterior-selective DRD4-DSGCs. However, the disparity may not be so great because it was subsequently reported[40] that the mature retina contains about 2,000 DRD4-DSGCs and, assuming a retinal area of 15 mm^2 (REFS 44,50), this equates to a density of approximately 130 DRD4-DSGCs per mm^2. Certainly, there is not a pronounced disparity in the dendritic-field size of the two subtypes: the DRD4-DSGCs have a dendritic-field diameter of 150–200 µm for each of the On and Off dendritic fields[40], whereas the HB9-DSGCs have a dendritic-field diameter of about 200 µm for both fields combined[42]. The dendritic field of all HB9-DSGCs was offset from the soma towards the temporal retina, corresponding to the preferred direction of the cells, similar to the asymmetric morphology of the BD-DSGCs[41] (BOX 1).

A population calcium-imaging study on a small patch of mouse retina revealed a local mosaic of 25 DSGCs composed of four physiological subtypes with orthogonal preferred directions (six northward, seven southward, eight eastward and four westward), but how these compass directions correspond to the four cardinal ocular directions was not specified[25] (FIG. 3e). These numbers equate to densities of 42–83 cells per mm^2 for each subtype, or up to 1,250 cells per retina, which is comparable to some of the molecular-labelling studies. Although the local mosaic of 25 DSGCs seemed to be irregular and perhaps incompletely mapped, all of the somata had a

nearest neighbour with a different preferred direction[25], thus providing no evidence that any of the subtypes were comprised of two independent arrays.

The recent studies on mouse retina strengthen the earlier evidence from rabbit retina that the On–Off DSGCs are comprised of four physiological subtypes, providing four independent maps of the direction of image motion. Although the finding that the posterior subtype can be separated into two populations indicates that there are actually five subtypes of DSGCs in mouse retina, it is not clear that the DRD4- and TRHR-DSGCs form independent cellular arrays, which is a principle criterion for distinguishing types or subtypes of RGCs[5]. The two populations have not been labelled in the same tissue, leaving open the possibility that they overlap to some extent[40]. Although the DRD4-DSGCs are twice as numerous as the TRHR-DSGCs, their dendritic trees are no smaller, indicating that the dendritic-field coverage factor of the DRD4-DSGCs is almost double that of the TRHR-DSGCs. However, dye injection of neighbouring cells of the same molecular subtype shows that for both the DRD4- and TRHR-DSGCs, the dendritic field extends approximately as far as the soma of the neighbouring cell[40]. It seems possible that there may be only a single array of posterior DSGCs, with most cells expressing GFP under the DRD4 promoter but only a minority expressing GFP under the TRHR promoter.

On DSGCs. On DSGCs respond preferentially to object movement in one of three directions aligned with the vestibular axes: anterior, superior with a posterior component and inferior with a posterior component[23]. It has recently been shown in rabbit retina that there are in fact two distinct types of On DSGCs, named 'sustained On DSGC' and 'transient On DSGC', that are each composed of three subtypes[51,52] (FIG. 2f–h). The transient On DSGCs were described in some earlier studies but were not recognized as a distinct type[53,54]. As well as exhibiting either relatively sustained or transient spike responses to both moving stimuli and stationary flashed stimuli, the two types have distinctive dendritic morphologies and stratify at different levels in the On sublamina of the IPL[52].

On DSGCs provide the major retinal projection to the MTN and perhaps also to the lateral and dorsal terminal nuclei of the accessory optic system; moreover, MTN-projecting RGCs, unlike On–Off DSGCs, do not project to the superior colliculus and probably not to the lateral geniculate nucleus[48]. Recent studies on mouse retina have established that MTN-projecting RGCs are mainly comprised of two subtypes of On DSGCs, each forming a regular somatic array in the retina[37,38] (FIG. 3f). One subtype responds to superior movement and can be selectively identified in neonatal retina as the only RGC population that transgenically expresses the secretory protein SPIG1 (SPARC-related protein containing immunoglobulin domains 1, which is encoded by follistatin-like 4 (*Fstl4*)) in the ventral nasal sector of mouse retina. The other subtype responds to inferior movement and does not express SPIG1. It seems that the MTN-projecting RGCs correspond morphologically and physiologically to the sustained On DSGCs[38,55,56].

Accessory optic system
(AOS). The AOS is the fourth primary visual system, after the thalamic, tectal and pretectal systems, and comprises the medial, lateral and dorsal terminal nuclei.

Varicosities
Swellings along neuronal processes that are the sites of *en passant* synapses.

Mechanisms of direction selectivity

The responses of DSGCs are shaped to a large extent by input from two types of retinal interneurons, cone bipolar cells and starburst amacrine cells (SACs) (see FIG. 1c for the laminar relationships of their somata and processes). Although both types of interneuron are present in high densities, the bipolar cells are small-field neurons with non-overlapping axonal fields, whereas the SACs are large-field neurons with widely overlapping dendritic fields. The bipolar cells are second-order neurons in the retina, receiving input from photoreceptors and providing direct excitatory drive through glutamatergic synapses to the SACs and DSGCs.

The SACs are the cholinergic neurons of the retina and are comprised of two mirror-symmetric types of amacrine cells, one with somata in the INL and dendrites stratifying in the adjacent Off sublamina of the IPL, and the other with somata in the GCL and dendrites stratifying in the adjacent On sublamina[57,58]. The cells have a distinctive radially symmetric morphology with prominent varicosities in the distal third of their dendritic tree, giving them the appearance of a starburst firework[59–61]. The INL and GCL SACs are depolarized at light Off and light On, respectively[62], and co-stratify precisely with the Off and On dendrites of the DSGCs[31,63]. Although the SACs and DSGCs have similar-sized dendritic fields, each type of SAC is present at much greater density than each subtype of DSGC, with the result that individual dendrites of a DSGC are overlapped by the dendritic fields of 25–70 SACs in rabbit retina[60,61] and about 30 SACs in mouse retina[50]. The dendrites of overlapping SACs run together in fascicles[64,65] that also co-fasciculate with the dendrites of DSGCs[66–68]. The SACs receive input synapses from bipolar and amacrine cells over the whole of their dendritic tree but make output synapses to RGCs only at the distal varicosities[25,69].

SACs inhibit DSGCs asymmetrically. SACs are atypical neurons in many ways: most surprisingly, they contain and release GABA as well as ACh[70–72]. The realization that SACs may be primarily inhibitory rather than excitatory in the direction-selective circuitry led to the proposal that the SACs may underlie the null-direction inhibition of DSGCs and that the centrifugal segregation of input and output synapses in SAC dendrites could provide the spatial asymmetry that is necessary for direction selectivity[70]. This would require that each subtype of DSGC receives inhibitory input selectively from those SAC dendrites with distal dendrites that point towards the null direction of the DSGC[66].

This model was built on an earlier proposal that the high density and extensive overlap of the SACs could account for the spatial properties of the direction-selective subunits in DSGCs[73]. DSGC dendrites that were separated by a distance equivalent to the intercellular spacing of SACs (40 μm in central rabbit retina) would receive inputs from different subsets of overlapping SACs, which would therefore provide the neuronal substrate for the high density of subunits[66,74]. Moreover, each subunit would be responsive to visual displacements as large as the radius of an SAC (100–150 μm in central

Box 2 | **Development of direction selectivity**

The demonstration that starburst amacrine cell (SAC) dendrites pointing in different directions provide input to different subtypes of direction-selective ganglion cells (DSGCs)[66,79–82] raises the question of how this asymmetric wiring arises during development. Evidence indicates that visual stimulation and neuronal activity do not have important roles in either the establishment of DSGC subtypes or the specification of their synaptic connections with SACs[124,125]. DSGCs show direction-selective spike responses to moving visual stimuli from the time that retinal ganglion cells (RGCs) are first responsive to light[24,126–128], even before eye-opening[128]. Furthermore, recordings from mouse DSGCs shortly after eye-opening already showed four discrete groups of preferred directions[24]. For both types of DSGCs, the early appearance of direction selectivity in the retina is unaffected by rearing the mice in the dark[24,38,127,128]. Consistent with this, the characteristic dendritic morphology of DSGCs and the regular somatic array of individual subtypes can be recognized early in development and are also unaffected by rearing in the dark[37,41,49,81,127–130].

In mice, the inhibitory inputs from SACs to both types of DSGCs become asymmetric between the first and second postnatal weeks[81,82]. Although this transition occurs in the absence of visual stimulation, the retina is subject to patterned stimulation before eye-opening from spontaneous waves that spread across the ganglion cell layer (GCL), producing correlated activation of the RGCs and SACs[131,132]. However, disrupting those waves that are mediated by acetylcholine (ACh) by knocking out nicotinic ACh receptors did not affect the direction selectivity of On–Off DSGCs[24]. The DSGCs are activated by waves moving in any direction across the retina, regardless of type or subtype[133], suggesting either that symmetrical cholinergic excitation overcomes the developing asymmetry in GABAergic inhibition before eye-opening or that retinal waves do not mimic the synaptic activation generated by light.

A recent study on mouse retina used repeated intravitreal injection of a GABA type A ($GABA_A$)-receptor agonist postnatally to block all spontaneous and evoked neural activity in the retina, but this did not prevent the development of direction selectivity in dopamine D4 receptor DSGCs (DRD4-DSGCs)[81]. Blocking endogenous GABAergic inhibition with a $GABA_A$-receptor antagonist was equally ineffective, and this finding was confirmed in a similar study on rat retina[26]. The rat study also showed that the direction selectivity of On–Off DSGCs was unaffected when a cholinergic agonist was used to block synchronous activity or when tetrodotoxin was used to block spiking activity[26].

These findings led to the proposal that the remarkable specificity in the synaptic connectivity is hard wired, and that it is reliant on the expression of different molecular signatures in different subtypes of DSGCs and in SAC dendrites pointing in different directions[125]. This hypothesis is supported by the finding that individual subtypes of DSGCs express unique molecular markers[22,37–41,49], although there is no evidence that these particular molecules underlie the specificity of the retinal connections or the central projections made by the subtypes. Although there is currently no indication that any molecule is distributed asymmetrically across the dendritic tree of a SAC, the cells would not require a different molecule for every subtype of DSGC. Two molecules might suffice, one showing a nasal–temporal gradient across the dendritic tree and the other a dorsal–ventral gradient; the different null directions of the four subtypes On–Off DSGCs and the three subtypes of On DSGCs would then arise from different vector sums of these two gradients.

Channelrhodopsin 2
A light-gated ion channel that can be genetically expressed in individual neurons or populations of neurons, enabling them to be depolarized selectively by photostimulation.

Serial block-face scanning electron microscopy
A technique for obtaining unbroken aligned series of images at sub-micron resolution by successively scanning then sectioning the face of the specimen block on the same apparatus.

rabbit retina), assuming that each dendrite of the SAC functions as an independent processing unit[75–78].

Direct evidence for an asymmetric inhibitory input from SACs to DSGCs was provided by four studies that recorded the currents elicited in a DSGC while stimulating SACs on different sides of the DSGC[79–82]. In the first of these studies, undertaken on rabbit retina, depolarization of an SAC on the side that is first stimulated by null-direction motion (the null side) elicited inhibition but not excitation of the DSGC, whereas depolarization of an SAC on the preferred side elicited neither inhibition nor excitation[79]. These findings were partly confirmed by a recent study that used a much larger sample of SAC–DSGC pairs in rabbit retina[80]. Depolarization of a null-side SAC elicited a pronounced $GABA_A$-mediated inhibitory current in the DSGC that was ninefold larger than that elicited by depolarization of a preferred-side SAC. In contrast to the earlier study, depolarization of the SACs also elicited a strong excitatory current, mediated by nicotinic cholinergic receptors, that was not statistically different for null- and preferred-side SACs. Although both the GABAergic and cholinergic currents in the DSGCs were elicited monosynaptically through calcium-dependent release mechanisms, GABA release and ACh release displayed different sensitivities to the concentration of external calcium, and to blockers that are selective for N-type or P/Q-type calcium channels, suggesting distinct release mechanisms[80].

These findings indicated that each DSGC receives symmetrical cholinergic inputs from SAC dendrites that point in all directions, and that connectional asymmetry arises only in the GABAergic inputs. The GABAergic asymmetry could arise during development through a decrease in the inhibitory input from preferred-side SACs or an increase in the inhibitory input from null-side SACs (BOX 2). These possibilities were investigated in two recent studies on mouse retina[81,82]. The first study examined the posterior subtype of On–Off DSGCs, using paired cell recordings from transgenically labelled SACs and DRD4-DSGCs, under conditions in which the excitatory inputs were blocked[81]. During the first postnatal week, stimulation of null- and preferred-side SACs elicited inhibitory inputs of equal strength in the DSGCs but, by the end of the second postnatal week, the null-side input increased significantly, resulting in a threefold difference in conductance.

The second study examined the superior subtype of On DSGC, using light stimulation of SACs expressing channelrhodopsin 2 to stimulate SPIG1-expressing RGCs[82]. Importantly, this study demonstrated that the pattern of SAC input to sustained On DSGCs follows the pattern established for On–Off DSGCs, with asymmetric GABAergic inputs from null-side SACs and symmetric cholinergic inputs from all SACs. The transition from symmetric to asymmetric inhibition occurred rapidly between postnatal days 6 and 9, and seemed to involve a significant increase in inhibitory strengths from null-side SACs.

Clear evidence for a structural asymmetry in the synaptic connectivity of DSGCs has been provided recently in a study that was a technical tour-de-force[25] in which two-photon population calcium imaging was combined with serial block-face scanning electron microscopy and neuronal reconstruction in a small patch of mouse retina[83,84]. The dendritic trees of six of the physiologically identified DSGCs were reconstructed, including at least one each of the four subtypes (FIG. 4a,b). Twenty-one overlapping SACs were also reconstructed, and putative synapses from SACs onto the DSGCs were identified (FIG. 4c,d). The DSGCs received 11 times as many putative synapses from null-side SACs than from preferred-side SACs, consistent with the conclusion that the putative synapses provide the substrate for null-direction GABAergic inhibition (FIG. 4e–g). This result suggests that the development of asymmetric inhibition arises from the refinement of synaptic number rather than changes in synaptic efficacy.

Although many of the reconstructed SAC dendrites exclusively contacted a single subtype of DSGC, the mapped synapses accounted for only a minority of the distal varicosities of the SAC dendrites[25]. Although some of these varicosities will contact overlapping DSGCs that were not reconstructed, they could also provide sites for interactions with overlapping SACs. Interestingly, the proportion of the Off SAC varicosities that contacted the reconstructed DSGCs seems no higher than that of the On SAC varicosities[25], which is surprising given that the On SACs provide synaptic input to three subtypes of On DSGCs in addition to four subtypes of On–Off DSGCs. It is possible that such issues may be addressed by expanding the present data set, which promises to provide insights into retinal circuitry to rival those gained when serial transmission electron microscopy was used to reconstruct the vertical pathways in cat retina 30 years ago[85].

Direction-selective inputs from SACs to DSGCs. As a result of the pattern of connectivity between SACs and DSGCs, the inhibitory receptive field of a DSGC is not only wider than the dendritic field of the DSGC but is also offset towards the null side of the DSGC. Consequently, preferred-direction motion first activates a fast-rising excitation that thus escapes the effects of the following inhibition, whereas null-direction motion first activates a long-lasting inhibition that overlaps and offsets the following excitation[79,86,87]. However, the spatiotemporal relationship between the excitatory and inhibitory inputs is only one of the mechanisms that shapes the direction selectivity of the spike responses. Somatic voltage-clamp recordings indicated that the excitatory and inhibitory inputs to DSGCs are themselves direction selective[79,87,88]: inhibitory currents are stronger when image motion is in the null direction and excitatory currents are stronger when motion is in the preferred direction, creating a push–pull effect on the DSGC. Therefore direction-selective visual responses arise presynaptically in retinal interneurons, and the key players in the direction-selective circuitry are the SACs[77,89]. Neurochemical ablation of SACs results in both the loss of direction-selective spike responses in DSGCs and the elimination of eye tracking of global image motion (optokinetic nystagmus)[90,91], which is thought to be driven by both the On DSGCs and the On–Off DSGCs[20].

As the dendrites of SACs are very thin, recordings from the soma may not faithfully track voltage changes in the distal dendrites, where the output synapses to RGCs are located. To overcome this problem, a landmark study combined visual stimulation and functional imaging of SACs filled with a calcium-indicator dye to show that SACs respond asymmetrically to moving stimuli[77]. The study revealed that the calcium transients in the distal varicosities of SACs are larger during centrifugal image motion (from the soma to the dendrites) than during centripetal motion (from the dendrites to the soma). This indicated that the calcium-dependent release of transmitter from individual SAC dendrites would be direction selective, with release being greatest for image motion in the direction that the dendrite is pointing. Consequently, the preferred direction of a DSGC is opposite to the preferred direction of the SAC dendrites that provide its inhibitory input.

The calcium-imaging experiments provided direct support for the hypothesis that the SAC dendrites function as independent processing units[75–78]. Their independence relies partly on the passive cable properties of SACs, which can also account to some extent for the larger calcium signals that are elicited by centrifugal motion compared with centripetal motion[92–95]. In response to a moving stimulus, the small-field bipolar cells that provide excitatory inputs at synapses located along a SAC dendrite will be activated successively. For centrifugal motion, the depolarizations that are generated in the proximal and medial dendritic segments will summate with that generated in the distal dendrite. For centripetal motion, the stimulus will activate the input to the distal dendrite before activating the inputs to the medial and proximal dendritic segments, resulting in a smaller peak depolarization in the distal dendrite. These differences seem to be amplified in a direction-selective manner by the regenerative activation of voltage-gated calcium channels and tetrodotoxin-resistant sodium channels[95,96]. The effect may also be augmented by the existence of a voltage gradient along SAC dendrites[97] arising from a tonic glutamatergic input from bipolar cells[98,99].

Inhibitory interactions in the SAC network. Experiments using two-spot apparent-motion stimuli had shown that both null-direction inhibition and preferred-direction facilitation contribute to the direction-selective spike responses of DSGCs[9]. Similarly, experiments using three-spot apparent-motion stimuli showed that both centrifugal facilitation and centripetal inhibition contribute to the direction-selective calcium responses of the distal dendrites of SACs in rabbit retina[89] (FIG. 5a–c). The centripetal inhibition of the SAC responses seems to be mediated mainly by reciprocal GABAergic inhibition between SACs (FIG. 5d). Paired recordings from overlapping SACs in mature rabbit retina showed that depolarization of one cell produces strong $GABA_A$-mediated inhibitory currents in the other cell, and vice versa, but no excitatory currents[89]. Mapping the inhibitory surround of SACs with visual stimuli indicated that the GABAergic inputs may be concentrated in the distal varicose zone of the SAC dendritic tree. As there is a tonic level of reciprocal inhibition between SAC dendrites pointing in opposite directions (known as anti-parallel dendrites), the feedback loop between SACs may serve to enhance centrifugal facilitation as well as centripetal inhibition.

For the strength of the inhibitory interactions between SAC dendrites pointing in the same direction (parallel dendrites) to be less than that between anti-parallel dendrites[89,100], it is not necessary to invoke an active mechanism in which SAC dendrites synapse selectively with anti-parallel dendrites or avoid synapsing with parallel dendrites. Instead, the asymmetric inhibition may simply be a consequence of the regular spacing between the dendritic trees of SACs, resulting in the most distal dendrites contacting anti-parallel dendrites

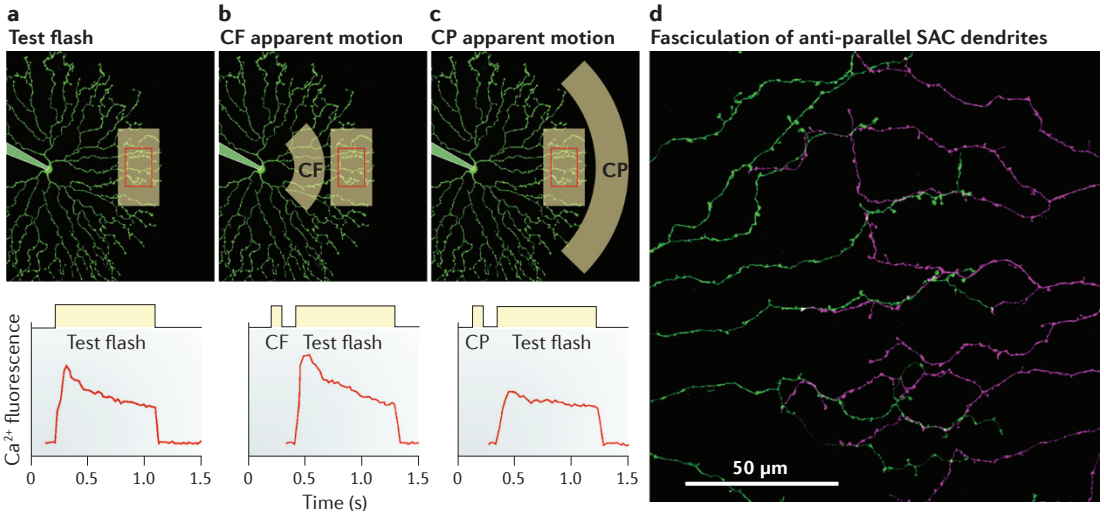

Figure 5 | **Direction-selective responses in starburst amacrine cells. a–c** | To examine the starburst amacrine cell (SAC) responses to visual stimuli moving in and out of the dendritic field, an apparent-motion paradigm was used to determine the effects of conditioning flashes on the calcium response produced by a test flash located over the distal varicose zone of an SAC in rabbit retina[89]. The figures show the outlines of the flashed stimuli (shown by the transparent yellow shapes) relative to the green dendritic tree of an SAC that was filled with a calcium-indicator dye through its soma, and the area from which the dye fluorescence was measured in response to visual stimulation (shown by the red box), as plotted in the graphs. The centripetal (CP) conditioning flash was placed to one side of the SAC, the test flash was placed over adjacent distal dendrites and the centrifugal (CF) conditioning flash was placed over proximal dendrites, closer to the soma. When the stimuli were flashed in the CF sequence (**b**), the calcium response to the test flash was greater than when flashed on its own (**a**). When the stimuli were flashed in the CP sequence (**c**), the calcium response to the test flash was less than when flashed on its own. **d** | The CP inhibition of SACs seems to be mediated by GABAergic synapses between the fasciculating dendrites of overlapping SACs (shown here for two SACs in rabbit retina injected with different dyes). Areas of close dendritic apposition are shown by white pixels. Parts **a–c** are modified, with permission, from REF. 89 © (2006) Cell Press.

but not parallel dendrites. Moreover, it has been proposed that there is a chloride gradient along the length of SAC dendrites that arises from the opposing gradients of two chloride co-transporters[101,102]. If there is a gradient in the chloride reversal potential along a SAC dendrite, GABAergic synapses that are located on the medial or proximal thirds of the dendrite would have less of an inhibitory effect, or could even be excitatory, as proposed in a related model of how direction-selective responses are created by GABAergic interactions in the network of SACs[103].

What remains unclear is the extent to which the direction selectivity of the DSGCs depends on the reciprocal GABAergic interactions between SACs. Direction-selective calcium transients persist in SACs under GABAergic block[77,104] and, moreover, a recent study on rabbit retina reported that visual stimuli that produce strong direction-selective signals in both SACs and DSGCs failed to produce a significant inhibitory input to SACs[96], consistent with the findings of some earlier studies[98,99,105]. These observations suggest that reciprocal inhibition between SACs is not essential for generating directional signals in the DSGCs, and underscore the idea that direction selectivity probably arises from a combination of diverse cellular mechanisms.

Reversal potential
The membrane potential at which the net ion current flow becomes zero.

Excitatory inputs to DSGCs. The excitatory inputs to DSGCs are made up of two components: glutamatergic inputs from small-field cone bipolar cells and nicotinic cholinergic inputs from large-field SACs. It is therefore surprising that the excitatory receptive-field centre of a DSGC is about the same size as the dendritic field[29], with the cholinergic excitatory field being no wider than the glutamatergic excitatory field[105], even though the dendritic fields of the overlapping SACs extend far beyond the dendritic field of the DSGC. This observation implies that ACh release only occurs when the visual stimulus overlies the distal dendrites of SACs, whereas GABA release also occurs when the visual stimulus overlies proximal and medial dendrites that are located outside the dendritic field of the DSGC (although only on the null side). However, the cholinergic receptive field of the DSGC is expanded during application of GABAergic antagonists, suggesting that visual stimulation of the proximal and medial dendrites provides sufficient drive for ACh release in the absence of GABAergic inhibition[105,106]. These observations are consistent with the idea that ACh release from SACs has a higher threshold than GABA release. Further support is provided by paired SAC–DSGC recordings showing that the GABAergic currents in DSGCs are evoked at both lower potentials and lower external calcium concentrations than the cholinergic currents[80].

Somatic voltage-clamp recordings from DSGCs indicate that both the cholinergic and glutamatergic currents are direction selective, as they are stronger in the preferred direction than the null direction[79,87]. Individual SAC dendrites would be expected to release more ACh in response to centrifugal motion than to centripetal motion, as is the case with the release of GABA. However,

given that paired-cell recordings indicate that the cholinergic inputs from SACs to a DSGC are isotropic, the combined input should be facilitated by image motion in any direction[106]. For moving stimuli to generate direction-selective cholinergic inputs, it would be necessary to selectively regulate only some of the cholinergic synapses that are made by an SAC dendrite on the basis of the subtype of the postsynaptic DSGC. This could be achieved by selectively inhibiting those cholinergic synapses that contact the DSGC subtype receiving GABAergic synapses from the same SAC dendrite[80]. Such circuitry would be remarkably intricate and, unlike the situation for the direction-selective GABAergic inputs, somatic recordings currently provide the only evidence that DSGCs receive direction-selective cholinergic inputs[80].

The apparent direction selectivity of the glutamatergic inputs from bipolar cells is even more puzzling. The axon terminals of each type of bipolar cell tile the IPL in a territorial manner, so there is little redundancy in their coverage. Consequently, each bipolar cell probably contacts all subtypes of DSGCs, and any direction-selective responses in the electrically compact bipolar cells would need to be confined to a branch of the axon terminal so that the glutamatergic signals to different subtypes of DSGCs are not mixed. It has been proposed that inhibition from null-side SACs, or other GABAergic amacrine cells, underlies the direction selectivity of the glutamatergic inputs from bipolar cells[105], although it is not clear whether SACs make synapses on bipolar cells[65,69,107,108]. Moreover, SAC dendrites that point in different directions would have to make specific synapses with different branches of the axon terminal of a bipolar cell on the basis of the subtype of DSGC that is postsynaptic to the branch.

The complex wiring that seems to be required to account for the direction selectivity of the cholinergic and glutamatergic inputs to the DSGCs raises the question of whether there is a much simpler explanation. We propose that the apparent direction selectivity of the excitatory inputs may arise simply from somatic voltage-clamp errors in both our own studies and those from other laboratories. DSGCs have extensive dendritic trees with many thin terminal dendrites, and it is likely that only the proximal dendrites are adequately clamped[109–111]. The errors will be a complex function of the magnitudes and relative time-course of the excitation and inhibition, and the relative locations of the inputs across the dendritic tree. Consequently, somatic voltage-clamp recordings cannot isolate the excitatory or inhibitory inputs to the distal dendrites, even if the membrane potential in the dendrites starts at the inhibitory or excitatory reversal potential, respectively[111].

The excitatory inputs to a DSGC have usually been estimated by voltage clamping the soma at the inhibitory reversal potential and then measuring the synaptic currents elicited by visual stimuli[30,80,105]. However, owing to the voltage-clamp errors, the accuracy of the excitatory currents recorded at the soma will be influenced not only by the excitatory inputs themselves but also by any concurrent inhibitory inputs[112]. Because this effect becomes more pronounced as the total synaptic conductance increases, the excitatory input will be underestimated more severely in the null direction, as the total amount of inhibition is greater in the null direction than in the preferred direction, and the peak inhibition tends to be coincident with the peak excitation in the null direction but not in the preferred direction. Consequently, if the excitatory inputs were equal in both directions, voltage-clamp errors would generate a directional asymmetry in the measured excitatory inputs, wrongly indicating that they are relatively greater in the preferred direction than in the null direction. The voltage-clamp errors would also lead to greater underestimation of the inhibitory inputs in the null direction than in the preferred direction and, therefore, an underestimation of the direction selectivity of the inhibitory inputs. It remains to be determined whether such voltage-clamp errors can account fully for the directional asymmetry in the excitatory currents measured at the soma.

Postsynaptic mechanisms. The spatiotemporal asymmetries in the excitatory and inhibitory inputs to the DSGC are clear-cut when viewed from the perspective of a terminal dendrite, which represents a single subunit (FIG. 6). However, the situation is not straightforward when the inputs to all subunits are viewed from the DSGC soma. Even for a simple dark-to-light edge moving through the receptive field in the preferred direction, the long-lasting inhibition from early activation of subunits on the preferred side of the DSGC will temporally coincide with the excitation from later activation of subunits on the null side, resulting in an overall reduction in the excitatory drive to the cell. Such discordant overlays of excitation and inhibition become more problematic for complex visual stimuli, such as gratings, which simultaneously activate inhibition and excitation at disparate locations throughout the dendritic arbor but nevertheless produce direction-selective spike responses[113–115].

However, if the inhibitory and excitatory inputs interact locally rather than being summed at the soma, then this problem does not arise. Before the synaptic inputs to DSGCs were shown to be direction selective, it was proposed that the direction-selective spike responses of DSGCs could be generated by a postsynaptic mechanism in which spatially asymmetric inhibitory inputs shunt nearby excitatory inputs but are effectively silent on their own[116,117]. An inhibitory shunt can act locally because the reversal potential is near the resting potential and, therefore, activation of the inhibitory conductance does not produce an opposing inhibitory postsynaptic potential but instead produces a localized increase in the membrane conductance of the dendrite that 'shunts' or reduces the depolarizing effect of local excitatory postsynaptic currents. The parameters for this model were constrained, particularly in the placement of the excitatory and inhibitory synapses, requiring null-direction inhibitory inputs to be excluded from proximal dendrites that passively channel excitatory currents from more distal dendrites.

The postsynaptic model has recently been extended with the observation that the dendrites of DSGCs produce voltage-gated self-propagating transients, termed

Inhibitory shunt
Suppression of excitatory postsynaptic potentials resulting from an increase in neuronal membrane conductance.

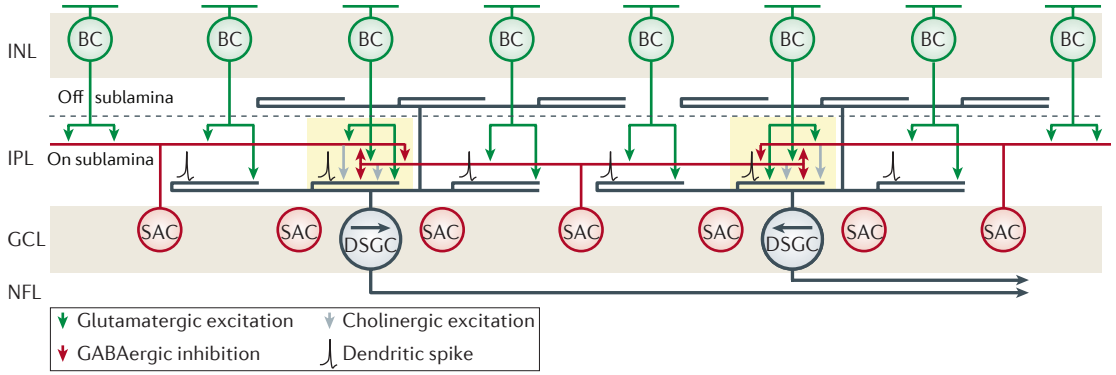

Figure 6 | **Circuit diagram of direction selectivity in the retina.** Two On–Off direction-selective ganglion cells (DSGCs) with opposite preferred directions are depicted (shown by black circles): the left-hand DSGC responds to rightwards image motion and the right-hand DSGC responds to leftwards image motion. For simplicity, only the synaptic connections in the On sublamina are shown, but there is a matching set of connections in the Off sublamina. The bipolar cells (BCs) provide excitatory inputs to both the starburst amacrine cells (SACs) and the DSGCs (shown by green arrows). The widely overlapping SACs symmetrically inhibit each other and asymmetrically inhibit the DSGCs, so that the leftwards- and rightwards-pointing dendrites of the SACs inhibit the rightwards- and leftwards-preferring DSGCs, respectively (shown by red arrows). The output of the asymmetric inhibitory synapses from SACs to the DSGCs is direction selective, and is greater for stimuli moving away from the SAC soma (centrifugal motion) than towards it (centripetal motion). Each DSGC also receives excitatory cholinergic inputs from both leftwards- and rightwards-pointing dendrites of the SACs (shown by grey arrows). This simplified model depicts neither the glutamatergic bipolar inputs nor the combined cholinergic SAC inputs to a DSGC as direction selective, although the release of acetylcholine (ACh) from an individual SAC dendrite may be greater for centrifugal motion than for centripetal motion. Each DSGC dendrite receives inputs from different BCs and SACs (shown by yellow boxes) and, together with the dendritic spike mechanism in the DSGC dendrite, this provides the microcircuit that generates direction selectivity in subunits of the DSGC's receptive field. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NFL, nerve fibre layer.

dendritic spikes[118]. Like the passive model described above, this active model also proposes localized interactions between excitation and inhibition; in this model, however, the important nonlinearity is a local threshold for dendritic-spike generation rather than a nonlinear shunting inhibition. Modelling shows that, once initiated, dendritic spikes are immune to shunting even by unreasonably large inhibitory inputs that are interposed between the spike and the soma[119]. However, this does not preclude a role for nonlinear shunting inhibition in suppressing the initiation of the dendritic spikes. Dendritic spike generation ensures that the response in each direction-selective subunit is transmitted faithfully to the soma, independently of where it is generated in the dendritic tree. The dendritic-spiking model also explains why direction-selective spike responses are obtained for stimuli that activate a small part of the receptive field, at least in rabbit retina[9]: such stimuli are unlikely to generate postsynaptic potentials that reach spike threshold at the soma, but they could efficiently generate dendritic spikes. The postsynaptic mechanisms could work together with presynaptic mechanisms and make the direction selectivity of the spike responses more robust over a range of stimulus conditions.

Summary. The elucidation of the microcircuit that underlies direction-selective subunits enables us to ascribe specific cellular mechanisms to the three general elements that are required for the generation of direction selectivity in the retina. First, the spatial asymmetry is dependent on both the centrifugal separation of the input and output synapses of the SACs — which affects neuronal processing both within SACs, between SACs and from SACs to DSGCs — and on the asymmetric connectivity of dendrites on different sides of SACs to different subtypes of DSGCs. Second, multiple nonlinearities contribute to the generation of direction-selective spike responses in DSGCs, reflecting both presynaptic and postsynaptic mechanisms. Although many details remain to be clarified, these nonlinearities seem to depend on threshold activation of voltage-gated processes, including neurotransmitter release, dendritic spike activation and action potential generation. Other nonlinear processes such as shunting inhibition also have a role. Third, the 'time delay' is less well characterized but seems to depend, at least in part, on the GABA that is released by SACs having a prolonged inhibitory effect on both the DSGCs and other SACs[80,89], with this effect perhaps mediated by specialized GABA receptors[120].

Future directions

The SACs were the first neurons to be shown to contain and release a fast-acting excitatory transmitter (ACh) and a fast-acting inhibitory transmitter (GABA)[70–72,121], but how this co-transmission shapes the receptive-field properties of DSGCs is still unclear. Although the role of the SACs in providing spatially asymmetric direction-selective inhibition to On–Off DSGCs has been established[25,79–82], the function of the cholinergic excitation is poorly understood. Electrical stimulation of either null- or preferred-side SACs elicits equivalent cholinergic excitatory postsynaptic currents in the DSGCs[80,82]; however, null-side SACs make 11 times as many putative synapses with a DSGC than preferred-side SACs[25],

raising the question of whether these synapses mediate the symmetrical cholinergic inputs in addition to the asymmetrical GABAergic inputs. This would require either that only 10% of the synapses from null-side SACs are excitatory or that the excitatory strength of each synapse from preferred-side SACs is ten times greater than that from null-side SACs. The putative synapses that are made by preferred-side SACs are few in number and irregularly distributed[25], and this belies the evidence that preferred-direction motion elicits strong cholinergic inputs in the DSGCs[80]. Although the cholinergic transmission seems to be both calcium-dependent and monosynaptic[80], it may be mediated either by neuronal contacts that are distinct from the mapped putative synapses or by a paracrine mechanism[25].

If the apparent direction selectivity of the excitatory inputs to DSGCs actually reflects errors in voltage-clamp recording, then the cholinergic inputs can be regarded as simply facilitating the responses of DSGCs to all moving stimuli, regardless of the direction of motion[106]. Such a mechanism could underlie the preferred-direction facilitation of spike responses that is observed with two-spot apparent-motion stimuli[9]. The facilitation may be more pronounced for episodic or textured visual stimuli because repetitive electrical stimulation of SACs results in strong facilitation of the cholinergic inputs to DSGCs but little facilitation of the GABAergic inputs[80]. However, more complex functions for the cholinergic inputs cannot be ruled out given earlier reports that cholinergic antagonists actually eliminated the direction selectivity of DSGCs when stimulated with slow-moving drifting gratings[113,122]. It will be important to test whether the excitatory inputs to DSGCs are direction selective using methods other than somatic voltage-clamp recordings. This may be easier for the glutamatergic inputs than for the cholinergic inputs because, in principle, it should be possible to use optical recording to monitor calcium responses in the axon terminal of bipolar cells and determine whether different branches respond preferentially to image motion in different directions, as done previously for the SACs[77].

Although most research has focused on the On–Off DSGCs, it has become clear that detailed study of the On DSGCs will provide important new perspectives on how direction selectivity is generated in the retina. The recent discovery that there are two distinct types of On DSGCs in rabbit retina[51,52] is particularly important because only the sustained On DSGCs co-stratify with the On SACs[31,52], whereas the transient On DSGCs stratify more distally in the IPL[52]. Consistent with this, the cholinergic agonist nicotine produces uncontrollable firing in the sustained On DSGCs but has little effect on the transient On DSGCs[52], suggesting that the SACs may not have an important role in the generation of direction selectivity in the transient On DSGCs.

Important questions also remain regarding differences in the excitatory and inhibitory inputs to the sustained On DSGCs and the On–Off DSGCs, which partly or completely co-stratify with the On SACs, respectively. The excitatory inputs to the On–Off DSGCs are fast-rising and transient, but those to the On DSGCs are relatively sustained, probably reflecting differences in the glutamatergic inputs from bipolar cells[115]. The On–Off DSGCs respond to a broad range of image velocities, whereas the On DSGCs only respond to slow velocities, and this is likely to reflect differences in their inhibitory inputs[12,115]. However, it is unclear whether the null-direction inhibition that confers the direction selectivity on these DSGCs also confers the velocity sensitivity, or whether this depends on other types of amacrine cells that may connect symmetrically to the cells. Indeed, the SACs account for only two of the dozens of types of GABAergic amacrine cells, which in turn account for only half of all amacrine cells; the other half are glycinergic amacrine cells[74,123]. A fuller understanding of the cellular mechanisms that underlie the generation of direction selectivity in the retina will require careful consideration of the other components in the circuitry.

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Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
W. Rowland Taylor's homepage: http://www.ohsu.edu/xd/health/services/casey-eye/research/research-faculty/taylor-lab.cfm

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