

 Open access • Posted Content • DOI:10.1101/2021.03.17.435642

An optimal population code for global motion estimation in local direction-selective cells — [Source link](#)

[Miriam Henning](#), [Miriam Henning](#), [Giordano Ramos-Traslosheros](#), [Giordano Ramos-Traslosheros](#) ...+3 more authors

Institutions: [University of Göttingen](#), [University of Mainz](#)

Published on: 17 Mar 2021 - [bioRxiv](#) (Cold Spring Harbor Laboratory)

Topics: [Population](#) and [Retinal ganglion](#)

Related papers:

- [Predictive encoding of motion begins in the primate retina](#)
- [Information-theoretical analysis of the neural code in the rodent temporal lobe](#)
- [Innate Visual Learning Through Spontaneous Patterns of Activity](#)
- [Flexible filtering by neural inputs supports motion computation across states and stimuli.](#)
- [Oscillatory neurocomputing with ring attractors: a network architecture for mapping locations in space onto patterns of neural synchrony](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/an-optimal-population-code-for-global-motion-estimation-in-16lo6lc1dy>

1 An optimal population code for global motion estimation in local direction-selective cells

2
3
4 Miriam Henning^{1,2}, Giordano Ramos-Traslosheros^{1,2}, Burak Gür^{1,2}, Marion Silies^{1*}

5
6
7
8
9 ¹Institute of Developmental Biology and Neurobiology, Johannes-Gutenberg University Mainz, 55128
10 Mainz, Germany.

11
12 ²Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences (GGNB) and
13 International Max Planck Research School (IMPRS) for Neurosciences at the University of Göttingen,
14 37077 Göttingen, Germany

15
16
17
18 *Corresponding author. Email: msilies@uni-mainz.de

19 20 21 Abstract

22
23 Nervous systems allocate computational resources to match stimulus statistics. However, the
24 physical information that needs to be processed depends on the animal's own behavior. For example,
25 visual motion patterns induced by self-motion provide essential information for navigation. How
26 behavioral constraints affect neural processing is not known. Here we show that, at the population
27 level, local direction-selective T4/T5 neurons in *Drosophila* represent optic flow fields generated by
28 self-motion, reminiscent to a population code in retinal ganglion cells in vertebrates. Whereas in
29 vertebrates four different cell types encode different optic flow fields, the four uniformly tuned T4/T5
30 subtypes described previously represent a local snapshot. As a population, six T4/T5 subtypes encode
31 different axes of self-motion. This representation might serve to efficiently encode more complex
32 flow fields generated during flight. Thus, a population code for optic flow appears to be a general
33 coding principle of visual systems, but matching the animal's individual ethological constraints.

34
35
36
37 Keywords: global motion processing, convergent evolution, population code, neuroethology, efficient
38 coding, direction-selectivity, *Drosophila*

39 Introduction

40

41 Evolution has matched neural resources to the sensory information that is available to the
42 animal (1, 2). This is particularly well studied in vision, where the sensitivity of photoreceptors
43 efficiently covers the range of intensities in the environment (3, 4), and many other specializations in
44 retinal circuitry match the statistics of visual information (5, 6). But how did evolution accommodate
45 sensory systems in which the physical distribution of information encountered depends on animal
46 behavior? For example, any visual animal that navigates through its environment needs to detect and
47 compute global motion patterns elicited on the eye, which will depend on the type of locomotion. In
48 vertebrates, such optic flow generated by self-motion is represented by the population of local motion-
49 sensitive retinal ganglion cells(7). Here, topographically organized directional tuning maps represent
50 optic flow that match the global motion patterns generated during walking. In insects, the first direction-
51 selective cells that encode local motion are the T4 and T5 neurons of the ON and OFF pathways. T4/T5
52 are thought to be uniformly tuned throughout the visual field, representing the four cardinal directions:
53 upward, downward, front-to-back and back-to-front motion(8–10). One synapse downstream of T4/T5
54 cells, optic flow patterns are then encoded by wide-field neurons that sample information globally
55 across visual space(11, 12). This suggests that the coding of optic flow is fundamentally different
56 between vertebrate and invertebrate visual systems. It is unclear why flies would have evolved a system
57 in which optic flow has to be computed through complex transformations from local motion detectors
58 with uniform tuning, to ultimately match the motion patterns generated during flight.

59

60 Insect wide-field neurons that are tuned to specific optic flow patterns generated by forward
61 movement or turns of the animal have been extensively characterized(13–15). Such wide field neurons,
62 the lobula-plate tangential cells (LPTCs), also exist in *Drosophila*(16–18), and are involved in the
63 control of optomotor responses, as well as in stabilizing gaze and forward walking(19–21). To extract
64 optic-flow information, wide-field neurons pool information from presynaptic local motion detectors.
65 In *Drosophila*, this is achieved by LPTCs receiving strong input from the columnar T4/T5 direction-
66 selective cells (8, 22). T4/T5 provide excitatory input to downstream LPTCs within the same layer and
67 inhibitory input via lobula plate-intrinsic neurons to LPTCs of the adjacent lobula plate layer with
68 opposite tuning, thus establishing motion opponency (22, 23). Most LPTCs extend their dendrites along
69 one layer of the lobula plate and thus pool information from one subtype of T4/T5 neurons (16, 17, 24),
70 although recently described LPTCs also project to more than one layer (18, 25). Additionally, local
71 motion signals are selectively amplified within the LPTC dendrites if they match the preferred global
72 motion pattern (26). However, it is not fully understood how the flow-field-encoding receptive fields in
73 the LPTCs are computed from presynaptic circuitry. The direction-selective T4/T5 cells respond to
74 local motion, together forming a retinotopic map (8, 9). Whether their tuning to four cardinal directions
75 generalizes over retinotopic locations is not known.

76

77 Here, we characterize the direction tuning distribution of T4/T5 neurons across anatomical and
78 visual space. We demonstrate that directional preference of T4/T5 subtypes changes gradually, forming
79 continuous maps of tuning. At the population level, T4/T5 cells in fact do not fall into four but six
80 subgroups that encode six diagonal directions of motion, matching the hexagonal lattice of the eye. The
81 six topographic tuning maps match optic flows field generated by self-motion of the fly. Therefore, the
82 organization of local direction-selective cells that represents self-motion parallels the retinal code for
83 optic flow, providing a striking example of convergent evolution. The specific types of optic flow that
84 are encoded differ between the mouse retina and the *Drosophila* visual system, arguing that evolution
85 matched neural resources to the different physical distribution of information encountered during
86 walking or flight.

87 **Results**

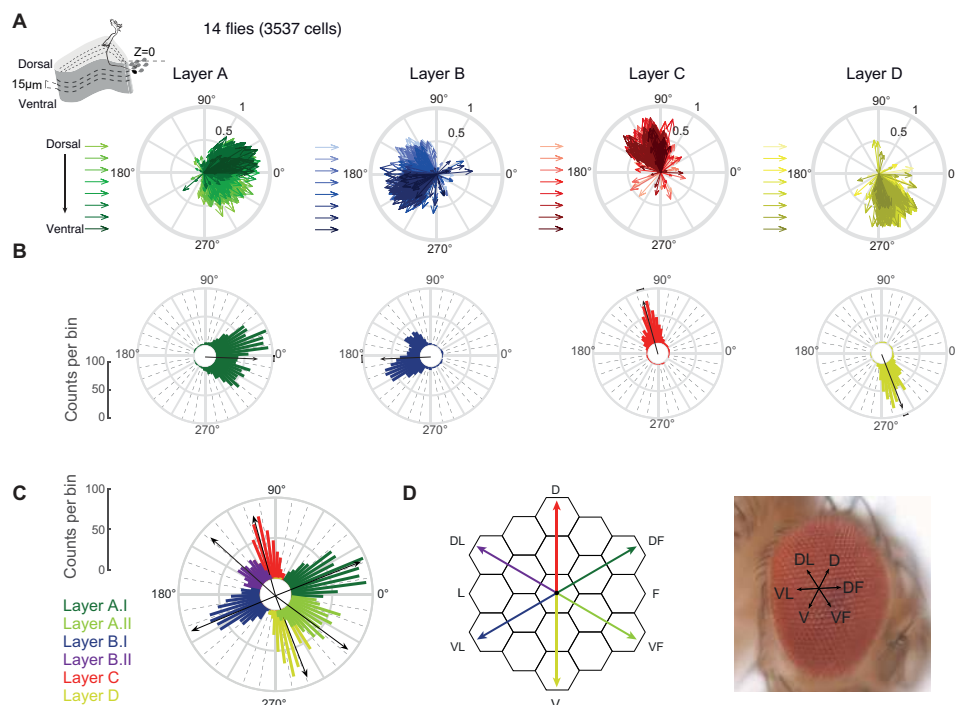
88

89 **T4/T5 population tuning clusters around hexagonal directions of motion**

90 To understand how the T4/T5 neurons contribute to downstream optic flow fields, it is
 91 necessary to have a detailed map of T4/T5 direction tuning across retinotopic space. We used *in vivo*
 92 two-photon calcium imaging to record motion responses from large populations of T4/T5 neurons in
 93 individual flies. We imaged GCaMP6f responses to ON and OFF edges moving in eight directions at
 94 different fly orientations relative to the screen, together subtending $\sim 150^\circ$ in azimuth and $\sim 60^\circ$ in
 95 elevation (**fig. S1, A and B**). Tuning across 3537 individual cells (1376 T4, 2161 T5), recorded in 14
 96 flies was broad, together spanning 360° of motion. Neurons in both layer A and B covered more than
 97 120° of tuning direction, and thus twice the range of cells in layers C and D, which were tuned to a
 98 range of $\sim 60^\circ$ (**Fig. 1A**). Dorsoventral location strongly impacted tuning direction in layers A and B
 99 (**Fig. 1A**). In layer A, cells that were more dorsally located in the lobula plate preferentially covered the
 100 300° - 360° range, whereas more ventral cells of the lobula plate showed tuning directions in the 0° - 60°
 101 range. In layer B, more dorsally located cells were tuned to the 120° - 180° range, and more ventrally
 102 located cells were tuned to 180° - 240° (**Fig. 1A**). Although the population of T4/T5 cells covered all
 103 directions of motion, the tuning distribution was non-uniform (*Circular Rayleigh test: $p < 0.0001$*).

104 Looking at the number of neurons sensitive to a certain motion direction, most neurons in layers
 105 A and B were tuned to the diagonal directions of motion, flanking the overall average orthogonal tuning
 106 of these layers (**Fig. 1B**). Cells in layers C and D each showed a unimodal directional tuning distribution
 107 in the upward or downward direction, respectively (**Fig. 1B**). The bimodal distribution in layers A and
 108 B were well fit by two Gaussians (**fig. S1C**). When thus assigning each cell to one of six subtypes,
 109 tuning of two subtypes in layer A and B split at 0° or 180° , respectively (**fig. S1D**). The population
 110 average of the A.I subtypes was tuned to diagonal upward motion ($\sim 30^\circ$) and the A.II subtype was tuned
 111 to diagonal downward motion ($\sim 330^\circ$). Layer B subtypes encoded the two opposite axes of motion
 112 direction (**fig. S1D**). Taken together, our data show that at the population level, T4/T5 neurons fall into
 113 six functional subtypes (**Fig. 1C and fig. S1D**) Average motion tuning within individual subtypes
 114 reveals sensitivity to six directions with each subtype spanning a 60° -range, matching the hexagonal
 115 arrangement of the fly compound eye (**Fig. 1, C and D**).

116



117

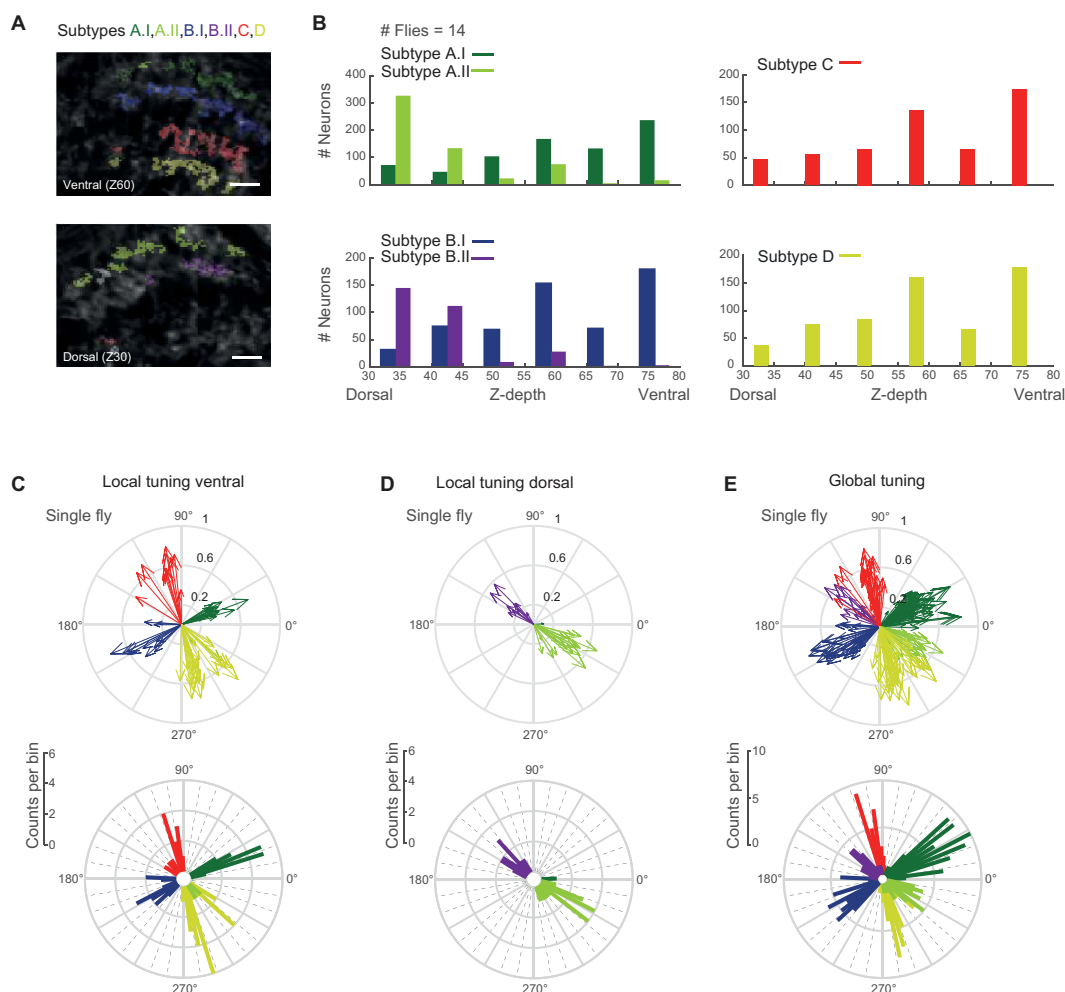
118

119

Fig. 1. Directional tuning clusters around hexagonal directions of motion. (A) Directional tuning of individual neurons from 3537 cells (Layer A: 479/926 T4/T5. Layer B: 252/662 T4/T5. Layer C: 365/220 T4/T5. Layer D: 280/353 T4/T5) in 14

120 flies. Motion responses were represented by a vector(27), whose direction depicts tuning, whereas its length indicates
 121 selectivity. Hue illustrates z-depth relative to a reference (the outermost T4/T5 cell bodies). **(B and C)** Circular histograms of
 122 neuronal tuning preference. Black vectors depict average tuning per layer (B) or subtype (C). **(D)** On average, tuning of the
 123 six subtypes matches the hexagonal arrangement of the fly eye.
 124

125 To understand the spatial organization of six T4/T5 subtypes projecting to four anatomically
 126 distinguishable lobula plate layers, we plotted cellular subtype identity back onto the anatomical
 127 structure of the lobula plate (**Fig. 2A** and **fig. S2**). T4/T5 cells of one subtype dominated one lobula
 128 plate layer recorded in one plane along the dorsoventral axis. At more ventral planes, subtype A.I and
 129 B.I as well as the single respective subtypes of layers C and D were found more frequently. Dorsal
 130 planes more prominently housed subtypes A.II and B.II, but hardly showed any layer C or D cell
 131 responses (**Fig. 2, A and B** and **fig. S2**). This argues for a spatial separation of layer A and B subtypes
 132 at the level of T4/T5 axon terminals. Importantly, local T4/T5 recordings in an individual fly
 133 preferentially showed either four subtypes (as e.g. described in(8, 9)), or two subtypes, each
 134 representing snapshots of the T4/T5 population (**Fig. 2, A to D**). Only a global analysis of tuning
 135 revealed the six T4/T5 subtypes encoding six diagonal directions of motion (**Fig. 2e**).
 136



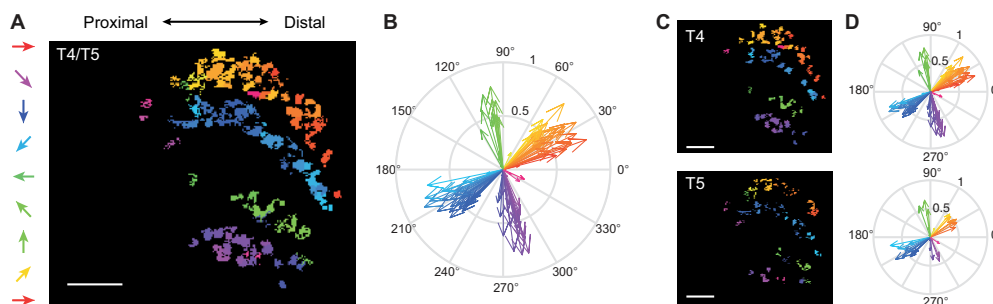
137
 138 **Fig. 2. Layer A and B subtype projections separate along the dorsoventral axis.** **(A)** *In vivo* two-photon calcium images
 139 of the lobula plate at two planes along the dorsoventral axis (Z30/Z60 = z-depth 30/60 μm). ROIs are color-coded based on
 140 their subtype identity. **(B)** Histograms displaying number of neurons from the different classes along the dorsoventral axis (z-
 141 depth). Scale bar 10 μm. **(C and D)** Tuning of individual neurons from one fly recorded in a ventral (Z60) (C), or dorsal (Z30)
 142 (D) plane of the lobula plate. Below: Same data plotted as circular histograms. **(E)** Tuning of all neurons recorded at different
 143 dorsoventral planes within one fly.

144
 145

146 T4/T5 neurons form topographic maps of directional tuning

147 We next asked if the $\sim 60^\circ$ distribution of directional tuning within one subtype was random, or
148 topographically organized. Color coding axon terminals based on their directional preference revealed
149 that the tuning of neighboring cells was similar and gradually changed along the distal-to-proximal axis.
150 As such, recording in one ventral plane of layer A (group A.I) revealed T4/T5 tuning ranging from
151 diagonally upward on the proximal end to front-to-back motion on the distal end of the lobula plate
152 (Fig. 3, A and B). T4/T5 cells of other subtypes also gradually changed tuning from proximal to distal.
153 Subtler changes in the tuning of neighboring cells within one subtype were also apparent along the
154 dorsoventral axis (fig. S3, A and B). This gradually distributed tuning existed for both T4 and T5 when
155 analyzed separately (Fig. 3, C and D and fig. S3C). Because T4/T5 neurons are retinotopically
156 organized, this directional tuning map suggests that the population of T4/T5 cells is sensitive to specific
157 global motion patterns.

158



159

160 **Fig. 3. T4/T5 neurons form topographic maps of directional tuning.** (A) Image of one layer of the lobula plate with ROIs
161 color coded according to their directional tuning. (B) Data from (A) shown in vector space. (C and D) Data shown separately
162 for T4 and T5 neurons. Scale bar = 10 μ m.

163

164

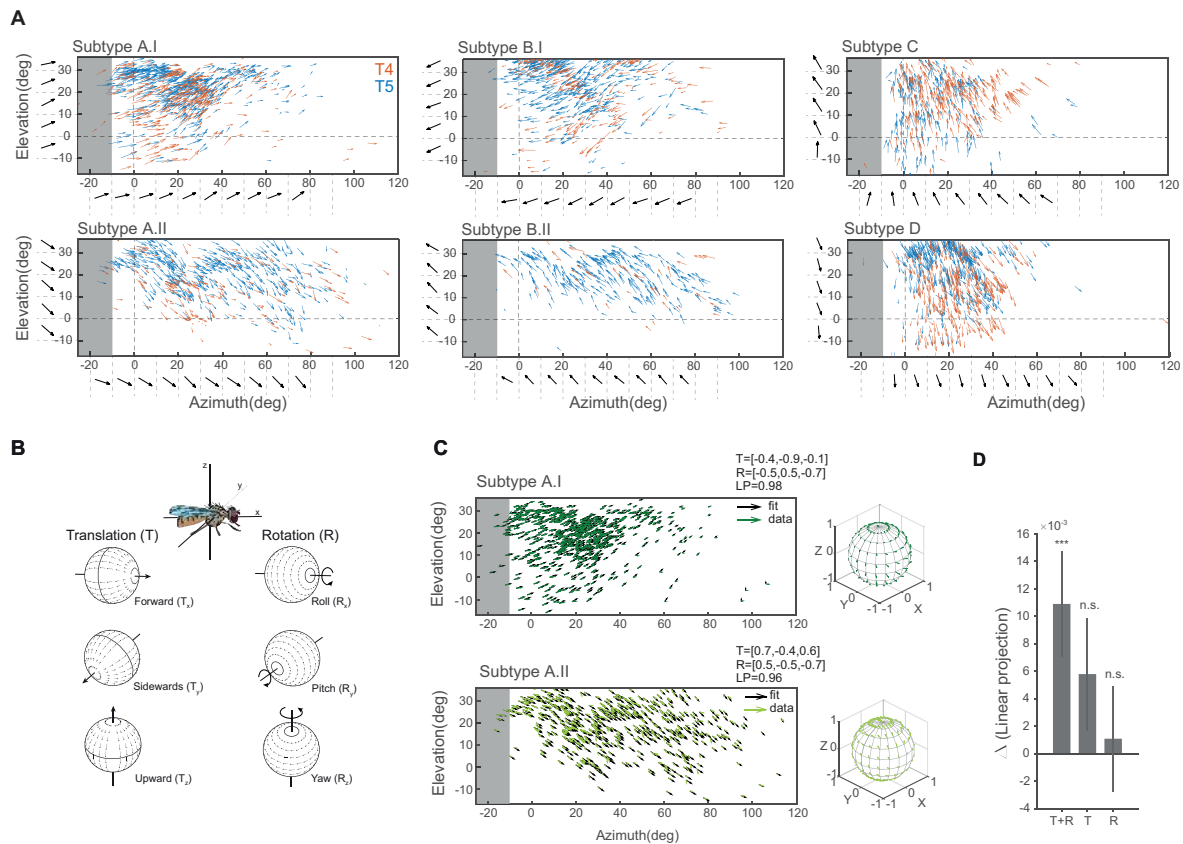
165 The six T4/T5 subtypes encode optic flow induced by self-motion

166 The local differences in the tuning preference within one T4/T5 subtype are reminiscent of
167 direction-selective ganglion cells in the vertebrate retina, where the population of cells encodes
168 translational optic flow generated by self-motion of the animal(7). We hypothesized that the differential
169 tuning measured within each subtype of T4/T5 cells in the fly visual system serves a similar function.
170 To relate tuning to the visual input, we mapped receptive-field centers (fig. S4, A and B) and plotted
171 tuning at each receptive-field location on the screen (fig. S4, C and D). This revealed that cells of one
172 subtype do not encode a uniform direction of motion, but rather that direction tuning of all cells within
173 one subtype change gradually across visual space (Fig. 4A and fig. S4E). These topographic tuning
174 maps resemble flow fields generated by different directions of self-motion in the fly. T4/T5 neurons in
175 layers C and D appear to encode optic flow generated by downward or upward movement of the fly,
176 whereas T4/T5 neurons in layers A and B seem to be tuned to diagonally upward or downward motion.
177 The two flow fields encoded by the two subtypes of layers A or B are vertically flipped versions of each
178 other (Fig. 4A). The successive change of tuning along azimuth and elevation matches the change of
179 tuning seen in the topographic maps in the lobula plate (Fig. 4A, Fig. 3, and fig. S4, E and F).

180

181 To investigate the type of self-motion encoded by the different subtypes, we trained an optic
182 flow model (28) to match the population receptive fields of T4/T5 neurons. We fitted the parameters
183 for the three axes of motion for both translation (T_x, T_y, T_z) and rotation (R_x, R_y, R_z) (Fig. 4B). Although
184 the population data did not fully cover the visual field of one eye (fig. S5A), optic flow fields were well-
185 matched filters for each of the six T4/T5 subtypes (Fig. 4C and fig. S5B). We compared performance
186 including models where the fly only turned (rotational optic flow) or moved straight (translational optic
187 flow). Across the six subtypes, only the model combining rotations and translations outperformed the
188 null model consisting of a uniform vector field, with larger performance in layers A.I, B.II, C and layer
189 D (Fig. 4D and fig. S4C). Thus, T4/T5 subtypes are tuned to optic flow generated by mixtures of
190 translational and rotational motion. For each T4/T5 subtype, a single component tended to dominate
191 the translational axis of motion, whereas the rotational axis was more distributed across the three
192 components (Fig. 4C and fig. S5A). Together, our data show that local direction-selective T4/T5

192 neurons display a population code for different types of self-motion of the fly. Populations of T4/T5
 193 cells are tuned to optic flow patterns, similar to their vertebrate counterparts(7), but representing six
 194 instead of four types of self-motion.
 195



196 **Fig. 4. The population of T4/T5 neurons encode optic flow induced by self-motion.** (A) Arrows indicate tuning direction
 197 of individual neurons plotted at their receptive field center coordinates in visual space. Length of vectors indicates direction
 198 and right visual hemispheres and the horizon, respectively. Black tuning vectors show mean across 10 degree-wide bins.
 199 Gray shaded areas indicate the visual space in the left hemisphere that cannot be seen by the right eye. (B) Schematic of rotational
 200 and translational flow fields around the three body axes of the fly (modified after (29)). (C) Flow fields of data from two
 201 subtypes and the fitted normalized optic-flow model. Model vectors are shown for each corresponding data vector. (D)
 202 Differences of the fit quality for three model types (translation + rotation [T+R], pure translation [T], pure rotation [R])
 203 compared to a uniform vector field model (**Wilcoxon: p<0.001).
 204
 205

206
 207

208 Discussion

209 In this study, we have demonstrated that the local motion detectors T4 and T5 are divided into
 210 six subtypes that encode specific optic flow pattern. T4/T5 appear to implement an optimal population
 211 code for global motion patterns containing information about translational and rotational self-motion of
 212 the fly.

213 Direction-selective T4/T5 neurons in *Drosophila* have been described to encode four cardinal directions
 214 of motion(8, 9). Population T4/T5 recordings now reveal average tuning to diagonal rather than cardinal
 215 motion directions, such that six subtypes of T4/T5 neurons exist. Only a global analysis of directional
 216 tuning reveals these six subtypes, but tuning to diagonal motion has been observed in
 217 electrophysiological recordings of an individual T4 neuron(30), and in optical recordings of T4/T5(9,
 218 31). T4/T5 neurons compute direction-selective signals across neighboring columns within the eye(32,
 219 33). Thus, motion can simply be computed along the internal organization of the fly eye, and the
 220 hexagonal arrangement of the eye does not need to be transformed into a cardinal coordinate system.

221 Individual directional preference of a T4/T5 neuron correlates with its dendrite orientation, which
222 manifests during development(34–36). Interestingly, developmental dendrite orientation for subtypes
223 A and B reveal two peaks of diagonal rather than orthogonal orientation of dendrites(36), consistent
224 with their distribution of direction selectivity. Our data show that each T4/T5 subtype retinotopically
225 covers overlapping regions in visual space. A comprehensive analysis of T4/T5 dendrite anatomy across
226 the visual system will be needed to clarify how adult dendrite orientation is distributed across the visual
227 system to represent the six subtypes. Furthermore, single-cell transcriptomics has assigned developing
228 T4/T5 cells to distinct clusters based on their genetic profiles(36–39), but genes involved in dendrite
229 development or the differentiation are expressed in narrow time windows(39). Interestingly, one recent
230 study identified a genetic subpopulation of T4 neurons, restricted to lobula plate layers A and B(37).
231 While it remains to be determined whether this corresponds to the functional layer A/B subtypes,
232 genetic access will help to better understand the development and anatomy of the individual subtypes.

233 While downstream of T4/T5, wide-field LPTCs are thought to encode self-motion(13, 17), our data
234 show that the population of T4/T5 cells already encodes optic flow generated by a combination of
235 rotational and translational self-motion of the fly. Within an optic flow field, single T4/T5 tuning
236 changes along the retinotopic map. This could be inherited by the spatial distribution of ommatidia
237 along the optical axis which varies with the curvature of the eye(40, 41). T4/T5 can then pass this
238 information to downstream LPTCs, which do not need to transform cardinal motion information into
239 complex flow fields. Further internal dendritic processing, such as suppression of adjacent local motion
240 signals, electrical coupling between LPTCs (17) and feedforward inhibition from lobula plate intrinsic
241 neurons (23) will support the computation of diverse optic flow fields(22, 23, 26).

242 The fly eye and the vertebrate retina both show differences between local and global directional
243 tuning(7, 42), and similarly compute visual signals generated by self-motion at the population level(7).
244 A population code for optic flow generated by self-motion might therefore be canonical and evolved
245 convergently during evolution. Such functionally driven convergence of neuronal circuit organization
246 argues for an optimal design of encoding self-motion. However, mice and flies differ in the number
247 and directions of optic flow encoded by local direction-selective cells. Flying animals might encode
248 more motion axes than walking animals to match the higher degrees of freedom encountered during
249 flight. This difference might therefore highlight adaptation to visuoecological niches of flying and
250 walking animals. We are just starting to understand how a population code in visual systems matches
251 the statistics of the visual environment(6, 7, 43–45), or animal behavior. Thus, this work is an important
252 step towards understanding how anatomy, ethological constraints, and neuronal function are ultimately
253 linked.

254

255 **Acknowledgements**

256 We thank Carlotta Martelli, Yvette Fisher, Axel Methner, and members of the Silies lab for
257 comments on the manuscript, and Christof Rickert for help with image analysis. This project has
258 received funding from the European Research Council (ERC) under the European Union’s Horizon
259 2020 research and innovation program (grant agreement No 716512).

260

261 **Author contributions**

262 MH and MS conceived the experiments. MH performed experiments and analyzed data. BG
263 helped with data analysis. GRT modeled the flow fields. MH and MS wrote the paper.

264 **Materials and Methods**

265

266 ***Drosophila* strains and fly husbandry**

267 *Drosophila melanogaster* were raised on molasses-based food at 25°C and 55% humidity in a 12:12 hr
268 light-dark cycle. For all imaging experiments female flies of the genotype w^+ ; *R59E08-LexA^{attP40}*,
269 *lexAop-GCaMP6f-p10^{su(Hw)attP5}*/*R59E08-LexA^{attP40}*, *lexAop-GCaMP6f-p10^{su(Hw)attP5}* were recorded 3-5
270 days after eclosion at room temperature (RT, 20°C). *R59E08-LexA^{attP40}* and *lexAop-GCaMP6f-*
271 *p10^{su(Hw)attP5}* were obtained from the Bloomington *Drosophila* Stock Center (BDSC #52832 and
272 #44277), recombined, and crossed into a w^+ background.

273

274 ***In vivo* two-photon calcium imaging**

275 *Fly preparation, experimental setup and data acquisition*

276 Prior to two-photon imaging, flies were anesthetized on ice and fit into a small hole in stainless-steel
277 foil, located in a custom-made holder. The head was tilted approximately 30° to expose the back of the
278 head. To fix the head of the fly, a small drop of UV-sensitive glue (Bondic) was used on the left side of
279 the brain and the thorax. The cuticle on the right eye, fat bodies and tracheae were removed using
280 breakable razor blades and forceps. To ensure constant nutrients and calcium supply flies were perfused
281 with a carboxygenated saline containing 103 mM NaCl, 3 mM KCl, 5 mM TES, 1mM NaH₂PO₄, 4 mM
282 MgCl₂, 1.5 mM CaCl₂, 10mM trehalose, 10mM glucose, 7mM sucrose, and 26mM NaHCO₃ (pH~7.3).
283 To record calcium activity, a two-photon microscope (Bruker Investigator, Bruker, Madison, WI, USA),
284 equipped with a 25x/1.1 objective (Nikon, Minato, Japan) was used. For excitation of GCaMP6f, the
285 excitation laser (Spectraphysics Insight DS+) was tuned to a wavelength of 920nm with <20mW of
286 laser power measured at the objective. Emitted light was filtered through an SP680 short pass filter, a
287 560 lpxr dichroic filter and a 525/70 emission filter and detected by PMTs set to a gain of 855V. Imaging
288 frames were acquired at a frame rate of ~15-20 Hz and 4-7 optical zoom using PrairieView software.
289 Each fly was recorded in at least three to five different focal planes (z-depth). We determined z-depth
290 position relative to cell bodies and started the first recording at a z-depth of 30µm from there. Planes
291 were then imaged every 15µm from there (Error! Reference source not found.**b**).

292 *Visual stimulation*

293 Visual stimuli were presented on an 8 cm x 8 cm rear projection screen in front of the fly covering a
294 visual angle of 60° in azimuth and elevation. To cover a larger part of the horizontal visual field of 150°
295 we rotated the fly with respect to the screen two times by 45° (Extended Data Fig. 1a). Stimuli were
296 filtered through a 482/18 bandpass filter (Semrock) and ND1.0 neutral density filter (Thorlabs) and
297 projected using a LightCrafter 4500 DLP (Texas Instruments, Texas, USA) with a frame rate of 100 Hz
298 and synchronized with the recording of the microscope as described previously(46). All visual stimuli
299 were generated using custom-written software using C++ and OpenGL.

300 *Moving OFF and ON edges*

301 Full-contrast dark or bright edges moving with a velocity of 20°/s across the full screen to four or eight
302 different directions. Each stimulus direction was presented at least twice in pseudo-random order. The
303 four-direction stimulus was merely used for the subsequent identification of T4 and T5 axon terminals.

304

305 **Data analysis**

306 *Preprocessing*

307 All data analysis was performed using MATLAB R2017a (The MathWorks Inc, Natick, MA) or
308 Python 2.7. Motion artifacts were corrected using Sequential Image Alignment SIMA, applying an
309 extended Hidden Markov Model(47).

310 *Automated ROI selection*

311 For the extraction of single T4 or T5 axon terminals we made use of their contrast- and direction-
312 selective responses to ON and OFF edges moving into four directions. First, the aligned images were
313 averaged across time and the average image intensity was Gaussian filtered ($s=1.5$) and then threshold-
314 selected by Otsu's method(48) to find foreground pixels suitable for further analysis. After averaging
315 responses across stimulus repetitions, we selected pixels that showed a peak response larger than the
316 average response plus two times the standard deviation of the full trace. These pixels were grouped
317 based on their contrast preference (ON or OFF pixels) and further assigned to four categories based on

318 their anatomical location within the lobula plate (layers A, B, C, or D). We further calculated a direction-
319 selectivity index (DSI) and contrast selectivity index (CSI) for each pixel as follows:

320

$$321 \quad DSI = \frac{PD_{max} - ND_{max}}{PD_{max}},$$
$$322 \quad CSI = \frac{PC_{max} - NC_{max}}{PC_{max}}.$$

323

324 where PD_{max} and ND_{max} denote the maximal response into the preferred direction (PD) and null
325 direction (ND) and PC_{max} and NC_{max} denote the maximum responses for the preferred contrast (PC)
326 and the non-preferred or inverse contrast (NC). We excluded all pixels that did not exceed the CSI
327 threshold of 0.2 to obtain clean T4 or T5 responses. For the final clustering we used the quantified DSI
328 and CSI parameter and the timing of the response to the PD. Based on these parameters the Euclidean
329 distance between each pair of pixels was calculated and average-linkage agglomerative hierarchical
330 clustering was performed. We further evaluated the optimal distance threshold that yielded most clusters
331 of the appropriate size between 1 and $2.5 \mu\text{m}^2$. All resulting clusters that fell outside this range were
332 excluded from further analysis. Cluster locations were saved and matched with subsequent recordings
333 of the same cells to other stimulus types.

334 *Moving OFF and ON stripes*

335 For dF/F calculation, baseline responses to $\sim 0.5\text{s}$ gray epoch were used. To quantify direction
336 selectivity (DS) of single cells, responses were trial averaged and the peak response to the eight different
337 directions of either increment or decrement bars was extracted for T4 and T5 cells respectively. We
338 further quantified the tuning of single cells by computing vector spaces as follows(27):

339

$$340 \quad L_{dir} = \left| \frac{\sum_k R(\theta_k) \exp(i\theta_k)}{\sum_k R(\theta_k)} \right|.$$

341

342 where $R(\theta_k)$ is the response to angle θ_k . The direction of the vector L_{dir} denotes the tuning angle of
343 the cell and the normalized length of the vector is related to the circular variance and thus represents
344 the selectivity of the cell.

345 *Receptive-field center extraction*

346 To extract receptive-field centers, we used a back-propagation algorithm to map the receptive fields of
347 T4 and T5 cells and to locate the center of the receptive fields(49). First, we imaged neural responses
348 to eight different directions and created two-dimensional images from one-dimensional response traces.
349 Neural latency and indicator dynamics introduce delays that will decrease the precision of receptive-
350 field position estimation. To account for this delay, we measured the spatial difference of the response
351 peaks between a static and a moving stimulus. We found an average of 9.6° delay for both T4 and T5
352 cells and shifted the traces for 9.6° before calculating the receptive-field map in our back-propagation
353 algorithm (Error! Reference source not found. **a, b**). These were rotated according to their corresponding
354 direction and averaged to obtain a receptive-field map. To find the center of the receptive field, we fitted
355 a two-dimensional gaussian and took its peak coordinate.

356 *Z-stack generation*

357 Images representing the location of single ROIs color coded by their directional preference were
358 generated in Matlab. Images containing data from different z-depth layers within the same fly were then
359 further processed in Illustrator to create pseudo z-stacks. For this, ROIs from the same lobula plate layer
360 were first compiled in a 3D structure and ROIs from different z-depth layers were stacked to better
361 represent the third dimension of the lobula plate.

362

363 **Statistics**

364 All statistics were done in Matlab using Circular Statistics Toolbox(50).

365 *SNOB analysis*

366 To extract underlying classes from the population of neurons found in layers A and B, we converted
367 data of each population to be linear in the range of directions that most neurons were selective to,
368 resulting in a scale from $-\pi$ to π for layer A, C and D and a scale from 0 to 2π for the data from layer
369 B. We used the finite mixture model SNOB(51) to predict the number of underlying Gaussians using
370 minimum message length criterion. We further used the statistical prediction from the model to assign

371 individual neurons to each of the underlying classes by choosing the class with the highest probability
 372 of the neuron's tuning preference (Error! Reference source not found.c,d).

373

374

375 **Model**

376 We fitted an optic flow field elicited from self-motion on the field of view at a constant distance from
 377 the observer, i.e., a spherical surface. Two coordinates describe the viewing direction: the azimuth θ
 378 and the elevation ϕ angles.

379 The self-motion flow-field vectors \vec{p}_i at each viewing location \vec{d}_i on the unit sphere were specified by
 380 the translation and rotation vectors, $\vec{v}_T = (v_{Tx}, v_{Ty}, v_{Tz})$ and $\vec{v}_R = (v_{Rx}, v_{Ry}, v_{Rz})$, respectively (28):

$$381 \quad \vec{p}_i = -(\vec{v}_T - (\vec{v}_T \cdot \vec{d}_i) \vec{d}_i) - \vec{v}_R \times \vec{d}_i$$

382 The flow-field vectors were then represented in spherical coordinates $\vec{p}_i = u_i \hat{e}_\theta + v_i \hat{e}_\phi + r \hat{e}_r$ to
 383 extract a vector tangential to the spherical surface $\vec{q}_i = (u_i, v_i)$ that could be matched to the direction-
 384 selectivity vectors from T4/T5 data. The uniform flow-field tangent to the spherical surface was
 385 specified by a single vector $\vec{v}_U = \vec{q}_i = (u, v)$ at every viewing position.

386 The comparison of data to model was done using the following *loss* function

$$387 \quad \mathcal{L}(\{\vec{q}_{i,data}, \vec{q}_{i,model} \mid i \in [1, N]\}) = \frac{\sum_{i=1}^N \vec{q}_{i,model} \cdot \vec{q}_{i,data}}{\sum_{i=1}^N \|\vec{q}_{i,model}\| \cdot \|\vec{q}_{i,data}\|}$$

$$= \frac{\sum_{i=1}^N \|\vec{q}_{i,model}\| \cdot \|\vec{q}_{i,data}\| \cos \eta_i}{\sum_{i=1}^N \|\vec{q}_{i,model}\| \cdot \|\vec{q}_{i,data}\|}$$

388 where η_i is the angle between the model and the data flow vectors at the location \vec{d}_i , for all N vectors
 389 in the dataset, and $\|\cdot\|$ indicates the magnitude of the vector. When all vectors match in both magnitude
 390 and direction this quantity is 1 and when all vectors match in magnitude but are in opposite directions
 391 this quantity is -1. To optimize for the vectors $\vec{v}_T = (v_{Tx}, v_{Ty}, v_{Tz})$ and $\vec{v}_R = (v_{Rx}, v_{Ry}, v_{Rz})$, and
 392 $\vec{v}_U = \vec{q}_i = (u, v)$ that maximize \mathcal{L} , the MATLAB function `fmincon` was used. The positive of the loss
 393 function is the linear projection (LP), shown in (Fig. 4c,d) and (Error! Reference source not
 394 found.a,c).

395 Four model variations were considered: fitting both \vec{v}_R and \vec{v}_T ; fitting \vec{v}_R with $\vec{v}_T = \vec{0}$; \vec{v}_T with $\vec{v}_R = \vec{0}$;
 396 and fitting the uniform model \vec{v}_U . For all cases the model was constrained to vectors of unit magnitude,
 397 to focus on the direction rather than the speed of self-motion, and because the T4/T5 vectors (direction-
 398 selectivity index) had magnitudes between zero and one.

399 The data was fitted using ten-fold cross-validation (CV), dividing the data into ten random subsets. In
 400 each fold, nine subsets were used for training and the remaining subset was used for testing the model
 401 fit. For each CV fold, the same training data was fit ten times starting from ten different random
 402 conditions, and the best fit was stored and used to calculate the performance on the test set. The same
 403 training and testing data were used for all models, resulting in repeated measures of the test performance
 404 across models. Statistical testing was done on the ten test-performance values obtained per model. A
 405 one-tailed nonparametric Wilcoxon signed-rank test was used to determine whether the performance of
 406 each of the self-motion models was higher than the performance of the uniform model, for tests pooling
 407 all subtypes (Fig. 4d) and tests of individual subtypes (Error! Reference source not found.c). A
 408 signed-rank test accounted for repeated measures, and a Bonferroni correction was applied to account
 409 for multiple testing ($p < 0.05/3$).

410 References

411

- 412 1. P. Sterling, S. Laughlin, *Principles of neural design* (MIT Press, 2015).
- 413 2. M. F. Land, D.-E. Nilsson, *Animal eyes* (Oxford University Press, 2012).
- 414 3. S. Laughlin, A simple coding procedure enhances a neuron's information capacity. *Zeitschrift*
415 *fur Naturforsch. - Sect. C J. Biosci.* **36**, 51 (1981).
- 416 4. D. A. Clark, J. B. Demb, Parallel Computations in Insect and Mammalian Visual Motion
417 Processing. *Curr. Biol.* **26**, R1062–R1072 (2016).
- 418 5. E. P. Simoncelli, B. A. Olshausen, Natural image statistics and neural representation. *Annu.*
419 *Rev. Neurosci.* **24**, 1193–1216 (2001).
- 420 6. T. Baden, T. Euler, P. Berens, Understanding the retinal basis of vision across species. *Nat.*
421 *Rev. Neurosci.* **21**, 5–20 (2020).
- 422 7. S. Sabbah, *et al.*, A retinal code for motion along the gravitational and body axes. *Nature* **546**,
423 492–497 (2017).
- 424 8. M. S. Maisak, *et al.*, A directional tuning map of Drosophila elementary motion detectors.
425 *Nature* **500**, 212–216 (2013).
- 426 9. Y. E. Fisher, M. Silies, T. R. Clandinin, Orientation Selectivity Sharpens Motion Detection in
427 Drosophila. *Neuron* **88**, 390–402 (2015).
- 428 10. G. Ramos-Traslosheros, M. Henning, M. Silies, Motion detection: Cells, circuits and
429 algorithms. *Neuroforum* **24**, A61–A72 (2018).
- 430 11. K. Hausen, Motion sensitive interneurons in the optomotor system of the fly. *Biol. Cybern.* **46**,
431 67–79 (1982).
- 432 12. H. G. Krapp, R. Hengstenberg, Estimation of self-motion by optic flow processing in single
433 visual interneurons. *Nature* **384**, 463–466 (1996).
- 434 13. H. G. Krapp, Estimation of self-motion for gaze and flight stabilization in flying insects.
435 *Navig. J. Inst. Navig.* **55**, 147–158 (2008).
- 436 14. H. G. Krapp, B. Hengstenberg, R. Hengstenberg, Dendritic structure and receptive-field
437 organization of optic flow processing interneurons in the fly. *J. Neurophysiol.* **79**, 1902–1917
438 (1998).
- 439 15. K. Hausen, M. Egelhaaf, “Neural Mechanisms of Visual Course Control in Insects” in *Facets*
440 *of Vision*, (1989), pp. 391–424.
- 441 16. M. Joesch, J. Plett, A. Borst, D. F. Reiff, Response Properties of Motion-Sensitive Visual
442 Interneurons in the Lobula Plate of Drosophila melanogaster. *Curr. Biol.* **18**, 368–374 (2008).
- 443 17. B. Schnell, *et al.*, Processing of horizontal optic flow in three visual interneurons of the
444 Drosophila brain. *J. Neurophysiol.* **103**, 1646–1657 (2010).
- 445 18. H. Wei, H. Y. Kyung, P. J. Kim, C. Desplan, The diversity of lobula plate tangential cells
446 (LPTCs) in the Drosophila motion vision system. *J. Comp. Physiol. A Neuroethol. Sensory,*
447 *Neural, Behav. Physiol.* **206**, 139–148 (2020).
- 448 19. V. Haikala, M. Joesch, A. Borst, A. S. Mauss, Optogenetic control of fly optomotor responses.
449 *J. Neurosci.* **33**, 13927–13934 (2013).

- 450 20. T. Fujiwara, T. L. Cruz, J. P. Bohnslav, M. E. Chiappe, A faithful internal representation of
451 walking movements in the *Drosophila* visual system. *Nat. Neurosci.* **20**, 72–81 (2017).
- 452 21. A. J. Kim, L. M. Fenk, C. Lyu, G. Maimon, Quantitative Predictions Orchestrate Visual
453 Signaling in *Drosophila*. *Cell* **168**, 280–294 (2017).
- 454 22. A. S. Mauss, M. Meier, E. Serbe, A. Borst, Optogenetic and Pharmacologic Dissection of
455 Feedforward Inhibition in *Drosophila* Motion Vision. *J. Neurosci.* **34**, 2254–2263 (2014).
- 456 23. A. S. Mauss, *et al.*, Neural Circuit to Integrate Opposing Motions in the Visual Field. *Cell* **162**,
457 351–362 (2015).
- 458 24. B. Schnell, S. V. Raghu, A. Nern, A. Borst, Columnar cells necessary for motion responses of
459 wide-field visual interneurons in *Drosophila*. *J. Comp. Physiol. A Neuroethol. Sensory, Neural,*
460 *Behav. Physiol.* **198**, 389–395 (2012).
- 461 25. K. M. Boergens, C. Kapfer, M. Helmstaedter, W. Denk, A. Borst, Full reconstruction of large
462 lobula plate tangential cells in *Drosophila* from a 3D EM dataset. *PLoS One* (2018)
463 <https://doi.org/10.1371/journal.pone.0207828>.
- 464 26. E. L. Barnhart, I. E. Wang, H. Wei, C. Desplan, T. R. Clandinin, Sequential Nonlinear
465 Filtering of Local Motion Cues by Global Motion Circuits. *Neuron* **100**, 229–243.e3 (2018).
- 466 27. M. Mazurek, M. Kager, S. D. Van Hooser, Robust quantification of orientation selectivity and
467 direction selectivity. *Front. Neural Circuits* **8**, 92 (2014).
- 468 28. J. J. Koenderink, A. J. van Doorn, Facts on optic flow. *Biol. Cybern.* **56**, 247–254 (1987).
- 469 29. M. Egelhaaf, “Visual processing in free flight” in *Encyclopedia of Computational*
470 *Neuroscience*, Encycloped, (2015).
- 471 30. E. Gruntman, S. Romani, M. B. Reiser, Simple integration of fast excitation and offset,
472 delayed inhibition computes directional selectivity in *Drosophila*. *Nat. Neurosci.* **21**, 250–257
473 (2018).
- 474 31. Y. Yue, S. Ke, W. Zhou, J. Chang, In vivo imaging reveals composite coding for diagonal
475 motion in the *Drosophila* visual system. *PLoS One* **11**, e0164020 (2016).
- 476 32. J. Haag, A. Arenz, E. Serbe, F. Gabbiani, A. Borst, Complementary mechanisms create
477 direction selectivity in the fly. *Elife* **5**, e17421 (2016).
- 478 33. A. S. Mauss, A. Vlasits, A. Borst, M. Feller, Visual Circuits for Direction Selectivity. **40**, 211–
479 230 (2017).
- 480 34. S. Y. Takemura, *et al.*, A visual motion detection circuit suggested by *Drosophila*
481 connectomics. *Nature* **500**, 175–181 (2013).
- 482 35. S. Y. Takemura, *et al.*, The comprehensive connectome of a neural substrate for ‘ON’ motion
483 detection in *Drosophila*. *Elife* **6**, e24394 (2017).
- 484 36. N. Hörmann, *et al.*, A combinatorial code of transcription factors specifies subtypes of visual
485 motion-sensing neurons in *Drosophila*. *Dev.* **147** (2020).
- 486 37. Y. Z. Kurmangaliyev, J. Yoo, J. Valdes-Aleman, P. Sanfilippo, S. L. Zipursky, Transcriptional
487 Programs of Circuit Assembly in the *Drosophila* Visual System. *Neuron* **108**, 1045–1057
488 (2020).
- 489 38. Y. Z. Kurmangaliyev, J. Yoo, S. A. Locascio, S. Lawrence Zipursky, Modular transcriptional
490 programs separately define axon and dendrite connectivity. *Elife* **8**, e50822 (2019).

- 491 39. M. N. Özel, *et al.*, Neuronal diversity and convergence in a visual system developmental atlas.
492 *Nature* **589**, 88–95 (2020).
- 493 40. R. Petrowitz, H. Dahmen, M. Egelhaaf, H. G. Krapp, Arrangement of optical axes and spatial
494 resolution in the compound eye of the female blowfly *Calliphora*. *J. Comp. Physiol. - A*
495 *Sensory, Neural, Behav. Physiol.* **186**, 737–746 (2000).
- 496 41. R. Heisenberg, M. & Wolf, *Vision on Drosophila: genetics of microbehavior* (1984).
- 497 42. J. Cafaro, J. Zylberberg, G. D. Field, Global motion processing by populations of direction-
498 selective retinal ganglion cells. *J. Neurosci.* **40**, 5807–5819 (2020).
- 499 43. M. J. Y. Zimmermann, *et al.*, Zebrafish Differentially Process Color across Visual Space to
500 Match Natural Scenes. *Curr. Biol.* **28** (2018).
- 501 44. A. Bleckert, G. W. Schwartz, M. H. Turner, F. Rieke, R. O. L. Wong, Visual space is
502 represented by nonmatching topographies of distinct mouse retinal ganglion cell types. *Curr.*
503 *Biol.* **24**, 310–315 (2014).
- 504 45. M. Zhou, *et al.*, Zebrafish Retinal Ganglion Cells Asymmetrically Encode Spectral and
505 Temporal Information across Visual Space. *Curr. Biol.* **30**, 2927-2942.e7 (2020).
- 506 46. L. Freifeld, D. A. Clark, M. J. Schnitzer, M. A. Horowitz, T. R. Clandinin, GABAergic Lateral
507 Interactions Tune the Early Stages of Visual Processing in *Drosophila*. *Neuron* **78**, 1075–1089
508 (2013).
- 509 47. P. Kaifosh, J. D. Zaremba, N. B. Danielson, A. Losonczy, SIMA: Python software for analysis
510 of dynamic fluorescence imaging data. *Front. Neuroinform.* **8**, 80 (2014).
- 511 48. N. Otsu, Threshold selection method from gray-level histograms. *IEEE Trans Syst Man*
512 *Cybern* **9**, 62–66 (1979).
- 513 49. M. Fiorani, J. C. B. Azzi, J. G. M. Soares, R. Gattass, Automatic mapping of visual cortex
514 receptive fields: A fast and precise algorithm. *J. Neurosci. Methods* **221**, 112–126 (2014).
- 515 50. P. Berens, CircStat : A MATLAB Toolbox for Circular Statistics. *J. Stat. Softw.* **31**, 1–21
516 (2009).
- 517 51. C. S. Wallace, *Statistical and Inductive Inference by Minimum Message Length* (2005).
- 518