Synaptic targets of photoreceptors specialized to detect color and skylight polarization in *Drosophila*

Emil Kind^{1,*}, Kit D. Longden^{2,*}, Aljoscha Nern^{2,*}, Arthur Zhao^{2,*}, Gizem Sancer^{1,3}, Miriam Flynn², Connor Laughland², Bruck Gezahegn², Henrique Ludwig², Alex Thomson², Tessa Obrusnik¹, Paula G. Alarcón¹, Heather Dionne², Davi D. Bock^{2,4}, Gerald M. Rubin², Michael B. Reiser^{2,#}, and Mathias F. Wernet^{1,#}

1 : Institut für Biologie – Abteilung Neurobiologie, Fachbereich Biologie, Chemie & Pharmazie, Freie Universität Berlin, Königin-Luise Strasse 1-3, 14195 Berlin, Germany.

2 : Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, USA

3: Current address: Department of Neuroscience, Yale University School of Medicine, Sterling Hall of Medicine C-400, 333 Cedar Street, New Haven, CT 06510, USA.

4: Current address: Department of Neurological Sciences, University of Vermont, Burlington, VT 05405, USA.

* These authors contributed equally

Corresponding authors:

reiser@janelia.hhmi.org

mathias.wernet@fu-berlin.de

Abstract

Color and polarization provide complementary information about the world and are detected by specialized photoreceptors. However, the downstream neural circuits that process these distinct modalities are incompletely understood in any animal. Using electron microscopy, we have systematically reconstructed the synaptic targets of the photoreceptors specialized to detect color and skylight polarization in *Drosophila*, and we have used light microscopy to confirm many of our findings. We identified known and novel downstream targets that are selective for different wavelengths or polarized light, and followed their projections to other areas in the optic lobes and the central brain. Our results revealed many synapses along the photoreceptor axons between brain regions, new pathways in the optic lobes, and spatially segregated projections to central brain regions. Strikingly, photoreceptors in the polarization-sensitive dorsal rim area target fewer cell types, and lack strong connections to the lobula, a neuropil involved in color processing. Our reconstruction identifies shared wiring and modality-specific specializations for color and polarization vision, and provides a comprehensive view of the first steps of the pathways processing color and polarized light inputs.

Introduction

Both the wavelength and the polarization angle of light contain valuable information that can be exploited by many visual animals. For instance, color gradients across the sky can serve as navigational cues, and skylight's characteristic pattern of linear polarization can also inform navigation by indicating the orientation relative to the sun (Heinze 2017; Figure 1A). The spectral content of light is detected by groups of photoreceptor cells containing Rhodopsin molecules with different sensitivities, often organized in stochastic retinal mosaics (Rister and Desplan 2011), and specialized, polarization-sensitive photoreceptors have been characterized in many species, both vertebrates and invertebrates (Nilsson and Warrant 1999). These two visual modalities, color and polarization vision, require the processing of signals over a wide range of spatial and temporal scales, and many questions remain about how the signals from functionally specialized photoreceptors are integrated in downstream neurons. Are color and polarization signal mixed at an early stage, or are they processed by different, modality-specific cell types? Do separate pathways exist that selectively process and convey information from photoreceptor types in the retinal mosaic to targets in the central brain? The full scope of the early synaptic stages of color and polarization circuitry is unknown in any animal, and the analysis of electron microscopy (EM) connectomes is ideally suited to exhaustively answer these questions, especially when corroborated with genetic labelling of cell types and circuit elements imaged with light microscopy. We make significant progress on the questions by mapping the neuronal connections of specialized, identified photoreceptors within the *Drosophila* Full Adult Fly Brain (FAFB) data set (Zheng et al. 2018) and validate many of these results by using the powerful genetic tools available in *Drosophila*.

While studies in many insects have contributed to the understanding of polarized light and color vision (Homberg 2015; Dacke and El Jundi 2018; Heinze 2017; Hempel de Ibarra et al. 2014), the visual system of *Drosophila* offers many advantages for the exploration of neural circuits (Wernet et al. 2014). Anatomical studies are facilitated by the stereotyped, repetitive structure of the optic lobes, with many cell types, the so-called columnar neurons, found in repeated circuit units, called visual columns, that are retinotopically arranged and

each correspond to one of the ~800 unit eyes (ommatidia) of the compound eye. Over one hundred optic lobe cell types have been described in classical Golgi work (Fischbach and Dittrich 1989), by more recent studies combining genetic labeling with light microscopy (Morante and Desplan 2008; Otsuna and Ito 2006; Wu et al. 2016; Nern et al. 2015) and, for some cell types, through EM reconstructions that have revealed not only cell morphologies but also detailed synaptic connectivity (Takemura et al. 2008, 2013, 2015; Meinertzhagen and O'Neil 1991; Rivera-Alba et al. 2011; Shinomiya et al. 2014). Furthermore, genetic tools (Jenett et al. 2012; Pfeiffer et al. 2008; Kvon et al. 2014; Dionne et al. 2018; Tirian and Dickson 2017), and, most recently, gene expression data (Davis et al. 2020; Konstantinides et al. 2018; Ozel et al. 2021; Kurmangaliyev et al. 2020) are available for many optic lobe cell types. An enabling feature of this emerging body of work is that in nearly all cases, distinct cell types can be reliably identified across data sets, such that new studies often directly enrich prior ones.

Each *Drosophila* ommatidium contains eight photoreceptors whose output is processed in a series of neuropils called the lamina, medulla, lobula and lobula plate that together form the optic lobes of the fly (Fischbach and Dittrich 1989). Outer photoreceptors R1-6 project to the lamina neuropil, and serve as the main input to the motion vision circuitry (Mauss et al. 2017); inner photoreceptors R7 and R8 pass through the lamina without connections and project directly to the deeper medulla neuropil, that also receives lamina projections (Fischbach and Dittrich 1989). In the main part of the retina, R7 and R8 differ in their axonal target layers, with R7 projecting to layer M6, and R8 to layer M3 (Fischbach and Dittrich 1989). R7 and R8 also differ in their rhodopsin expression, being sensitive to short wavelength UV (R7) and blue (R8), respectively, in so-called 'pale' ommatidia (Chou et al. 1996; Papatsenko et al. 1997), and to long wavelength UV (R7) and green (R8) in 'yellow' ommatidia (Salcedo et al. 1999; Huber et al. 1997). Pale and yellow ommatidia are distributed randomly throughout the main part of the retina (Feiler et al. 1992; Fortini and Rubin 1990), at an uneven ratio that is conserved across insects (Wernet, et al. 2015; Kind et al. 2020). Meanwhile, along the dorsal rim area (DRA) of the eye, the ommatidia are morphologically and molecularly specialized for detecting skylight polarization (Wernet et al. 2003, 2012; Wada

1974a), that *Drosophila* can use to set a heading (Mathejczyk and Wernet 2019, 2020; Warren et al. 2018; Weir and Dickinson 2012). In the DRA, the inner photoreceptors express the same UV rhodopsin (Rh3; Fortini and Rubin 1991, 1990) and detect perpendicular angles of polarized UV light (Weir et al. 2016). In contrast to the rest of the medulla, R7-DRA and R8-DRA project to the same medulla layer (M6; Chin et al. 2014; Pollack and Hofbauer 1991; Fischbach and Dittrich 1989), where their targets include polarization-specific cell types (Sancer et al. 2019, 2020; Hardcastle et al. 2021). Across insects, a ‘compass pathway’ connects the DRA to the central brain via the anterior optic tubercle (AOTU; Homberg 2015; Hardcastle et al. 2021; Pfeiffer and Kinoshita 2012). Anatomical and functional data from *Drosophila* suggests that the central medulla is also connected to the compass pathway (Omoto et al. 2017; Hardcastle et al. 2021; Otsuna et al. 2014), potentially forming parallel pathways for processing different celestial cues (Timaeus et al. 2020; Tai et al. 2021).

EM studies have already revealed some of the circuitry downstream of R7 and R8 (Takemura et al. 2013, 2015; Gao et al. 2008). For example, axons of R7 and R8 from the same ommatidium are reciprocally connected with inhibitory synapses (Takemura et al. 2013), leading to color-opponent signals in their presynaptic terminals (Schnaitmann et al. 2018). Interestingly, R7-DRA and R8-DRA also inhibit each other (Weir et al. 2016). Other known R7 and R8 targets in the main medulla include local interneurons (e.g. Dm8; Gao et al. 2008; Karuppudurai et al. 2014; Pagni et al. 2021; Menon et al. 2019) and projection neurons that provide connections to deeper optic lobe regions (e.g. Tm5 and Tm20 neurons; Karuppudurai et al. 2014; Meinertzhagen et al. 2009; Gao et al. 2008). A previous light microscopy study (Karuppudurai et al. 2014) identified a single cell type, Tm5a, that is specific for yellow medulla columns; this neuron has been used to identify pale and yellow columns in an EM volume (Menon et al. 2019; Takemura et al. 2015; Karuppudurai et al. 2014). Using genetic labelling techniques, four classes of TmY cells have also been reported as specific targets of pale versus yellow photoreceptors (Jagadish et al. 2014), yet previous connectomic studies did not reveal similar cells. The currently most comprehensive EM study of the medulla reconstructed the connections between neurons in 7 neighboring medulla columns (Takemura et al. 2015),

revealing a detailed, yet incomplete, inventory of cell types connected to R7-8. This data set, now publicly available (Clements et al. 2020), is remarkable for its dense reconstruction of columnar circuits, but could not be used to identify many multicolumnar neurons, that were cut-off at the edge of the data volume, leaving ~40% of R7-8 synapses onto unidentified cell types. In addition, no EM-based reconstructions of DRA neurons and their connections is currently available.

Here we present a comprehensive reconstruction of all inner photoreceptor synaptic outputs and inputs, from pairs of pale and yellow columns and from three DRA columns in the FAFB data set. These reconstructions were carried out within a full brain volume, such that, for the first time, nearly all neurons connected to these photoreceptors were identified. We discovered, through light microscopy, a large visual projection neuron with distinctive morphology, named accessory medulla cell type 12 (aMe12), that selectively innervates pale ommatidia across the medulla. We then reconstructed and then used this cell in the FAFB data set to identify pale and yellow columns, from which we enumerated the connectivity of R7 and R8 with known and novel cell types within the optic lobes and projecting to the central brain, including more cells with pale-yellow specificity, and synapses on axons between neuropils. In the DRA, we show that cellular diversity is reduced, with local interneurons and projection neurons to the AOTU dominating, and connections to the lobula virtually missing. We identify circuit motifs shared between DRA and central columns, and describe modality-specific cell types, including cells with interhemispheric connections and projections to the central brain. Together, we identify the connected neurons that account for 96% of these inner photoreceptor synapses, a comprehensive set of the neurons that comprise the first step of the pathways through which color and polarization signals are transduced to the rest of the brain.

Results

Systematic reconstruction of all synaptic targets of inner photoreceptor subtypes

We reconstructed R7 and R8 photoreceptor axons in the central region of the right optic lobe, as well as inner photoreceptors from 27 DRA columns from the FAFB data set (Zheng et al. 2018; Figure 1C). From these, we selected seven ‘seed columns’ (four central, three DRA), for which all pre- and postsynaptic sites of the photoreceptor axons were identified and manually reconstructed, that is annotated and assembled across EM images (Figure 1F; Material and Methods). There were 422 skeletons, corresponding to individual cells, contacted by the seed column photoreceptors, and we focused our analysis on the 245 individual cells with >2 synapses to or from the photoreceptors. We reconstructed the morphology of these cells with a sufficient degree of completeness to ascribe a unique cell type to 92% (225/245) or to identify a cell class (Dm, Mi, Tm, or TmY cell class) for an additional 6.5% (16/245), so that 98% (241/245) of these cells were identified. We also traced and uniquely identified the cell type of all 30 cells that provided >2 synaptic inputs to the photoreceptors. In total, we identified 4043 synaptic connections from the seed column R7 and R8 photoreceptors, and 970 synaptic inputs to them, summarized in Figure 1H.

To distinguish between pale and yellow columns we took advantage of our discovery of one specific cell type from the accessory medulla that widely arborised in the medulla with ascending branches that align with the photoreceptors (Figure 1Ei-iii). The cell type was selective for R8 input and showed a strong preference for innervating pale columns (Figure 1D, Eiii). We identified three of these cells per hemisphere (hereinafter referred to as aMe12), that had heavily overlapping arbors, but only rarely shared ascending branches in the same columns (3/779 columns). Using the presence of aMe12 branches, we were able to assign as pale 38% (297/779) of the medulla columns, in good agreement with studies on the retinal mosaic of *Drosophila* (Feiler et al. 1992; Fortini and Rubin 1990; Bell et al. 2007; Hilbrant et al. 2014). Since further analysis identified a total of 42 DRA columns (see below), we assigned the remaining columns as nominally yellow (Figure 1C). Our reconstructions also revealed another new pale-selective visual projection neuron, that we named ML-VPN1, and confirmed the previously reported yellow-selectivity of Tm5a cells (Menon et al. 2019; Karuppururai et

al. 2014), findings that further supported the designations of pale and yellow seed columns (see below).

After mapping the pale and yellow columns, we selected four adjacent central seed columns, two nominally pale and two nominally yellow (Figure 1C). Across the four central seed columns, the absolute number of both synaptic inputs and outputs for R7 and R8 photoreceptors were in good agreement with previous reports analyzing a medulla FIB-SEM (focused ion beam scanning electron microscopy) data set (Takemura et al. 2015; Figure 1 – figure supplement 2A-D). As we had access to the complete brain volume, we additionally found substantial numbers of synapses in the axon bundles projecting between the lamina and the medulla, which accounted for 14% and 12% of the output synapses for R7 and R8 respectively, and 16% and 27% of their input synapses (Figure 1 F, Figure 1 – figure supplement 2G).

We readily identified columns in the DRA region of the medulla since it is only there that both inner photoreceptors terminate in medulla layer M6 (Figure 1F, arrow; Pollack and Hofbauer 1991; Fortini and Rubin 1991; Sancer et al. 2019; Chin et al. 2014; Fischbach and Dittrich 1989). The morphology of DRA-specific Dm-DRA1 cells differs significantly between central and polar positions of the DRA (Sancer et al. 2019), and we therefore chose one polar DRA seed column and two more equatorial columns (Figure 1C). Beyond the 27 DRA columns with manually traced R7-DRA and R8-DRA photoreceptors we mapped the full extent of the DRA region of the medulla by systematically probing for the presence of two inner photoreceptor profiles in layer M6 without reconstruction (see Materials and Methods), which resulted in a total of 42 identified DRA columns (Figure 1C). Direct comparison of the absolute number of pre- versus postsynaptic sites between central and DRA photoreceptors revealed drastic differences: while the numbers of R7 and R7-DRA presynapses were comparable (mean 279, range 262 – 284 for R7-DRA vs mean 297, range 274 - 322 for R7), the number of R8-DRA presynapses was reduced by ~60% compared to those of R8 (mean 166, range 157 - 172 for R8-DRA vs mean 380, range 356 - 417 for R8). In particular, there was a striking

reduction in the number of synapses from R8-DRA to cells intrinsic to the medulla and to cells that projected to the lobula compared to R8 (see below and Figure 1G, H).

We have endeavored to make this comprehensive data set as accessible and navigable as possible. In the following sections we first describe the connections of the central column R7 and R8 cells (Figure 2 – 5), following the sequence of cell classes in Figure 1G-H, before presenting the connections of the DRA (Figures 6 – 9). For brevity, we refer to the region of the medulla innervated by DRA inner photoreceptors as the DRA region, and for clarity we refer to the central column inner photoreceptors as R7 and R8, and the DRA inner photoreceptors as R7-DRA and R8-DRA. The synaptic outputs from and inputs to the central pale and yellow R7-8 cells are summarized in Tables 1-2, for R7-DRA and R8-DRA in Tables 3-4, and presentations of individual cell types include summaries of total photoreceptor synapse counts (e.g., Figure 1Eiii for aMe12). The text highlights downstream cell types that are selective for inputs from either R7 or R8 or are preferentially found in pale or yellow columns. We used 65% as the threshold to consider an input selective and tables of connectivities of the individual cells are arranged by cell type and collected together in Supplementary File 1. The main figures show the morphology of reconstructed exemplars, with the many full reconstructions noted (and listed in Materials and Methods), while the morphologies of individual cells are collected together in Supplementary File 2. The numbers of synapses outside the medulla are summarized in Supplementary File 3. For the cell types for which we are unaware of published, detailed descriptions, we endeavored to produce light microscopic images of single cell clones which were matched largely based on the cell body location, the extent and neuropil-layer-specific branching patterns, and comparisons to any other known cell types (see Materials and Methods). In a few cases, we also use light microscopy to illustrate specific features of known neurons or to explore additional properties (such as direct co-labeling with markers for pale and yellow columns) that are not readily accessible by EM.

Synapses between R7 and R8, and with lamina cell types

The inner photoreceptors inhibit each other via histaminergic synapses, a key mechanism for converting their sensitivity to specific wavelengths into presynaptic color-opponency (Schnaitmann et al. 2018). We annotated a large proportion of the synapses between R7 and R8 along the axons between the lamina and the medulla (Figure 2A, Figure 1 – figure supplement 2G). For R8, ~80% of the synaptic input from R7 was found outside the medulla (mean 11.5 synapses per cell, range 9-17), and the R8 inputs to R7 in this region were as frequent (mean 10.3 synapses per cell, range 5-13). These results were not anticipated by prior EM reconstructions of individual neuropils, and our counts are substantial increases in the numbers of inter-photoreceptor synapses (Figure 1 – figure supplement 2D; Takemura et al. 2015; Meinertzhagen and O'Neil 1991).

R7-8 pass through the lamina without forming synapses (Meinertzhagen and O'Neil 1991), and instead form synapses with lamina cell types in the medulla (Takemura et al. 2008, 2013). Our reconstructions revealed that 58% of R7-8 inputs to the lamina cells L1 and L3 were also located within the optic chiasm (Figure 1 – figure supplement 1B, Figure 1 – figure supplement 2G, Figure 2 – figure supplement 1A-B).

R7-8 synapses with distal medulla (Dm) cell types

The Dm neurons included the strongest targets of R7-8 (Table 1). Dm9 is glutaminergic and provides excitatory feedback to R7 and R8, regulating their gain and augmenting their color-opponency (Davis et al. 2020; Uhlhorn and Wernet 2020; Gao et al. 2008; Heath et al. 2020). It accounted for more than 90% all the synaptic inputs to R7-8 that were not from photoreceptors (333/369 synapses, Table 2). Dm9 cells span multiple columns and tile the medulla (Nern et al. 2015; Takemura et al. 2015; Figure 2B). We reconstructed six Dm9 cells that were contacted by our seed column R7-8s, which received 16% of all R7-8 synapses, and provided 57% of the total seed column photoreceptor synaptic input (Tables 1, 2). Of the six Dm9 cells, one cell occupied our seed columns and formed the bulk of the synapses between R7-8 and Dm9 in these columns. The other five cells received far fewer connections and only in medulla layers M1 and M6. The well-connected Dm9 cell that we fully

reconstructed received 92 synapses per seed column (mean, range 78-102), and provided 70 synapses per seed column (mean, range 64-78) offering the most detailed picture of Dm9 connectivity yet (Supplementary File 1—Dm9 outgoing). While its R7-8 inputs were biased towards R8 (31% R7 vs 69% R8), the output synapses were balanced (55% R7 vs 45% R8), and pale and yellow columns were equally contacted (outputs from R7 and R8: 54% pale vs 46% yellow; inputs from R7 and R8: 53% pale vs 47% yellow). In total, 8% of the synapses from R7-8 to Dm9 were outside the medulla (34/445 of all R7-8 synapses onto Dm9), on processes following the photoreceptor axons (Figure 2Bii, arrow).

The most strongly connected R7 target cell type was Dm8, a central component of *Drosophila*'s circuitry mediating color vision and wavelength-specific phototaxis (Gao et al. 2008; Melnattur et al. 2014; Pagni et al. 2021). Individual Dm8 cells cover multiple columns in medulla layer M6 (Gao et al. 2008; Nern et al. 2015; Luo et al. 2020), and extend processes vertically, with one characteristic process usually reaching higher than the others, up to layer M3, defining a so-called 'home' column (Figure 2Ci, arrow). In our reconstructions, we analyzed the connectivity of each Dm8 cell with the surrounding pale and yellow R7 photoreceptors in relation to its home column.

Dm8 cells were exclusively R7 targets, with no synapses from our seed column R8s. Individual R7 cells synapsed onto 8 Dm8 cells (mean, range 6-10), contacting 15 Dm8s altogether, and there were five Dm8 cells with processes spanning our seed column R7s and receiving >40 synapses from them. Two of these cells had a pale home column and no input from yellow seed column R7s, and two had a yellow home column and received 13% and 20% of their seed column input from pale R7s (Supplementary File 1—Dm8 outgoing). The fifth cell had a pale home column outside the seed column area, and its seed column inputs were divided evenly, 55% from pale and 45% from yellow. Collectively, these data revealed that Dm8 cells selectively innervated columns around the home column, but not simply the closest columns.

In view of the importance of Dm8 cells for processing R7 output, we extended our reconstructions to include all the photoreceptor inputs to three Dm8s, one with a pale home

seed column, one with a yellow home seed column, and one with a yellow home column outside the seed columns (Figure 2Cii). All three cells were most densely innervated by R7 in their home column: the yellow home column cells received 26% and 23% of their R7 input in the home column, and the pale home column cell received 52% (Figure 2Ciii). The innervated columns outside the home column were not the nearest neighbors of the home column, but formed a more irregular, idiosyncratic spatial pattern. Together, our data are consistent with Dm8 cells having a central, strong R7 input from either a pale or a yellow cell in its home column and a surround that integrates spatially varied pale and yellow R7 inputs.

Two additional Dm cell types, Dm11 and Dm2, were prominent inner photoreceptor targets. Dm11 cells tile the medulla in layer M6, with each cell covering ~9 columns (Nern et al. 2015; Figure 2Di), and sending vertical processes that reach far up into the optic chiasm, tracking the R7 and R8 photoreceptor axons (Courgeon and Desplan 2019; Figure 2Dii, Diii, arrows). Dm11 was R7-selective, with 87% of its seed column photoreceptor inputs originating from R7s (Figure 2Dii). Two-thirds of the Dm11 seed column R7-8 inputs were located outside of the medulla (44/63 synapses), and this was another cell type innervated by photoreceptors much more than previously reported (Figure 1 – figure supplement 2D; Clements et al. 2020; Takemura et al. 2015). One Dm11 cell squarely occupied the four seed columns, receiving 52 synapses from the seed column photoreceptors, and this cell showed no clear bias for pale or yellow inputs (40% pale, 60% yellow; Supplementary File 1—Dm11 outgoing).

Dm2 is a columnar cell type that spans 1-2 columns and has processes that reach from layer M6 up to layer M3 to receive synaptic input from R8 cells (Figure 2Ei, Eii; Takemura et al. 2013; Nern et al. 2015). We reconstructed four Dm2s that received 77% of their seed column photoreceptor input from R8 (Figure 2Eii) and 81% from pale cells. Three of the Dm2 cells were centered on seed columns, with two in the pale seed columns receiving 36 and 35 synapses, and the cell in a yellow seed column receiving 18; the fourth cell was centered on a neighboring yellow column and innervated one of the pale seed columns (Supplementary File 2—Dm2). In the columns of the medulla FIB-SEM data set that have been identified as pale and yellow (Menon et al. 2019; see Materials and Methods), Dm2 was also R8-selective

(70% of photoreceptor input from R8; Takemura et al. 2015; Clements et al. 2020). The cell was missing from one of the pale columns in that data set (Takemura et al. 2015), but in the columns where it was present, the cells received ~50% more synapses per column in the pale columns (18 mean, range 16-20 in pale columns, versus 12, mean, range 10-15 in yellow columns).

R7-8 synapses with medulla intrinsic (Mi) and medulla tangential intrinsic (Mti) cell types

The Mi cells connect the distal with the proximal medulla (Fischbach and Dittrich 1989). These two medulla zones are separated by the serpentine layer M7, containing the processes of large medulla tangential cells, including those intrinsic to the medulla which we refer to as Mti cells. There were three Mti cells contacted by our seed column R7-8, and we matched these cells to light microscopy images of two separate, previously undescribed cell types using the locations of cell bodies, sizes of arbors and layer expression (Figure 3A,B). Both cell types received a modest number of synapses from our seed-column R7-8 (Mti1: 7 synapses; Mti2: 10 and 2 synapses, respectively; Supplementary File 1—Mti outgoing), yet were likely to be substantial targets overall since their processes covered large sections of the medulla (Figure 3A, B). The first cell type, which we refer to as Mti1, had a cell body located in the cell body layer distally to the medulla, similar to most medulla neurons. Its processes were asymmetrically oriented mainly along the dorsoventral axis, with dendrites that occupied layers M3-M6, and axonal processes oriented dorsoventrally that spread laterally in layers M7-M8 (Figure 3A). The second cell type, here named Mti2 (Figure 3B), had cell bodies located at the anterior ventral edge of the medulla, adjacent to where its processes entered and coursed through layer M7 going on to make elaborations in layer 6 and small, further vertical processes that reach vertically up to layers M3 and M4 (Figure 3Biii and 3Bvi, arrows). In our EM reconstructions, two cells shared these properties and overlapped around our seed columns (Figure 3Bv).

Mi15 is the only local dopaminergic medulla cell type identified in *Drosophila* so far (Meissner et al. 2019; Davis et al. 2020). Its morphology and connectivity in the medulla have

been described in previously (Takemura et al. 2013, 2015), and it has a long process that extends into the optic chiasm and tracks photoreceptor axons. Our reconstructions revealed that this process was a locus of photoreceptor input (Figure 3Cii, black arrow), and that Mi15 cells were R8-selective, with 91% of their seed column photoreceptor input drawn from R8. The Mi15 cells received 47% of their seed column photoreceptor input outside the medulla (mean of 15/32 synapses per column; Figure 1 – figure supplement 2G), and R8s characteristically formed synapses with Mi15 cells at locations where they also made synapses with R7 (Figure 3 – figure supplement 1A).

The columnar cell types Mi1, Mi4 and Mi9 are integral components of the ON-motion pathway (Strother et al. 2017); prior EM reconstructions have found they receive significant input from R7-8, a result confirmed by our data (Takemura et al. 2013, 2015; Figure 3 – figure supplement 1B-D). In the identified pale and yellow columns of the medulla FIB-SEM data set, Mi9 was selective for R8 (65% R8 vs 35% R7 input), and 53% of its photoreceptor input originated from pale R8 (Takemura et al. 2015). Our data reinforced the observation that Mi9 show a bias for R8 input: 68% of our reconstructed Mi9 input originated from pale seed column R8; the cells in pale columns received 15 and 14 R7-8 synapses, while those in yellow columns received 6 and 4 (Figure 3 – figure supplement 1D).

R7-8 synapses with cell types projecting to the lobula

The lobula receives diverse inputs from the medulla, including from cell types required for wavelength-specific phototaxis and learned color discrimination (Gao et al. 2008; Karuppudurai et al. 2014; Melnattur et al. 2014; Otsuna et al. 2014). Many of these cell types express the histamine receptor *ort*, notably the transmedulla (Tm) cell types Tm5a, Tm5b, Tm5c and Tm20 (Gao et al. 2008), indicating they receive direct input from the histaminergic R7 or R8 (Pantazis et al. 2008). We found all of these Tm cell types and they were highly connected with the seed column R7-8, with a mean of 35 seed column photoreceptor inputs per cell for Tm20, 27 for Tm5a, 23 for Tm5c, and 24 for Tm5b (Figure 4, Figure 4 – figure supplement 1).

The cholinergic Tm5a cell type was the first cell type described to show pale versus yellow selectivity, being preferentially innervated by yellow R7, as revealed by genetic methods and light microscopy (Karuppururai et al. 2014). It was identified in the medulla FIB-SEM data set (Menon et al. 2019) and used for identifying putative yellow columns therein (see Materials and Methods). Its medulla processes are centered on one yellow column, with a single main dendrite from which branches spread laterally reaching some of the neighboring columns in M3, M6 and M8, and an axon terminating in lobula layer 5B manifesting a characteristically hooked final reversal of direction (Gao et al. 2008; Figure 4A). In both our yellow seed columns there was a Tm5a cell, whose seed column photoreceptor input was exclusively from the R7 in the home column (Figure 4Aiv). Reassuringly, there were also no Tm5a cells centered on pale columns or innervated by pale photoreceptors. Thus, the Tm5a cells supported the designation of our seed columns as pale or yellow, that was based on the presence or absence of aMe12 vertical processes. One more cell that we refer to as a Tm5a-like had the morphological features of a Tm5a cell, and was selective for yellow seed column input, but from R8 (Figure 4 – figure supplement 1A). There was no cell with a similar morphology or connectivity in the medulla FIB-SEM data set, as explored using NeuPrint (Clements et al. 2020). The cell received its R8 input on three different dendritic branches, indicating that they were unlikely to have resulted from a reconstruction error (Figure 4 – figure supplement 1Aiv). Overall, our results confirmed the selectivity of Tm5a cells for yellow columns and the reliability of finding R7-selective Tm5a cells in yellow columns.

The cholinergic Tm5b cell type is similar to Tm5a, but with ~2-3 vertical main dendrites spanning ~5 columns, from which branches spread laterally in M3, M6 and M8 (Gao et al. 2008; Karuppururai et al. 2014; Meinertzhagen et al. 2009; Figure 4B). A recent analysis of the medulla FIB-SEM data set has proposed that Tm5b cells are pale-specific (Menon et al. 2019). Our reconstructions revealed two Tm5b cells that were innervated by pale R7, and not yellow R7 (Figure 4B), with only a minor input from seed column R8 (19 and 20 R7 inputs, vs 7 and 2 R8 inputs, respectively). We also identified 3 more cells with a morphology matching the Tm5b cell type, but innervated by predominantly yellow inputs, which we refer to as Tm5b-

like (Figure 4 – figure supplement 1B). In these yellow-specific cells, R8 input dominated, with 9.0 R8 synapses (mean, range 4-16), versus 4.7 R7 synapses (mean, range 3-7; Figure 4 – figure supplement 1Biv). In particular, one Tm5b-like cell had >20 synapses, and innervated all four seed columns, and so was well covered in our reconstructions (Figure 4 – figure supplement 1B).

The Tm5c cells are glutaminergic neurons, spanning ~8 columns with single vertical dendrite in the medulla, from which lateral branches spread out in M1, as well as M3 and M6, while the axons terminate near the boundary between lobula layers 5 and 6, often with a branch in layer 4 (Gao et al. 2008; Karuppudurai et al. 2014; Meinertzhagen et al. 2009; Figure 4C); one cell axon terminated deep in layer 6 (Supplementary File 2—Tm5c). Our reconstructions confirmed they were selective for R8, as indicated previously (Takemura et al. 2013, 2015; Karuppudurai et al. 2014; Figure 1 – figure supplement 2D). Six Tm5c cells were innervated by seed column photoreceptors, and they all received many synapses from multiple columns, ranging from 11 to 44, consistent with R7-8 input over a large spatial receptive field. One Tm5c was centered on our seed columns, with processes in all of them (Figure 4Cii), and only targeted by yellow seed column photoreceptors, predominantly R8. This bias towards yellow R8 inputs was maintained over the population (Figure 4Civ), yet there were two Tm5c cells with pale selectivity, and it remains possible that different Tm5c subtypes exist (Supplementary File 1—outgoing Tm5c). In the identified pale and yellow columns of the FIB-SEM data set, there were two Tm5c cells reliably contacted by R7-8, with > 10 synapses (Takemura et al. 2015; Clements et al. 2020). These two cells also showed a bias for yellow R8 input, with respectively 18 and 11 synapses from yellow R8 cells, versus 5 and 0 synapses from pale R8 and R7 combined.

The Tm20 cell type is a known columnar target of R8 (Takemura et al. 2013, 2015; Gao et al. 2008), and the four Tm20 cells located in our seed columns were indeed selectively connected to R8 (Figure 4 – figure supplement 1C, Figure 1 – figure supplement 2D). The Tm20 and Tm5c cell types together received 140 synapses from seed column photoreceptors, making them two of the most targeted cell types (Table 1). We also found one TmY10 cell that

received 7 synapses from our seed column photoreceptors (Supplementary File 1 - TmY10). In addition, we partially reconstructed 10 other Tm cells with a mean of 4.4 photoreceptor synapses per cell. We did not fully reconstruct these lightly innervated Tm cells; Tm subtypes may manifest subtle differences in morphology and connectivity that require multiple examples to distinguish (Jagadish et al. 2014; Gao et al. 2008; Fischbach and Dittrich 1989), which was beyond the scope of our targeted reconstruction.

We found one more prominent cell type projecting to the lobula, which we refer to as the medulla-to-lobula cell type ML1 (Figure 4D). We completely reconstructed one cell and used light microscopy data to explore the anatomy of the population (Figure 4Dv-vi). These cells projected to the deepest layer of the lobula and also to the adjacent central brain, with cell bodies in the anterior medulla cell body rind (Figure 4Dv). They were morphologically similar to a putative *ort*-expressing cell identified by Gao et al. (2008; see their Figure S6). ML1 dendrites covered ~20 medulla columns with overlap, and the population covered the medulla (Figure 4Dii,vi), ramifying from vertical processes in layers M1-4, and also in M8 (Figure 4Diii,v). Unlike the Tm cells, the ML1 axons exited the distal surface of the medulla and traveled anteriorly to enter and terminate in or near lobula layer 6 (Figure 4Diii, v, vi), providing an alternate pathway connecting the medulla to the lobula. Light microscopy suggests that a fraction of the cells also formed synapses in the central brain, in the posterior lateral protocerebrum (Figure 4Dvi, arrow). The input to the lobula was non-columnar, and although it was not strictly retinotopically organized, it was spatially organized, with two axon bundles originating in the dorsal and ventral medulla terminated in two general locations, incompletely covering the lobula (Figure 4 – figure supplement 2). Our seed columns contacted four ML1 cells, making an average of 25 synapses per ML1 cell. The cell was exclusively targeted by R8s from both pale and yellow columns (Figure 4Div).

Visual projection neurons connecting R7-8 with the central brain

Nearly 10% of the synapses made by the central seed column R7-8 were with cells projecting directly to the central brain (Table 1). Of these, the strongest targets were the MeTu

cells, which resemble Tm cells, but instead of sending axons to the lobula, projected to the AOTU via the anterior optic tract (Fischbach and Lyly-Hunerberg 1983; Otsuna et al. 2014; Otsuna and Ito 2006; Omoto et al. 2017; Timaeus et al. 2020; Tai et al. 2021; Figure 5A). Our reconstructions confirmed that MeTu cells were targets of R7 (Timaeus et al. 2020), and that their photoreceptor input was exclusively from R7 (Figure 5Aiv). In total MeTu cells received 15% of all the seed column R7 synapses (Table 1). We found 7 such neurons and reconstructed the complete morphology of one. Their dendrites overlapped and covered ~20-30 columns (Figure 5Aii), while the axons all targeted the lateral tip of the AOTU (Timaeus et al. 2020; Omoto et al. 2017; Tai et al. 2021; Figure 5Ai).

The presence of MeTu cells was the largest discrepancy by synapse count between our list of R7 targets and those of the medulla FIB-SEM data set (Clements et al. 2020; Takemura et al. 2015; Figure 1 – figure supplement 2E), which was focused on columnar cell types and did not identify MeTu cells. In the FIB-SEM data set, we identified a partially reconstructed Tm cell (Figure 1 – figure supplement 2F) and several cells annotated as Dm7 as potential matches to MeTu cells. We did not identify any Dm7 cells postsynaptic to R7 in our data. We confirmed details of the connectivity of our MeTu neurons that were found for the FIB-SEM putative Dm7 neurons (including strong inputs from Dm2 and Mi15 neurons), and we propose that the partially reconstructed Tm cells and also Dm7 cells in the 7-column data set were most likely MeTu cells, resolving the largest (numerical) discrepancy between the data sets, and underscoring the benefit of reconstructing small circuits in whole-brain EM volumes.

Amongst the photoreceptor target cells projecting to the central brain, we identified a second cell type whose axonal projections follow the anterior axonal tracts of the ML1 neuron, that we refer to here as ML-VPN1 (Figure 5B). The cell bodies were located near the anterior proximal medulla, and, based on our match using light-microscopic data, their dendrites overlapped, with each cell spanning tens of columns, collectively covering the medulla (Figure 5Ci-vi). The dendrites ramified along the border of M7 and M8, with vertical processes reaching up and spreading laterally in M3 and again vertically up to M1 (Figure 5Bii-iii, Ciii).

Although the ML-VPN1 axons followed the ML1 axonal tract, they innervated the posterior lateral protocerebrum (PLP) of the ipsilateral hemisphere, in a region just posterior to the optic glomeruli (Figure 5Bi, Ci). The ML-VPN1 cells were almost exclusively targeted by the pale R8, with 8.7 seed pale R8 inputs per cell (mean, range 5-14), and just one yellow R8 input across the three cells connected to our seed column photoreceptors (Figure 5Biv). Light microscopy of co-labelled ML-VPN1 neurons and either yellow R8 axons (labelled for Rh6 in Figure 5Cvi) or pale R8 axons (labelled for Rh5 in Figure 5 – figure supplement 1) supported the pale preference seen in the EM data: ~90% of columns in which ML1 arbors and photoreceptors appeared to overlap were pale. Together, our data indicate that ML-VPN1 neurons have a strong bias for pale R8 input.

The aMe12 cells were also R8 targets, and the two contacted cells received 15 and 11 R8 synapses, and 2 and 2 R7 synapses, respectively in the seed columns (Figure 1D, Eiii, Supplementary File 1—aMe12 outgoing). Their axons also targeted the PLP, although in different locations to ML-VPN1, but they did so in both hemispheres (Figure 5D). In addition, aMe12 cells also projected to the mushroom body calyces of both hemispheres, as well as to the contralateral and ipsilateral accessory medulla and superior clamp (Figure 5Di, Dii). All three aMe12 cells shared a characteristic branching pattern, but only one of them additionally innervated the contralateral accessory medulla (Figure 5Dii). The aMe12 cell type is present in the hemibrain connectome, where it is also a strong input to γ d Kenyon cells (Li et al. 2020; Scheffer et al. 2020).

There were 5 additional tangential cells projecting to the central brain (Mt-VPNs) targeted by seed column R7-8, collectively receiving 25 synapses (1 neuron in Figure 5E, others in Figure 5 – figure supplement 2). The most targeted cell, which we refer to as Mt-VPN1, had processes in layer M7, with branches reaching up into layers M5-M6, where it received inputs from both seed column yellow R7s, and projected an axon to the posterior lateral protocerebrum (Figure 5E). Despite the exclusive seed connections with yellow R7, Mt-VPN1 was not specific for yellow R7: we traced Mt-VPN1 in 20 columns, in which it received input from 13 yellow R7, and 7 pale R7 cells. The remaining tangential cells included a match

to an octopaminergic neuron (OA-AL2i3; Busch et al. 2009; Figure 5 – figure supplement 2A). This cell had processes in the distal medulla in layers M1-M2 as well as processes reaching out into the chiasm which received photoreceptor input (Figure 5 – figure supplement 2Aii-iii), and an axon that ipsilaterally innervated the inferior and superior posterior slope, and the flange (Figure 5 – figure supplement 2Ai). This cell was one of the rare cell types that formed synapses onto photoreceptors, making 5 synapses onto the R7-R8, but only in one yellow column (Supplementary File 1—Mt-VPN). The remaining Mt-VPN cells were very lightly innervated by the seed column photoreceptors in multiple (Figure 5 – figure supplement 2Cii) and single columns (Figure 5 – figure supplement 2 Bii, Dii), but may integrate large numbers of photoreceptor inputs as they covered many columns. Collectively they innervated the ipsilateral superior posterior slope, accessory medulla, and lobula, and in both hemispheres the inferior posterior slope and lobula plate (Figure 5 – figure supplement 2B-D), revealing multiple pathways for transmitting direct photoreceptor signals into the central brain.

Synapses between R7-DRA and R8-DRA, and with medulla cell types

The following four sections present the first EM connectomic data for targets of the polarization-sensitive photoreceptors in the DRA of *Drosophila*. We first describe the cell types targeted by photoreceptors in the DRA region that were also found in the central columns (Figure 6), followed by modality-specific Dm cell types connected exclusively to DRA inner photoreceptors (Figure 7), visual projection neurons connecting the DRA region and central brain (Figure 8), and finally cell types only found in the DRA region (Figure 9).

The anatomical arrangement of synapses between R7-DRA and R8-DRA strikingly resembled that of central R7 and R8. The number of R7-DRA synapses onto R8-DRA was similar (13.7 mean, range 10 – 16), accounting for 4.9% (41/837) of the total R7-DRA output (Figure 6 – Figure supplement 1A, Table 3), and the number of R8-DRA synapses onto R7-DRA was also similar (36.0 mean, range 33-38; Figure 6 – Figure supplement 1B, Table 3). Many of the synapses between the inner photoreceptors were also located outside the medulla (15% of R8-DRA synapses to R7-DRA, 16/108, mean 5.3 synapses per cell, range

3-8; and 48% of R7-DRA synapses to R8-DRA, 19/41, mean 6.3 synapses per cell, range 4-8; Figure 6Ai, arrow, Supplementary File 3).

The Dm9 cells in the DRA region were prominent synaptic targets of both inner photoreceptors, and provided strong synaptic feedback onto them (Figure 6B, Table 3, 4). They received 31.7 R7-DRA synapses per column (mean, range 26 – 38), corresponding to 11.3% (95/837) of the total R7-DRA output (Table 3), and R8-DRA was more strongly connected with 38.3 synapses (mean, range 36 – 43), corresponding to 23% (115/499) of the total R8-DRA output. Although the total number of inner photoreceptor inputs to Dm9 per seed column were fewer in the DRA region compared to central seed columns (70 mean, range 62 – 81), their relative strength was similar to central columns (Supplementary File 1). The distribution of R8 and R8-DRA synapses onto Dm9 was also different, with the greatest density of R8-DRA synapses in layer M6, reflecting the characteristic morphology of R8-DRA (Figure 6Bi). Our prior light microscopic study suggested that marginal Dm9 cells might receive both DRA and non-DRA inputs (Sancer et al. 2020). We therefore reconstructed R7 and R8 in three non-DRA columns innervating the same DRA Dm9, and found that cell indeed received input from both color and polarized light-sensitive photoreceptors (Figure 6Biv).

Dm2 was an R8 target in the central columns, but in the DRA region there were drastically fewer R8-DRA inputs, with equal contacts from R7-DRA and R8-DRA (7.0 mean, range 5 – 10 for R7-DRA, 9.0 mean, range 5 -15 for R8-DRA synapses per column; Figure 6Ci-ii, Table 3; (Sancer et al. 2020). The Dm11 cell type was an R7 target in central columns, but in the DRA region it received minor, balanced input from both R7-DRA and R8-DRA and was only found in one of the three DRA seed columns (4 from R7-DRA and 8 from R8-DRA; Figure 6 – figure supplement 2D, Table 3). Thus, while a reduction in R8-DRA input to cells targeted by R8 in the central columns was a repeated feature of the DRA region, as exemplified by Dm2, it was also seen in cells targeted by R7, such as Dm11.

The connectivity of inner photoreceptors with lamina cells was maintained in the DRA region, including with a substantial fraction of synapses outside the medulla (Figure 6 – figure supplement 2A,B; Figure 1 – figure supplement 2G; Supplementary File 3), but there were

markedly fewer synapses to Mi cells from R8-DRA than from R8, including to Mi1 and Mi9, while Mi4 was not contacted in the seed DRA columns at all (Mi1: 3 mean, range 2 – 5; Mi9: 4 mean, range 0 – 9; Figure 6 – figure supplement 2C,E). Of all the Mi cell types, Mi15 alone was a significant photoreceptor target in the DRA region, receiving synapses from both R7-DRA and R8-DRA (respectively, 7 mean, range 4 – 9, and 6 mean, range 3 – 10; Figure 6Di-ii, Table 3). Compared to the central column R7-8 input, Mi15 cells gained R7-DRA synapses in the DRA region, and they maintained their high fraction of inputs outside the medulla (Figure 6D; Figure 1 – figure supplement 2G; Supplementary File 3).

Only one Tm cell type, Tm20, was connected to inner photoreceptors with more than 2 synapses throughout all three DRA seed columns. This cell type was selective for R8-DRA, but was much more weakly connected (5.3 mean, range 4 – 8 for R8-DRA; Table 3, Supplementary File 1) than it was to R8 in the central columns. There was only one other strongly connected Tm cell, which we refer to as Tm5-like, that had 12 synaptic inputs from both R7-DRA and R8-DRA inputs in one of the three DRA columns (Figure 6 – figure supplement 2F). Together, these four cells highlight the absence of projections to the lobula, as compared to the central columns with 27 identified Tm cells (Table 1).

Dm8-like photoreceptor targets in the DRA region

The strongest target of R7-DRA cells was Dm-DRA1 (Sancer et al. 2019). Together, 20 Dm-DRA1 cells received 70.1 (mean, range 66 – 77) synapses per DRA seed column, corresponding to 25.3% (212/837) of total R7-DRA output (Table 3, Supplementary File 1). There were no synaptic inputs from R8-DRA, and synapses from R7-DRA to Dm-DRA1 were consistently restricted to the proximal edge of layer M6 where R8-DRA are not present (Figure 7Ai, Aii). There were few synapses in neighboring non-DRA columns (Figure 7 – figure supplement 1A), and only one of the contacting R7 cells made a substantial number (11) of synapses. The inner photoreceptors choose their DRA fates independently, and therefore this photoreceptor could be an R7-DRA, even though the R8 in the same column terminated in M3; low numbers of such mixed ommatidia consistently occur at the DRA boundary (Wernet

et al. 2003; Wada 1974b). Dm-DRA1 cells have deep projections that leave the DRA region and stratify below layer M6, and these did not receive strong photoreceptor input (Figure 7Aii, compare light microscopy of clone in 7Aiii), nor did they preferentially occupy either pale or yellow columns (Figure 7 – figure supplement 2).

To gain a better understanding of the synaptic connectivity between R7-DRA and Dm-DRA1, we identified all the DRA photoreceptor inputs to three fully reconstructed Dm-DRA1 cells (Figure 7B). Each cell was connected to 9 (mean, range 8 – 10) neighboring R7-DRA, receiving a total of 94 synapses (mean, range 66 – 112) from R7-DRA, but almost no connections from R8-DRA (2 R8-DRA form 3 and 2 synapses with the posterior Dm-DRA1; Figure 7Bi). Dm-DRA1 cells have been proposed to be DRA-specific equivalents of Dm8 (Courgeon and Desplan 2019; Sancer and Wernet 2021), but we never detected vertical protrusions resembling Dm8 ‘home columns’. The smoothed distribution of synapses along a linear projection of the DRA covered ~50 μ m with the strongest input in the center, but without a single, dominant home column (Figure 7Biv, Figure 7 – figure supplement 3).

The Dm-DRA2 cell type was the strongest R8-DRA target after Dm9 and R7-DRA (Table 3), with 34.3 (mean, range 19 – 44) seed columns synapses to nine cells (Figure 7C, Supplementary File 1; Sancer et al. 2019). Each Dm-DRA2 cell had multiple characteristic vertical projections, whose lengths were variable but markedly longer than the single vertical processes of Dm8 home columns, often reaching layer M1 (Figure 7Ci, Ciii; Sancer et al. 2019), and these vertical projections received R8-DRA synapses (Figure 7Cii, Ciii, Eiii). To quantify the pattern of their photoreceptor inputs, we fully reconstructed three Dm-DRA2 cells. These cells were innervated by 9.3 (mean, range 8 – 11) R8-DRA and received minor inputs from R7-DRA (3.7 mean, range 3 – 4 cells per Dm-DRA2 with an average of 5.3, range 3 – 7 synapses; Figure 7D, Supplementary File 1). The distribution of R8-DRA synapses to each Dm-DRA2 cell covered ~50 μ m with a peak in its center and without a single, obvious home column (Figure 7Di, Dii). Thus, Dm-DRA2 directly integrate photoreceptor inputs over multiple

columns, with stronger input at the center of their receptive field, much like we found for Dm-DRA1.

The reconstructed Dm-DRA1 cells highly overlapped along the DRA region, as did the Dm-DRA2 cells (Figure 7Ei, Eii; Sancer et al. 2019). The population of all Dm-DRA1 cells connected to the same R7-DRA seed photoreceptor (8.3 mean, range 6 - 11) spanned 17 DRA columns (mean, range 13 - 19), corresponding to just under half the length of the DRA region. In comparison, the number of Dm-DRA2 targets of one R8-DRA was fewer (3.3 mean, range 2 – 5 Dm-DRA2 cells per column), and the length of the DRA region covered was also shorter at 10.7 columns (mean, range 10 -12). These differences in connectivity may have implications for how the two Dm-DRA cell types differently compute skylight polarization information originating from the same visual field. Finally, comparison of inputs to Dm-DRA1 and Dm-DRA2 from the same location along the DRA revealed a separation of R7-DRA and R8-DRA synapses into different sublayers of M6 (Figure 7Eiii), confirming prior observations from light microscopy (Sancer et al. 2019).

Visual projection neurons connecting R7-DRA with the central brain

The second most prominent R7-DRA target was a cell type that we refer to as MeTu-DRA (Figure 8A). These cells exclusively received R7-DRA synapses, with no input from R8-DRA, and are modality-specific. Both the reconstructed cells and the matching light microscopy MeTu-DRA clones projected long axons to the small unit of the AOTU (Figure 8Aii), identifying them as specialized MeTu cells (Omoto et al. 2017; Panzer et al. 2016; Otsuna et al. 2014; Tai et al. 2021). They closely resemble cells described in the DRA region of optic lobes from larger insects (Pfeiffer and Kinoshita 2012; el Jundi et al. 2011). All 30 reconstructed MeTu-DRA cells received 61.3 (mean, range 49 – 85) R7-DRA synapses per seed column, corresponding to 22.0% (184/837) of the total R7-DRA output (Figure 8Aiii, Table 3, Supplementary File 1). Synapses from R7-DRA to MeTu-DRA were restricted to the photoreceptor tips at the proximal edge of layer M6, as we found for Dm-DRA1 (Figure 8Aiv). MeTu-DRA cells also innervated the dorsal medulla outside the DRA region (Figure 8Av), but

avoided non-DRA photoreceptors by stratifying below M6, and showed no obvious pale or yellow preference (Figure 8 – figure supplement 1A). We visualized single MeTu-DRA cell clones in the DRA and found their photoreceptor contacts were always restricted to the DRA region (Figure 8Av, arrow). The medulla branches of all MeTu-DRA skeletons connected to R7-DRA from the same DRA seed column (11.3 mean, range 8 – 16) widely overlapped, covering 15 DRA columns (mean, range 12 – 17; Figure 8Avi). Thus, the MeTu-DRA cells receive skylight polarization information from R7-DRA over similar portions of the sky as Dm-DRA1 cells.

Since the axons of the central region MeTu cells projected to a discrete subdomain of the small unit of the AOTU (Figure 5Ai), we assessed whether they intermixed with MeTu-DRA terminals, and found they do not. The MeTu-DRA and the MeTu cells we reconstructed from central columns terminate in separate, adjacent AOTU compartments (Figure 8vii, inset).

We identified one additional, previously uncharacterized visual projection neuron type connecting the DRA region with the central brain, which we refer to as VPN-DRA. These cells send axonal projections to the PLP (Figure 8Bi, Bii). There were 6 VPN-DRA cells with >2 synapses contacted by the DRA seed columns, and they received 10 (mean, range 6 – 13) R7-DRA synapses per seed column (corresponding to 3.6%, 30/837, of total R7-DRA output), with no R8-DRA synapses (Figure 8Biii, Table 3, Supplementary File 1). The main branches in the medulla of VPN-DRA cells followed the DRA region, with smaller branches innervating non-DRA columns (Figure 8Biv). Double labeling of photoreceptors and a driver line with apparent VPN-DRA expression indicated that photoreceptor input to VPN-DRA cells might be dominated by DRA inner photoreceptors, with only fine processes contacting non-DRA photoreceptors (Figure 8Bv).

VPN-DRA cells form an overlapping group of neurons covering the entire DRA region of a given hemisphere (Figure 8Bvi). There was morphological heterogeneity within the population, with some cells having additional processes, both in the medulla, as well as in the central brain and a different cell body position (Figure 8 – figure supplement 1B). Therefore, there exist different subtypes of VPN-DRA.

Other newly identified photoreceptor targets in the DRA region

Two more previously uncharacterized cell types received photoreceptor input in the DRA seed columns. The first, which we refer to as MeMe-DRA, were large heterolateral medulla cells that connected the DRA regions of both hemispheres, crossing the central brain without forming synapses there (Figure 9A). We reconstructed two MeMe-DRA cells, and could not find additional ones, and we matched the morphology of the cell type using light microscopy (Figure 9Aiv). Morphologically, these cells resemble the polarization-sensitive MeMe1 cells characterized in locusts (el Jundi et al. 2011; el Jundi and Homberg 2010). They were exclusively innervated by R7-DRA, receiving 12.0 (mean, range 0 – 21) synapses per seed column, corresponding to 4.9% of total R7-DRA output (Figure 9Aii, Aiii, Table 3, Supplementary File 1). The MeMe-DRA processes in the ipsilateral hemisphere appear to be primarily dendritic, whereas the contralateral processes appear to be axonic (Figure 9Aiii, Aiv). The putative dendritic processes appeared to contact DRA photoreceptors, based on light microscopy labeling, with processes reaching layer M6 exclusively in the DRA region (Figure 9Aiv). Intriguingly, the area of medulla columns innervated by a given cell is flipped along the anterior-posterior axis between the two hemispheres, so that the anterior half of the ipsilateral medulla is connected, via the MeMe-DRA cell, with the posterior half of the contralateral medulla and vice versa (Figure 9Av).

Another newly identified set of DRA photoreceptor targets were morphologically diverse Mti cells that covered the dorsal medulla (Figure 9B, C, Figure 9 – figure supplement 1), for which we also found putative light microscopy matches. All 10 of these Mti-DRA cells were innervated exclusively by R7-DRA, but there were two distinct morphological populations, which we refer to as Mti-DRA1 (Figure 9Bi-Biv) and Mti-DRA2 (Figure 9Ci-Civ). There were six Mti-DRA1 cells with ventral cell bodies (Figure 9Bi) that received together 12.3 (mean, range 10 – 14) synapses per seed column, corresponding to 4.4% of total R7-DRA output (Figure 9Bii, Supplementary File 1). Individual reconstructed Mti-DRA1 cells had main processes along the DRA region with short processes innervating non-DRA columns (Figure

9Biii, Biv). Mti-DRA2 cells had dorsally located cell bodies (Figure 9Ci) and were more weakly connected to R7-DRA, with four Mti-DRA2 neurons making 5 (mean, range 3 – 8) synapses per seed column, corresponding to 1.8% of R7-DRA total output, (Figure 9Cii– figure supplement 1, Table 3, Supplementary File 1). The main processes of Mti-DRA2 skeletons also innervated the DRA region but these cells formed longer processes extending further ventrally into non-DRA columns (Figure 9Ciii, Civ).

Comparison of inner photoreceptor connectivity of central and the DRA columns

Taken together, our reconstructions of the synapses of the inner photoreceptors specialized for color and polarization vision revealed specific stereotypical similarities, as well as striking differences. The pattern of connectivity between photoreceptor terminals from the same ommatidium, and with Dm9, was highly consistent in columns processing color and polarization, indicating that the mechanisms of lateral inhibition between photoreceptors and gain modulation by Dm9 can serve both modalities (Schnaitmann et al. 2018; Weir et al. 2016; Figure 10A).

A second striking similarity was the location of synapses located outside the medulla (Figure 1 – figure supplement 2G). In both the central and DRA columns, more than half of the synapses between the photoreceptors and the inputs to the lamina cells L1 and L3 were outside the medulla (Figure 1 – figure supplement 2G, Figure 2 – figure supplement 1, Figure 6 – figure supplement 1A, B, Supplementary File 3). In both cases, the strongest lamina monopolar target was L3, receiving 80% of its inner photoreceptor inputs outside the medulla (Figure 2 – figure supplement 1C, Figure 6 – figure supplement 1B). L3 could contribute to color processing through innervation of Dm9, Tm20, and Tm5c (Takemura et al. 2015), in addition to the ON and OFF pathways computing motion (Silies et al. 2013). Our reconstructions therefore provide further support for the early convergence of motion and color channels, which have until recently been believed to operate separately (Heisenberg and Buchner 1977; Yamaguchi et al. 2008; Wardill et al. 2012; Pagni et al. 2021; Li et al. 2021).

Amongst Dm cell types, both R7-DRA and R8-DRA connect to their own modality-specific amacrine-like cell type, Dm-DRA1 and Dm-DRA2, respectively (Sancer et al. 2019). Such connections never occur in the central seed columns, where only R7 connects to Dm8 (Figure 10B). Recent studies point towards Dm-DRA1 (and possibly Dm-DRA2) as developmentally similar duplicates of Dm8 cells (Courgeon and Desplan 2019; Sancer and Wernet 2021). Many of the connections to Mi cells are weaker or missing in the DRA region (Figure 10C). Given the role of Mi1, Mi4 and Mi9 in computing motion (Strother et al. 2017), this could indicate a stricter separation between computations of motion and polarization, as compared to motion and color. In contrast, Mi15 cells are targeted by both central and DRA inner photoreceptors. Its role is not known, but its partly dopaminergic neurotransmitter phenotype suggests a modulatory role (Davis et al. 2020). We reconstructed morphologically different Mti cells both from central and DRA columns, and while the Mti cells are R8 targets in the central columns (Figure 3A, B), they are targets of R7 in the DRA region with Mti-DRA1 cells having a greater input per seed column than the Mti cells (Figure 9B, C).

A striking difference between the central and DRA columns is the absence of most lobula-projecting cells targeted by photoreceptors (Figure 10D). We confirm Tm5a, Tm5b, Tm5c, and Tm20 as targets of central R7 or R8 (Meinertzhagen et al. 2009; Gao et al. 2008; Melnattur et al. 2014), and we also confirm the lack of synaptic connections with these cell types in the DRA region (Sancer et al. 2020). In addition to Tm cells, we identify the new cell type ML1, also connecting the medulla to the lobula but without the precise retinopy of the Tm cell arrays, and with additional branches in the central brain (Figure 10Dii). The lack of Tm and ML1 connectivity in the DRA region is reflected in the overall lower number of R8-DRA synapses (Table 3).

R7 and R7-DRA cells are strongly connected to respectively different classes of MeTu cells, whose axon terminals are spatially segregated in the small unit of the AOTU (Figure 10E; Timaeus et al. 2020). These data support previous proposals of parallel pathways to the central brain via the AOTU (Otsuna et al. 2014; Omoto et al. 2017; Timaeus et al. 2020; Tai et al. 2021). Besides the AOTU, photoreceptor targets in the central and DRA columns both

project to the PLP, but otherwise they show little overlap in the central brain areas they project to (Figure 10F). The additional contacts of DRA region projection neurons are focused in the lateral horn and the contralateral medulla, while the central column projection neurons innervate widely across the central brain, including the mushroom bodies, and the contralateral lobula plate. A key modality-specific feature of the visual projection neurons is the dominance of R8 input in the central columns, and R7 in the DRA region (Figure 11A).

Cell types distinguishing between pale and yellow inputs

The fly's retinal mosaic can support circuits with different chromatic sensitivities by selective sampling of pale and yellow photoreceptors. For example, cells contrasting pale and yellow R7 input would be expected to have high chromatic sensitivity around ~350 nm, where the spectral sensitivity of Rh3 and Rh4 overlap (Salcedo et al. 1999). Our reconstructions revealed several cases of connectivity patterns that could support such selective sampling. For R7, these included two cell types previously reported to be pale- or yellow-selective: Tm5a for yellow R7 (Karuppudurai et al. 2014), and Tm5b for pale R7 (Menon et al. 2019; Figure 11Bi). By completely reconstructing several Dm8 cells for the first time (Figure 2C), we found that Dm8 neurons were most highly innervated by R7 in their home columns, with approximately equal sampling of pale and yellow R7 input in the surrounding columns. Unlike in the DRA region, there were no cells projecting to the central brain that were substantial targets of R7. Examining the encoding of chromatic signals in Tm5a/b and Dm8 cells, and their other synaptic partners, is therefore a promising avenue for addressing the open question of how, or if, UV information from yellow ommatidia is processed differently from UV signals downstream of pale R7 cells.

We found more pale-yellow selective cells among the R8 targets. Two visual projection neurons were newly identified as selective for pale R8 input, the aMe12 and ML-VPN1 cells. ML-VPN1 provides input to the PLP, while aMe12 has presynaptic sites in the accessory medulla, the PLP, and the mushroom body, where it provides input to γ d Kenyon cells (Li et al. 2020; Scheffer et al. 2020). These connectivity patterns suggest roles in circadian

entrainment and learning and memory, respectively (Figure 11Bii). *Drosophila* pale R8 cells are most sensitive to blue wavelengths (Sharkey et al. 2020; Salcedo et al. 1999; Schnaitmann et al. 2018; Heath et al. 2020), and the projections of aMe12 and ML-VPN1 demonstrate that blue light is processed and used by the central brain within one synapse. Little is known about the central processing of color in *Drosophila* (Longden 2016), but the detection of blue light plays an important role in the circadian avoidance of bright light (Lazopulo et al. 2019). Flies can also learn to discriminate between large areas of blue and green light and a group of visual projection neurons have been reported to provide input to γ d Kenyon cells and be required for this ability (Vogt et al. 2014).

While near perfect specificity for pale versus yellow inputs appears to be limited to a few cell types, several strongly connected cell types show strongly biased inputs: For example, Dm2 favors pale over yellow R8 (60:15), and Tm5c favors yellow over pale R8 (103:20) a result that corroborates and extends the findings of the 7-column medulla connectome. Individual cells, in particular Tm20 neurons, that span just one column, are also constrained to be selective for pale and yellow photoreceptor input. Such selectivity could in principle be exploited at the level of the synaptic targets of these neurons. Surprisingly, Mi9 cells, another cell type present in every column of the eye, also revealed strong pale/yellow biases in our reconstructions, both with R7 inputs (3:9), as well as R8 inputs (26:1). Given the known role of Mi9 in shaping direction-selective responses on T4 dendrites (Strother et al. 2017; Takemura et al. 2017), these data identify yet another level of crosstalk between chromatic and motion-sensitive channels. Overall, it is an unexpected finding that most projections from the medulla to the lobula from photoreceptor target neurons convey biased pale/yellow inputs. These differences could mediate much broader effects of pale/yellow differences in medulla and lobula circuits than currently appreciated, or these specializations could be blunted by combinations at the next synaptic layer.

Discussion

Our systematic reconstruction of all synaptic inputs and outputs of identified, functionally specialized *Drosophila* photoreceptors (pale and yellow R7-8, R7-DRA and R8-DRA) provides a comprehensive inventory of the first steps of the color and polarization pathways, from which all the computations of the dependent behaviors stem. These data revealed core connectomic motifs shared across column types (Figure 10A-C), multiple new photoreceptor targets, and uncovered additional cell types as being connected to specific photoreceptor subtypes conveying specific color and polarization information to the central brain (Figure 10E, F).

Inner photoreceptor connections outside the medulla neuropil

We confirmed previously reported synaptic partners of the inner photoreceptors in the central medulla (Figure 1-supplement 2) and identified new photoreceptor targets. As prior reconstructions were incomplete (Takemura et al. 2015), it was unclear whether the unidentified connections were mainly onto new target neurons or represented more connections onto known cell types; our reconstructions revealed both types of omissions. One functionally important set of missed connections are the synapses between inner photoreceptors from the same central and DRA ommatidia, which we found to be stronger than previously reported (Takemura et al. 2008, 2015), due to significant numbers of synapses outside the medulla. These synapses likely contribute to color-opponent responses seen in central R7 and R8 terminals (Schnaitmann et al. 2018) and the polarization-opponent signals measured from DRA photoreceptors (Weir et al. 2016). Our reconstructions also support a larger scale opponent process mediated by multi-columnar Dm9 cells (Heath et al. 2020), which also formed some synapses outside the medulla neuropil.

Other cell types also received inner photoreceptor input outside the medulla, notably the lamina monopolar cells L1 and L3. These lamina connections indicate that chromatic comparisons arising from R7 and R8 may feed into the motion vision pathway, and identifies a new site for interplay between the ‘color’ and ‘motion’ pathways (Wardill et al. 2012; Schnaitmann et al. 2013; Pagni et al. 2021; Li et al. 2021). Together, these observations

suggest that synapses in an unexpected location, outside the main synaptic layer of the medulla, could play a significant role in early visual processing.

Newly identified targets of central versus DRA inner photoreceptors

In the central medulla, we found, for the first time in an EM study, strong synaptic connections between R7s and MeTu cells that project to the AOTU (Figure 11Ai), confirming previous claims based on light microscopy (Timaeus et al. 2020). This finding may reconcile disparate observations, such as a role in wavelength-specific phototaxis for cells matching MeTu morphology (Otsuna et al. 2014), as well as measurements of color-sensitive signals in the AOTU of bees (Mota et al. 2013). Previous anatomical studies partitioned MeTu cells into distinct subclasses that terminate in discrete subdomains of the AOTU (Omoto et al. 2017; Timaeus et al. 2020; Tai et al. 2021). Here, we identified modality-specific MeTu-DRA cells that only integrate from the polarization-sensitive R7-DRA photoreceptors, while avoiding synaptic contacts with color-sensitive pale or yellow R7s. Our MeTu and MeTu-DRA cells target adjacent subdomains within the small unit of the AOTU (Figure 10E), in agreement with proposals that parallel channels convey different forms of visual information from the eye to the central complex via the AOTU (Hardcastle et al. 2021; Hulse et al. 2020; Timaeus et al. 2020; Omoto et al. 2017).

We also identified connections from inner photoreceptors to several cell types either not previously described, or not known to be photoreceptor targets, thus setting up a clear expectation that these cells should contribute to color or polarization vision. ML1 is a new, major target of R8, a cell type that connects the medulla to the lobula via a previously unknown, non-columnar pathway, and the central brain (Figure 4D, Figure 11Aii). Previous studies have identified an important role for Tm5a/b/c and Tm20 cell types for chromatic processing in the lobula (Lin et al. 2016; Gao et al. 2008), and we have confirmed that these cell types are targets of R7-8. Whether these Tm neurons and ML1 cells have common targets in the lobula, feed into shared central pathways or contribute to separate channels remain

open questions; the lobula arbors of the Tm and ML1 cells are mostly in different layers arguing against direct synaptic interactions between the cells.

The reconstruction of DRA photoreceptor targets confirmed the modality-specific connectivity of Dm-DRA cell types within layer M6: Dm-DRA1 to R7-DRA, and Dm-DRA2 to R8-DRA (Figure 11A). The R7-DRA and R8-DRA cells respond to orthogonal orientations of polarized light at each location of the DRA, so Dm-DRA1 and Dm-DRA2 likely process orthogonal e-vector orientations spatially averaged by pooling over ~10 ommatidia (Weir et al. 2016). Our data also revealed additional DRA pathways into the PLP in the central brain via VPN-DRA cells, as well as to the contralateral DRA, via MeMe-DRA cells (Figure 10F). Such interhemispheric connections have been demonstrated in larger insects (el Jundi et al. 2011; Labhart 1988), but not in *Drosophila*, and their synaptic input was not known. Interocular transfer contributes to navigation by desert ants that can see the celestial polarization but not visual landmarks (Wehner and Muller 1985), but the interactions between the DRA regions remain poorly understood. The identification of MeMe-DRA neurons may enable the mechanisms of such phenomena to be explored.

For color vision, we have confirmed the pale-yellow specificity of Tm5a and Tm5b cells (Figure 11Bi). We have additionally identified as pale-specific the aMe12 and ML-VPN1 cell types, and the yellow bias of Tm5c and the pale bias of Mi9 (Figure 11Bii). Our detailed analysis of Dm8 inputs confirmed that these neurons receive most of their photoreceptor input in a central home column, consistent with pale and yellow subtypes of Dm8 cells having distinct chromatic properties (Courgeon and Desplan 2019, Menon et al. 2019, Li et al. 2021, Pagni et al. 2021). The selective photoreceptor input to these cell types, combined with input to columnar cells from a single photoreceptor subtype, indicate that wavelength-specific information is maintained in the medulla and lobula (Figure 11Bi). The projections of aMe12 and ML-VPN1 to the central brain indicate the possibility that wavelength-specific photoreceptor responses are directly conveyed into the central brain by these cells, although they likely integrate input from other chromatically sensitive cell types (Figure 11Bii).

878

879 Limitations of our approach

880 By focusing on a small number of columns, we have delivered a near complete picture
881 of local connectivity, but we cannot rule out the possibility that certain cell types may have
882 been overlooked. For instance, we chose an arbitrary threshold of 2-3 synapses below which
883 we did not endeavor to reconstruct synaptic targets to the extent required to uniquely identify
884 them. As a result we may, in principle, have missed large cells that receive small, but
885 significant inputs across many columns. Furthermore, we cannot rule out regional
886 specializations in the optic lobes, such that specific cell types might be found outside of our
887 seed columns. For example, some MeTu cells are only found in the dorsal third of the medulla
888 (Omoto et al. 2017; Otsuna et al. 2014), where incoming R7 cells are known to co-express
889 Rh3 and Rh4 Rhodopsins (Mazzoni et al. 2008).

890

891 Complementary usage of multiple connectomic data sets and outlook

892 Taken together, the data presented here provides access to the full complement of R7
893 and R8 photoreceptor targets from functionally specialized optical units. By reconstructing
894 these local circuits within a full-brain EM volume, we were able to establish the complete
895 morphology of large, multi-columnar cell types, that are strongly connected to photoreceptors,
896 but had eluded previous connectomic reconstruction efforts (Takemura et al. 2013, 2015). The
897 sparse tracing approach we have implemented and described here enabled efficient
898 identification of the complete set of upstream and downstream partners of the inner
899 photoreceptors, in a manner that is complementary to the dense connectomes generated from
900 smaller-scale medulla volumes. As an example of this synergistic usage of complementary
901 data sets, we have returned to the 7-column data (Takemura et al. 2015; Clements et al. 2020)
902 and used our whole-cell morphologies to match the bodies of previously unidentified
903 photoreceptor targets. In that process we have established strong candidates for MeTu, ML1,
904 and perhaps for aMe12 (see Materials and Methods) and confirmed several aspects of their
905 connectivity in FAFB. In so doing, we now have access to the additional connectivity data

provided by the 7-column dense reconstruction. While full exploration and follow-up analyses of these combined data is beyond the scope of this work, the FIB-SEM/FAFB combination revealed several intriguing connectivity patterns, including new candidate paths for the integration of output from different photoreceptor types. For example, the MeTu cells that are postsynaptic to R7 also receive indirect R8 input via the R8 target Mi15, and ML1 combines direct R8 input with indirect input from outer photoreceptors via lamina neurons. As further connectome data sets are completed, this comparative interplay between data sets with unique advantages and limitations, will be an important step in both cross-validating and extending the applicability of all related data sets.

Our reconstruction of the DRA photoreceptor targets provides the first EM-based connectomic data set for modality-specific cell types likely to process skylight information in any insect and will be important for developing refined models of skylight navigation (Gkaniats et al. 2019). Core motifs shared between DRA and central columns such as opponency are prime candidates for circuit elements that perform computations, such as establishing opponency, that are key for both polarization and color processing, whereas cell types with preferential connections to either pale or yellow columns are promising candidates for the study of specific aspects of color processing in the insect brain. This comprehensive catalog of the neurons carrying signals from R7 and R8 photoreceptors deeper into the brain establishes a broad foundation for further studies into the mechanistic basis of color vision and its contributions to perception and behavior.

926 Materials & Methods

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
genetic reagent (<i>Drosophila melanogaster</i>)	MCFO-1; "pBPhsFlp2::PEST in attP3; ;pJFRC201-10XUAS-FRT>STOP>FRT-myr::smGFP-HA in VK0005, pJFRC240-10X-UAS-FRT>STOP>FRT-myr::smGFP-V5-THS-10XUAS-FRT>STOP>FRT-myr::smGFP-FLAG in su(Hw)attP1"	https://doi.org/10.1073/pnas.1506763112	RRID:BDSC_64085	
genetic reagent (<i>Drosophila melanogaster</i>)	pJFRC51-3XUAS-IVS-Syt::smHA in su(Hw)attP1, pJFRC225-5XUAS-IVS-myr::smFLAG in VK00005	https://doi.org/10.1073/pnas.1506763112		
genetic reagent (<i>Drosophila melanogaster</i>)	SS02978	This study	SS02978	For further details on driver lines see Table S1. Driver lines will be available via https://www.janelia.org/split-GAL4 .
genetic reagent (<i>Drosophila melanogaster</i>)	SS01015	This study	SS01015	For further details on driver lines see Table S1. Driver lines will be available via https://www.janelia.org/split-GAL4 .
genetic reagent (<i>Drosophila melanogaster</i>)	SS28175	This study	SS28175	For further details on driver lines see Table S1. Driver lines will be available via https://www.janelia.org/split-GAL4 .
genetic reagent (<i>Drosophila melanogaster</i>)	R56F07	Bloomington Drosophila Stock Center	RRID:BDSC_39160	
genetic reagent (<i>Drosophila melanogaster</i>)	ortc2b-Gal4	https://doi.org/10.1016/j.neuron.2013.12.010		

genetic reagent (<i>Drosophila melanogaster</i>)	20XUAS-CsChrimson-mVenus in attP18	https://doi.org/10.1038/nmeth.2836	RRID:BDSC_55134	
antibody	anti-chaoptin mouse monoclonal 24B10	DSHB	RRID:AB_528161	
antibody	anti-Brp mouse monoclonal nc82	DSHB	RRID:AB_2314866	
antibody	anti-dsRed rabbit polyclonal	Clontech Laboratories, Inc.: 632496	RRID:AB_10013483	
antibody	Rat cadherin, DN-Ex #8(extracellular domain)	Developmental Studies Hybridoma Bank	RRID:AB_528121	(1:100)
antibody	anti-HA rabbit monoclonal C29F4	Cell Signaling Technologies: 3724S	RRID:AB_1549585	
antibody	anti-FLAG rat monoclonal DYKDDDDK Epitope Tag Antibody [L5],	Novus Biologicals: NBP1-06712	RRID:AB_1625981	
antibody	DyLight 550 conjugated anti-V5 mouse monoclonal	AbD Serotec: MCA1360D550 GA	RRID:AB_2687576	
antibody	DyLight 549 conjugated anti-V5 mouse monoclonal	AbD Serotec: MCA1360D549 GA	RRID:AB_10850329	
antibody	anti-GFP rabbit polyclonal	ThermoFisher: A-11122	RRID:AB_221569	
antibody	anti-GFP mouse monoclonal 3E6	ThermoFisher: A-11120	RRID:AB_221568	
Software, algorithms	FluoRender	http://www.sci.uta.h.edu/software/flurorender.html		

Software, algorithms	VVDviewer	https://github.com/takashi310/VVDViewer		
Software, algorithm	Python 3	http://www.python.org	RRID:SCR_008394	
Software, algorithm	pymaid	https://github.com/schlegelp/PyMaid		python library
Software, algorithm	FAFBseg	https://github.com/flyconnectome/fafbseg-py		python library
Software, algorithm	R	https://www.r-project.org/		
Software, algorithm	R Studio	https://www.rstudio.com/		
Software, algorithm	Natverse	https://natverse.org	DOI: 10.7554/eLife.53350	collection of R packages for neuroanatomical analysis
Software, algorithm	Tidyverse	https://www.tidyverse.org/		R package
Software, algorithm	knitr	https://cran.r-project.org/package=knitr		R package
Software, algorithm	kableExtra	https://cran.r-project.org/package=kableExtra		R package

Software, algorithm	gridExtra	https://cran.r-project.org/package=gridExtra		R package
Software, algorithm	png	https://cran.r-project.org/package=png		R package
Software, algorithm	RColorBrewer	https://cran.r-project.org/package=RColorBrewer		R package
Software, algorithm	alphashape3d	https://CRAN.R-project.org/package=alphashape3d		R package
Software, algorithm	Fiji	http://fiji.sc	RRID:SCR_002285	
Software, algorithm	MATLAB			
Software, algorithm	CATMAID	www.catmaid.org		neuron reconstruction
other	FAFB	Zheng et al. 2018; https://v2.virtualflybrain.org/		EM data set
other	FAFB-FFN1	Li et al. 2020; http://fafb-ffn1.storage.googleapis.com/data.html		EM auto-segmentation
other	FlyWire	Dorkenwald et al. 2020; https://flywire.ai/		EM auto-segmentation

Sequence-based reagent	Rh3 F	This paper	PCR primer	CACC GGT ACG CTT ATG ATA ACC TTC G
Sequence-based reagent	Rh3 R	This paper	PCR primer	GCT CCG GTC TGC GGG CCA AGA
Sequence-based reagent	Rh5 F	This paper	PCR primer	CACC GGT GAT TAA TGC GAA TGT AGC TGC
Sequence-based reagent	Rh5 R	This paper	PCR primer	ACT GCT TGA TCC GCT CCA AAA TC
Sequence-based reagent	Rh6 F	This paper	PCR primer	CA CCA ACA TGA TGG CGG ACA TCA C
Sequence-based reagent	Rh6 R	This paper	PCR primer	TTC GAA TGG CTG GTA CTG GTG

927

928 EM reconstruction

929 The FAFB data set is a serial section transmission EM volume of a female *Drosophila*
930 *melanogaster* brain (Zheng et al. 2018). We manually reconstructed neurons in this volume in
931 the CATMAID environment, which includes a browser-based annotation interface and a server
932 hosting a copy of the data (Saalfeld et al. 2009). We also searched for neurons using two
933 recent auto-segmentations FAFB-FFN1 (Li et al. 2020) and FlyWire (Dorkenwald et al. 2020),
934 importing some of the FAFB-FFN1 auto-segmentation fragments afterwards into the
935 CATMAID environment, a process that facilitated the identification of a small number of
936 neurons. We followed established guidelines for manually tracing neuron skeletons,
937 annotating synaptic connections, and reviewing reconstructed cells (Schneider-Mizell et al.
938 2016). We completely reconstructed the morphology of pairs of R7 and R8 cells in 7 “seed”
939 columns, 4 adjacent columns chosen to be near the center of the retinal mosaic, and 3

columns in the dorsal rim area (Figure 1C). On the R7 and R8 cells, we annotated as a “pre-synapse” all the locations where there was a T-bar (Figure 1 – figure supplement 1A). Associated with each pre-synapse we annotated a “post-synapse” connection with every synaptic partner. These seed neurons and their pre-synapses were completely proofread by independent team members, a process that amounted in small (<10%) amendments to the connectivity data presented here.

In total, we identified 4043 connections from the seed column R7 and R8 cells to 422 postsynaptic skeletons (the vast majority of which correspond to individual neurons), including the 14 R7-8 cells. We reconstructed their morphology to be able to identify the cell type (e.g. Dm8, Mi1, Tm5a) or cell class (e.g. Dm, Mi, Tm) of 299 cells, whose synapses accounted for 96.2% (3888/4043) of the total synaptic output. For all but one cell, we applied a threshold of >2 synapses to focus our reconstructions on reliable connections – the exception was the Mt-VPN2 cell, which had a large dendritic field and so may integrate many sparse R7 and R8 inputs (Figure 5 – figure supplement 1). There were 244 cells with >2 synapses, of which 224 were identified to cell type, 16 were identified to cell class, giving a total of 240 identified cells (224 + 16 cells), and the remaining 4 were unidentified (Tables 1 and 3). The 240 identified cells with >2 synapses accounted for 94% (3803/4043) of the total synapses. The 16 cells reconstructed to cell class were sparsely connected, with 4.9 synapses per cell (mean, 79 synapses total). The R7 and R8 cells make infrequent synaptic contacts with glia as well as neurons (Figure 1 – figure supplement 1C), but we did not systematically trace the glia connections.

The 244 cells with >2 synapses were the focus of our analysis, and their connections are summarized in Tables 1 and 3; the connections of individual cells are also organized by cell type for incoming and outgoing synapses in Supplementary File 1. In addition, the morphologies and connections of individual cells with >1 synapses are shown in the gallery figures (Supplementary File 2). Most neurons could be uniquely identified with incomplete reconstructions. Nevertheless, it is useful to establish a reference morphology data and so we completely reconstructed individual examples of specific cell types, in addition to the seed R7

and R8 cells, and in some cases multiple examples. In the central columns, there were 14 completely reconstructed neurons: Dm2, Dm8 (3 cells), Dm9, Dm11, MeTu, Mi15, ML1, ML-VPN1, Tm5a, Tm5b, Tm5c (2 cells). In the DRA, there were 10 completely reconstructed neurons: Dm-DRA1 (4 cells), Dm-DRA2 (3 cells), and MeTuDRA (3 cells). We believe that a number of these cell types we reconstructed have not been previously described: aMe12, ML1, ML-VPN1, and ML-VPN2 in the central columns; MeMe-DRA, MeTu-DRA, DRA-VPN and Mti-DRA in the DRA region. For one cell whose morphology was similar to the described Tm5a cell type, but whose connectivity was markedly different from the others, we annotated this cell as Tm5a-like. Likewise, for three cells whose morphology was similar to the described Tm5b cell type, but whose connectivity was markedly different from that reported by (Menon et al. 2019), we annotated these cells as Tm5b-like. There were numerous tangential cells contacted in the central and DRA columns that do not match those characterized in previous anatomical studies (Fischbach and Dittrich 1989). We have annotated these as medulla tangential intrinsic (Mti) cells when the cells have processes intrinsic to the medulla, and as medulla tangential visual projection neurons (Mt-VPNs) when they project to neuropils outside the medulla.

We additionally identified 970 incoming synapses to the seed column R7-8 cells from 56 cells, including the 14 R7-8 cells. All the 30 cells providing >2 synapses were reconstructed so that we could ascribe a cell type, and these cells provided 96% (936/970) of the total incoming synapses (Tables 2,4). In total, we identified the presynaptic cell type of 99% (963/970) of the synapses onto the R7-8 cells (including the 1-synapse contributing cells). All but 1 of the cells providing >2 synapses were themselves contacted by the seed column R7-8 cells, the exception being a DRA C2 cell that provided 5 synapses (Supplementary File 1). Including this C2 cell, there were 245 cells with >2 input or output synapses with the seed column photoreceptors (244 + 1 cells).

This report was focused on identifying the synaptic inputs and outputs of the R7 and R8 cells of the central and DRA columns, and it was not feasible to also detail the circuit connectivity for the cells targeted by these photoreceptors. For exceptional cell types,

however, we traced more than the cells' connections to the seed column R7 and R8 cells. 1) We traced three aMe12 cells to near completion, to determine the columns that contained the cell type's characteristic vertical processes that we used to determine whether a column was pale or not (see below). 2) We traced all the photoreceptor inputs to three Dm8 cells in the central columns (Figure 2Cii-iii). 3) For the Mt-VPN1 cell, we reconstructed the photoreceptor inputs in 20 medulla columns to discount the possibility that this was a pale-specific neuronal target in the central columns. 4) For three fully reconstructed Dm-DRA1 cells and three fully reconstructed Dm-DRA2 cells we traced all their R7-DRA and R8-DRA inputs (Figure 6E,F). 5) For one Dm9 cell innervated by R7-DRA and R8-DRA, we traced in non-DRA columns to confirm that Dm9 cells in the DRA also receive non-DRA photoreceptor input (Figure 6Cvi).

Defining pale, yellow and DRA medulla columns

To identify medulla columns, we used a map of Mi1 neurons. This Mi1 map, which we have generated for a separate, ongoing study, includes nearly all Mi1 neurons in the medulla of the right hemisphere of the FAFB volume. The Mi1 cell type is columnar, with one cell per column and unambiguous arborizations in medulla layer 1, 5, 9 and 10 (Fischbach and Dittrich 1989). We defined the location of a column in the medulla as the center-of-mass of the Mi1 dendrite in layer 5 (Figure 1C, 2A).

We traced the vertical dendritic branches of all three aMe12 cells in the data set. Their vertical branches innervate pale columns (Figure 1D), and so we labelled the columns they occupied (by proximity to the nearest Mi1-defined column) as pale columns (Figure 1C). The aMe12 cells have short vertical branches reaching from M6 to M3 and longer vertical branches reaching up to M1 (Figure 1Dii, Eiii). Both the long and short vertical processes were used to assign pale medulla columns. Nearly all the assigned pale columns were innervated by one aMe12 cell, but three columns were innervated by two cells. Using this system, the columns not innervated by aMe12 were yellow candidates (Figure 1C). Despite extreme care and multiple reviews of the aMe12 neurons, errors of omission are always possible in the manual reconstruction procedure, and so we are confident that the 'pale' identified columns are

innervated by aMe12, however, it is possible that a small number of the ‘yellow’ columns are mis-classified. The aMe12 cells innervated just 1 of the 42 identified DRA columns (Figure 1C). While most medulla columns innervated by R7-8 are pale or yellow, there are a small number of ommatidial cartridges with other identities, for example rare (~1-2 per fly) ommatidial pairs of R7 and R8 cells expressing Rh3 and Rh6, respectively (Chou et al. 1996). The congruence of the pale- and yellow-specificity we observed for Tm5a, aMe12 and ML-VPN1 cell types indicated that our allocation of pale columns in the central columns was robust, and consistent with the previous report of the yellow-specificity of Tm5a cells (Karuppudurai et al. 2014).

To identify the DRA columns, we reconstructed the R7 and R8 cells in 27 columns along the dorsal-rim-corresponding margin of the medulla and ascertained whether the R8 cell terminated in the same layer as the R7 cell (Figure 1E, arrow; Figure 1 – figure supplement 1A). The R8-DRA cells terminate before the R7-DRA cells in this layer, allowing the two cell types to be distinguished. By careful visual inspection of the M6 layer of all medulla columns located close to the dorsal edge of the medulla we identified 15 additional DRA columns resulting in a total of 42 DRA columns which is in good agreement with the previously reported average number of 39 DRA columns (Weir et al. 2016; Figure 1C).

Analysis of synapse locations

To define the medulla and lobula layers, we first used the R package collection “natverse” (Bates et al. 2020) to transform pre-defined neuropil meshes (Jenett et al. 2012; Bogovic et al. 2019) into the FAFB space. Our meshes for the medulla and lobula computed in this way defined the top and bottom layers of these neuropils. We then interpolated the initial internal layer boundaries using demarcations established in prior studies (Takemura et al. 2015; Wu et al. 2016). Finally, we refined the layer boundaries using the characteristic arborizations patterns of the Mi1, C2, Tm5 and Tm20 cell types (Gao et al. 2008; Fischbach and Dittrich 1989). For the medulla, the demarcations were: 0%, 8.2%, 26.2%, 36.1%, 45.9%, 54.1%, 62.3%, 67.2%, 76.2%, 91.0%, 100%. For the lobula the layer demarcations were: 0%,

4.2%, 11.3%, 22.4%, 37.4%, 49.4%, 64.7%, 100%. The layer designations are therefore guides to aid interpretations and comparisons, and not measurements of the neuropils themselves.

To plot the histograms of the synaptic depths in the medulla (e.g. Figure 1Eiii, we took the projection of the synapse locations along the columnar axis, and smoothed this distribution of synaptic depths with a zero-phase Gaussian filter with a standard deviation of 0.4 μm . For the histograms of distances of R7 synapses to each Dm8 cell's home column (Figure 2Ciii), we took the projection of the synapse locations onto the plane perpendicular to the columnar axis and calculated the distance in that plane to the column center. The resulting distribution of distances was then smoothed with a zero-phase Gaussian filter with a standard deviation of 0.6 μm .

For the histograms of the distances along the DRA of photoreceptor synapses to Dm-DRA1 and Dm-DRA2 cells (Figure 7B, D), we first fitted an ellipse to the centers of DRA columns, using least squares regression (Figure 7 – figure supplement 3Ai). We then calculated the location of the perpendicular projection of every synapse along the fitted ellipse (Figure 7 – figure supplement 3Aii-iv). Finally, these distances were filtered with a zero-phase Gaussian filter with a standard deviation of 0.6 μm .

Comparison with 7-column medulla FIB-SEM data set

To compare the connectivity of R7 and R8 of our central column reconstructions with the connectivity identified in the medulla FIB-SEM data set (Takemura et al. 2013, 2015), we took advantage of the identification of pale and yellow columns in that data set used by (Menon et al. 2019). That study used the presence or absence of Tm5a to indicate yellow and pale columns, respectively, and excluded columns that contained Tm5 neurons that were ambiguous for being Tm5a or Tm5b neurons. Using this scheme, columns B, D and H are pale, and columns A, E and F are yellow (here using the column references used for the 7-column data set). The 7-column medulla data set is now publicly available and accessible with

the release of NeuPrint (Clements et al. 2020), and we used NeuPrint to compile the connectivity of cells with pale and yellow R7 and R8 cells from the data set (Figure 1 – figure supplement 2). The unidentified cells, including cells annotated as output ('out') and fragments of putative tangential cells ('tan') in the six pale and yellow columns are 38.1% for R7 (518/1342 synapses over 6 columns) and 38.9% for R8 (726/1854 synapses over 6 columns).

We also searched the FIB-SEM data set for neurons with shapes and patterns of connectivity similar to aMe12, ML1, and MeTu neurons and identified the following putative matches (NeuPrint medulla7column identifiers in parenthesis): aMe12 (54028), ML1(16666), MeTu (11770; annotated as an unknown Tm; 35751 and other reconstructions annotated as 'Dm7'). The ML1 and MeTu matches are further supported by multiple inputs other than R-cells (e.g. L3, L4 and Dm9 to ML1 and Mi15 to MeTu) that are present in both data sets. These matches provide examples of how the combination of our complete but focused reconstructions and the dense but strongly volume-limited FIB-SEM data can together enable insights that go beyond each individual data set.

Genetics and molecular biology

Fly genotypes

Fly genotypes used throughout the manuscript are listed organized by Figure Panel in Supplementary File 4.

Driver lines

Split-GAL4 lines were constructed and characterized as in previous work (Wu et al. 2016). Briefly, we tested candidate AD and DBD hemidriver pairs (Tirian and Dickson 2017; Dionne et al. 2018). for expression in cell types of interest and assembled successful combinations into stable fly strains that were then used for subsequent experiments. Split-GAL4 lines generated in this study and images of their expression patterns will be made available online (<https://www.janelia.org/split-GAL4>). aMe12, ML1 and ML-VPN1 were initially identified by light microscopy, allowing us to generate split-GAL4 driver lines targeting these

previously undescribed cell types. Images of cells labeled by these driver lines were subsequently matched to the EM reconstructions. Split-GAL4 lines labeling Dm11, Mi15, L2 and VPN-DRA were from prior work (Davis et al. 2020; Tuthill et al. 2013; Wu et al. 2016). The candidate VPN-DRA driver (OL0007B) was originally described as a split-GAL4 driver for a different cell type (LC12; Wu et al. 2016) but also labels neurons highly similar or identical to VPN-DRA. We also used GAL4 driver lines from the Janelia and Vienna Tiles collections (Jenett et al. 2012; Tirian and Dickson 2017).

Rhodopsin-LexA reporter constructs

To construct Rh3-, Rh5- and Rh6-LexA driver lines, we amplified previously characterized promoter regions (Mollereau et al. 2000; Pichaud and Desplan 2001; Tahayato et al. 2003; Chou et al. 1996; Papatsenko et al. 1997; Huber et al. 1997) by PCR from genomic DNA. Primer sequences are listed in the Key Resources Table. The PCR products were TOPO-cloned into pENTR-D-TOPO (Invitrogen) and transferred to [pBPnIsLexA::GADfIUw](#) (addgene #26232) using standard Gateway cloning. Transgenic flies were generated by phiC31-mediated integration into the attP40 landing site (injections were done by Genetic Services, Inc).

Histology

To characterize the neurons labeled by split-GAL4 lines, we visualized both overall expression patterns and individual cells. For the former, we used we pJFRC51-3XUAS-IVS-Syt::smHA in su(Hw)attP1 and pJFRC225-5XUAS-IVS-myr::smFLAG in VK00005 (Nern et al., 2015) or 20XUAS-CsChrimson-mVenus in attP18 (Klapoetke et al. 2014) as reporters; the latter was achieved by stochastic labeling of individual cells with MCFO (Nern et al. 2015). Specimens were processed and imaged by the Janelia FlyLight Project team following protocols that are available online (<https://www.janelia.org/project-team/flylight/protocols> under “IHC - Anti-GFP”, “IHC - Polarity Sequential”, “IHC – MCFO and “DPX mounting”). Additional MCFO images of cells labeled by GAL4 (instead of split-GAL4) driver lines were

generated in the same way. Images were acquired on Zeiss LSM 710 or 780 confocal microscope using 20x 0.8 NA or 63x 1.4 NA objectives.

The specimens shown in Figures 7Aiii, 7Ciii, 8Aii and 8v were prepared and imaged as previously described (Sancer et al 2019).

For the combined labeling of aMe12, ML-VPN1 and VPN-DRA with photoreceptor markers, fly brains were processed using standard immunolabeling protocols as previously described in (Davis et al. 2020). Briefly, flies were dissected in insect cell culture medium (Schneider's Insect Medium, Sigma Aldrich, #S0146) followed by fixation with 2% PFA (w/v; prepared from a 20% stock solution, Electron Microscopy Sciences: 15713) in cell culture medium for 1 h at room temperature. This fixation step and the subsequent primary and secondary antibody incubations were each followed by several washes with PBT (0.5 % v/v TX-100, Sigma Aldrich: X100, in PBS). To block nonspecific antibody binding, brains were incubated in PBT-NGS (5% Goat Serum ThermoFisher: 16210-064 in PBT) for at least 30 min prior to addition of primary antibodies in PBT-NGS. Primary and secondary antibody incubations were at 4°C overnight, all other steps were at room temperature. Brains were mounted in SlowFadeGold (ThermoFisher: S36937).

Primary antibodies were anti-GFP rabbit polyclonal (ThermoFisher: A-11122, RRID:AB_221569; used at 1:1000 dilution), anti-GFP mouse monoclonal 3E6 (ThermoFisher: A-11120, RRID:AB_221568; dilution 1:100), anti-dsRed rabbit polyclonal (Clontech Laboratories, Inc.: 632496, RRID:AB_10013483; dilution 1:1000), anti-chaoptin mouse monoclonal 24B10 (Fujita et al., 1982; DSHB: RRID:AB_528161, dilution 1:20) and anti-Brp mouse monoclonal nc82 (Wagh et al., 2006; DSHB:RRID:AB_2314866; dilution 1:30). Secondary antibodies were from Jackson ImmunoResearch Laboratories, Inc. Images were acquired on a Zeiss LSM 880 or confocal microscope using a 40x 1.3 NA objective.

Image processing

Most anatomy figures show reconstructed views of cells of interest generated from confocal stack using FluoRender (<http://www.sci.utah.edu/software/fluorender.html>). Some

1163 images were manually edited to only show the cell relevant for the comparison to the EM
 1164 reconstructions. Some panels in Figure 5 are overlays of registered images with either the
 1165 template brain used for registration (Bogovic et al. 2019) or the pattern of a second registered
 1166 expression pattern (L2 lamina neurons terminal in Figure 5Cv). Images with rhodopsin reporter
 1167 (Rh3-, Rh5 or Rh6-LexA) labeling show single sections or maximum intensity projections
 1168 through a small number of adjacent slices. For these images, other processing was limited to
 1169 adjustments of brightness and contrast across the entire field of view for each channel.

Acknowledgements

The authors thank: Lou Scheffer, Shinya Takemura, and Kazunori Shinomiya for access to data, advice and discussions; Ruchi Parekh for managing the CAT team and coordinating the tracing effort; the FAFB tracing community for supportive and open sharing of methods and data; Janelia Fly Core for fly care and Janelia FlyLight Project Team for help with preparation and imaging of light microscopy samples; and the Janelia Visitor program for facilitating early stages of this collaboration. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) through grants WE 5761/2-1 (M.F.W.) and SPP2205 (M.F.W.), AFOSR grant FA9550-19-1-7005 (M.F.W), with support from the Fachbereich Biologie, Chemie & Pharmazie of the Freie Universität Berlin (M.F.W.) and by the Howard Hughes Medical Institute through its support of the Janelia Research Campus (D.D.B, M.B.R. and G.M.R.).

Author Contributions

Emil Kind: Conceptualization, Methodology - neuronal reconstructions, Methodology – analysis, Software, Validation, Formal Analysis, Investigation – light microscopy, Investigation – neuronal reconstructions, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization.

Kit D. Longden: Methodology – analysis, Validation, Formal Analysis, Writing – original draft preparation, Writing – review and editing, Visualization.

Aljoscha Nern: Conceptualization, Methodology - neuronal reconstructions, Methodology – analysis, Validation, Formal Analysis, Investigation – light microscopy, Resources, Writing – original draft preparation, Writing – review and editing, Visualization.

1196 Arthur Zhao: Methodology - neuronal reconstructions, Methodology – analysis, Software,
 1197 Validation, Formal Analysis, Data Curation, Writing – original draft preparation, Writing –
 1198 review and editing, Visualization.
 1199
 1200 Gizem Sancer: Methodology - neuronal reconstructions, Investigation – light microscopy,
 1201 Investigation – neuronal reconstructions.
 1202
 1203 Miriam Flynn: Methodology - neuronal reconstructions, Investigation – neuronal
 1204 reconstructions.
 1205
 1206 Connor Laughland: Methodology - neuronal reconstructions, Investigation – neuronal
 1207 reconstructions.
 1208
 1209 Bruck Gezahegn: Methodology - neuronal reconstructions, Investigation – neuronal
 1210 reconstructions.
 1211
 1212 Henrique Ludwig: Methodology - neuronal reconstructions, Investigation – neuronal
 1213 reconstructions.
 1214
 1215 Alex Thomson: Methodology - neuronal reconstructions, Investigation – neuronal
 1216 reconstructions.
 1217
 1218 Tessa Obrusnik: Methodology - neuronal reconstructions, Investigation – neuronal
 1219 reconstructions.
 1220
 1221 Paula G. Alarcón: Methodology - neuronal reconstructions, Investigation – light microscopy,
 1222 Investigation – neuronal reconstructions.
 1223

1224 Heather Dionne: Investigation – molecular constructs, Resources.
1225
1226 Davi D. Bock: Methodology - neuronal reconstructions, Resources, Data Curation, Funding
1227 Acquisition.
1228
1229 Gerald M. Rubin: Resources, Writing – review and editing, Supervision, Funding Acquisition.
1230
1231 Michael B. Reiser: Conceptualization, Methodology - neuronal reconstructions, Methodology
1232 – analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review
1233 and editing, Visualization, Supervision, Project Administration, Funding Acquisition.
1234
1235 Mathias F. Wernet: Conceptualization, Methodology - neuronal reconstructions,
1236 Methodology – analysis, Resources, Writing – original draft preparation, Writing – review
1237 and editing, Visualization, Supervision, Project Administration, Funding Acquisition.

1238 **Tables**

1239 Table 1: Synaptic targets of seed column R7 and R8

1240 Table 2: Cells that synapse onto seed column R7 and R8

1241 Table 3: Synaptic targets of seed column R7-DRA and R8-DRA

1242 Table 4: Cells that synapse onto seed column R7-DRA and R8-DRA

1243

1244 **Supplementary Files**

1245 Supplementary File 1:

1246 Tables of all seed column R7, R8, R7-DRA and R8-DRA target cells by type

1247 Supplementary File 2:

1248 Gallery plots of all seed column R7, R8, R7-DRA and R8-DRA target cells by type

1249 Supplementary File 3

1250 Tables of seed column synapses outside the medulla

1251 Supplementary File 4

1252 Fly genotypes organized by Figure panel

Figure 1

Figure 1: Systematic reconstruction of all synaptic targets of inner photoreceptor subtypes

A. Simplified schematic summarizing some of the most salient visual stimuli of a fly (center): celestial cues (sun), color gradients (distribution of green versus UV wavelengths) and skylight polarization (as defined by degree of polarization and angle of polarization) can be used for navigation, as well as more or less colorful landmarks (tree). **B.** Schematic representation of the fly visual system. Left: In the retina, inner photoreceptor R7 (distal) and R8 (proximal) rhodopsin expression differs across three functionally specialized subtypes pale (p), yellow (y), and DRA (d). Rh3 and Rh4 opsins are both UV-sensitive, whereas Rh5 and Rh6 are more sensitive to blue and green wavelengths, respectively. R1-6 express Rh1 and are broadly sensitive to green and UV wavelengths. Only in the DRA, both R7 and R8 axons terminate in the same layer of the medulla neuropil (M6), which is the target layer of R7 cells outside the DRA, and non-DRA R8 cells terminate in layer M3. Right: Overview of the main four optic lobe neuropils. Only the lamina and medulla receive direct photoreceptor input. **C.** Distribution of medulla columns downstream of either p (light blue), y (yellow), and DRA (red) photoreceptors reconstructed from the FAFB data set. Pale columns were assigned via presence of aMe12 long vertical projections (see below). Seven seed columns used for systematic reconstruction are highlighted with black circles. The colored axes indicate the orientation of the displayed view: green for lateral, magenta for dorsal, and blue for posterior axes. Scale bar: 20 μ m. **D.** Double labeling of aMe12 vertical processes and yellow R8 cells. See Materials and Methods and Supplementary File 4 for fly genotypes and other details of this and other light microscopy panels. Left (Di): Confocal image showing the array of medulla columns with labeling of aMe12 neurons (purple) and yellow R8 axons (green). Note that the two patterns appear near-mutually exclusive. Right (Dii): Side view of aMe12 vertical projections and pale R7 axons (green). Di-ii Scale bars: 20 μ m. **E.** Left (Ei): Skeleton of the optic lobe part of a fully reconstructed aMe12 neuron (gray), with processes leaving the medulla through the accessory medulla (pink). Eii: Across the four central seed columns (darker shading), synaptic input from R7 (purple dots) and R8 photoreceptors (green dots) to aMe12 is specific to the

designated pale columns. Eiii, Left: Sideview depicting the distribution of R7 and R8 inputs into aMe12 across medulla layers (color code of pale and yellow columns as before). Right: Same distribution plotted as synapses/ μm , for both pale and yellow columns (color code as before). Ei-iii: Scale bars: 10 μm . **F.** Reconstructed pale R7 and R8, yellow R7 and R8, and R7-DRA and R8-DRA terminals with R7 presynapses in purple and R8 presynapses in green. Note the termination of R8-DRA in the R7 target layer M6 (arrow). Scale bars: 10 μm . **G.** Illustrations of reconstructed neuron classes, including lamina monopolar (L), distal medulla (Dm), medulla intrinsic (Mi), transmedulla (Tm), medulla tangential intrinsic (Mti), visual projection neurons targeting the central brain (example: aMe12), and medulla-to-tubercle (MeTu) cells projecting to the anterior optic tubercle (AOTU). **H.** For the synapses from seed column inner photoreceptors to cells with ≥ 3 synapses, we identified the cell type or cell class of 99.5% (3803/3821 synapses). Overview of the relative strength of these connections with different neuron classes (color code as before), including with the unidentified cells (Tables 1-4), across pale, yellow, and DRA columns (pre- and postsynaptic). Area of circles corresponds to the number of synapses per R7 and R8 cell.

Figure 1 – figure supplement 1

Figure 1 - figure supplement 1: Example EM-images

A. Example EM-images of photoreceptor synapses in medulla layer 6. Top: R7 synapse (T-bar labelled) with postsynaptic Dm2, Dm8, Dm9 and Tm5b cells. Bottom: R8-DRA synapse, with postsynaptic Dm9 and Dm-DRA2 cells. R8 synapses in layer 6 were diagnostic of DRA columns (see Materials and Methods). **B.** Images of synapses outside the medulla in the central (top) and DRA (bottom) seed columns. **C.** Two examples of photoreceptor synapses with glia, with the arrows indicating the postsynaptic density in the glial cell. Scale bars: 500 μm .

Figure 1 – figure supplement 2

Figure 1 – figure supplement 2: Comparison with medulla FIB-SEM data

A. Left: Comparison of total numbers of synaptic inputs to R7 (purple, circles) and R8 (green, squares) in our data set (filled symbols) and a medulla FIB-SEM data set (open symbols), with mean values are indicated by black symbols. These plotting conventions are used throughout the figure. We used the six columns identified as pale and yellow in the FIB-SEM data set (Takemura et al. 2015) by Menon et al. (2019), made available in Clements et al. (2020), see Materials and Methods. Right: The R7-8 photoreceptors and Dm9 inputs dominate the synaptic input to R7 (purple, circles) and to R8 (green, squares); the increased number of synapses outside medulla (see panel G) likely accounts for the higher number of inputs to our R7-8 cells. **B.** Comparison of the total number of output synapses from R7 and R8. We reconstructed more output synapses, per cell, than in the FIB-SEM data set. **C.** Comparison of total numbers of R7-8 output synapses disaggregated by cell classes. To enable like-for-like comparisons, we used only cell classes and cell types annotated in both data sets, with the cell types shown in D. To quantify the completeness of cell type identification, we counted all other identified cell types found each data set in the ‘Other’ category. Our reconstruction found a large increase in R7 synapses to multicolumnar Dm cells, and increases in R8 synapses to multicolumnar cell classes, such as Tm cells and cells like aMe12 in the ‘Other’ class. **D.** Comparison of total numbers of R7 (top) and R8 (bottom) output synapses disaggregated by cell type, with cell types grouped from left to right by their cell class categories in C. For R7, there were increases in synapses to R8 and L3, which receive synapses outside the medulla, and Dm8 cells, which are multicolumnar. For R8, there were increases in synapses to R7 and Mi15, which receive synapses outside the medulla, and multicolumnar Tm5c and ‘Other’ cell types, and also Tm20 cells, which may be surprising because this cell type is columnar. **E.** Comparison of R7-8 synapses to (FIB-SEM) Dm7 and (current study) MeTu cells. These two cell types were the only cell type discrepancies between the data sets: Dm7 cells were major targets of R7 in the FIB-SEM data, but not in ours, while MeTu cells were major targets in our data but not in the FIB-SEM data. **F.** Example Tm cell (brown, Tm #11770) in the FIB-SEM data whose dendritic morphology matched MeTu cell.

1333 Two R7 cells (green, left, and purple, right), are also shown, along with R7 synapses (green
 1334 circles) and Mi15 synapses (purple). We propose that some MeTu cells are included in the
 1335 FIB-SEM data but are currently annotated as unidentified Tm or Dm7 cells. **G.** Percentages
 1336 of synapses outside the medulla for R7 (left) and R8 (right) with cell types in the central (top)
 1337 and DRA (bottom) seed columns. L3 in particular is almost exclusively innervated outside the
 1338 medulla. Tables of synapses outside the medulla are listed for each cell type in Supplementary
 1339 File 3.

Figure 2

Figure 2: Synapses between R7 and R8, and with Dm neurons in the central medulla

A. Synapses between central R7 and R8 cells. Left: Side view of R7 (purple) and R8 (green), with R7→R8 synapses (purple points) and R8→R7 synapses (green points). Note synapses outside the medulla neuropil (distal to layer M1). Scale bar: 10 μm. Right: Synapse distribution (in synapses/μm) in the yellow and pale seed columns. **B.** Synapses between R7 and R8 and Dm9 cells. Bi: Top view of a fully reconstructed Dm9 skeleton (grey) covering all four central seed columns (darker shading) with all R7 (purple) and R8 (green) inputs. Bii, Left: Side view of R7 (purple) and R8 synapses (green) to the same Dm9 cell across medulla layers. Right: Layer distribution of photoreceptor inputs to six Dm9 cells. Biii, Left: Side view of Dm9→R7 (purple) and Dm9→R8 (green) feedback synapses from the same Dm9 cell. Right: Layer distribution of feedback synapses from six Dm9 occupying the seed columns. Most of these synapses are from the illustrated Dm9 cell with other Dm9 cells contributing a small number of connections in M1 and M6. **C.** Synapses between R7 and Dm8 cells. Ci, Left: Side view of R7 synapses (purple) to a fully reconstructed Dm8 cell, with one pale R7 cell is shown in purple. Note the characteristic vertical projections of Dm8 in its 'home column' (arrow; yellow R7 cell not shown). Right: Layer distribution of R7 inputs from 14 Dm8 cells innervating the four seed columns. Cii: Full reconstructions of three Dm8 cells (labeled 1,2,3) innervating the four seed columns, with all R7 synapses (purple), including inputs beyond the seed columns. Individual Dm8 home columns (h) are marked. Ci-ii Scale bars: 10 μm. Ciii: R7→Dm8 synapses/μm as a function of their distance from the home column, for the three Dm8 cells (1,2,3 above). The cells receive dense innervation in their home column and weaker inputs from both pale and yellow R7 cells in their periphery. **D.** Synapses between R7 and Dm11 cells with R7 (purple) and R8 (green) synapses from seed columns. Di: Top view of a reconstructed Dm11 skeleton. Dii, Left: Side view of the layer distribution of photoreceptor synapses, including synapses outside the medulla (arrow). Di-ii Scale bars: 10 μm. Right: Layer distribution of R7 and R8 inputs to three Dm11 cells. Diii: Rendering of a confocal image of MultiColor FlpOut (MCFO) labelled Dm11 cells. Note the characteristic vertical projections

1367 leaving the medulla neuropil (arrow). The dashed line marks the approximate boundary of the
 1368 medulla neuropil. Scale bar: 20 μ m. **E.** Synapses between R7 and R8 and Dm2 cells. Ei: Top
 1369 view of a Dm2 skeleton, with R7 (purple) and R8 (green) synapses. Eii, Left: Side view of layer
 1370 distribution of photoreceptor synapses onto the same Dm2 cell. Ei-ii Scale bars: 10 μ m. Right:
 1371 Layer distribution of R7 and R8 inputs to four Dm2 cells.
 1372

Figure 2 – figure supplement 1

1373 Figure 2 – figure supplement 1: Lamina cell types targeted by central column R7-8

1374 **A.** Left: Side view of a reconstructed L1 cell (gray) with R8→L1 synapses (green). Right:

1375 Layer distribution (in synapses/μm) of all R7 and R8 inputs to 4 L1 cells. **B.** Left: Side view of

1376 a reconstructed L3 cell (gray), with R7→L3 (purple) and R8→L3 synapses (green). Right:

1377 Layer distribution (in synapses/μm) of all R7 and R8 inputs to 4 L3 cells. **C.** Left: Side view of

1378 a reconstructed C2 cell (gray), with R7→C2 (purple) and R8→C2 synapses (green). Right:

1379 Layer distribution (in synapses/μm) of all R7 and R8 inputs to 2 C2 cells. **A-C.** All scale bars:

1380 10 μm.

Figure 3

Figure 3: R7-8 synapses with medulla intrinsic (Mi) and medulla tangential intrinsic (Mti) cell types

A. Synapses of central R7 and R8 with Mti1 cells. Ai: Top view of an Mti1 skeleton with synapses from seed column R8 cells (green). Scale bar: 10 μ m. Aii: Top view of an MCFO-labeled cell matching overall Mti1 morphology. Light microscopy images (Aii,Aiv,Bii,Biv,Ciii) were manually segmented to focus on the cells of interest. Scale bar: 20 μ m. Aiii: Side view of the same Mti1 cell as in Ai with R8 (green) synapses. Scale bar: 10 μ m. Aiv: Side view rendering of the same light microscopy Mti1 image (green). Scale bar: 20 μ m. **B.** Synapses from R7 and R8 to Mti2. Bi: Top view of a reconstructed Mti2 cell, with R7 (purple) and R8 (green) synapses. Scale bar: 10 μ m. Bii: Top view of an MCFO-labeled cell matching overall Mti2 morphology. Scale bar: 20 μ m. Biii: Side view of the same Mti2 skeleton as in Bi with R7 (purple) and R8 (green) synapses on characteristic vertical projections (arrow). Scale bar: 10 μ m. Biv: Side view of the cell shown in Bii. Arrow indicates vertical projections. Scale bar: 20 μ m. Bv: Top view of a second reconstructed Mti2 cell (dark gray) overlaid on the first (light gray), with R8 (green) synapses. Bvi: Side view of the second Mti2 skeleton, with R8 (green) synapses again located on vertical projections (arrow). Bv-vi Scale bars: 10 μ m. **C.** Synapses of R7 and R8 with Mi15 cells. Ci: Top view of a fully reconstructed Mi15 skeleton, with R7 (purple) and R8 (green) synapses. Cii, left: Side view depicting the layer distribution of photoreceptor synapses (same color code) onto the same Mi15 skeleton, with synapses outside the medulla (arrow). Ci-ii Scale bars: 10 μ m. Right: Layer distribution of R7 and R8 inputs to four Mi15 cells. Ciii: Side view rendering of a light microscopy image (MCFO) of an Mi15 cell. Note the long process leaving the medulla (arrow). Scale bar: 10 μ m.

Figure 3 – figure supplement 1

Figure 3 – figure supplement 1: Mi cell types targeted by R7-8

A. Colocalization of R8→Mi15 synapses with R8→R7 synapses. Ai. Example EM-image of Mi15 and R7 cells as postsynaptic partners of the same R8 presynapse. Scale bar: 1 μ m. Aii. Magnified view of skeletons of R8, Mi15, and R7 cells in the optic chiasm, illustrating the colocalization of R8→Mi15 synapses with R8→R7 synapses at 5 successive locations. Scale bar: 1 μ m. **B.** Left: Side view of a reconstructed Mi1 cell (gray) with R7→Mi1 (purple) and R8→Mi1 synapses (green). Right: Layer distribution (in synapses/ μ m) of all R7 and R8 inputs to 4 Mi1 cells. **C.** Left: Side view of a reconstructed Mi4 cell (gray) with R7→Mi4 (purple) and R8→Mi4 synapses (green). Right: Layer distribution (in synapses/ μ m) of all R7 and R8 inputs to 4 Mi4 cells. **D.** Left: Side view of a reconstructed Mi9 cell (gray) with R7→Mi9 (purple) and R8→Mi9 synapses (green). Right: Layer distribution (in synapses/ μ m) of all R7 and R8 inputs to 4 Mi9 cells. In the pale seed columns, Mi9 cells received many R8 synapses, but only received 1 R8 synapse in the yellow seed columns. **B-D.** Scale bars: 10 μ m.

Figure 4

Figure 4: R7-8 synapses with cell types projecting to the lobula

A-D. Synapses between R7 and R8 and Tm5a (A), Tm5b (B), Tm5c (C) and ML1 (D) cell types. Panels **i-iii**: Anatomy of fully reconstructed cells. **i**: Side view. **ii**: Top view. **iii**, Left: Side view of axon terminals in the lobula. Right: Side view of medulla branches. R7 synapses are indicated in purple, R8 synapses in green in **ii**, **iii** (Right). **iv**: Layer distribution of R7 and R8 inputs. Scale bars: 10 μ m. **A.** Tm5a. The two Tm5a cells were exclusively targeted by yellow R7 cells. **B.** Tm5b. The two Tm5b cells were highly biased to pale R7 inputs. **C.** Tm5c. The five Tm5c cells were highly biased to yellow R8 inputs. **D.** ML1. Dv: Side view of a single MCFO-labeled ML1 cell (light microscopy). Note this cell has terminals both at the base of the deepest lobula layer and in the central brain; not all examined ML1 cells have terminals in the central brain. Scale bar: 20 μ m. Dvi: Side view showing the distribution of a membrane marker (green) and presynaptic marker (synaptotagmin-HA, purple) in ML1 cells imaged as a population. The combined patterns (Left), and the presynaptic marker alone (Right) are shown. Arrow indicates central brain terminals. Scale bar: 20 μ m.

Figure 4 – figure supplement 1

Figure 4 – figure supplement 1: Additional connections of R7-8 to cell types projecting to the lobula

A. R7 and R8 connections with the Tm5a-like cell, that has a morphology similar to a Tm5a cell, but a different pattern of connectivity. The same reconstructed cell is shown in Ai-iii. Ai: Side view. Aii: Top view with R7 (purple) and R8 (green) synapses. Aiii: Left: The axon terminals in the lobula. Right: Side view with R7 (purple) and R8 (green) synapses. Aiv: Layer distribution (in synapses/ μm) of all R7 and R8 inputs from four seed columns to the Tm5a-like cell. **B.** R7 and R8 connections with three Tm5b-like cells, that each have a morphology similar to a Tm5b cell, but a different, shared pattern of connectivity. The same reconstructed Tm5b-like cell is shown in Bi-iii. Bi: Side view. Bii: Top view with R7 (purple) and R8 (green) synapses. Biii: Left: The axon terminals in the lobula. Right: Side view with R7 (purple) and R8 (green) synapses. Biv: Layer distribution (in synapses/ μm) of all R7 and R8 inputs from four seed columns to the three Tm5b-like cells. Unlike the Tm5b cells, the Tm5b-like cells are predominantly targeted by yellow R8 photoreceptors. **C.** R7 and R8 connections with Tm20 cells. The same reconstructed Tm20 cell is shown in Ci-iii. Ci: Side view. Cii: Top view with R7 (purple) and R8 (green) synapses. Ciii: Left: The axon terminals in the lobula. Right: Side view with R7 (purple) and R8 (green) synapses. Civ: Layer distribution (in synapses/ μm) of all R7 and R8 inputs from four seed columns to the four Tm20 cells. **A-C.** Scale bars: 10 μm .

Figure 4 – figure supplement 2

Figure 4 – figure supplement 2: ML1 arbor distribution in the medulla and lobula

Scale bars in (Ai,Aii) represent 20 μ m. The other panels are shown at a similar scale. Images show views generated from image stacks (see Materials and Methods). Images show a top view of the array of column in the lobula (top panels, lower part of each image) or the medulla (lower panels), respectively. **A.** As a population, ML1 cells appear not to cover all visual columns in the lobula (Ai) but do cover the entire medulla (Aii). Note absence of the green ML1 membrane label near the dorsal and ventral margin of the lobula but not the medulla. **B,C.** Lobula (Bi,Ci) and medulla (Bii,Cii) arbors of two groups of MCFO-labeled ML1 cells. The retinotopic array of ML1 cells in the medulla (Bii,Cii) is only partly reflected in the positions of their axon terminals (Bi,Ci) in or near the lobula. For example, cells near the medulla edge have terminals away from the margin of the lobula.

Figure 5

Figure 5: Visual projection neurons connecting R7-8 with the central brain

A,B,E. Synapses between R7 and R8 and MeTu (A), ML-VPN1 (B) and Mt-VPN1 (E) cells. Panels **i-iii**: Anatomy of reconstructed cells. **i**: Side view. **ii**: Top view. **iii**: Side view of medulla branches. R7 synapses are indicated in purple, R8 synapses in green in **ii**, **iii**. **iv**: Layer distribution of R7 and R8 inputs. Scale bars: 10 μ m. **A.** The MeTu cell shown was fully reconstructed. **Ai.** Inset shows a magnified view of axon projections to the AOTU. **Aiv:** All seven MeTu cells were exclusively R7 targets. **B.** The ML-VPN1 cell shown was fully reconstructed. **Bi.** ML-VPN1 cells project to the PLP. **Biv.** Both ML-VPN1 cells were pale R8 targets. **Ci-vi:** Light microscopy of ML-VPN1 anatomy. **Ci,Cii:** Side view (**Ci**) and top view (**Cii**) of the population of ML-VPN1 cells. **Ciii:** Side view of a single MCFO-labeled ML-VPN1 cells. **Civ:** Top view of multiple MCFO-labeled ML-VPN1 cells. **Cv:** Overlay of arbors of a single cell ML-VPN1 cell with L2 terminals (gray) indicating medulla columns. Images in **Ci-Cv** show overlays of aligned confocal images with the standard brain used for registration (**Ci-iv**) or a second registered image showing L2 terminals (**Cv**). **Cvi:** Confocal substack projection showing medulla columns at approximately the level of R8 terminals. ML-VPN1 (red) and photoreceptor axons (blue) are shown without (top) and with (bottom) labelling of yellow R8 axons (Rh6 > green). Overlap between ML1 and photoreceptors is largely limited to pale columns (i.e. columns without the Rh6 marker). **Ci-vi** Scale bars: 20 μ m. **D.** Reconstructions of three aMe12 cells (orange, turquoise, purple) covering the entire medulla, with axons leaving via the accessory medulla and innervating the mushroom body calyces (Calyx) and the PLP and accessory medulla (aMe) of both hemispheres. **Di:** Frontal view. **Dii:** Dorsal view. **E.** Mt-VPN1 cells. **Eiv:** Tracing of Mt-VPN1 photoreceptor synapses in 16 additional columns did not confirm yellow specificity found in the seed columns.

Figure 5 – figure supplement 1

Figure 5 – figure supplement 1: Pale-specificity of ML-VPN1

A. Side view of R7 and R8 photoreceptors (blue) and ML-VPN1 cells (red), without (Ai) and with (Aii) Rh5 expression (green), from confocal microscopy. Ai. Four vertical processes that traverse layers 1-3 are labelled by arrows; in our EM reconstructions the photoreceptor synapses were largely located on such characteristic processes (Figure 5Biii). Most vertical processes that followed photoreceptors (long arrows, left) were in pale (Rh5-positive) columns; vertical processes that did not follow photoreceptors (short arrows, right) were frequently found in yellow (Rh5-negative) columns or could not be readily assigned to a column. **B.** Projection through a few adjacent confocal slices showing ML-VPN1 and photoreceptor axons at approximately the level of R8 terminals, with color-coded image channels as in A, without (Bi) and with (Bii) pale R8 columns labelled by the Rh5 marker. Overlap between ML1 and R-cells is largely limited to pale columns. For example, out of 43 putative overlaps circled (Biii), only four did not overlap with pale R8 expression (Biv, yellow thick circles), indicating that 93% of putative innervated columns were pale in this image. **A-B** Scale bars: 20 μ m.

Figure 5 – figure supplement 2

Figure 5 – figure supplement 2: All additional neurons connecting R7-8 to the central brain with ≥ 3 synapses.

A. R7 and R8 connections with a neuron matching the anatomy of a known octopaminergic cell type (OA-AL2i3, skid 14286406). The same reconstructed cell is shown in Ai-iii. Ai: Side view, showing the projections to the flange (Fla), inferior posterior slope (IPS), and superior posterior slope (SPS). Aii: Top view with R7 (purple) and R8 (green) synapses. Aiii: Side view with R7 (purple) and R8 (green) synapses. The cell has processes leaving the medulla that track the photoreceptors, where it received synaptic input in our seed columns. Synapse totals: 1 yellow R7, 3 yellow R8. **B.** R8 connections with the reconstructed Mt-VPN cell with skid 11453464. Bi: Side view, showing the projection to the SPS. Bii: Top view with R8 (green) synapses. Biii: Side view with R8 (green) synapses. Synapse totals: 11 yellow R7. **C.** R8 connections with the reconstructed Mt-VPN cell with skid 11469481l. Ci: Side view, showing the projections to the lobula (Lob) and accessory medulla (aMe). Cii: Top view with R8 (green) synapses. Ciii: Side view with R8 (green) synapses. Synapse totals: 5 yellow R8. **D.** R8 connections with the reconstructed Mt-VPN cell with skid 3509520, which is also a heterolateral lobula late tangential cell. Di: Side view, showing the projections to the lobula plate (LP) and IPS in both hemispheres. Dii: Top view with R8 (green) synapses. Diii: Side view with R8 (green) synapses. Synapse totals: 3 pale R8. **A-D.** Scale bars: 10 μm .

Figure 6

Ai R7-DRA & R8-DRA

Bi Dm9

Bii

Ci Dm2

Cii

Biii

Biv

Di Mi15

Dii

Ei Tm20

Eii

Figure 6: Synapses between R7-DRA and R8-DRA, and with medulla cell types

A. Reciprocal synapses between R7-DRA and R8-DRA. Left: Side view of an R7- and R8-DRA cell with R7-DRA→R8-DRA (purple points) and R8-DRA→R7-DRA (green points) Right: Layer distribution of R7-DRA and R8-DRA reciprocal synapses in three columns. **B.** Reciprocal synapses between Dm9 in the DRA region and R7-DRA and R8-DRA. Bi, Left: Side view of a reconstructed Dm9 skeleton (grey) in the DRA region, with R7-DRA→Dm9 synapses (purple) and R8-DRA→Dm9 synapses (green). Right: Layer distribution of DRA photoreceptor inputs into Dm9. Bii: Top view of Dm9 cell that connects to photoreceptors in both DRA (light red) and non-DRA columns (grey). Biii, Left: Feedback synapses from Dm9 to R7-DRA (purple) and R8-DRA (green). Right: Layer distribution of Dm9 inputs into R7-DRA and R8-DRA. Biv: DRA and non-DRA R7 (purple) and R8 (green) synapses to the same Dm9 cell. **C.** Synapses between R7-DRA, R8-DRA and Dm2. Ci, Left: Side view of a Dm2 cell with synapses from R7-DRA (purple) and R8-DRA (green). Right: Layer distribution of R7-DRA and R8-DRA synapses onto 4 Dm2 cells. Cii: Top view of Dm2 skeleton in the DRA region. **D.** Synapses between R7-DRA, R8-DRA and Mi15. Di, Left: Side view of a reconstructed Mi15 cell with R7-DRA→Mi15 synapses (purple) and R8-DRA→Mi15 synapses (green). Right: Layer distribution of R7-DRA and R8-DRA synapses to 4 Mi15 cells. Dii: Top view of Mi15 skeleton in the DRA region. **E.** Synapses between R7-DRA, R8-DRA and Tm20. Ei, Left: Side view of a reconstructed Tm20 cell with R8-DRA→Tm20 synapses in green. Right: Layer distribution of R8-DRA input to 3 Tm20 cells. Eii: Top view of Tm20 skeleton in the DRA region. (All scale bars: 10 μm).

Figure 6 – figure supplement 1

1540 Figure 6 - figure supplement 1: Synapses between inner photoreceptors in both the central
1541 and DRA columns
1542 **A.** Total number of R7 synapses onto R8 for all full reconstructed cells in the central columns
1543 (4) and R7-DRA onto R8-DRA in the DRA columns (3). **B.** Total number of R8 synapses onto
1544 R7 for all full reconstructed cells in the central columns (4) and R8-DRA onto R7-DRA in the
1545 DRA columns (3). **C.** Fraction of photoreceptor-to-photoreceptor synapses of the total output
1546 of R7 and R8 in the central columns, and of R7-DRA and R8-DRA in the DRA columns.
1547 Individual counts in colored circles mean for each group in black circle.

Figure 6 – figure supplement 2

Figure 6 – figure supplement 2: Additional cell types connected to R7-DRA and R8-DRA

A. Synapses between R7-DRA, R8-DRA and L1. Ai, Left: Side view of a reconstructed L1 skeleton (dark grey), together with R7-DRA (purple) and R8-DRA (green) from the same column plus R7-DRA→L1 synapses (purple) and R8-DRA→L1 synapses (green). Right: Synapse distribution (in synapses/μm) of all three reconstructed L1 cells from the three DRA seed columns across medulla layers. Aii Top view of the same L1 cell with R7-DRA→L1 synapses (purple) and R8-DRA→L1 synapses (green). **B.** Synapses between R7-DRA, R8-DRA and L3. Bi, Left: Side view of a reconstructed L3 skeleton (dark grey), together with R7-DRA (purple) and R8-DRA (green) from the same column plus R7-DRA→L3 synapses (purple) and R8-DRA→L3 synapses (green). Right: Synapse distribution (in synapses/μm) of all three reconstructed L3 cells from the three DRA seed columns across medulla layers. Bii Top view of the same L3 cell with R7-DRA→L3 synapses (purple) and R8-DRA→L3 synapses (green). **C.** Synapses between R7-DRA, R8-DRA and Mi1. Ci, Left: Side view of a reconstructed Mi1 skeleton (dark grey), together with R7-DRA (purple) and R8-DRA (green) from the same column plus R7-DRA→Mi1 synapses (purple) and R8-DRA→Mi1 synapses (green). Right: Synapse distribution (in synapses/μm) of all three reconstructed Mi1 cells from the three DRA seed columns across medulla layers. Cii Top view of the same Mi1 cell with R7-DRA→Mi1 synapses (purple) and R8-DRA→Mi1 synapses (green). **D.** Synapses between R7-DRA, R8-DRA and Dm11. Di, Left: Side view of a reconstructed Dm11 skeleton (dark grey), together with R7-DRA (purple) and R8-DRA (green) plus R7-DRA→Dm11 synapses (purple) and R8-DRA→Dm11 synapses (green). Right: Synapse distribution (in synapses/μm) of one reconstructed Dm11 cell across medulla layers. Note that synapses between R7-DRA and R8-DRA→Dm11 were only found in one of the three DRA seed columns. Dii Top view of the same Dm11 cell with R7-DRA→Dm11 synapses (purple) and R8-DRA→Dm11 synapses (green). **E.** Synapses between R8-DRA and Mi9. Ei, Left: Side view of a reconstructed Mi9 skeleton (dark grey), together with R8-DRA (green) from the same column plus R8-DRA→Mi9 synapses (green). Right: Synapse distribution (in synapses/μm) of two reconstructed Mi9 cells

1575 across medulla layers. Note that only in two of the three DRA seed columns R8-DRA→Mi9
 1576 synapses were found. Eii Top view of the same Mi9 cell with R8-DRA→Mi9 synapses (green).
 1577 **F. Synapses between R8-DRA and a Tm5-like cell.** Fi, Left: Side view of a reconstructed Tm5-
 1578 like skeleton (dark grey), together with R8-DRA (green) plus R8-DRA→ Tm5-like synapses
 1579 (green). Right: Synapse distribution (in synapses/μm) of one Tm5-like cell across medulla
 1580 layers. Note that in only one of the three DRA seed columns was such a cell found. Fii: Top
 1581 view of the same Tm5-like cell with R8-DRA→Tm5-like synapses (green). Fiii: Lobula
 1582 innervation of the Tm5-like cell.

Figure 7

Figure 7: Dm8-like photoreceptor targets in the DRA region

A. Synapses between photoreceptors and Dm-DRA1 cells. Ai, Left: Side view of a reconstructed Dm-DRA1 (grey) innervated by R7-DRA (purple). Right: Distribution of R7-DRA synapses (purple) onto 20 Dm-DRA1 cells, plotted across medulla layers. Aii: Top view of the same fully reconstructed Dm-DRA1 skeleton and R7-DRA inputs from the (polar) seed column. Aiii: Top view of a light microscopic Dm-DRA1 single cell clone with processes leaving the DRA region. **B.** R7-DRA inputs into Dm-DRA1 cells. Bi-iii: Three skeletons of fully reconstructed Dm-DRA1 cells (grey) at different positions along the DRA (posterior, polar and anterior) with all R7-DRA synapses (purple) originating from an average of 11 columns. Biv: Distribution of R7-DRA synapses onto the three Dm-DRA1 cells from Bi-iii along the DRA region. **C.** Synapses between photoreceptors and Dm-DRA2 cells. Ci, Left: Side view of one reconstructed Dm-DRA2 skeleton (grey) innervated by R8-DRA (green). Right: Layer distribution of DRA photoreceptor synapses onto 9 Dm-DRA2 cells. Cii: Top view of the same fully reconstructed Dm-DRA2 skeleton and photoreceptor inputs from the (polar) seed column. Ciii: Side view of a light microscopic Dm-DRA2 single cell clone with vertical processes (arrows in Ci and Ciii indicate vertical projections). **D.** Photoreceptor inputs to Dm-DRA2 cells. Di-iii: Three skeletons of fully reconstructed Dm-DRA2 cells (grey) at different positions along the DRA (posterior, polar and anterior) with all R8-DRA (green) synapses originating from an average of 11 columns. Div: Distribution of R8-DRA (and few R7-DRA) synapses onto the three Dm-DRA2 cells from Di-iii along the DRA region. **E.** Comparison of Dm-DRA1 and Dm-DRA2 connectivity. All reconstructed Dm-DRA1 skeletons connected to R7-DRA in the polar seed column (circled column, arrow). The saturation of blue color indicates strength of connectivity (from 3 to 15 synapses). Eii: All Dm-DRA2 skeletons connected to R8-DRA in the polar seed column (circled column, arrow), blue color indicates the strength of connectivity (from 2 to 19 synapses). Eiii: Side views of overlapping Dm-DRA1 and Dm-DRA2 cell skeletons with all R7-DRA and R8-DRA synapses (same cells as in Bii and Dii). (All scale bars: 10 μ m).

Figure 7 – figure supplement 1

1611 Figure 7 – figure supplement 1: Non-DRA photoreceptor inputs to Dm-DRA cells

1612 **A.** Top view (Ai-Aiii) of three fully reconstructed Dm-DRA1 cells (same as in Figure 7B) with
 1613 all non-DRA R7 inputs marked (purple dots), as well as distribution of synapses along the
 1614 DRA region (Aiv). Note the strong inputs into the posterior Dm-DRA1 cell, originating from an
 1615 R7 cell at the DRA/non-DRA boundary, with a putative ‘mixed’ fate (Ai). **B.** Top view (Bi-Bii)
 1616 of two fully reconstructed Dm-DRA2 cells (same as in Figure 7D) with all non-DRA R7 inputs
 1617 marked (purple dots), as well as distribution of synapses along the DRA region (Biii). Note that
 1618 no non-DRA R7 synapses were found for the posterior Dm-DRA2.

Figure 7 – figure supplement 2

1619 Figure 7 – figure supplement 2: Deep projections of Dm-DRA1 cells

1620 **A.** Top view (Ai-Aiii) of three fully traced Dm-DRA1 cells (same as in Figure 7B) with pale and
 1621 yellow fates of all non-DRA columns indicated. Deep projections of Dm-DRA1 cells do not
 1622 seem to show any obvious preference for pale or yellow columns. (All scale bars: 10 μ m).

Figure 7 – figure supplement 3

Figure 7 – figure supplement 3: Fitting a linear distance of synapses along the DRA region

Ai. The lateral locations of the 42 DRA columns (pink circles) were used to fit an ellipse (black line) along the DRA, where the lateral x- and y-coordinates are points in a plane that is approximately parallel to the medulla layers, and this plane does not take into account the curvature of the medulla. At the dorsal pole, there are DRA columns in two rows, and the ellipse passes between these. **Aii.** The alignment of R7 (purple) synapses to Dm-DRA1 cells and R8 (green) synapses to Dm-DRA2 vary along an axis that is approximately perpendicular to the ellipse, partly due to differences in the locations of the synapses, and partly an effect of the curvature of the medulla. For all the synapses, the projection onto the fitted DRA ellipse provides a good description of the location along the DRA region. **Aiii.** Synapses from R7 (purple) to Dm-DRA1 cells shown with the Mi1 columns (pink circles) to appreciate their locations in the columns. The synapses are concentrated around layer 6, and this narrow depth profile generates tightly clustered synaptic locations in columns. **Aiv.** Synapses from R8 (green) to Dm-DRA2 cells shown with the DRA columns (pink circles) to appreciate their locations in the columns. The synapses are spread between the chiasm and layer 6, and this diffuse depth profile contributes to the wide, linear clusters of synapses as the medulla curves away from the plane containing the fitted DRA ellipse.

Figure 8

Figure 8: Visual projection neurons connecting R7-DRA with the central brain

A. Synapses of R7-DRA onto MeTu-DRA cells. Ai: The complete skeleton of a fully reconstructed MeTu-DRA cell with an axon projecting to the AOTU. Aii: Light microscopic single cell clone of a MeTu-DRA cell. Aiii, Left: Side view of the same MeTu-DRA skeleton (grey) as in Ai innervated by R7-DRA (purple). Right: Layer distribution of R7-DRA synapses (purple) onto 30 MeTu-DRA cells. Aiv: Top view of the MeTu-DRA skeleton depicting its medulla processes innervating both DRA (red circles), and non-DRA columns. Avi: Light microscopic side view of a MeTu-DRA single cell clone (green) with exclusive contacts to DRA photoreceptor terminals (white arrowhead). Avii: All MeTu-DRA skeletons connected to R7-DRA in the polar seed column (circled column, arrow). The saturation of blue color indicates the strength of connectivity (from 3 to 14 synapses). Inset: MeTu-DRA axon terminations in the AOTU in orange and reconstructed MeTu cells from the central columns in cyan. **B.** Synapses between R7-DRA and visual projection neurons VPN-DRA. Bi: The entire skeleton of a reconstructed VPN-DRA cell with its axon projecting to the PLP. Bii: Light microscopic image of a VPN-DRA single cell clone shown in green and neuropil reference in grey (Nc82). Biii, Left: Side view of one VPN-DRA skeleton (grey) innervated by R7-DRA (purple). Right: Layer distribution of R7-DRA synapses onto 5 VPN-DRA cells. Biv: Top view of medulla processes formed by one reconstructed VPN-DRA skeleton (grey) with all R7-DRA synapses from the seed column (purple). Bv: Double labeling of several VPN-DRA cells (green) with R7 and R8 photoreceptors (purple, anti-Chaoptin). VPN-DRA processes overlap with DRA photoreceptors (white arrowhead) but also appear to show some contacts to non-DRA photoreceptors. Bvi: Skeletons of all reconstructed VPN-DRA cells covering the dorsal medulla. (All scale bars: 10 μ m).

Figure 8 – figure supplement 1

1664 Figure 8 – figure supplement 1: Visual projection neuron morphology in the DRA region

1665 **A.** Top view of the fully traced MeTu-DRA cell (same as in Figure 7A) with pale and yellow

1666 fates of all non-DRA ommatidia indicated. Medulla processes of MeTu-DRA do not seem to

1667 show any obvious preference for pale or yellow columns. Scale bar 10 μ m. **B.** Reconstructed

1668 skeleton (Bi) of an unusual VPN-DRA cell with additional processes in the central brain

1669 (putative VPN-DRA2). Light microscopic image of a cell with similar morphology (Bii).

Figure 9

Figure 9: Other newly identified photoreceptor targets in the DRA region

A. Synapses from R7-DRA to bilaterally projecting MeMe-DRA neurons. Ai: A reconstructed MeMe-DRA cell with projections to the dorsal periphery of the contralateral medulla. Aii, Left: Side view of one MeMe-DRA skeleton (grey) innervated by R7-DRA (purple). Right: layer distribution of R7-DRA synapses onto 2 MeMe-DRA cells. Aiii: Top view of medulla processes of the MeMe-DRA skeleton (grey) with all R7-DRA synapses from the seed columns (purple). Aiv: Light microscopic image of a MeMe-DRA single cell clone shown (in green) with processes to the contralateral medulla (neuropil reference in grey, Nc82). Inset: Medulla cross section showing branches of a MeMe-DRA cell. Note that the most dorsal arbors innervate the distal medulla (arrowhead). Av: Two reconstructed MeMe-DRA skeletons, both with cell bodies on the left-side (cyan and orange), connecting both medullas in a reciprocal manner, innervating opposite DRA regions along the anterior-posterior axis

B. Synapses from R7-DRA onto Mti-DRA-1. Bi: A reconstructed Mti-DRA-1 cell with processes covering the dorsal periphery of the medulla. Bii, Left: Side view of one Mti-DRA-1 skeleton (grey) innervated by R7-DRA (purple). Right: Layer distribution of R7-DRA synapses onto 6 Mti-DRA-1 cells. Biii: Top view of medulla processes of one reconstructed Mti-DRA-1 cell (grey) with all R7-DRA synapses from the (polar) seed column (purple). Biv: Light microscopic image of a putative morphological single cell match of Mti-DRA-1 shown in green.

C. Synapses from R7-DRA onto Mti-DRA-2. Ci: A reconstructed Mti-DRA-2 cell with processes covering dorsal parts of the medulla. Cii, Left: Side view of one Mti-DRA-2 skeleton (grey) innervated by R7-DRA (purple). Right: Layer distribution of R7-DRA synapses onto 4 Mti-DRA-2 cells. Ciii: Top view of medulla processes formed by one reconstructed Mti-DRA-2 cell (grey) with all R7-DRA synapses from the (polar) seed columns (purple). Cv: Light microscopic image of a putative morphological single cell match of Mti-DRA-2 shown in green. (All scale bars: 10 μ m).

Figure 9 – figure supplement 1

1694 Figure 9 – figure supplement 1: Mti cells in the DRA region

1695 **A.** All Mti-DRA-1 skeletons (6x) covering the dorsal medulla. **B.** All Mti-DRA-2 skeletons (4x)
 1696 covering the dorsal medulla. **C.** Separation of putative Mti-DRA subtypes. One population with
 1697 cell bodies located ventrally (putative cell type Mti-DRA-1, cyan, arrow), and several cells with
 1698 dorsally located cell bodies (putative Mti-DRA-2, orange, arrow). (All scale bars: 10 μ m).

Figure 10

Figure 10: Summary of inner photoreceptor connectivity of central and the DRA columns

A. Schematic summary of synaptic connections between central R7-8 or R7-DRA and R8-DRA, as well as other cell types providing synaptic feedback to them (blue). In all panels of this figure, arrow widths are proportional to the numbers of synapses per seed column, and weak connections were excluded for clarity by a threshold of >4 synapses per column per photoreceptor type. Despite the differences in modality, the circuit organization of inputs to R7-8 cells is conserved in the inputs to R7-DRA and R8-DRA. **B.** Synapses between R7-8 and Dm cell types in central and DRA columns. Note the presence of a second Dm8-like cell type downstream of R8 only in the DRA (Dm-DRA2). **C.** Summary of inner photoreceptor connections with lamina, Mi and Mti cells in central and DRA columns; in the central columns, the Mti connections were below the threshold of 4 synapses per column and are not shown. The connections from R8 to these cells were very reduced in the DRA. **D.** Summary of lobula connections. Di: Schematic of R7-8 versus R7-DRA and R8-DRA connections with Tm5a, Tm5b, Tm5c, Tm20, ML1 cells, and cells we identified as belonging to the Tm cell class (Tm). Note the virtual absence of lobula connectivity in the DRA region. Dii: Front view (left) and top view (right) of all reconstructed central Tm and ML1 skeletons (claret) and Tm skeletons from the DRA (orange). **E.** Summary of MeTu cell connections. Ei: Schematic of R7 and R7-DRA cells targeting different MeTu populations. Eii: Front view (left) and DRA view (right) of all central MeTu skeletons (claret) and MeTu-DRA skeletons (orange), with axons following the same tract but terminating with spatially separated axon target areas in the AOTU. **F.** Summary of other visual projection neurons. Fi: Diagram summarizing VPN connectivity in central and DRA columns. While R8 input dominates in central columns, R7 input dominates in the DRA region. Fii: Front view (left) and top view (right) of VPN target areas in the central brain. VPNs from both central and DRA columns project to the posterior lateral protocerebrum (PLP, golden yellow) and the AOTU (red). The central column VPNs additionally project ipsilaterally to the mushroom body calyx (mauve), accessory medulla (aMe, pink), superior clamp (SC, dark blue), flange (Fla, green), superior posterior slope (SPS, brown), inferior posterior slope (IPS, cyan), and contralaterally to the PLP, mushroom body calyx, aMe, and

- 1727 lobula plate (LP, cream). The DRA VPNs project ipsilaterally to the AOTU, PLP, and lateral
- 1728 horn (LH, turquoise), and contralaterally to the medulla (Me, lime green).

Figure 11

Figure 11: Comparison of central versus DRA, and pale versus yellow pathways

A. Graphical comparison of central and DRA synaptic pathways. In all panels, the arrow widths are proportional to the numbers of synapses per seed column, and weak connections were excluded for clarity by a threshold of >4 synapses per column. Ai: Arrows indicating the relative weight of R7 connections (cell types postsynaptic to pale and yellow R7) and R7-DRA. Aii: Arrows indicating the relative weight of R8 connections (sum of pale and yellow R8) and R8-DRA region. Connections to the lobula neuropil are dominated by R8 targets in the central columns. Lobula connections are virtually absent in the DRA region (synapse numbers below 1% of total synapse count), where connectivity of R8-DRA is dramatically reduced, limited to local computations in the medulla. The projections to the central brain that are not to the AOTU are driven by R8 in the central columns, but by R7-DRA in the DRA region. **B.** Graphical comparison of central pale and yellow-specific synaptic pathways. Bi: Arrows indicating the relative weight of pale R7 versus yellow R7 connections in central seed columns. Columnar cell types such as lamina cells have the capacity to preserve pale and yellow information, and Dm8 cells are most densely innervated by their home column input. Tm5a cells were selective for yellow R7, Tm5b cells were selective for pale R7, and the Tm5a-like and Tm5b-like cells were selective for yellow R8, but also received yellow R7 synapses. These diagrams omit cells such as the MeTu neurons since they receive nearly balanced input from pale and yellow R7s. Bii: Arrows indicating the relative weight of pale R8 versus yellow R8 connections in central seed columns. Columnar medulla cells that have the capacity to preserve pale and yellow information were targets of R8 cells. The aMe12 and ML-VPN1 cell types were specific for pale R8 input, while Tm5c had a strong bias for yellow R8, along with the Tm5a-like and Tm5b-like cells. ML1s are omitted from these diagrams since they received nearly balanced input from pale and yellow R8s.

Table 1: Synaptic targets of seed column R7 and R8

Type	No.	pR7	yR7	pR8	yR8	Sum	%R7	%R8	%p	%y	%Total	%Total R7	%Total R8
Dm9	6	94	66	142	143	445	36.0	64.0	53.0	47.0	16.4	13.5	18.8
Dm8	15	235	161	0	0	396	100.0	0.0	59.3	40.7	14.6	33.4	0.0
MeTu	7	82	93	0	0	175	100.0	0.0	46.9	53.1	6.5	14.7	0.0
R7	5	0	3	75	82	160	1.9	98.1	46.9	53.1	5.9	0.3	10.3
Tm5c	6	8	9	20	103	140	12.1	87.9	20.0	80.0	5.2	1.4	8.1
Tm20	4	5	3	67	65	140	5.7	94.3	51.4	48.6	5.2	0.7	8.7
Mi15	4	6	7	38	81	132	9.8	90.2	33.3	66.7	4.9	1.1	7.8
Mi4	4	0	0	58	58	116	0.0	100.0	50.0	50.0	4.3	0.0	7.6
ML1	4	0	0	58	41	99	0.0	100.0	58.6	41.4	3.7	0.0	6.5
Dm2	4	18	3	60	15	96	21.9	78.1	81.2	18.8	3.5	1.8	4.9
Dm11	2	28	27	4	4	63	87.3	12.7	50.8	49.2	2.3	4.6	0.5
L3	4	19	17	12	13	61	59.0	41.0	50.8	49.2	2.3	3.0	1.6
Mi1	4	0	0	31	28	59	0.0	100.0	52.5	47.5	2.2	0.0	3.9
R8	4	31	26	1	0	58	98.3	1.7	55.2	44.8	2.1	4.8	0.1
Tm5a	2	0	53	0	0	53	100.0	0.0	0.0	100.0	2.0	4.5	0.0
Tm5b	2	39	0	3	6	48	81.2	18.8	87.5	12.5	1.8	3.3	0.6
Tm	9	17	4	7	16	44	47.7	52.3	54.5	45.5	1.6	1.8	1.5
Tm5b-like	3	0	14	0	27	41	34.1	65.9	0.0	100.0	1.5	1.2	1.8
Mi9	4	3	9	26	1	39	30.8	69.2	74.4	25.6	1.4	1.0	1.8
L1	4	2	1	21	14	38	7.9	92.1	60.5	39.5	1.4	0.3	2.3
aMe12	2	4	0	26	0	30	13.3	86.7	100.0	0.0	1.1	0.3	1.7
Dm	5	11	7	6	5	29	62.1	37.9	58.6	41.4	1.1	1.5	0.7
ML-VPN1	3	0	0	26	1	27	0.0	100.0	96.3	3.7	1.0	0.0	1.8
C2	2	7	13	6	0	26	76.9	23.1	50.0	50.0	1.0	1.7	0.4
Mt-VPN	4	0	12	3	8	23	52.2	47.8	13.0	87.0	0.8	1.0	0.7
Mti	3	2	0	10	7	19	10.5	89.5	63.2	36.8	0.7	0.2	1.1
Tm5a-like	1	0	1	0	16	17	5.9	94.1	0.0	100.0	0.6	0.1	1.1
TmY10	1	1	0	6	0	7	14.3	85.7	100.0	0.0	0.3	0.1	0.4
Mi10	1	0	0	0	5	5	0.0	100.0	0.0	100.0	0.2	0.0	0.3
Mi	1	2	1	0	0	3	100.0	0.0	66.7	33.3	0.1	0.3	0.0
C3	1	0	0	0	3	3	0.0	100.0	0.0	100.0	0.1	0.0	0.2
Identified <3	26	5	14	14	4	37	51.4	48.6	51.4	48.6	1.4	1.6	1.2
Unidentified >=3	2	0	0	5	3	8	0.0	100.0	62.5	37.5	0.3	0.0	0.5
Unidentified <3	62	9	15	22	24	70	34.3	65.7	44.3	55.7	2.6	2.0	3.0
Total	211	628	559	747	773	2707	43.8	56.2	50.8	49.2	100.0	100.0	100.0

Table 2: Cells that synapse onto seed column R7 and R8

Type	No.	pR7	yR7	pR8	yR8	Sum	%R7	%R8	%p	%y	%Total	%Total R7	%Total R8
Dm9	6	90	92	79	72	333	54.7	45.3	50.8	49.2	57.0	51.3	65.9
R8	4	75	82	1	0	158	99.4	0.6	48.1	51.9	27.1	44.2	0.4
R7	4	0	0	31	26	57	0.0	100.0	54.4	45.6	9.8	0.0	24.9
Mt-VPN	1	0	2	0	3	5	40.0	60.0	0.0	100.0	0.9	0.6	1.3
C2	1	2	3	0	0	5	100.0	0.0	40.0	60.0	0.9	1.4	0.0
L3	1	0	0	4	0	4	0.0	100.0	100.0	0.0	0.7	0.0	1.7
Identified <3	11	4	2	4	8	18	33.3	66.7	44.4	55.6	3.1	1.7	5.2
Unidentified >=3	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unidentified <3	4	1	2	1	0	4	75.0	25.0	50.0	50.0	0.7	0.8	0.4
Total	32	172	183	120	109	584	60.8	39.2	50.0	50.0	100.0	100.0	100.0

Table 3: Synaptic targets of seed column R7-DRA and R8-DRA

Type	No.	R7-DRA	R8-DRA	Sum	%R7	%R8	%Total	%Total R7	%Total R8
Dm-DRA1	20	212	0	212	100.0	0.0	15.9	25.3	0.0
Dm9	6	95	115	210	45.2	54.8	15.7	11.3	23.0
MeTu-DRA	30	184	0	184	100.0	0.0	13.8	22.0	0.0
R7-DRA	3	1	108	109	0.9	99.1	8.2	0.1	21.6
Dm-DRA2	9	3	103	106	2.8	97.2	7.9	0.4	20.6
Dm2	4	21	27	48	43.8	56.2	3.6	2.5	5.4
R8-DRA	3	41	1	42	97.6	2.4	3.1	4.9	0.2
Mi15	4	21	18	39	53.8	46.2	2.9	2.5	3.6
Mti-DRA-1	6	37	0	37	100.0	0.0	2.8	4.4	0.0
MeMe-DRA	2	36	0	36	100.0	0.0	2.7	4.3	0.0
L3	3	16	16	32	50.0	50.0	2.4	1.9	3.2
VPN-DRA	6	30	0	30	100.0	0.0	2.2	3.6	0.0
L1	3	9	9	18	50.0	50.0	1.3	1.1	1.8
Tm20	3	0	16	16	0.0	100.0	1.2	0.0	3.2
Mti-DRA-2	4	15	0	15	100.0	0.0	1.1	1.8	0.0
Mi1	3	6	9	15	40.0	60.0	1.1	0.7	1.8
MeTu	2	13	0	13	100.0	0.0	1.0	1.6	0.0
Tm5-like	1	0	12	12	0.0	100.0	0.9	0.0	2.4
Mi9	2	0	12	12	0.0	100.0	0.9	0.0	2.4
Dm11	1	4	8	12	33.3	66.7	0.9	0.5	1.6
aMe12	1	3	1	4	75.0	25.0	0.3	0.4	0.2
TmY	1	0	3	3	0.0	100.0	0.2	0.0	0.6
ML-VPN2	1	3	0	3	100.0	0.0	0.2	0.4	0.0
C2	1	2	1	3	66.7	33.3	0.2	0.2	0.2
Identified <3	33	34	14	48	70.8	29.2	3.6	4.1	2.8
Unidentified >=3	2	5	5	10	50.0	50.0	0.7	0.6	1.0
Unidentified <3	57	46	21	67	68.7	31.3	5.0	5.5	4.2
Total	211	837	499	1336	62.6	37.4	100.0	100.0	100.0

Table 4: Cells that synapse onto seed column R7-DRA and R8-DRA

Type	No.	R7-DRA	R8-DRA	Sum	%R7	%R8	%Total	%Total R7	%Total R8
Dm9	4	105	106	211	49.8	50.2	54.7	46.1	67.1
R8-DRA	3	108	1	109	99.1	0.9	28.2	47.4	0.6
R7-DRA	3	1	41	42	2.4	97.6	10.9	0.4	25.9
C2	2	4	4	8	50.0	50.0	2.1	1.8	2.5
Mi15	1	2	2	4	50.0	50.0	1.0	0.9	1.3
Identified <3	9	6	3	9	66.7	33.3	2.3	2.6	1.9
Unidentified >=3	0	0	0	0	0.0	0.0	0.0	0.0	0.0
Unidentified <3	2	2	1	3	66.7	33.3	0.8	0.9	0.6
Total	24	228	158	386	59.1	40.9	100.0	100.0	100.0

References

- Bates AS, Manton JD, Jagannathan SR, Costa M, Schlegel P, Rohlfing T, and Jefferis GS. 2020. 'The natverse, a versatile toolbox for combining and analysing neuroanatomical data', *Elife*, 9:e53350.
- Bell ML, Earl JB, and Britt SG. 2007. 'Two types of *Drosophila* R7 photoreceptor cells are arranged randomly: a model for stochastic cell-fate determination', *J Comp Neurol*, 502: 75-85.
- Bogovic JA, Otsuna H, Heinrich L, Ito M, Jeter J, Meissner G, Nern A, Colonell J, Malkesman O, Ito K., and Saalfeld S. 2019. 'An unbiased template of the *Drosophila* brain and ventral nerve cord': *PLoS One* 15: e0236495.
- Busch S, Selcho M, Ito K, and Tanimoto H.. 2009. 'A map of octopaminergic neurons in the *Drosophila* brain', *J Comp Neurol*, 513: 643-67.
- Chin AL, Lin CY, Fu TF, Dickson BJ, and Chiang AS. 2014. 'Diversity and wiring variability of visual local neurons in the *Drosophila* medulla M6 stratum', *J Comp Neurol*, 522: 3795-816.
- Chou WH, Hall KJ, Wilson DB, Wideman CL, Townson SM, Chadwell LV, and Britt SG. 1996. 'Identification of a novel *Drosophila* opsin reveals specific patterning of the R7 and R8 photoreceptor cells', *Neuron*, 17: 1101-15.
- Clements J, Dolafi T, Umayam L, Neubarth NL, Berg S, Scheffer LK, and Plaza SM. 2020. 'neuPrint: Analysis Tools for EM Connectomics': *bioRxiv* 2020.01.16.909465.
- Courgeon M, and Desplan C. 2019. 'Coordination between stochastic and deterministic specification in the *Drosophila* visual system', *Science*, 366: eaay6727.
- Dacke M, and El Jundi B. 2018. 'The Dung Beetle Compass', *Curr Biol*, 28: R993-R97.
- Davis FP, Nern A, Picard S, Reiser MB, Rubin GM, Eddy SR, and Henry GL. 2020. 'A genetic, genomic, and computational resource for exploring neural circuit function', *Elife*, 9: e50901.
- Dionne H, Hibbard KL, Cavallaro A, Kao JC, and Rubin GM. 2018. 'Genetic Reagents for Making Split-GAL4 Lines in *Drosophila* ', *Genetics*, 209: 31-35.
- Dorkenwald S, McKellar C, Macrina T, Kemnitz N, Lee K, Lu R, Wu J, Popovych S, Mitchell E, Nehoran B, Jia Z, Bae JA, Mu S, et al. 2020. 'FlyWire: Online community for whole-brain connectomics': *bioRxiv* 2020.08.30.274225.
- el Jundi B, and Homberg U. 2010. 'Evidence for the possible existence of a second polarization-vision pathway in the locust brain', *J Insect Physiol*, 56: 971-9.
- el Jundi B, Pfeiffer K, and Homberg U. 2011. 'A distinct layer of the medulla integrates sky compass signals in the brain of an insect', *PLoS One*, 6: e27855.
- Feiler R, Bjornson R, Kirschfeld K, Mismar D, Rubin GM, Smith DP, Socolich M, and Zuker CS. 1992. 'Ectopic expression of ultraviolet-rhodopsins in the blue photoreceptor cells of *Drosophila*: visual physiology and photochemistry of transgenic animals', *J Neurosci*, 12: 3862-8.
- Fischbach KF, and Dittrich APM. 1989. 'The Optic Lobe of *Drosophila melanogaster* .1. A Golgi Analysis of Wild-Type Structure', *Cell Tissue Res*, 258: 441-75.
- Fischbach KF, and Lyly-Hunerberg I. 1983. 'Genetic dissection of the anterior optic tract of *Drosophila melanogaster*', *Cell Tissue Res*, 231: 551-63.

Fortini ME, and Rubin GM. 1990. 'Analysis of Cis-Acting Requirements of the Rh3 and Rh4 Genes Reveals a Bipartite Organization to Rhodopsin Promoters in *Drosophila melanogaster*', *Gene Dev*, 4: 444-63.

———. 1991. 'The Optic Lobe Projection Pattern of Polarization-Sensitive Photoreceptor Cells in *Drosophila melanogaster*', *Cell Tissue Res*, 265: 185-91.

Fujita SC, Zipursky SL, Benzer S, Ferrús A, and Shotwell SL. 1982. 'Monoclonal antibodies against the *Drosophila* nervous system', *Proc Natl Acad Sci USA*, 79: 7929-33.

Gao S, Takemura SY, Ting CY, Huang S, Lu Z, Luan H, Rister J, Thum AS, Yang M, Hong ST, Wang JW, Odenwald WF, White BH, Meinertzhagen IA, and Lee CH. 2008. 'The neural substrate of spectral preference in *Drosophila*', *Neuron*, 60: 328-42.

Gkaniyas E, Risse B, Mangan M, and Webb B. 2019. 'From skylight input to behavioural output: A computational model of the insect polarised light compass', *PLoS Comput Biol*, 15: e1007123.

Hardcastle BJ, Omoto JJ, Kandimalla P, Nguyen BM, Keles MF, Boyd NK, Hartenstein V, and Frye MA. 2021. 'A visual pathway for skylight polarization processing in *Drosophila*', *Elife*, 10: e63225

Heath SL, Christenson MP, Oriol E, Saavedra-Weisenhaus M, Kohn JR, and Behnia R. 2020. 'Circuit mechanisms underlying chromatic encoding in *Drosophila* photoreceptors'. *Curr Biol*. 30(2):264-275.e8.

Heinze S. 2017. 'Unraveling the neural basis of insect navigation', *Curr Opin Insect Sci*, 24: 58-67.

Heisenberg M, and Buchner E. 1977. 'The role of retinula cell types in visual behavior of *Drosophila melanogaster*', *J Comp Physiol*, 117: 127-62.

Hempel de Ibarra N, Vorobyev NM, and Menzel R. 2014. 'Mechanisms, functions and ecology of colour vision in the honeybee', *J Comp Physiol A*, 200: 411-33.

Hilbrant M, Almudi I, Leite DJ, Kuncheria L, Posnien N, Nunes MD, and McGregor AP. 2014. 'Sexual dimorphism and natural variation within and among species in the *Drosophila* retinal mosaic', *BMC Evol Biol*, 14: 240.

Homberg, U. 2015. 'Sky Compass Orientation in Desert Locusts-Evidence from Field and Laboratory Studies', *Front Behav Neurosci*, 9: 346.

Huber A, Schulz S, Bentre J, Groell C, Wolfrum U, and Paulsen R. 1997. 'Molecular cloning of *Drosophila* Rh6 rhodopsin: the visual pigment of a subset of R8 photoreceptor cells', *FEBS Lett*, 406: 6-10.

Hulse BK, Haberkern H, Franconville R, Turner-Evans DB, Takemura S, Wolff T, Noorman M, Dreher M, Dan C, Parekh R, Hermundstad AN, Rubin GM, and Jayaraman V. 2020. 'A connectome of the *Drosophila* central complex reveals network motifs suitable for flexible navigation and context-dependent action selection': *bioRxiv* 2020.12.08.413955.

Jagdish S, Barnea G, Clandinin TR, and Axel R. 2014. 'Identifying functional connections of the inner photoreceptors in *Drosophila* using Tango-Trace', *Neuron*, 83: 630-44.

Jenett A, Rubin GM, Ngo TT, Shepherd D, Murphy C, Dionne H, Pfeiffer BD, Cavallaro A, Hall D, Jeter J, Iyer N, Fetter D, Hausenfluck JH, et al. 2012. 'A GAL4-driver line resource for *Drosophila* neurobiology', *Cell Rep*, 2: 991-1001.

Karuppudurai T, Lin TY, Ting CY, Pursley R, Melnattur KV, Diao F, White BH, Macpherson LJ, Gallio M, Pohida T, and Lee CH. 2014. 'A hard-wired glutamatergic circuit pools

1841 and relays UV signals to mediate spectral preference in *Drosophila*', *Neuron*, 81: 603-
1842 15.

1843 Kind E, Belušič G, and Wernet MF. 2020. 'Retinal Mosaics Across Fly Species: Variations on
1844 a Theme.' in, *Reference Module in Neuroscience and Biobehavioral Psychology*
1845 (Elsevier).

1846 Klapoetke NC, Murata Y, Kim SS, Pulver SR, Birdsey-Benson A, Cho YK, Morimoto TK,
1847 Chuong AS, Carpenter EJ, Tian Z, Wang J, Xie Y, Yan Z, et al. 2014. 'Independent
1848 Optical Excitement of Distinct Neural Populations', *Nat Methods*, 11: 338-346.

1849 Konstantinides N, Kapuralin K, Fadil C, Barboza L, Satija R, and Desplan C. 2018. 'Phenotypic
1850 Convergence: Distinct Transcription Factors Regulate Common Terminal Features',
1851 *Cell*, 174: 622-35 e13.

1852 Kurmangaliyev YZ, Yoo J, Valdes-Aleman J, Sanfilippo P, and Zipursky SL. 2020.
1853 'Transcriptional Programs of Circuit Assembly in the *Drosophila* Visual System',
1854 *Neuron*, 108: 1045-57 e6.

1855 Kvon EZ, Kazmar T, Stampfel G, Yanez-Cuna JO, Pagani M, Schernhuber K, Dickson BJ,
1856 and Stark A. 2014. 'Genome-scale functional characterization of *Drosophila*
1857 developmental enhancers in vivo', *Nature*, 512: 91-5.

1858 Labhart T. 1988. 'Polarization-Opponent Interneurons in the Insect Visual-System', *Nature*,
1859 331: 435-37.

1860 Lazopulo S, Lazopulo A, Baker JD, and Syed S. 2019. 'Daytime colour preference in
1861 *Drosophila* depends on the circadian clock and TRP channels', *Nature*, 574: 108-11.

1862 Li F, Lindsey JW, Marin EC, Otto N, Dreher M, Dempsey G, Stark I, Bates AS, Pleijzier MW,
1863 Schlegel P, Nern A, Takemura SY, Eckstein N, et al. 2020. 'The connectome of the
1864 adult *Drosophila* mushroom body provides insights into function', *Elife*, 9: e62576.

1865 Li Y, Chen PJ, Lin TY, Ting CY, Muthurulan P, Pursley R, Ilić M, Pirih P, Drews MS, Menon
1866 KP, Zinn KG, Pohida T, Borst A, and Lee CH. 2021. 'Neural mechanism of spatio-
1867 chromatic opponency in the *Drosophila* amacrine neurons', *Curr Biol*, S0960-
1868 9822(21)00615-1.

1869 Lin TY, Luo J, Shinomiya K, Ting CY, Lu Z, Meinertzhagen IA, and Lee CH. 2016. 'Mapping
1870 chromatic pathways in the *Drosophila* visual system', *J Comp Neurol*, 524: 213-27.

1871 Longden KD. 2016. 'Central Brain Circuitry for Color-Vision-Modulated Behaviors', *Curr Biol*,
1872 26: R981-8.

1873 Luo J, Ting CY, Li Y, McQueen P, Lin TY, Hsu CP, and Lee CH. 2020. 'Antagonistic regulation
1874 by insulin-like peptide and activin ensures the elaboration of appropriate dendritic field
1875 sizes of amacrine neurons', *Elife*, 9: e50568.

1876 Mathejczyk TF, and Wernet MF. 2019. 'Heading choices of flying *Drosophila* under changing
1877 angles of polarized light', *Sci Rep*, 9: 16773.

1878 ———. 2020. 'Modular assays for the quantitative study of visually guided navigation in both
1879 flying and walking flies', *J Neurosci Methods*, 340: 108747.

1880 Mauss AS, Vlasits A, Borst A, and Feller M. 2017. 'Visual Circuits for Direction Selectivity',
1881 *Annu Rev Neurosci*, 40: 211-30.

1882 Mazzoni EO, Celik A, Wernet MF, Vasilaiuskas D, Johnston RJ, Cook TA, Pichaud F, and
1883 Desplan C. 2008. 'Iroquois complex genes induce co-expression of rhodopsins in
1884 *Drosophila*', *PLoS Biol*, 6: e97.

- 1885 Meinertzhagen IA, and O'Neil SD. 1991. 'Synaptic organization of columnar elements in the
1886 lamina of the wild type in *Drosophila melanogaster*', *J Comp Neurol*, 305: 232-63.
- 1887 Meinertzhagen IA, Takemura SY, Lu Z, Huang S, Gao S, Ting CY, and Lee CH. 2009. 'From
1888 form to function: the ways to know a neuron', *J Neurogenet*, 23: 68-77.
- 1889 Meissner GW, Nern A, Singer RH, Wong AM, Malkesman O, and Long X. 2019. 'Mapping
1890 Neurotransmitter Identity in the Whole-Mount *Drosophila* Brain Using Multiplex High-
1891 Throughput Fluorescence in Situ Hybridization', *Genetics*, 211: 473-82.
- 1892 Melnattur KV, Pursley R, Lin TY, Ting CY, Smith PD, Pohida T, and Lee CH. 2014. 'Multiple
1893 redundant medulla projection neurons mediate color vision in *Drosophila*', *J
1894 Neurogenet*, 28: 374-88.
- 1895 Menon KP, Kulkarni V, Takemura SY, Anaya M, and Zinn K. 2019. 'Interactions between
1896 Dpr11 and DIP-gamma control selection of amacrine neurons in *Drosophila* color
1897 vision circuits', *Elife*, 8: e48935.
- 1898 Mollereau B, Wernet MF, Beaufils P, Killian D, Pichaud F, Kuhnlein R, and Desplan C. 2000.
1899 'A green fluorescent protein enhancer trap screen in *Drosophila* photoreceptor cells',
1900 *Mech Dev*, 93: 151-60.
- 1901 Morante J, and Desplan C. 2008. 'The color-vision circuit in the medulla of *Drosophila* ', *Curr
1902 Biol*, 18: 553-65.
- 1903 Mota T, Gronenberg W, Giurfa M, and Sandoz JC. 2013. 'Chromatic processing in the anterior
1904 optic tubercle of the honey bee brain', *J Neurosci*, 33: 4-16.
- 1905 Nern A, Pfeiffer DB, and Rubin GM. 2015. 'Optimized tools for multicolor stochastic labeling
1906 reveal diverse stereotyped cell arrangements in the fly visual system', *Proc Natl Acad
1907 Sci USA*, 112: E2967-76.
- 1908 Nilsson DE., and Warrant EJ. 1999. 'Visual discrimination: Seeing the third quality of light',
1909 *Curr Biol*, 9: R535-7.
- 1910 Omoto JJ, Keles MF, Nguyen BM, Bolanos C, Lovick JK, Frye MA, and Hartenstein V. 2017.
1911 'Visual Input to the *Drosophila* Central Complex by Developmentally and Functionally
1912 Distinct Neuronal Populations', *Curr Biol*, 27: 1098-110.
- 1913 Otsuna H, and Ito K. 2006. 'Systematic analysis of the visual projection neurons of *Drosophila
1914 melanogaster*. I. Lobula-specific pathways', *J Comp Neurol*, 497: 928-58.
- 1915 Otsuna H, Shinomiya K, and Ito K. 2014. 'Parallel neural pathways in higher visual centers of
1916 the *Drosophila* brain that mediate wavelength-specific behavior', *Front Neural Circuits*,
1917 8: 8.
- 1918 Ozel MN, Simon F, Jafari S, Holguera I, Chen YC, Benhra N, El-Danaf RN, Kapuralin K, Malin
1919 JA, Konstantinides N, and Desplan C. 2021. 'Neuronal diversity and convergence in a
1920 visual system developmental atlas', *Nature*, 589: 88-95.
- 1921 Pagni M, Haikala V, Oberhauser V, Meyer PB, Reiff DF, and Schnaitmann C. 2021.
1922 'Interaction of "chromatic" and "achromatic" circuits in *Drosophila* color opponent
1923 processing', *Curr Biol*, 31, 1687-1698.
- 1924 Panser K, Tirian L, Sculze F, Villalba S, Jefferis GSXE, Bühler K, and Straw AD. 2016.
1925 'Automatic Segmentation of *Drosophila* Neural Compartments Using GAL4 Expression
1926 Data Reveals Novel Visual Pathways', *Curr Biol*, 26: 1943-54.
- 1927 Pantazis A, Segaran A, Liu CH, Nikolaev A, Rister J, Thum AS, Roeder T, Semenov E,
1928 Juusola M, and Hardie RC. 2008. 'Distinct roles for two histamine receptors (hclA and
1929 hclB) at the *Drosophila* photoreceptor synapse', *J Neurosci*, 28: 7250-9.

1930 Papatsenko D, Sheng G, and Desplan C. 1997. 'A new rhodopsin in R8 photoreceptors of
1931 *Drosophila*: evidence for coordinate expression with Rh3 in R7 cells', *Development*,
1932 124: 1665-73.

1933 Pfeiffer BD, Jenett A, Hammonds AS, Ngo TT, Misra S, Murphy C, Scully A, Carlson JW, Wan
1934 KH, Lavery TR, Mungall C, Svirskas R, Kadonaga JT, Doe CQ, Eisen MB, Celniker
1935 SE, and Rubin GM. 2008. 'Tools for neuroanatomy and neurogenetics in *Drosophila*',
1936 *Proc Natl Acad Sci USA*, 105: 9715-20.

1937 Pfeiffer K, and Kinoshita M. 2012. 'Segregation of visual inputs from different regions of the
1938 compound eye in two parallel pathways through the anterior optic tubercle of the
1939 bumblebee (*Bombus ignitus*)', *J Comp Neurol*, 520: 212-29.

1940 Pichaud F, and Desplan C. 2001. 'A new visualization approach for identifying mutations that
1941 affect differentiation and organization of the *Drosophila* ommatidia', *Development*, 128:
1942 815-26.

1943 Pollack I, and Hofbauer A. 1991. 'Histamine-like immunoreactivity in the visual system and
1944 brain of *Drosophila melanogaster*', *Cell Tissue Res*, 266: 391-8.

1945 Rister J, and Desplan C. 2011. 'The retinal mosaics of opsin expression in invertebrates and
1946 vertebrates', *Dev Neurobiol*, 71: 1212-26.

1947 Rivera-Alba M, Vitaladevuni SN, Mishchenko Y, Lu Z, Takemura SY, Scheffer L,
1948 Meinertzhagen IA, Chklovskii DB, and de Polavieja GC. 2011. 'Wiring economy and
1949 volume exclusion determine neuronal placement in the *Drosophila* brain', *Curr Biol*,
1950 21: 2000-5.

1951 Saalfeld S, Cardona A, Hartenstein V, and Tomancak P. 2009. 'CATMAID: collaborative
1952 annotation toolkit for massive amounts of image data', *Bioinformatics*, 25: 1984-6.

1953 Salcedo E, Huber A, Henrich S, Chadwell LV, Chou WH, Paulsen R, and Britt SG. 1999. 'Blue-
1954 and green-absorbing visual pigments of *Drosophila*: ectopic expression and
1955 physiological characterization of the R8 photoreceptor cell-specific Rh5 and Rh6
1956 rhodopsins', *J Neurosci*, 19: 10716-26.

1957 Sancer G, Kind E, Plazaola-Sasieta H, Balke J, Pham T, Hasan A, Munch LO, Courgeon M,
1958 Mathejczyk TF, and Wernet MF. 2019. 'Modality-Specific Circuits for Skylight
1959 Orientation in the Fly Visual System', *Curr Biol* 29: 2812-2825.e4.

1960 Sancer G, Kind E, Uhlhorn J, Volkmann J, Hammacher J, Pham T, Plazaola-Sasieta H, and
1961 Wernet MF. 2020. 'Cellular and synaptic adaptations of neural circuits processing
1962 skylight polarization in the fly', *J Comp Physiol A*, 206: 233-46.

1963 Sancer G, and Wernet MF. 2021. 'The development and function of neuronal subtypes
1964 processing color and skylight polarization in the optic lobes of *Drosophila*
1965 *melanogaster*', *Arthropod Struct Dev*, 61: 101012.

1966 Scheffer LK, Xu CS, Januszewski M, Lu Z, Takemura SY, Hayworth KJ, Huang GB, Shinomiya
1967 K, Maitin-Shepard J, Berg S, Clements J, Hubbard P, Katz W, et al. 'A Connectome
1968 and Analysis of the Adult *Drosophila* Central Brain', *eLife* 9: e57443.

1969 Schnaitmann C, Garbers C, Wachtler T, and Tanimoto H. 2013. 'Color discrimination with
1970 broadband photoreceptors', *Curr Biol*, 23: 2375-82.

1971 Schnaitmann C, Haikala V, Abraham E, Oberhauser V, Thestrup T, Griesbeck O, and Reiff
1972 DF. 2018. 'Color Processing in the Early Visual System of *Drosophila*', *Cell*, 172: 318-
1973 30 e18.

- 1974 Schneider-Mizell CM, Gerhard S, Longair M, Kazimiers T, Li F, Zwart MF, Champion A,
1975 Midgley FM, Fetter RD, Saalfeld S, and Cardona A. 2016. 'Quantitative neuroanatomy
1976 for connectomics in *Drosophila* ', *Elife*, 5: e12059.
- 1977 Sharkey CR, Blanco J, Leibowitz MM, Pinto-Benito D, and Wardill TJ. 2020. 'The spectral
1978 sensitivity of *Drosophila* photoreceptors', *Sci Rep*, 10: 18242.
- 1979 Shinomiya K, Karuppudurai T, Lin TY, Lu Z, Lee CH, and Meinertzhagen IA. 2014. 'Candidate
1980 neural substrates for off-edge motion detection in *Drosophila* ', *Curr Biol*, 24: 1062-70.
- 1981 Silies MD, Gohl M, Fisher YE, Freifeld L, Clark DA, and Clandinin TR. 2013. 'Modular use of
1982 peripheral input channels tunes motion-detecting circuitry', *Neuron*, 79: 111-27.
- 1983 Strother JA, Wu ST, Wong AM, Nern A, Rogers EM, Le JQ, Rubin GM, and Reiser MB. 2017.
1984 'The Emergence of Directional Selectivity in the Visual Motion Pathway of *Drosophila* ',
1985 *Neuron*, 94: 168-82 e10.
- 1986 Tahayato A, Sonnevile R, Pichaud F, Wernet MF, Papatsenko D, Beauvils P, Cook T, and
1987 Desplan C. 2003. 'Otd/Crx, a dual regulator for the specification of ommatidia subtypes
1988 in the *Drosophila* retina', *Dev Cell*, 5: 391-402.
- 1989 Tai CY, Chin AL, and Chiang AS. 2021. 'Comprehensive map of visual projection neurons for
1990 processing ultraviolet information in the *Drosophila* brain', *J Comp Neurol*, 529: 1988-
1991 2013.
- 1992 Takemura S, Bharioke A, Lu ZY, Nern A, Vitaladevuni S, Rivlin PK, Katz WT, Olbris DJ, Plaza
1993 SM, Winston P, Zhao T, Horne JA, Fetter RD, et al. 2013. 'A visual motion detection
1994 circuit suggested by *Drosophila* connectomics', *Nature*, 500: 175-181.
- 1995 Takemura SY, Lu ZY, and Meinertzhagen IA. 2008. 'Synaptic circuits of the *Drosophila* optic
1996 lobe: The input terminals to the medulla', *J Comp Neurol*, 509: 493-513.
- 1997 Takemura SY, Nern A, Chklovskii DB, Scheffer LK, Rubin GM, and Meinertzhagen IA. 2017.
1998 'The comprehensive connectome of a neural substrate for 'ON' motion detection in
1999 *Drosophila* ', *Elife*, 6: e24394.
- 2000 Takemura SY, Xu CS, Lu Z, Rivlin PK, Parag T, Olbris DJ, Plaza S, Zhao T, Katz WT, Umayam
2001 L, Weaver C, Hess HF, Horne JA, et al. 2015. 'Synaptic circuits and their variations
2002 within different columns in the visual system of *Drosophila* ', *Proc Natl Acad Sci USA*,
2003 112: 13711-6.
- 2004 Timaeus L, Geid L, Sancer G, Wernet MF, and Hummel T. 2020. 'Parallel visual pathways
2005 with topographic versus non-topographic organization connect the *Drosophila* eyes to
2006 the central brain', *bioRxiv*: 2020.04.11.037333.
- 2007 Tirian L, and Dickson BJ. 2017. 'The VT GAL4, LexA, and split-GAL4 driver line collections for
2008 targeted expression in the *Drosophila* nervous system': *bioRxiv* 198648.
- 2009 Tuthill JC, Nern A, Holtz SL, Rubin GM, and Reiser MB. 2013. 'Contributions of the 12 neuron
2010 classes in the fly lamina to motion vision', *Neuron*, 79: 128-40.
- 2011 Uhlhorn J, and Wernet MF. 2020. 'Colour Vision: Self-Centered Fly Photoreceptors
2012 Communicate over Distances', *Curr Biol*, 30: R78-R81.
- 2013 Vogt K, Schnaitmann C, Dylla KV, Knapek S, Aso Y, Rubin GM, and Tanimoto H. 2014.
2014 'Shared mushroom body circuits underlie visual and olfactory memories in *Drosophila* ',
2015 *Elife*. 3: e02395.
- 2016 Wada S. 1974a. 'Special Marginal Ommatidia of Flies (Diptera-Brachycera) - Architecture and
2017 Distribution in Compound Eyes', *Z Morphol Tiere*, 77: 87-125.

2018 ———. 1974b. 'Spezielle randzonale ommatidien von *Calliphora Erythrocephala* meig.
2019 (diptera calliphoridae): Architektur der zentralen rhabdomeren-kolumne und
2020 topographie im komplexauge', *Int J Insect Morphol*, 3: 397-424.

2021 Wagh DA, Rasse TM, Asan E, Hofbauer A, Schwenkert I, Dürbeck H, Buchner S, Dabauvalle
2022 MC, Schmidt M, Qin G, Wichmann C, Kittel R, Sigrist SJ, Buchner E. 2006. 'Bruchpilot,
2023 a protein with homology to ELKS/CAST, is required for structural integrity and function
2024 of synaptic active zones in *Drosophila*', *Neuron*, 49: 833-44.

2025 Wardill TJ, List O, Li X, Dongre S, McCulloch M, Ting CY, O'Kane CJ, Tang S, Lee CH, Hardie
2026 RC, and Juusola M. 2012. 'Multiple spectral inputs improve motion discrimination in
2027 the *Drosophila* visual system', *Science*, 336: 925-31.

2028 Warren TL., Weir PT, and Dickinson MH. 2018. 'Flying *Drosophila melanogaster* maintain
2029 arbitrary but stable headings relative to the angle of polarized light', *J Exp Biol*, 221.

2030 Wehner R, and Muller M. 1985. 'Does Interocular Transfer Occur in Visual Navigation by Ants',
2031 *Nature*, 315: 228-29.

2032 Weir PT, and Dickinson MH. 2012. 'Flying *Drosophila* Orient to Sky Polarization', *Current*
2033 *Biology*, 22: 21-27.

2034 Weir PT, Henze MJ, Bleul C, Baumann-Klausener F, Labhart T, and Dickinson MH. 2016.
2035 'Anatomical Reconstruction and Functional Imaging Reveal an Ordered Array of
2036 Skylight Polarization Detectors in *Drosophila* ', *J Neurosci*, 36: 5397-404.

2037 Wernet MF, Huberman AD, and Desplan C. 2014. 'So many pieces, one puzzle: cell type
2038 specification and visual circuitry in flies and mice', *Genes Dev*, 28: 2565-84.

2039 Wernet MF, Labhart T, Baumann F, Mazzoni EO, Pichaud F, and Desplan C. 2003.
2040 'Homothorax switches function of *Drosophila* photoreceptors from color to polarized
2041 light sensors', *Cell*, 115: 267-79.

2042 Wernet MF, Perry MW, and Desplan C. 2015. 'The evolutionary diversity of insect retinal
2043 mosaics: common design principles and emerging molecular logic', *Trends Genet*, 31:
2044 316-28.

2045 Wernet MF, Velez MM, Clark DA, Baumann-Klausener F, Brown JR, Klovstad M, Labhart T,
2046 and Clandinin TR. 2012. 'Genetic dissection reveals two separate retinal substrates
2047 for polarization vision in *Drosophila* ', *Curr Biol*, 22: 12-20.

2048 Wu M, Nern A, Williamson WR, Morimoto MM, Reiser MB, Card GM, and Rubin GM. 2016.
2049 'Visual projection neurons in the *Drosophila* lobula link feature detection to distinct
2050 behavioral programs', *Elife*, 5: e21022.

2051 Yamaguchi S, Wolf R, Desplan C, and Heisenberg M. 2008. 'Motion vision is independent of
2052 color in *Drosophila*', *Proc Natl Acad Sci USA*, 105: 4910-5.

2053 Zheng Z, Lauritzen JS, Perlman E, Robinson CG, Nichols M, Milkie D, Torrens O, Price J,
2054 Fisher CB, Sharifi N, Calle-Schuler SA, Kmecova L, Ali IJ, et al. 2018. 'A Complete
2055 Electron Microscopy Volume of the Brain of Adult *Drosophila melanogaster*', *Cell*, 174:
2056 730-43 e22.