1 # A retinal code for motion
2 # along the gravitational and body axes

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4 Shai Sabbah[1], John A. Gemmer[2], Ananya Bhatia-Lin[1], Gabrielle Manoff[1], Gabriel Castro[1], Jesse

5 K. Siegel[1], Nathan Jeffery[3] and David M. Berson[1]

6

7 [1] Department of Neuroscience, Brown University, Providence, RI, 02912, USA

8 [2] Department of Mathematics and Statistics, Wake Forest University, Winston Salem, NC,

9   27109, USA

10 [3] Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, L69 3GA, UK

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1 **<u>Summary</u>**

2 Self-motion triggers complementary visual and vestibular reflexes supporting image-stabilization

3 and balance. Translation through space produces one global pattern of retinal image motion

4 (optic flow), rotation another. We show that each subtype of direction-selective ganglion cell

5 (DSGC) adjusts its direction preference topographically to align with specific translatory optic

6 flow fields, creating a neural ensemble tuned for a specific direction of motion through space.

7 Four cardinal translatory directions are represented, aligned with two axes of high adaptive

8 relevance: the body and gravitational axes. One subtype maximizes its output when the mouse

9 advances, others when it retreats, rises, or falls. ON-DSGCs and ON-OFF-DSGCs share the

10 same spatial geometry but weight the four channels differently. Each subtype ensemble is also

11 tuned for rotation. The relative activation of DSGC channels uniquely encodes every translation

12 and rotation. Though retinal and vestibular systems both encode translatory and rotatory self-

13 motion, their coordinate systems differ.

14

15

__Introduction__

Direction-selective retinal ganglion cells (DSGCs) encode visual motion. Earlier work has probed how they do so[1-9]. Here, we relate global patterns of direction preference to visual reafference during self-motion. When animals move, visual and vestibular feedback drive postural adjustments, image-stabilizing eye and head movements, and cerebellar learning[10]. Self-motion can be decomposed into *translatory* and *rotatory* elements — movement along, or rotation about, an axis. The vestibular apparatus achieves this biomechanically. The relative activation of otolithic organs and semicircular canals uniquely encodes every translation or rotation[11].

Movement through space also produces global patterns of retinal image motion called optic flow. Translation (Fig. 1a) induces optic flow that *diverges from a point* in extrapersonal space ('direction of heading;' asterisk), follows *lines of longitude* in global visual space, and converges at a diametrically opposed singularity. Rotational optic flow follows *lines of latitude, circulating around a point* in visual space (Fig. 1b). These motion trajectories are imaged on the hemispheric retinal surface (Figs. 1c,d). Rotatory and translatory optic flows evoke different behaviors, implying divergent encoding mechanisms and output circuits.

DSGCs encode optic flow locally within small receptive fields[12] (~1% of the monocular field; Fig. 1c, red circle). Most belong to two canonical classes — ON-DSGCs and ON-OFF-DSGCs — differing in gene expression, structure, projections, functional properties and roles[1].

1   ON-OFF-DSGCs innervate retinotopic targets mediating gaze shifts and conscious motion

2   perception[13-15]. They comprise four subtypes, each preferring one of four cardinal directions[16-18].

3   The polar distribution of directional preferences among ON-OFF-DSGCs appears cruciform,

4   with four lobes separated by 90° (Fig. 1k).

5

6   How do the directional preferences of ON-OFF-DSGCs relate to the spherical geometry of optic

7   flow? If the cruciform pattern is universal, as widely assumed, a surprising corollary follows:

8   one pair of subtypes must prefer motion along meridians as in translatory optic flow (Fig. 1a),

9   while the other pair follows orthogonal lines of latitude, like rotatory flow (Fig. 1b). The

10   dorsal/ventral pair matches translatory flow in Fig. 1e,f, but could match rotatory flow instead,

11   provided the nasal/temporal pair also switches, to translatory flow.

12

13   By intensive global mapping of DS, we refute this model. Instead, we find that all four

14   ON-OFF-DSGC subtypes align their preferences everywhere with one of four cardinal

15   translatory optic flow fields, thereby encoding self-motion along two specific axes — the

16   gravitational and body axes. Each subtype forms a panoramic, binocular ensemble best activated

17   when the animal rises, falls, advances or retreats. We expected the other DSGC class — ON-

18   DSGCs — would exhibit a distinct geometry (Supplementary Note 1). Surprisingly, they proved

19   to adhere to the same translatory optic-flow geometry as ON-OFF-DSGCs, and to comprise four

20   subtypes, not three[18,19]. Though optimally tuned for translatory flow, DSGC ensembles also

21   respond differentially to rotatory flow. Any translation or rotation is uniquely encoded in the

22   relative activation of these channels, permitting the brain to differentiate translation from rotation

23   by simple global summation or subtraction of channels.

Results

Mapping global direction preferences of DSGCs

We mapped the direction preferences of >2400 DSGCs at known locations in flattened mouse

retinas *in vitro* (from >33,900 neurons; 26 retinas; Fig. 1g,h). DSGCs were identified from Ca^{2+}

responses to moving stimuli, imaged by two-photon microscopy of virally expressed GCaMP6f

(Methods; Extended Data Fig. 1a-d). All were ganglion cells (immunopositive for RNA-binding

protein with multiple splicing[20]; $n = 706$; Extended Data Fig. 2a-f). ON-OFF-DSGCs were

discriminated from ON-DSGCs by unsupervised clustering, exploiting their faster, more

transient ON responses (Extended Data Fig. 3a-h). ON-OFF-DSGCs greatly outnumbered ON-

DSGCs (ON-OFF: n=1949; $6.6 \pm 2.1\%$ of imaged cells per retina vs. ON: n=497; $1.8 \pm 0.9\%$;

mean $\pm$ s.d., Supplementary Note 2). No OFF-DSGCs were encountered. Each cell's location

was mapped to spherical coordinates, permitting comparisons across retinas using standardized

displays as projected or flattened spherical surfaces (Extended Data Fig. 4, Supplementary

Equations).

ON-OFF-DSGCs align direction preferences with optic flow produced by translation along

two cardinal axes

Like all imaged cells, ON-OFF-DSGCs were widely distributed but concentrated centrally due to

preferential viral infection (Fig. 1g,h). Their DS preferences, pooled across retinas and displayed

in polar format, were markedly disordered (Fig. 1i). Four lobes marking the cardinal directions

were apparent, but broad and blurred, without expected gaps. Correcting errors in retinal

orientation (Fig. 1j) did not resolve this (Extended Data Fig. 5e). The disorder derived instead

mainly from systematic topographic variation in DS preferences. Four distinct lobes emerged

1   when sampling only central retina (Fig. 1k), but elsewhere the lobes tilted relative to cardinal

2   retinal directions and one another (Fig. 1l). Such topographic dependence is not an artefact of

3   retinal flattening; it is even more apparent when viewed in reconstructed three-dimensional form

4   (Fig. 1m). Thus, plots of DS preference are not ubiquitously cruciform (Fig. 1e,f).

5

6   Consider the ON-OFF-DSGC subtype we call "*V-cells,*" after their preference for *ventral* motion

7   on the retina, or of the mouse ('upward' motion in the visual field; red lobes in Fig. 1m). V-cells

8   preferred motion toward a center of contraction in the ventral retina, and everywhere paralleled a

9   ventrally directed translatory optic flow field (Fig. 1m, red meridians). We extended this analysis

10   by targeted patch recording of GFP-tagged V-cells in Hb9-GFP mice[21]. They preferred motion

11   toward a ventral singularity (Fig. 2a, black arrows; Extended Data Fig. 2l-r), just as imaged

12   V-cells did (Fig. 1m), aligned everywhere with the same translatory optic flow (Figs. 1m and 2a,

13   red meridians). Dendritic-field asymmetries of V-cells, which correlate with preferred

14   direction[21] (Extended Data Fig. 2q), also aligned with this flow field (Figs. 2a, gold arrowheads).

15   Thus, as an ensemble, V-cells respond best to a specific translatory optic flow, produced by

16   roughly downward motion.

17

18   Other subtypes of ON-OFF-DSGCs also adhered to translatory optic-flow geometry. In

19   Trhr-GFP mice[15], we targeted "*N-cells,*" preferring *nasal* retinal motion, for recordings. N-cells

20   preferred motion away from a center of expansion in temporal retina (Fig. 2b), following a

21   distinct translatory flow field (blue meridians). To assess a third subtype, "*T-cells,*" preferring

22   *temporal* retinal motion (Fig. 2c), we analyzed imaged ON-OFF-DSGCs that were

23   immunonegative for CART (Cocaine and Amphetamine Regulated Transcript)[22,23]. T-cell

1   preferences too were aligned with a translatory optic flow field (green meridians), here with a

2   center of contraction in the temporal retina (Fig. 2c).

3

4   To quantify how well the DS preferences of a cell sample align with any single optic flow field,

5   we devised a *concordance index* — the percentage of cells preferring directions within 10° of

6   local flow. Repeating such template-matching for many possible translatory axes ($n = 2701$; 5°

7   resolution), we generated a spherical tuning plot, displaying concordance as a function of the

8   axis of translation. Hotspots show directions of translation inducing flow best aligned with

9   observed DS preferences and thus driving maximal net output of this neuronal ensemble

10   (Extended data Fig. 5a-d; Methods).

11

12   Fig. 2d shows such a translatory flow-tuning plot for patch-recorded Hb9 V-cells. Longer spikes

13   and warmer map colors indicate better concordance and point to a location in extrapersonal

14   space, the center of flow convergence. To facilitate comparisons, we cartographically flattened

15   these plots (Fig. 2j,k). The flattened plot for V-cells (Fig. 2g) exhibits one hotspot at the visual

16   coordinates of the optimal center of contraction. So do the other two molecularly defined

17   ON-OFF subtypes (Trhr N-cells; CART-negative T-cells; Fig. 2b,c,e,f,h,i). Each subtype thus

18   has a single best translation. The N- and T-cell hotspots are separated by ~180° in polar

19   direction (abscissa), suggesting they share a common axis, while the V-cell hotspot (Fig. 2g) is

20   offset 90°, indicating preference for translation along an orthogonal axis (Supplementary

21   Note 3).

22

1   The flow-tuning plot for all imaged ON-OFF-DSGCs featured four prominent hotspots separated

2   by ~90° around the margin of the visual field or retina (Fig. 3a-c). Three of them correspond to

3   V-, N-, and T-cell hotspots (Fig. 2d-f), the last evidently to D-cells, preferring dorsal retinal

4   motion. The best axes of N- and T-cells are effectively aligned, as are those for D- and V-cells

5   (Figure 3b). Thus ON-OFF-DSGCs comprise two pairs of subtypes, each pair preferring

6   translation along the same axis, but in opposite directions.

7

8   How well does this geometric description predict the DS preferences of DSGCs? We modeled

9   four ON-OFF-DSGC channels adhering to the inferred translatory-flow-matching geometry.

10   Each channel comprised modeled cells with locations matched to imaged DSGCs but with

11   preferences aligned exactly with one of the four cardinal translatory flow fields. Angular jitter

12   (~10°) was added to mimic biological and experimental variability. As expected, flow-tuning

13   plots for single modeled channels (Fig. 3e-h) exhibited one hotspot (i.e., best axis; cf. Fig. 2g-i).

14   The ~90° intervals between hotspots reflect the orthogonality of the two cardinal axes.

15   Randomizing modeled preferences yielded a plot of uniformly mediocre concordance (Fig. 3i).

16   We differentially weighted each channel to reproduce its apparent abundance in our sample

17   (Extended data Fig. 5f-j; Methods) and summed them. The flow-tuning plot for the best-fitting

18   model (Fig. 3d) was strikingly similar to that for the real data (Fig. 3c; $R^2 = 0.97$). The fit was

19   optimal when the relative abundance of subtypes was N > V > D or T (Fig. 3k). Local polar plots

20   of DS preference for modeled cells (Fig. 3j; black) faithfully reproduced those for real cells

21   (gold).

22

The model also recapitulated the behavior of other subsets of ON-OFF-DSGCs (Hb9; Trhr;

CART–negative or –positive; Fig. 3l-p; see meridians in Fig. 2a-c). Weighting coefficients (Fig.

3l-p) provide estimates of relative abundances of subtypes in each sample; three molecularly

defined samples effectively comprised single subtypes (Fig. 3l-o).

ON-DSGC preferences are aligned with the same translatory optic-flow fields

ON-DSGCs ostensibly comprise three subtypes preferring directions ~120° apart[18,19], implying a

global architecture distinct from that of ON-OFF-DSGCs. We suspected single ON-DSGC

ensembles might prefer rotatory optic flow because they innervate the accessory optic system

(AOS), which encodes rotatory slip and drives optokinetic image-stabilization[24,25]. Surprisingly,

ON-DSGCs proved virtually identical in global architecture to ON-OFF-DSGCs (Fig. 4). They,

too, comprised four subtypes (N-, T-, D-, and V-type ON-DSGCs). Each subtype aligned its DS

preferences everywhere with essentially the same translatory flow field as its ON-OFF-DSGC

counterpart. As before, four lobes ~90° apart were discernable in the polar plots of all

ON-DSGCs (Fig. 4b) and became more distinct when the central retina was selectively sampled

(Fig. 4c).

The unexpected similarity between ON- and ON-OFF-DSGCs is not an artefact of

misidentification (Supplementary Discussion; Extended Data Figs. 1e-l, 3h, 6, and 7i-n). Why,

then, do we find four ON-DSGC subtypes instead of three? ON-DSGC N-cells were less

common (Fig. 4b,c) and less well-tuned than other ON-DSGC subtypes (Fig. 4e). A stringent

direction-selectivity-index (DSI) criterion excluded virtually all of them, resulting in a three-

lobed polar plot reminiscent of the classic one (Supplementary Note 4).

1

2 Like ON-OFF-DGSCs, ON-DSGCs aligned their preferred directions with optic flow produced

3 by translation along one of two mutually orthogonal axes. Each is served by a pair of subtypes,

4 generating paired hotspots at diametrically opposed global locations (Fig. 4i) or separated by

5 180° in flattened maps (Fig. 4j). Cardinal translatory axes were virtually identical for ON- and

6 ON-OFF-DGSCs (Fig. 4i).

7

8 An adaptation of the model developed for ON-OFF-DSGCs faithfully recapitulated the

9 flow-tuning plot for ON-DSGCs (Figs. 4j,k; R^2=0.95) and topographic variations in local DS

10 preference (Figs. 4h). Real and modeled ON-DSGCs consistently matched ON-OFF-DSGCs in

11 DS preference (Fig. 4h). However, the weighting of individual subtypes differed (Fig. 4m);

12 D-cells were the most abundant ON-DSGC subtype. Retrograde tracing revealed that ON-DSGC

13 subtypes project differentially to components of the AOS (Extended Data Fig. 2g-k, Extended

14 Data Fig. 3i-m).

15

16 **DS subtypes panoramically encode optic flow induced by translation along the body and**

17 **gravitational axes**

18 From resting eye and head positions, we determined the orientation of these cardinal translatory

19 axes of DSGCs relative to the visual environment (Methods). The N/T-cell axis approximated

20 the anteroposterior (longitudinal body) axis, whereas the D/V-cell axis corresponded closely to

21 the gravitational axis (Fig. 5a). Because N-cells, as an ensemble, respond best to forward

22 translation along the body axis (ambulation), it is appropriate to call them "advance cells." By

23 extension, T-, V-, and D-cells are "retreat", "fall", and "rise" cells. The cardinal translatory axes

10

1   are virtually identical for the two eyes (Fig. 5b), so members of one subtype prefer the same

2   global flow regardless of their location in either retina. Together, they form a binocular,

3   near-panoramic cell array responding best to a specific translatory optic flow field and thus to

4   self-motion in a cardinal direction (Fig. 5c,d).

5

6   **Retinal DSGCs also encode rotatory optic flow**

7   It is surprising that ON-DSGCs adhere to translatory optic-flow geometry because they supply

8   directional information to the AOS and flocculus, which encode rotatory flow[25]. However, an

9   ensemble aligned with translatory optic flow (Fig. 5e, blue) will also respond differentially to

10   rotatory optic flow (Fig. 5e-f; Extended Data Fig. 8o-s; Supplementary Note 5).   Might DSGC

11   ensembles actually adhere to a rotatory flow geometry and, only secondarily, exhibit tuning for

12   translation? Apparently not. The translatory model significantly outperformed the best rotatory

13   one for every sample tested, including all imaged cells ON-OFF-DSGCs ($R^2 = 0.97$ vs. 0.92) and

14   ON-DSGCs ($R^2 = 0.95$ vs. 0.87) (Supplementary Note 6; Extended Data Fig. 8a-n),

15

16   **The brain can infer self-motion by comparing DS channels**

17   Thus, a single DSGC ensemble's pooled output provides ambiguous information about self-

18   motion. However, the brain could distinguish translation from rotation by comparing the relative

19   activation of DS-subtype ensembles in the two eyes. For example, forward translation activates

20   the same subtype (N-cells) in both eyes, whereas leftward rotation activates opposing subtypes

21   (right eye: N; left: T). More generally, every possible translatory or rotatory optic flow produces

22   a unique pattern of activation across the eight DS channels (2 axes ✕ 2 directions ✕ 2 eyes;

23   Extended Data Fig. 9a-e). Indeed, a simple decoder using only relative activation of channels

can distinguish translation from rotation and infer the motion axis (Extended Data Fig. 9f,g). Similarly, modeled postsynaptic cells differentially tuned for rotation or translation were easily constructed by summing or subtracting specific subtype signals from the two eyes (Extended Data Fig. 10).

Relationship between visual and vestibular channels

We have shown how retinal DS channels decompose reafferent visual motion signals into translatory (otolith-like) and rotatory (canal-like) components. Tomographic reconstruction revealed that cardinal vestibular cardinal axes and planes lie near, but do not precisely match, their DSGC analogs, (Fig. 5g-k; Supplementary Methods; Supplementary Notes 7, 8).

<u>Discussion</u>

All canonical DSGCs organize their directional preferences around the geometry of optic flow produced by translatory self-motion along two axes of high behavioral relevance: the body axis and the gravitational axis. They comprise 16 channels altogether: four cardinal directions of translation encoded per eye x 2 eyes x 2 DSGC classes (ON and ON-OFF). Subtype ensembles also respond differentially to rotatory optic flow. Any arbitrary translation or rotation will activate these 16 channels in a unique pattern the brain presumably decomposes into translatory and rotatory components. A table summarizing the properties of DSGC subtypes, their preferred translations and rotations, and their relation to previously reported DSGC subtypes and reporter mice is provided (Supplementary Table 1).

1   Eye movements in freely moving rodents appear driven largely by the vestibulo-ocular reflex

2   (VOR)[26] and optokinetic nystagmus (OKN), which compensate for head movements to stabilize

3   the retinal image. The alignment between visual and vestibular axes, imperfect even at rest (Fig.

4   5j,k), would be further degraded by such deviations of the eye. The brain presumably

5   compensates when coupling these two reafferent streams to common motor outputs. On the

6   other hand, because VOR and OKN are image-stabilizing eye movements, they defend the

7   alignment between DSGC cardinal translatory axes and the gravitational and body axes when the

8   head rotates[26]. Gaze shifts transiently reorient the cardinal translatory directions in extrapersonal

9   space, but eye and head typically return promptly to primary position[26], reestablishing the

10   alignment of cardinal visual directions with gravitational and body axes.

11

12   The matching of DS geometry to translatory optic flow may reflect the earlier evolutionary

13   origins of translation-sensitive as compared to rotation-sensitive vestibular organs[27]. Retinotopic

14   information from DS ensembles is preserved in mappings to the colliculus and geniculocortical

15   system[13], permitting higher-order computations based on retinal motion.

16

17   Anatomical asymmetries of starburst amacrine-cell inhibition generate direction preference in

18   DSGCs[8]. Our data imply that these asymmetries are the same for ON- and ON-OFF-DSGCs, and

19   are organized around two retinal singularities, corresponding to the centers of expansion or

20   contraction of cardinal translatory flow fields (Fig. 5l, m).

21

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Supplementary Information is available in the online version of the paper.

Author Contributions

D.M.B. and S.S. designed the study and developed the theoretical framework. S.S. performed all imaging, electrophysiological recordings, and intracellular dye-fills. S.S., A.B.L., G.M., G.C.

1   and J.K.S performed intracranial and intraocular injections, immunostaining of retinas and

2   brains, and calcium imaging data processing. J.A.G. developed the mathematical methods for

3   interconverting locations on flattened retinas to hemispherical ones and to global extrapersonal

4   space.   N.J. performed the tomographic analysis of the vestibular system. S.S., D.M.B., J.A.G.

5   and N.J. analyzed the data. D.M.B., S.S., J.A.G. and N.J. wrote the paper.

6

7   **Author Information**

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10   The authors declare no competing financial interests.

11

12   Correspondence and requests for materials should be addressed to david_berson@brown.edu or

13   shai_sabbah@brown.edu

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Main figure legends

Figure 1. Directional preferences of ON-OFF-DSGCs are topographically dependent

a-d, Optic flow induced by animal's translation (**a,c**) or rotation (**b,d**) (pink arrows) and illustrated as apparent motions (blue arrows) in the visual space around the animal (**a,b**) and projected onto the retina, after flattening (**c,d**). Asterisks: flow fields' center of expansion (**a,c**) or rotation (**b,d**). Red circle in (c): DSGC receptive-field size. **e,f,** Inferred geometry of ON-OFF-DS preferences assuming cardinal directions remain orthogonal everywhere. One pair of types (orange, red) follows longitudinal (translatory-flow) geometry, the other (blue, green) latitudinal (rotatory-flow) geometry. **g,h**. Location of calcium-imaged cells (**g**) and imaged ON-OFF-DSGCs (**h**). **i-k,** Polar plots of DS preference among imaged ON-OFF-DSGCs, one line per cell. Polar histograms are overlaid. Cells pooled from whole retina (**i, j**) or only from the small circled central region (**k**). **l,** Topographic dependence of ON-OFF-DSGC local directional preferences, displayed as polar plots on a standardized flattened retinal map. **m,** Same as (**l**) but in reconstructed 3D view, corrected for histological distortions. Cells preferring ventral retinal movement (red lobes; 'V-cell' subtype) prefer motion toward a ventral singularity (center of contraction) and align with optic flow produced by downward translation (red meridians in [**m**]; cf. pink arrows in [**e,f**]).

Figure 2. Genetically defined ON-OFF-DSGC subtypes align DS preferences with translatory optic flow

a-c, DS preferences of molecularly defined ON-OFF-DSGC subtypes, assessed electrophysiologically (black arrows) and plotted on standard flattened (above) and reconstructed

hemispheric retinas viewed from two perspectives (below). **a**, V-cells, preferring ventral motion, targeted in Hb9-GFP mice. Gold arrowheads indicate orientations of dendritic asymmetry. **b**, N-cells, preferring nasal motion, targeted in Trhr-GFP mice. **c,** T-cells, preferring temporal motion, identified by immunonegativity for CART. Colored lines in (**a-c**) indicate best-fitting translatory optic flow. **d-k**, Quantitative assessment and cartographic representation of best translatory axes for DSGC ensembles. **d.** Concordance index (goodness of fit) as a function of axis of translation for recorded Hb9 V−cells, plotted as a spherical flow-tuning map. Optic axis (projection of optic disk into central visual field) points downward in this view. Hotspot and longest spike (red) point to visual-field location of the center of contraction of the translatory flow field best aligned with the direction preferences of sampled cells. **g**, Cartographically flattened (*plate carré*) projection of the flow-tuning plot in (**d**). The spherical (**d**) and flattened plots (**g**) both exhibit the same hot spot near the superior (S) edge of the visual field. Equivalent plots shown for N-cells (**e,h;** e is rotated 90° to display hotspot**)** and T-cells **(f,i),** with optimal optic flow either diverging from (N-cells) or converging at a singularity in the anterior visual field. **j-k,** Schematic explication of cartographic flattening of earth (**j**) or spherical translatory tuning plots (**k**) by *plate carré* projection.

Figure 3. Direction preferences of ON-OFF-DSGCs align globally with two axes of translatory optic flow.

a, Spherical translatory-flow tuning plot for all ON-OFF-DSGCs, from four perspectives. Four hotspots are apparent, one per subtype (cf. Fig. 2d-f). **b**, Best translatory axes are aligned for D- and V-cells (orange; red), and for N- and T-cells (blue; green). **c**, Flattened version of translatory-flow-tuning plot in (**a**) (cf. Figs. 2g-k) is recapitulated by a model (**d-h**) comprising

21

1 four subtype ensembles (**e-h**), weighted as in (**k**). **i**, Control flow-tuning map (randomized DS

2 preferences). See Supplementary Note 3 for clarification. **j**, Local polar plots of DS preference

3 for modeled cells (black) recapitulate the same topographic variations for imaged cells (gold).

4 Colored meridians plot the two best-fitting translatory optic flows. **l-o**, Left column: flattened

5 flow-tuning maps for effectively pure single-subtype samples. **l, m**, V-cells (Hb9-GFP); **n,** N-

6 cells (Trhr-GFP); **o,** T-cells (CART-immunonegative). **p,** Same, but for all subtypes but T-cells

7 using CART-immunopositivity. Best-fitting models (right column of plots) reproduce the actual

8 plots; optimal weighting factors (bar plots) confirm subtype purity.

9

10

11 **Figure 4. ON-DSGCs unexpectedly match global geometry of ON-OFF-DSGCs: Four**

12 **channels; four cardinal translatory directions**

13 Locations (**a**) and DS preferences (**b-d**) of imaged ON-DSGCs. **b**, all cells; **c,d,** central retina

14 (square in **a**). Four subtypes (lobes) are apparent in the central retina (**c**) but N-cells are less well-

15 tuned for direction (**e**), so N-cell lobe vanishes under a more stringent tuning criterion (**d,f,g**).

16 **h**, Topography of DS preference among ON-DSGCs (gold) resembles that of ON-OFF-DSGCs

17 (purple) and of modeled ON-DSGCs (black) aligned with optimal orthogonal translatory optic

18 flow fields (colored meridians). **i**, Spherical flow-tuning plot for all ON-DSGCs; four

19 perspectives. Best axes for the four subtypes (colored) form two co-linear pairs defining two

20 perpendicular cardinal translatory axes closely matching those of ON-OFF-DSGCs (black). **j-m**,

21 Flow tuning plot for all ON-DSGCs (**j**) reveals four channels well described by the model (**k**)

22 and closely resembling the equivalent plot for imaged ON-OFF-DSGCs (**l**) (from Fig. 3c) except

23 for the relative weighting of channels (**m**).

1

Figure 5. Global geometry of retinal DS: ensemble coding of translatory and rotatory self-motion.

a, Orientation of best translatory axes in extrapersonal space, roughly midaxial and gravitational.

b, Orientation of best translatory axes is identical for both eyes; thus a single translatory optic flow optimally activates the N-cell ensemble in both the right and left eyes (**c,d**). **e,** Geometric basis of sensitivity of DS ensembles to rotatory optic flow. In central retina, "advance" or N-cells (blue arrows) align their preferences with optic flow (gold lines and arrowheads) produced by rotation around a vertical axis (gold) orthogonal to their best translatory axis (blue).

f, Best rotatory axes of N- and T-cells (green) and V- and D-cells (orange) in the two eyes; left-eye axes striped. **g-i**, MicroCT reconstruction of vestibular system in context from side (**g**), above (**h**), or in isolation (**i**). See Supplementary Note 7 for abbreviations. **j**, Relationship of cardinal DSGC translatory axes to otolithic planes. **k**, Relationship of best rotatory axes of DSGCs (thick) to those of semicircular canals (thin); axes for left eye and labyrinth are striped.

l, Asymmetric starburst amacrine cell wiring underlying retinal DS. **m,** Current findings imply wiring asymmetries for a given DS subtype (blue) vary topographically and are oriented toward or away from a singularity.

18

19

Methods

Animals

All procedures were in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee at Brown University. We used three strains of adult mice of either sex, 2 - 2.5 months old: wildtype C57BL/6J (Jackson Laboratory), or Hb9:GFP (B6.Cg-Tg(Hlxb9-GFP)1Tmj/J; Jackson Laboratory) or Trhr:GFP (Tg(Trhr-EGFP)HU193Gsat; MMRRC) reporter mice.

Intravitreal injections of calcium indicator

Mice (C57BL/6J) were anesthetized with isoflurane (3% in oxygen; Matrx VIP 3000, Midmark). A viral vector inducing expression of the calcium indicator GCaMP6f (AAV2/1.hSynapsin.GCaMP6f; Vector Core, UPenn; 1.5 –2 µl of ~3 x 10^{13} units/ml) was injected into the vitreous humor of the right eye through a glass pipette using a microinjector (Picospritzer III, Science Products GmbH). Animals were killed and retinas harvested 14-21d later. GCaMP6f was expressed mainly in RGCs and amacrine cells of the ganglion-cell layer, most densely near central retinal blood vessels.

Retrograde labeling

Mice were anesthetized as for eye injections and secured in a stereotaxic apparatus. Respiration and body temperature were monitored. A micropipette (1B100-4; World Precision Instruments) filled with a retrograde tracer (1µl; cholera toxin beta-subunit conjugated to CF568; CtB-568; 1µg/µl PBS; Biotium #00071) was positioned stereotaxically. Tracer was injected pneumatically (Picospritzer; Parker Hannifin; 10 PSI; 400 ms pulses) and the pipette slowly withdrawn after 10

1 min. The wound was sutured and the mouse monitored until able to remain upright. Analgesia

2 (Buprenex SR, subcutaneous) minimized postoperative pain.

3

4 **Tissue harvest and retinal dissection**

5 The right eye was removed and immersed in oxygenated Ames medium (95% O_2, 5% CO_2;

6 Sigma-Aldrich; supplemented with 23 mM $NaHCO_3$ and 10 mM D-glucose). In retrograde-

7 labeling experiments, the brain was also removed and fixed by immersion in 4%

8 paraformaldehyde overnight. Under dim red light, the globe was cut along the ora serrata, and

9 cornea, lens and vitreous removed. Four radial relieving cuts were made in the eyecup, the

10 largest centered on the insertions of the lateral and medial recti, useful later as a reference axis.

11 The other two were deliberately asymmetric (roughly dorsotemporal and ventral) to

12 disambiguate retinal orientation. The retina was flat-mounted on a custom-machined hydrophilic

13 polytetrafluoroethylene membrane (cell culture inserts, Millicell[28]) using gentle suction, and

14 secured in a chamber on the microscope stage. Retinas were continuously superfused with

15 oxygenated Ames' medium (32–34°C). The left eye was enucleated and photographed for

16 measurement of arc length from optic disk to ora serrata, a parameter needed for mapping

17 flatmounted retina data to spherical coordinates (see Extended Data Fig. 5 and Supplementary

18 Equations).

19

20 **Immunohistochemistry of retina and brain**

21 After imaging or recording, retinas were fixed (4% paraformaldehyde, 30 min, 20°C) and

22 counterstained with one or more antibodies: 1) rabbit anti-CART (Cocaine and Amphetamine

23 Regulated Transcript; H00362, Phoenix Pharmaceuticals) - a specific marker for most ON-OFF

1 DS cells [23]; 2) guinea pig anti-RBPMS (RNA-binding protein with multiple splicing; 1832-

2 RBPMS, PhosphoSolutions), a pan-ganglion-cell marker[20]; or 3) chicken anti-GFP (Abcam), to

3 enhance the fluorescence of the GFP-based GCaMP6f indicator, which helped us to align images

4 captured during live imaging with subsequent histology. Using a custom-designed rotating stage,

5 we oriented the processed retina in the multiphoton microscope to match that during calcium

6 imaging, then acquired confocal one-photon Z-stacks of all fluorophores (including retrograde

7 tracers) in the RGC layer. Brains were embedded in 4% agarose, cut coronally (50 μM) on a

8 vibrating microtome and immunostained using rabbit anti-GFP (Life Technologies, A-11122) to

9 enhance GCaMP6f fluorescence in retinal axons, useful for localizing deposits in relation to

10 fasciculi and terminal nuclei of the AOS.

11

12 **Patch recording and dye filling of ganglion cells**

13 Whole-cell patch-clamp recordings of isolated flat-mount retinae were performed under current-

14 clamp using a Multiclamp 700B amplifier, Digidata 1550 digitizer, and pClamp 10.5 data

15 acquisition software (Molecular Devices; 10 kHz sampling). Pipettes were pulled from thick-

16 walled borosilicate tubing (P-97; Sutter Instruments). Tip resistances were 4–8 MΩ when filled

17 with internal solution, which contained (in mM): 120 K-gluconate, 5 NaCl, 4 KCl, 2 EGTA, 10

18 HEPES, 4 ATP-Mg, 7 phosphocreatine-Tris, and 0.3 GTP-Tris, pH 7.3, 270–280 mOsm). We

19 added red fluorescent dye (Alexa Fluor 568; Invitrogen) for visual guidance during two-photon

20 imaging and intracellular dye-filling.

21

22 Selected calcium-imaged cells were dye-filled (Alexa Fluor 568 hydrazide; Invitrogen) using

23 fine glass pipettes (~50 MΩ resistance) guided by two-photon imaging. A current pulse (-20 nA;

26

1   100 ms) triggered cell penetration. Dye was iontophoretically injected (20 - 60 biphasic current

2   pulses; -2000 pA for 500 ms and +500 pA for 400 ms) until dendrites were well filled. After all

3   calcium imaging was completed, filled cells were documented in Z-stacks acquired in confocal

4   (single-photon) mode.

5

6   **Two-photon functional imaging**

7   Imaging was conducted on an Olympus FV1200MPE BASIC (BX-61WI) microscope equipped

8   with a 25x, 1.05 NA water-immersion objective (XLPL25XWMP, Olympus) and an ultrafast

9   pulsed laser (Mai Tai DeepSee HP, Spectra-Physics) tuned to 910 nm. Epifluorescence emission

10   was separated into "green" and "red" channels with a 570nm dichroic mirror and a 525/50

11   bandpass filter (FF03-525/50-32, Semrock, green channel) and 575-630nm bandpass filter

12   (BA575-630, Olympus, red channel), respectively. The microscope system was controlled by

13   FluoView software (FV10-ASW v.4.1). Images of 256 x 128 pixels representing 256 x 128 μm

14   on the retina were acquired at 15 Hz (zoom setting of 2).

15

16   **Visual stimulation**

17   Patterned visual stimuli, synthesized by custom software using Psychophysics Toolbox under

18   Matlab (The MathWorks), were projected (AX325AA, HP) and focused onto photoreceptor

19   outer segments through the microscope's condenser. The projected display covered 1.5 x 1.5

20   mm; each pixel was 5×5 μm. The video projector was modified to use a single UV LED lamp

21   (NC4U134A, Nichia). The LED's peak wavelength (385 nm) shifted to 395 nm after

22   transmission through a 440 nm short-pass dichroic filter (FF01-440/SP, Semrock), a dichroic

23   mirror (T425lpxr, Chroma), and various reflective neutral density filters (Edmund Optics).

1 Photoisomerization rates were derived from the stimulus spectrum (measured using an

2 absolute-irradiance-calibrated spectrometer [USB4000-UV-VIS-ES, Ocean Optics]), estimated

3 rod (0.85 μm^2) and cone ($1\mu m^2$) collecting areas[29]; and spectral absorbances of mouse rod and

4 cone pigments[30]. Rates were very similar among rods and cones [~10^4 photoisomerizations/s

5 (R*/photoreceptor/s)], independent of their relative expression of S- and M-cone pigments[31].

6

7 To probe ON and OFF responses, we used a bright bar on a dark background (bar width=1500

8 μm, inter-stimulus duration=5 sec) drifting perpendicular to the bars long axis in 8 randomized

9 directions (45° interval, speed=300 μm/sec, 4 repetitions). To assess directional tuning, we used

10 a sinusoidal grating spanning two spatial periods (spatial frequency=0.132 cycle/degree,

11 Michelson contrast=0.95, stimulus duration=3.65 sec, inter-stimulus duration=5 sec at uniform

12 mean grating luminance) drifted in 8 randomized directions (45° interval, drift speed=4.5

13 degree/sec, 4 repetitions). Grating and bar parameters were optimized for ON-DSGCs[22]. Frames

14 of the stimulus movie appeared for 50 μs during the short 185 μs interval between successive

15 sweeps of the imaging laser; thus, no stimulus was presented during the interval of laser scanning

16 and associated imaging (300 μs / sweep). The very rapid flickering of the visual stimulus (>2000

17 Hz) was well above critical fusion frequency in mice [32].

18

19 **ROI selection and data analysis**

20 Somatic Ca^{2+} responses were analyzed using FluoAnalyzer [33] and custom Matlab scripts. In each

21 imaged field, we manually defined many regions of interest (ROIs; >500 pixels each); each ROI

22 consisted of one soma expressing enough GCaMP6f to support functional imaging. The space-

23 averaged pixel intensity within such ROIs was the activity readout for the associated cell, a

1 proxy for its spike rate [34]. Fluorescence responses are reported as normalized increases as

2 follows:

3

4 $$\frac{\Delta F}{F} = \frac{F - F_0}{F_0},$$ (1)

5

6 where F denotes the instantaneous fluorescence and F_0 the mean fluorescence over a 1-second

7 period immediately preceding stimulus onset.

8

9 The preferred direction (PD) of a cell was estimated as the angle of the vector sum following [35]:

10

11 $$PD = \arg \sum_\varphi r(\varphi)e^{i\varphi}$$ (2)

12 where r is the response amplitude to stimuli moving at direction φ (0, 45,…,315). The direction

13 selectivity index (DSI) of cells which may range between 0 (no direction selectivity) and 1

14 (highest direction selectivity) was calculated as:

15

16 $$DSI = \left| \sum_\varphi r(\varphi)e^{i\varphi} \right| \bigg/ \sum_\varphi r(\varphi).$$ (3)

17

18 The response amplitude r represented the average calcium response from the stimulus onset to 2

19 sec following the termination of the stimulus, to capture the OFF responses and to account for

20 the slow decay time of the calcium response [36].

21

1   **Unsupervised clustering and the diversity of DSGCs**

2   For decades, seven canonical subtypes of DSGCs have been recognized: 3 ON-DSGCs and 4

3   ON-OFF-DSGCs. Recently, however, several new DSGC types or subtypes have been identified

4   or suggested, including previously unknown OFF-DS types and novel forms of ON- and ON-

5   OFF DSGCs (Supplementary Discussion).

6

7   To assess how many subtypes might be differentiable in our sample based on calcium-response

8   kinetics, we performed unsupervised clustering using Gaussian Mixture Models. From each

9   cell's response to bar motion in the optimal tested direction, we extracted two features. The first

10   was a response-latency measure. We estimated when the leading edge of the stimulus would first

11   arrive at the receptive-field (assuming a diameter of 300 μm and accounting for cell position

12   within the imaged field), then measured the subsequent delay to the peak of the ON response.

13   The second feature extracted was the slope of the decay during the first 300 msec following the

14   ON peak. For clustering, we used the full covariance matrix, regularized by the addition of a

15   constant (0.001) to guarantee that the estimated covariance matrix is positive. To avoid local

16   minima, we repeated the algorithm using 50 different sets of initial values, with the accepted fit

17   being that with the largest log likelihood. The optimal model (the model with the optimal number

18   of clusters), was estimated based on the Bayesian information criterion:

19

20    $BIC = -2\log(L) + k\,log(n)$ (4)

21

22   where L represents the maximum of the likelihood function of the model, k represents the

23   number of clusters in the model (0-10 clusters), and n stands for the number of observations. The

1 model with the lowest BIC was considered optimal, and any model with a BIC no higher than 6

2 than the optimal was considered an acceptable alternative [37]. Running the clustering routine on

3 all DSGCs (n=2446) yielded an optimal model with six clusters, and a seven-cluster model that

4 was essentially as good. An alternative approach, the Akaike information criterion (AIC),

5 yielded an optimal model with eight clusters and acceptable alternatives with three, four, and

6 seven clusters. Altogether, this analysis provides evidence for 3 - 8 DSGC clusters, dependent

7 on criteria selected.   The relationship between these clusters and known or proposed DSGC

8 subtypes is difficult to work out, though the canonical types are certainly among them, and the

9 novel OFF-DGSCs are not (see Supplementary Discussion). In this report we therefore fall back

10 on the classical and widely used binary division of DSGCs into ON-DSGC and ON-OFF-DSGCs

11 classes. Of course, both classes population comprise multiple subtypes. Our findings indicate the

12 presence of at least eight subtypes in total (ON vs. ON-OFF classes; four subtypes each,

13 distinguishable by their topographically dependent direction preferences), in good agreement

14 with the BIC and AIC analysis above. Because our analysis considered a limited set of

15 potentially distinguishing parameters, it does not preclude further subdivision of DSGCs.

16

17 **Inferring the form of the generic directional tuning curve for DSGCs**

18 To generate the generic directional tuning function for DSGCs (Extended Data Fig. 9a), we

19 expressed the directional preferences of 56 ON-DSGCs and 141 ON-OFF-DSGCs in terms of

20 normalized spike output as a function of stimulus direction relative to the optimal (determined as

21 described earlier). Each of these tuning curves was fit with a von Mises distribution[38]:

22 $$r = r_{max} e^{\left(\frac{k * \cos(\varphi) * \frac{\pi}{180°}}{e^k} \right)} \tag{5}$$

1   where r_{max} is the peak amplitude, k is the width of the distribution, r is the calcium response

2   amplitude to stimuli moving in angle φ (0°, 45°,…, 315°) away from the preferred direction.

3   The bandwidths of ON-DSGC tuning curves, assessed as full width half maximum (FWHM), did

4   not differ significantly from those of ON-OFF-DSGCs (Welch t-test for unequal variances, $t = $ -

5   1.65, d.f. = 86.2, p = 0.101 [two-tailed]; average±s.d., 131°±16° for ON DSGCs and 135°±13°

6   for ON-OFF-DSGCs). Therefore, we estimated the directional tuning of DSGCs, regardless of

7   class and subtype, by averaging the normalized tuning curves of all imaged DSGCs (Extended

8   Data Fig. 9a). The bandwidth of this function (FWHM) was 135°±14°.

9

10   **Registration of calcium imaging data**

11   Initially, retinas were oriented relative to the line connecting nasal and temporal relieving cuts

12   made through rectus muscle insertions. We made a post-hoc adjustment to this orientation by

13   exploiting the stereotyped form across retinas of flow-tuning maps for ON-OFF-DSGCs (four

14   'hot' bands, roughly 90° apart along the direction axis – abscissa; see Extended Data Fig. 5). We

15   assumed that the slight variation in the exact position (direction) of these bands resulted from

16   experimental error in orienting the retina, though we cannot exclude contributions from

17   biological variability. To correct for this presumed rotatory error, we measured the displacement

18   of each retina's flow-tuning map from a reference map of the same form. The reference map was

19   generated by averaging four flow tuning plots maps in which the hot bands were clear and

20   appeared in similar positions (Extended Data Fig. 5e). For each retina ($n = 26$), the rotatory error

21   was taken to be the phase offset of its flow-tuning plot from this standard, determined by

22   convolving the two along a single dimension (direction; abscissa) at a resolution of 5°. The

23   correction factor was the phase shift maximizing the total amount of energy (brightness) in the

1   convolution matrix. Computationally correcting these rotatory errors registered all retinal

2   datasets, allowing us to pool directional data across retinas. Such offsets had a median value of

3   2.5°± 11.5° (s.d.). We corrected for these when transforming retinotopic data to global

4   extrapersonal coordinates.

5

6   **Estimating the relative abundance of single subtypes in samples of DSGCs**

7   For ON- and ON-OFF-DSGCs independently, we generated a model comprising four DS

8   subtypes (Fig. 3e-h), each of which aligned its DS preferences everywhere with the optic flow

9   produced by translation along a single, empirically determined best axis (Fig. 3b). Coordinates of

10   these four best axes were derived from the four local maxima (hotspots) in flow-tuning plots. We

11   asked what weighting of these subtypes could best reproduce the translatory-flow-tuning plot for

12   all DSGCs (Fig. 3c,d). Formally, the best fitting flow-tuning map for modeled cells (M_f) was

13   derived from the weighted sum of four (i=1,2,…,4) single-subtype maps M_i (Fig. 3e-h):

14

15   $$M_f = \sum_{i=1}^{4} C_i M_i \tag{6}$$

16

17   where C_i denotes the weighting allocated to individual subtype maps. We used least-squares

18   fitting (regress function in Matlab) and the multiple correlation coefficient (R^2) for regression

19   models without a constant term[39] to assess the goodness of fit. Two-tailed 95% confidence

20   intervals for R^2 values were calculated using bootstrapping, resampling DS cells with

21   replacement (Supplementary Note 6).

22

1 **Transforming retinal coordinates into global egocentric coordinates**

2 The retinocentric coordinate system we used was monocular, had its origin at the nodal point of

3 the eye, and specified retinal or visual-field location by two coordinates: eccentricity and

4 direction (Extended Data Fig. 5). *Eccentricity* was defined as the visual angle between the point

5 of interest and the optic axis (the projection of optic disk). Eccentricity is 0° at the blind spot and

6 90° at the margin of the visual hemifield, corresponding to the retinal margin. Eccentricities of

7 90°- 180° correspond to spatial locations outside the visual hemifield. *Direction* refers to the

8 direction of displacement of the point of interest from the optic axis, where nasal = 0°, dorsal =

9 90°, temporal = 180° and ventral = 270°.

10

11 For representations of global extrapersonal visual space, the origin was considered to lie at a

12 point halfway between the two eyes (we neglected the small displacement of the eyes from the

13 midline) (Extended Data Fig. 9). The horizontal plane (elevation=0°) was defined as the plane

14 perpendicular to the gravitational axis that passes through the nodal points of the eyes. To

15 translate retinocentric spatial locations to global ones, we assumed that the mouse's head was in

16 a typical ambulatory position[40] which puts the lambda–bregma axis of the skull at an angle of

17 29° (bregma lower) relative to the anteroposterior axis. Locations in global extrapersonal space

18 were defined by two coordinates: azimuth and elevation. Azimuth corresponds to the angle

19 between a vector pointing straight ahead and a second vector defined by the intersection of two

20 planes: the horizontal, and a vertical plane passing through the eye and the point of interest

21 (azimuth = 0° at the vertical meridian of the visual field). At the horizon, azimuth = 90° lies

22 directly to the right, -90° lies directly to the left, and 180° lies directly behind the animal.

1   Iso-elevation lines follow lines of latitude in the same global coordinate system, and range from

2   90° at the zenith, through 0° at the horizontal plane, to -90° at the nadir.

3

4   We transformed retinal or visual field locations from spherical retinocentric coordinates to global

5   egocentric coordinates using two key additional values. The first was the orientation of the optic

6   axis in the head of ambulating mice, drawn from Oommen and Stahl [40], namely at global

7   egocentric coordinates (as defined here) of 22° elevation and 64° azimuth. These values

8   accounted for the tilt maculo-ocular reflex (tiltMOR), which tilts the eye upward in visual space

9   when the nose tilts downward. The second value was the torsional orientation of the eyes

10   measured from the positions of the insertions of the lateral and medial rectus muscles relative to

11   the stereotaxic lambda-bregma axis. To obtain this value, mice ($n = 5$) were anesthetized as for

12   survival surgery, mounted in a stereotaxic apparatus, the skull exposed, and the mouse's head

13   tilted about the interaural axis so that the lambda-bregma axis lay within a stereotaxic horizontal

14   plane. Using a stereotaxically mounted fine probe as a depth guide, we used a cautery pen to

15   make two small burn marks on the cornea, both within the same stereotaxic horizontal plane, but

16   one at the rostral margin of the cornea, and the other at its caudal limit. The line connecting the

17   two burn marks thus paralleled the lambda-bregma axis. We then measured the angle between

18   this line and that connecting the insertions on the globe of the lateral and medial rectus muscles.

19   This angle averaged 35.6±2.3° (average±s.d.), with the medial rectus insertion lying above the

20   lateral one.

21

Tomography of vestibular and ocular systems. See Supplementary Methods and

Supplementary Table 2.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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2 (2009).

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Extended Data Figure Legends

Extended Data Figure 1. Direction-selectivity of ON- and ON-OFF-DSGCs revealed by their calcium and voltage responses.

a-d, Calcium transients of representative ON-OFF-DSGCs (**a,b**) and ON-DSGCs (**c,d**) in response to bright bars (**a,c**) or sinusoidal contrast gratings (**b,d**) drifting in eight directions at 45° intervals. Preferred direction and direction selectivity index (DSI) were determined from grating responses, cell class (ON- or ON-OFF-DSGC) from the bar responses. Traces plot somatic GCaMP6f Ca^{2+} signal over time; red = mean; gray = single trials. Black marker indicates when stimulus bar was within the cell's receptive field. Polar plots show response amplitude (normalized to maximum) for each direction (bold curves = mean of four repetitions; thin = single trials). Red vectors show preferred direction (bold, mean; thin, single trials; N, nasal; D, dorsal; T, temporal; V, ventral). Direction-selectivity index (DSI) was higher for gratings than bars, especially for ON-DSGCs (Supplementary Note 9). **e-k**, Morphological and functional validation of DSGC subtypes inferred from Ca^{2+} imaging. **e**, GCaMP6f fluorescence (top right) revealed DS tuning in a cell that was then targeted for patch recording (top left) and dye filling (bottom left; see Extended data Fig. 3i for representative morphological data). **f,g**, Calcium (**f**) and voltage (**g**) traces for representative ON- and ON-OFF-DSGCs in response to a drifting bright bar. **h,i**, mean calcium responses (**h**) and peristimulus time spike histograms (PSTH) (**i**) for patched ON- ($n = 8$) and ON-OFF-DSGCs ($n = 6$) in response to a drifting bright bar (gray bands: ±1 s.d.). The bar's trailing edge evoked a small OFF Ca^{2+} transient in ON-DSGCs (**h**; see also **f**, left trace) and a very slight uptick in the PSTH (**i**). **j-k**, DS preference inferred from Ca^{2+} signal closely matches that inferred from spiking for both ON-DSGCs (red) and ON-OFF-

1   DSGCs (black) (**j**), despite having significantly broader tuning (**k**; lower DSI; paired *t*-test, *t*

2   = -4.068, p<0.001). **l**, Bath application of a selective ON-channel blocker (L-AP4) abolished the

3   ON response, but left an OFF transient of reduced amplitude in ON-DSGCs (*n*=10) and

4   ON-OFF-DSGCs (*n*=43) alike. OFF responses of ON-DSGCs are presumably mediated by

5   excitatory input from OFF bipolar cells to the sparse OFF dendritic arbors of ON-DSGCs[22].

6

7

8   **<u>Extended Data Figure 2</u>. Anatomical analyses: correlating calcium with**

9   **immunofluorescence, retrograde labeling, and morphological asymmetries.**

10 **a-f**, Correlating Ca^{2+} imaging and post-hoc immunofluorescence in a single imaged field. **a**, A

11 2-photon image of GCaMP6f-expressing cells in live retina, in vitro. Numbers indicate regions

12 of interest (ROIs) for assessing somatic DS responses. Cell-free zones are blood vessels. **b-f**,

13 Confocal image of the same field after fixation, immunolabeling, and alignment with the live

14 image (**a**). **b**, Immunolabeling for CART, a specific marker for 3 of 4 ON-OFF-DSGC subtypes

15 (N-, V- and D-cells). **c**, Anti-GFP immunofluorescence, to enhance GCaMP6f signal which

16 faded after fixation. **d**, Immunofluorescence for RBPMS, a marker for all RGCs. **e,f**, Merged

17 images combining GCaMP6f (anti-GFP) signal with either RBPMS (**e**) or CART (**f**). **g-j**, Single

18 imaged field demonstrating that identification of cells labeled by retrograde transport from the

19 AOS can be linked to specific Ca^{2+}-imaged cells. **g,** 2-photon image of GCaMP6f fluorescence

20 in live retina in vitro; ROIs marked as in (**a**). **h,i**, Live confocal images of same field, showing

21 (**h**) two cells retrolabeled from the MTNd (**h;** fluorescent cholera toxin beta-subunit, CTb) and

22 GCaMP6f fluorescence (**i**). **j**, Merged image of **h** and **i**. **k**, Confocal images of coronal sections

23 showing retrograde-tracer deposits (red) in 12 different mice, three mice each (#1-3) for four

1   AOS targets (rows): superior fasciculus of the accessory optic tract (SF-AOT); inferior

2   fasciculus of the AOT (IF-AOT); dorsal division of the medial terminal nucleus (MTNd); and

3   nucleus of the optic tract (NOT). Far right column shows enlarged images from third column.

4   These deposits spared retinorecipient nuclei outside the AOS, visible from nonspecific labeling

5   by GCaMP6f fluorescence (green). **l-s**, Structure and function of GFP-labeled DSGCs in Hb9-

6   GFP mice. **l.** Voltage responses to drifting gratings (conventions as for Extended Data Fig. 1b,d).

7   **m,n**, Spike responses to a bright bar moving in the preferred direction. Dashed line indicates

8   roughly when the stimulus overlapped the receptive field. **m**, Voltage response of representative

9   Hb9-GFP cell on a single trial. **n**, Mean peristimulus time histogram (PSTH) for all recorded

10   Hb9-GFP cells ($n = 32$; 4 retinas). Gray band represents ± 1 s.d. **o**, Preferred directions of all

11   recorded GFP+ cells ($n = 32$ cells; 4 retinas). All point generally ventral, but scatter is

12   substantial. (Arrow length not scaled to DSI). **p-s**, Dendritic asymmetry in Hb9 cells correlates

13   with preferred direction. **p**, Typical Hb9 cell dye-filled during patch recording; maximum-

14   intensity projected (MIP) confocal image. Blue vector indicates displacement of centroid of

15   dendritic arbor (red dot) from the soma (black dot), a measure of the magnitude and direction of

16   asymmetry. **q**, Dendritic asymmetry correlates strongly with physiologically determined DS

17   preference ($n = 12$). **r**, Assessment of dendritic-field asymmetry in Hb9-GFP cells based on GFP

18   fluorescence alone. In this MIP image, colored polygons show estimated dendritic-field

19   envelopes for four cells. Vectors mark magnitude and direction of asymmetry as in (**p**). **s**, Polar

20   plot of direction and magnitude of dendritic asymmetry among a large sample of Hb9-GFP cells

21   ($n = 82$ cells; 3 retinas) estimated as in (**r**). Estimates made blinded to retinal location. Vector

22   length indicates magnitude of arbor asymmetry (displacement of dendritic-field centroid from

1   soma, normalized by the arbor circumference). Nearly all markedly asymmetric fields were

2   displaced in a ventral direction from their somatic location, but with substantial scatter.

3

4

5   **Extended Data Figure 3.   ON-DGSCs: Differentiation from ON-OFF-DSGCs by**

6   **unsupervised clustering and projections to accessory optic system .**

7   **a**, DSGCs can be clustered into two classes (ON and ON-OFF) based on two parameters of Ca^{2+}

8   responses to moving bars: 1) the latency of the ON peak (relative to the arrival of the bar at the

9   receptive-field; assumes 300 μm field diameter and accounts for cell position within the imaged

10 field); and 2) the slope of decay for 300 msec following the ON peak, a measure of the

11 transience. Each cell is shown by a point colored to represent its posterior probability of

12 belonging to the ON-OFF-DSGC cluster. Most cells are very likely to be one type or the other.

13 **b-g**, Average Ca^{2+} signals ($\Delta F/F$; mean [red] ± s.d. [gray]) evoked by light bar moving in the

14 preferred direction for various samples of DSGCs (ON-OFF-DSGCs: red traces; ON-DSGCs:

15 blue): **b**, all imaged DSGCs; **c**, morphologically identified (dye-filled) DSGCs; **d**, CART-

16 immunopositive DSGCs (comprising mainly N-, D-, and V-type ON-OFF-DSGCs); **e-g**, DSGCs

17 retrolabeled from three components of the AOS: superior (**e**) and inferior fasciculi (**f**) of the

18 AOT, and dorsal division of the MTN (**g**). See Supplementary Note 10 for additional details. **h**,

19 Representative morphology of ON-OFF-DSGCs (left) and ON-DSGCs (right) revealed by dye

20 injection after Ca^{2+} imaging, and illustrated as MIP confocal images, projected onto the retinal

21 plane (top) and an orthogonal one (bottom). ON-OFF-DSGCs stratify about equally in ON and

22 OFF sublaminae; ON-DSGCs mainly in the ON sublamina. **i-m**, Ca^{2+} imaging of retrolabeled

23 cells shows ON-DSGCs subtypes project differentially to AOS. Each panel includes DS

1   preferences plotted on a standard flat retina with superimposed best-fitting optic flow for each

2   subtype (left); translatory optic-flow-tuning plots (middle), one for the actual data [top] and a

3   second for the best-fitting model [bottom]); and model-derived weighting coefficients (right),

4   providing an estimate of relative subtype abundance. D- and V-cells selectively innervated the

5   medial terminal nucleus (MTN) (**i-k**), while T- and N-cells supplied the nucleus of the optic tract

6   (NOT)/ dorsal terminal nucleus (DTN) (**l**). The superior fasciculus of the AOT (SF-AOT)

7   apparently carries only D-cell axons (**j**), whereas the inferior fasciculus (IF-AOT) and dorsal

8   MTN (MTNd) contain mixed V and D fibers (**k**) (but see[42]). Thus, two cardinal translatory axes

9   are separately represented in the AOS, one in the NOT/DTN and the other in the MTN.

10

11

12   **Extended Data Figure 4**. **Mapping cell locations in standard retinal coordinates.**

13   **a**, Representative experimental retina after GCaMP6f imaging (confocal image). Bright patches

14   (mostly central) are GCaMP6f fluorescence. Magenta vectors show locations and DS preferences

15   of imaged DSGCs. Four radial relieving cuts were made to promote flattening; blue circles mark

16   their termini. The nasal and temporal cuts were made at medial and lateral rectus insertions; the

17   line connecting these (the 'horizontal reference line') effectively parallels the horizontal plane in

18   ambulating mice. Curl at retinal margins developed gradually and was taken into account. **b**,

19   Map of estimated strain energy density, reflecting local stretching during flattening . **c-f**,

20   Mapping of cell locations onto a standardized spherical coordinate system for pooling data

21   across retinas and display in flattened (**d**) or hemispherical form (**f**). **c**, Data in (**a**) remapped into

22   standardized spherical coordinates, followed by remapping into flattened form using modeled

23   relieving cuts approximating the actual ones; close similarity to (**a**) indicates these

1   transformations are accurate and reversible. **d**, Same as (**c**), but in the form of a standardized flat-

2   mounted retina (four virtual radial cuts, 90° apart, extending 60% of the distance from retinal

3   margin to optic disk). **e,f**, Schematic illustration of the mathematical approaches used for

4   mapping to, and transformation between, coordinate systems. See Supplementary Equations.

5   Parametrization x : $\Omega \rightarrow \mathbf{R}^3$ of the spherical retina S. *s:* arc length as measured from the optic

6   disk. *θ:* the longitudinal coordinate; 0° corresponds to the nasal terminus of the horizontal

7   reference line, 90° to the dorsal retina, and so forth. Four red meridional arcs show the

8   reconstructed positions of the four radial cuts in (**a**) and (**c**). These have angular coordinates of θ_i

9   and an arc length of $M - m_i$. M was approximated from the flat-mount as the average length of

10 lines extending from the optic disk to the retinal margin (green lines, including the curled

11 portions in **a**). m_i was estimated as the distance on the flat-mount from optic disk to the terminus

12 of the corresponding radial cut [blue lines in (**a**), (**e**) and (**f**)]. Numerical reconstruction of the

13 flat-mounted retina allowed empirical cell locations to be mapped to the spherical retina via the

14 mapping x $\circ$ F $^{-1}$.

15

16

17 **Extended Data Figure 5.** **Flow-tuning plots, correction for errors of rotatory orientation *in**

18 ***vitro*, and development of a flow-tuning model.**

19 **a-d**, Generation of flow-tuning plots. **a**, DS preferences of all imaged ON-OFF-DSGCs mapped

20 onto standard flattened retina. Each black vector marks one cell's location and preferred

21 direction. How well do these DS preferences aligned with the local retinal optic flow produced

22 by the mouse's translation along specific axes? Two flow fields are shown (blue and red lines

23 and arrowheads); many more were tested (2701 axes; 5° intervals of spherical angle). For each

1   tested translation, we measured the angle θ between each cell's preferred direction and the local

2   direction of optic flow (inset: θ_1 for the blue flow field, θ_2 for the red one). N: nasal; D: dorsal;

3   T: temporal; V: ventral. **b**, Distributions of angles θ_1 (top) and θ_2 (bottom) among all cells in **a**.

4   *Concordance index* comprises the percentage of cells with DS preferences differing <10° from

5   the local motion in a specific flow field (green rectangles). Alignment was much greater with

6   'blue' than 'red' optic flow (concordance index = 11.7 and 2.6, respectively). **c**, Spherical

7   translatory-flow-tuning plot displaying concordance index as a function of the axis of the

8   animal's translation through space. Location of data for "blue" and "red" optic flows are

9   indicated.   **d**, Schematic representation of translatory optic flow induced in visual space (bottom

10   hemisphere) and on retina (upper hemisphere) by animal's (and eye's) translation along the

11   indicated axis (best axis of V-cell subtype). Translation along the indicated axis yields flow with

12   a center of contraction in the ventral retina (as for V-cells; Fig. 1m and 2a) and in the

13   corresponding locus in superior visual field. **e**, Correcting for errors of rotatory orientation of

14   retina in chamber. Flattened translatory-flow-tuning plots for ON-OFF-DSGCs in four different

15   retinas with large samples of imaged cells. Plots are highly stereotyped in form, but exhibit some

16   variation in phase (i.e., horizontal position; compare positions of hotspots to the arbitrary red

17   vertical reference line). To correct for this experimental error, we phase-shifted each retina's heat

18   map (i.e., offset it along the x-axis) to produce the best match with a reference heat map (an

19   average of the four maps in **e**). See Methods for details. **f-i**, Stepwise development of the model

20   of global DS geometry. Note the progressive improvement in the agreement between the flow-

21   tuning plots of modeled and actual imaged ON-OFF-DSGCs (**j**). **f**, Basic version of the model.

22   Modeled DSGCs were uniformly distributed over the retina. DS preferences set to local flow

23   produced by translation in one direction along one of two orthogonal axes, derived in Fig. 3b. **g**,

45

1   After restricting modeled cells to the locations of actual imaged cells. Smearing of hotpots

2   reflects degraded certainty about the position of the best axes due to undersampling of cells in

3   retinal regions near the singularities (centers of expansion or contraction). **h**, After mimicking

4   biological and experimental variability by adding angular noise (standard deviation = 10°) to the

5   preferred directions of cells used in **g**.   **i**, After accounting for the unequal abundances of

6   subtypes by differentially weighting them before summation. Below: local polar plots of

7   preferred directions of modeled DSGCs. Equivalent plots for cells in the final, refined model (i)

8   are shown in Fig. 3j (black). **k-n**, Evidence for the predictive power of the model across data

9   sets. ON-OFF-DSGC cells were arbitrary divided into two samples, a training set (flow tuning

10   plot in **k**) and a test set (**l**). Best translatory axes derived from the training set were used to

11   generate a 4-subtype translatory flow-matching model of the same form as in **i**. **m,** flow-tuning

12   plot for these modeled cells closely resembles that for imaged cells in the test set (**l**; R^2=0.95). **n**,

13   A model with translatory axes derived from ON-OFF-DSGCs predicts the DS preferences of

14   imaged ON-DSGCs cells (R^2=0.83). Weighting coefficients giving the best fit recapitulated

15   those from direct modeling of ON-DSGC (Fig. 4j,m). See Supplementary Note 11 for details.

16

17

18   <u>**Extended Data Figure 6**</u>. **Four ON-DSGC subtypes are still apparent when stringent**

19   **criteria are used to distinguish them from ON-OFF-DSGCs.**

20   Even when we apply a more stringent criterion to differentiate ON- from ON-OFF-DSGCs, we

21   detect four ON-DSGC subtypes rather than the expected three (Fig. 4). **a**, Cluster analysis

22   identical to Extended Data Fig. 3a but including only cells very likely to belong to the ON- or

23   ON-OFF-DSGC classes (posterior probabilities >0.95 of membership in one of the classes).

1   This excluded 457 cells from analysis. **b**, Average calcium signals ($\Delta F/F$; mean±s.d.) to moving

2   bars for these stringently classified samples of ON-OFF-DSGCs (red traces) or ON-DSGCs (blue

3   traces). See Extended Data Fig. 3c for details. **c,d**, Four subtypes remain evident in both ON

4   DSGCs (d) and ON-OFF DSGCs (c) after application of the stringent classification criterion.

5   These subtypes are evident in the four lobes of local polar plots of preferred direction (left) and

6   in the four hotspots in the associated flattened translatory-flow-tuning plots (center). Bar plots

7   (right) display the weighting coefficients of the best fitting model, a measure of the relative

8   abundance of the four subtypes. These weighting coefficients were similar to those obtained

9   using the standard posterior-probability criterion (>0.5), suggesting that the cells excluded due to

10   the stringent criteria were distributed roughly equally across the four subtypes for each class.

11

12   **<u>Extended Data Figure 7</u>. Selectivity for direction varies among DSGC subtypes.**

13   **a**, Polar plots of local DS preference among ON-DSGCs, plotted on standard flat retinas (above)

14   and in reconstructed hemispherical form (below). Increasing the stringency of the criterion for

15   direction-selectivity index (DSI) from 0.3 to 0.5 reduced the number of N-cells (top left) by 95%,

16   but other subtypes by only 42-60% (sample zone: red rectangle). Thus, N-type ON-DS cells are

17   unusually poorly tuned, and may have been excluded on that basis in previous studies reporting

18   only three ON-DSGC subtypes. **b**, Left panels of (**a**) reproduced with color coded subtypes.

19   Each cell's subtype assignment determined by which of the four cardinal translatory flow fields

20   was most closely aligned with its preferred direction. N, blue; T, green; D, orange; V, magenta.

21   **c**, Line histograms showing the distribution of DSIs among each ON-DSGC subtype. The

22   distribution for N-type ON-DSGCs is shifted to lower DSI values. **d**, Median DSI and first and

23   third quartiles for each ON-DSGC subtype. N-cells were significantly less well tuned. **e-h,** same

1 analysis as **a**-**d**, but for ON-OFF DSGCs. There were significant differences among subtypes but

2 the stringency of the DSI criterion did not affect the relative abundance of subtypes. **i,j**, Mean

3 calcium responses (*n*=497) to preferred direction of bar motion of individual subtypes of

4 ON-DSGCs (**i**) and ON-OFF-DSGC (**j**) . **k,l**, Histograms (**k**) and mean values (plus 1st and 3rd

5 quartiles) (**l**) of latency to ON peak for each DSGC subtype, measured from estimated time of

6 arrival bar edge at receptive field (see Methods). Latency differed significantly between each

7 ON-DSGC subtype and its matching (homonymous) ON-OFF-DSGC subtype. **m,n** Histograms

8 (**n**) and median plus 1st and 3rd quartiles (**n**) of the slope following the ON peak for each subtype

9 of ON- and ON-OFF-DSGCs (see Methods). See Supplementary Note 12 for statistics and

10 further details.

11

12 **<u>Extended Data Figure 8</u>. Direction preferences of both ON- and ON-OFF-DSGCs are**

13 **better aligned with translatory optic flow fields than with rotatory ones.**

14 **a**, Flattened *rotatory* flow-tuning plot illustrating the concordance of DS preferences of all

15 imaged ON-OFF-DSGCs with *rotatory* optic flow fields as a function of rotatory axis orientation

16 (*plate carré projection;* cf. Fig. 2j,k; Supplementary Note 13). **b**, Same as (**a**) but using modeled

17 cells drawn from the best-fitting model. Model as for Extended Data Fig. 5i, but with subtypes

18 (**d**) aligning DS preferences with rotatory instead of translatory optic flow fields (Supplementary

19 Note 13). **c**, Weighting coefficients for the four subtypes in the best-fitting model. **d.** Rotatory-

20 flow-tuning plots for the four individual rotatory-flow-matching subtypes used in the model,

21 each with DS preferences aligned (±10° noise) with one of the four cardinal rotatory flow fields.

22 **e-g**, Local polar plots of DS preferences predicted for two sets of modeled DS cells, aligning

23 their DS preferences either with translatory optic flow (Fig. 3d-h) or with rotatory optic flow

1   [this figure, (**b**) and (**d**)]. **e**, Comparison of translatory and rotatory flow-matching models (green

2   and blue polar plots, respectively). Predictions are similar in the central retina but diverge

3   sharply in the periphery, especially ventrally and temporally. **f,** Observed preferred directions of

4   imaged ON-OFF-DSGCs (red vectors; n = 1953) compared with those predicted by the model

5   comprising four *translatory*-flow-matching channels (black vectors; n = 8100). **g**, Same as (**f**),

6   but with preferred directions predicted from the *rotatory*-flow-matching model (black vectors; n

7   = 8100). Optic flow preferred by each channel shown in pastel lines and arrowheads (N-cells,

8   blue; D, green; T, red; V, magenta). **h-n**, Same as for (**a-g**), but comparing modeled and imaged

9   ON-DSGCs. **o**, Comparison of real and modeled responses to translatory and rotatory flow for an

10   ensemble comprising a single ON-OFF-DSGC subtype (T-cells). Modeled cells were aligned

11   with their respective canonical translatory optic flows and were uniformly distributed over the

12   retina. Top: Response of modeled T-cell ensemble to *translatory* optic flow. Middle: Modeled

13   ensemble response of same cells to *rotatory* optic flow. Bottom: same as middle, but for real

14   imaged T-cells. **p-s**, Flow-tuning plots for all imaged ON-OFF-DSGCs (**p,r**) and ON-DSGCs

15   (**q,s**) probed with translatory (**p,q**) or rotatory (**r,s**) flow. **t-w**, Translatory-flow-matching model

16   outperforms its rotatory equivalent overall, but especially temporally (green sector in **t** and **v**),

17   where model predictions are most divergent (see **e**). **u,w,** Bar plots of R^2 for fit to data of

18   translatory (grey) and rotatory (black) models, assessed separately for the retina overall (left) or

19   the temporal sample region alone. Similar trends were also apparent near the ventral translatory

20   singularity (not shown).

21

1   **Extended Data Figure 9. Ensemble coding by DSGCs of all possible translatory and**

2   **rotatory optic flows, mapped in retinal or global extrapersonal coordinates.**

3   **a**, Average directional tuning curve of imaged DSGCs (mean Ca^{2+} response, normalized to

4   maximum, as a function of the angular offset from preferred directions). Tuning curves did not

5   differ for ON- and ON-OFF-DSGC classes (see Methods). **b**, Flattened map of summed spike

6   output of a single modeled ON-OFF-DSGC subtype — the D-cells — as a function of direction

7   of the animal's translation, expressed in retinal coordinates. Hotspot indicates location in retinal

8   space of the center of contraction of the preferred translatory flow field (Supplemental Note 14).

9   **c**, Alternative flow-tuning plot for the same subtype but plotting the concordance index (as

10   elsewhere in this report; cf. Fig. 2d-k; Supplementary Note 14). **d**, Flow-tuning plots of

11   estimated summed ensemble spike output for each of four modeled translatory-flow-matching

12   DSGC subtypes in response to optic flow generated by translation along (left column) or rotation

13   about any axis (right column). **e**, Same as (**d**) but remapped in extrapersonal space and shown

14   separately for the left and right eyes. Azimuth of zero is anterior; elevation of 90º is overhead.

15   Hotspots in maps for translatory optic flow (first and third columns) indicate the best egocentric

16   direction of heading for activating that DS subtype ensemble (Supplementary Note 14). **f, g,**

17   Decoder model showing the brain could discriminate rotatory from translatory optic flow and

18   identify the axis of self-motion by exploiting unique patterns of relative activation of the eight

19   DS-channels (4 subtypes X 2 eyes) induced by specific optic flow fields. **f.** Left column: heat

20   plots showing, as a function of axis orientation, the Euclidean distance (dissimilarity) between

21   two patterns of relative activation of the eight DS channels induced by: 1) translation along that

22   axis; and 2) translation along a single "input" axis (the unknown to be inferred by decoding,

23   indicated at left, in green). Each probed axis is represented by a single pixel in the global map.

1   Coldest color marks the coordinates in extrapersonal space of the axis of translation evoking

2   8-channel outputs closest in Euclidean distance (black number, upper right), and thus highest in

3   similarity, to the 8-channel outputs evoked by the reference (unknown) axis. Right column of

4   plots: same as left except that the eight-channel outputs induced by the input translatory optic

5   flow are now compared to those of the same eight channels when induced by *rotation* about any

6   possible axis. The coldest spot in this plot marks the orientation of the rotatory axis producing

7   an 8-channel output pattern most similar to that produced by translation along the input

8   (unknown) axis. **g**, Same as (**f**) except that the input axis listed at left (the unknown) is an axis of

9   rotation, rather than of translation. In each case, the flow field generating eight-channel outputs

10 most like those induced by rotation about the input axis (minimum Euclidean distance and

11 darkest blue) is rotatory (right column of plots), not translatory (left column), and the orientation

12 of the best-matching rotatory axis corresponds to that of the input axis of rotation. See

13 Supplementary Note 14 for further explanation and interpretation.

14

15

16 **<u>Extended Data Figure 10</u>. Modeled postsynaptic cells differentially tuned for rotation or**

17 **translation are easily generated by summing or subtracting specific DS channels in the two**

18 **eyes.**

19 **a**, We devised a 'tuning index' to quantify the strength of tuning of single modeled subtype-

20 ensembles for specific optic flow fields (Extended Data Fig. 9). The index consisted simply of

21 the range of the Euclidean-distance values for the individual modeled DSGC ensembles.

22 Individual DSGC subtype ensembles are nearly as well tuned to a specific axis of rotation as they

23 are to their best axis of translation (Extended Data Figs. 8a,h; 9e). **b**, Design of a simple model

1   for generating postsynaptic cells with a selective preference for translation over rotation (T1-T4),

2   or for rotation over translation (R1-R6), through linear summation or subtraction of multiple

3   DSGC channels in the two eyes (see Supplementary Note 15 for details on model design).

4   **c**, Selectivity of modeled postsynaptic cell types for specific translations (T1-T4) or rotations

5   (R1-R6), reflected in the tuning index as in **a**; note the enhanced discriminability of translation

6   from rotation relative to the input DSGCs (**a**). **d**, Flattened spherical ensemble-output plots,

7   showing variations in net excitation of specific modeled postsynaptic cell classes as a function of

8   the axis of translation or rotation, all in egocentric (global) coordinates (see Extended Data

9   Figure 9e). In heat maps, red and blue represent maximum and minimum channel activation,

10   respectively. The model thus demonstrates that arithmetic combinations of signals from specific

11   retinal DS channels could allow the brain to decompose optic flow into its translatory and

12   rotatory components, paralleling the biomechanical decomposition of self-motion into these

13   components by the vestibular labyrinth.

optic flow

a

b

visual
space

translation

rotation

cardinal directions

e

c

D

T ←→ N

V

d

retina

f

all cells

ON-OFF-DSGCs

g

h

i

j

k

D

N

T

V

global

global corrected

local

l

m

V cells

a Hb9 **b** Trhr **c** CART-

D
T ↔ N
V

d V cells (Hb9)
blind hemifield
visual hemifield
best axis of translation
optic axis

e N cells (Trhr)

f T cells (CART-)

Concordance index (%)

g 0   20   40   60
Eccentricity (deg.)
anti-OA
135
margin
45
OA
45 S 135 A 225 I 315 P 45
Direction (deg.)
V

h 0   10   20   30   40
45 S 135 A 225 I 315 P 45
Direction (deg.)
N

i 0   20   40   60
45 S 135 A 225 I 315 P 45
Direction (deg.)
T

j pole
equator
pole

k anti-optic axis
margin of visual field
optic axis
best axis
center of contraction
blind hemifield
visual field
sup ant inf post

a All ON-OFF DSGCs

Concordance index (%)

Single subtype models

b

c data
d model

e model
f
g
h
i control

Eccentricity (deg)

anti-OA
135
margin
45
OA

Direction (deg.)

45 S 135 A 225 I 315 P 45
S A I P

j

data
model

D
T ← → N
V

k $R^2 = 0.97$

all ON-OFF-DS

ON-OFF DS subtypes
data   model

l Hb9 physiology
m Hb9 morphology
n Trhr physiology
o CART-negative
p CART-positive

Direction (deg.)

Fraction

N T D V

$R^2 = 0.78$
$R^2 = 0.71$
$R^2 = 0.72$
$R^2 = 0.88$
$R^2 = 0.95$

N T D V
Subtype

a

b

c DSI > 0.3

d DSI > 0.5
central retina only

e
DSI
0.6

0.4

N T D V
subtype

f *n* = 497

g *n* = 210

DSI > 0.3

DSI > 0.5

h
ON-DS
data
model
ON-OFF
data

D
T ← → N
V

i
N
D
D
T
N
V
V
T

j Concordance index (%)
0   10   20

anti-OA   ON
135   N
margin   V   T   D
45
OA   data

k
Eccentricity (deg.)
0   ON
                            model

l
0   10   20

anti-OA   ON-OFF
135   N
margin   V   D
45   T
OA   data
         45 S 135 A 225 I 315 P 45
         Direction (deg.)

m
Weighting
ON   ON-OFF
0.4 R^2= 0.95 R^2= 0.97
0.2
0   N T D V   N T D V

a
D - rise
T - retreat
N - advance
V - fall

b
D
right eye
left eye
left eye
right eye
T
N
V

c
right eye
left eye
binocular

d
binocular
(overhead)
N,T (left)

e

f
N,T (right)
N,T (left)
D,V (right)
D,V (left)

g
1 mm

h
1 mm

i
CC
ASCx
ASCa
PSCx
UM
LSCx
PSC+
LSCa
SM
LSCv

j
D,V
UM
N,T
SM
D,V

k
N, T
LSC
PSC
ASC
D,V

l
starburst
amacrine
null
ON-OFF
DSGC
preferred

m