Supplementary Information

1. Supplementary Figures

Supplementary Fig. 1. Profiles of neurons reconstructed from EM in a dorsal view looking ventral. The full figure contains 379 neurons representing anchor bodies that were relatively completely reconstructed (i.e. containing a large fraction of the neurites within the the 37 µm x 37 µm medulla region of interest) after proofreading and anchor body refinement (Supplementary Methods). In all cases, the neurons were reconstructed to the farthest extent possible within the medulla region of interest. For some neurons, whose arbors extend outside of the region, this resulted in only a partial reconstruction. The multiple profiles of each cell type provide an understanding of the inter-column variation among individual cell types that is useful in cell identification (Supplementary Methods). Horizontal lines mark the borders between the 10 medulla strata (M1-M10) (Fig. 1d). The neurons are grouped by similarity in their stratum arborizations, and assigned to a proposed cell type. A total of 56 of the more confidently identified cell types are detailed in Supplementary Table 2. The full figure is included as a separate file, with 54 pages: Supplementary Fig. 1 – Neuron Reconstructions.docx. The first page of the figure is included here: (see following page). Scale bars: 10µm.
Supplementary Fig. 2. Reconstructed EM profiles (from Supplementary Fig. 1) and corresponding neurons identified from GSC labeling, compared to confirm the existence of cell types for which no matches exist in the collection of Golgi impregnated cell types. Wherever possible, we have shown multiple examples from the EM reconstruction for each cell type. In some cases (such as Mi14 and Dm10), the cells reconstructed from EM appear to have fewer processes than the light microscopy images. This relative sparsening results from difficulties in connecting the fine processes of the arborizations to the main body of the neuron during EM reconstruction. For other cases (such as TmY14, Dm9, and Dm10), the full extent of the neuronal arborization is not seen in the EM profiles, despite the profiles being largely complete within our medulla region of interest. This is because the limited volume of tissue reconstructed by EM contains only a portion of the complete neuron. Scale bars: 10μm.

(see following 3 pages)
Supplementary Fig. 3. The fraction of synaptic connections between Mi1 / Tm3 and T4 neuron pairs (derived from sparse proofreading) which have a connection weight greater than indicated.
Supplementary Fig. 4. Receptive field components for 19 T4s, ordered by the depth of their arborizations within the lobula plate (Fig. 6b) (blue: inputs through Mi1 neurons; red: inputs through Tm3 neurons). The color within each hexagon is computed by placing the mass corresponding to the compound synaptic weight from L1 through one of Mi1 (blue) or Tm3 (red) to T4 (Supplementary Methods) at the center of the corresponding hexagon. Purple cells represent overlapping receptive field components. The center of mass of the Mi1 component (blue circle, computed as shown schematically in Fig. 4e) is displaced relative to the center of mass of the Tm3 component (red circle). Three T4s, the axons of which were not traceable, are in the bottom row.
Supplementary Fig. 5. Directionality of dendrite branches, colored as in Fig. 6. The cells are ordered according to their lobula plate axon arborization depth (as in Supplementary Fig. 4), except for T4-4, T4-14, and T4-5 (right of dotted lines in Layers 1 and 2). These three cells with untraced axons have been assigned to layers based on their dominant direction of arborization. T4-4 (P = 0.09) and T4-14 (P = 0.09) are classified into Layer 1, and T4-5 (P = 2.1 x 10^-4) is classified into Layer 2 (Supplementary Methods). Scale bar: 5 µm.
Supplementary Fig. 6. Arbor overlap (quantified by Peters’ rule) provides poor quantitative predictions of connection weight. (a) The overlap between the outer surfaces of a neuron pair, compared with the synaptic weight between the pair, found through EM reconstruction. The most completely reconstructed cells were chosen to compute overlap. Since overlap does not define directionality, the synaptic weight for each pair is summed in both directions. There is a positive correlation ($R^2 = 0.79$, $P<0.01$) overall, but quantitative predictions of the number of synaptic contacts based on overlapping area are poor for many pairs: 13% of neuron pairs lie outside of the 95% confidence intervals (yellow) computed from the known rate of tracing terminations outside of an identified body. In order to compute the extent of this region, we first make the assumption (as in the text) that the false negative rate applies uniformly to all synapses. We then compute a line of best fit for our data, i.e. to obtain the best fit
estimate of Peters’ rule, and sample, over a range of overlapping areas, from a binomial
distribution with: 1) p = 0.4 (known for our consensus connectome), and 2) n chosen to
center the mode of the distribution on the Peters’ rule estimate of synaptic strength (as
in our Monte Carlo Error Estimate (Supplementary Methods)). (b) An example for cells
involved in the candidate motion detection circuit: T4-5, Tm3-a, and Tm3-p arborizations
in M10. These pairs of neurons are circled in (a). Both Tm3 neurons have an overlap
with T4-5 of ~0.6 µm², yet the number of connections is different. Selective sparse
tracing (Supplementary Methods) revealed that Tm3-a was presynaptic to T4-5 at 6
contacts, while Tm3-p was presynaptic to T4-5 with only 3 contacts.
2. Supplementary Methods

Tissue Preparation and Transmission EM (TEM). The head of a wild-type Oregon R female fly, 5 days post-eclosion, was dissected in 2.5% glutaraldehyde and 2.2% formaldehyde, as paraformaldehyde, in 0.1M cacodylate at pH 7.3 to expose the brain. The brain was then prepared for high pressure freezing, as in 61. Briefly, the brain was fixed for a total of 5-10 min, then transferred to 20% aqueous bovine serum albumin for a few minutes, before being carefully loaded into a 200 µm deep specimen carrier and high-pressure frozen in a Wohlwend HPF Compact 01 High Pressure Freezing Machine (Wohlwend GmbH, Sennwald, Germany). The brain was then freeze-substituted62 in a Leica EM AFS2 system in 1% osmium tetroxide, 0.1% uranyl acetate and 5% water in 100% acetone with 1% methanol, for 3 more days, starting at -140°C inc. to -90°C for 8h, and after 38h gradually increasing to -20°C for 14h overnight, then finally warmed to 20°C for 4h, before embedding in Poly/Bed 812 epoxy.

The right part of the brain, oriented as in 10, was serially sectioned in a plane tangential to the surface of the medulla using a microtome set at 40nm section thickness (total sectioning time: 144 hours). Ribbons of sections were collected on Pioloform-coated 1 x 2 µm slot grids. The first 1460 sections of the series contained the medulla, with a further set of 1309 sections containing the deeper inner chiasm, lobula and lobula plate. 17% of the sections contained large (≥ 300 nm wide) folds, large enough to make the identification of connected processes across the fold difficult, and a further 18% of the sections contained small folds (< 300 nm wide), too small to significantly obscure cellular processes. We used the smallest of these small folds to estimate section thickness (using the “small-fold method”)63 and determined that the section thickness was 42 nm with a S.D. of 6 nm between sections. We confirmed this by making a second, independent measure of average thickness, utilizing a modified version of the method in 64, dividing the caliber of a longitudinally sectioned neurite by the number of sections that intersect the neurite. We arrived at an estimate of 44 ± 13 nm.

The series of sections was imaged as mosaics of 4k x 4k micrographs, at a magnification of 5,000x, using an FEI Tecnai 20 operated at 80 kV equipped with a Gatan UltraScan 4000 camera controlled by Leginon software65 (taking a total of 1560 hours). Three sections were lost during imaging and 18 sections were contaminated with uranyl acetate deposits during post-contrast staining; these sections all lay outside the first 1460 sections (containing the medulla) and instead within the deeper 1309 sections. In total, the dataset consisted of >200,000 micrographs (>2Tb in size). Within this stack, we chose a reference column, located ~12 rows from the anterior margin and 16 rows from the dorsal margin of the medulla.

Proofreading/Reconstruction. The five key steps within the reconstruction procedure are as follows:

1) Volume proofreading. We divided the central region of the image dataset (4k x 4k pixels throughout the depth of the medulla) containing the reference column and its
immediate surround (including part of the six nearest-neighbor columns) into multiple subvolumes, each comprising 150 sections. A proofreader examined each subvolume’s segmentation, proofreading segments by size in descending order until 70-90% of the pixels had been assigned to fragments of neuronal arbors. To complete this step, each proofreader used elementary operations in Raveler, most commonly the re-assignment of a super-pixel to a different neuronal fragment. This process took ~2490 person-hours in total. The resulting fragments were inspected for false connections and manually stitched together across the large subvolumes to form large bodies within the entire, connected medulla. This step was performed by an expert; (ST) (total time: ~150 hours). Because only a limited number of neurites had to be connected between each pair of subvolumes, this stitching task was unambiguous.

2) Synapse annotation. Experts (ST and ST2) annotated all the synapses within the central 4k x 4k region spending ~300 person-hours. Presynaptic terminals were identified by the presence of a T-bar ribbon\textsuperscript{66}. Postsynaptic sites were identified by the presence of a postsynaptic density (PSD) proximal to the T-bar (Fig. 2c). In rare cases (< 1%), a postsynaptic site that did not contain a PSD, but was directly adjacent to the presynaptic T-bar pedestal, was also annotated, justified purely by its proximity to the T-bar. The identification of T-bars and associated PSDs was validated by having both ST and ST2 independently annotate synapses within two sub-volumes of neuropil (4k x 4k pixels, and 10 sections in depth). Comparing the result of combining both experts (consensus decision) vs. including all T-bars and PSDs found by either expert (inclusive decision), we find that the both experts agree on an average of 79% of the T-bars found, and on an average of 72% of the PSDs found (associated with the fraction of T-bars that they agree on).

3) Postsynaptic tracing. Initially, we relied on ST to associate the postsynaptic sites identified in step 2 with the anchor bodies constructed in step 1. After completing less than 10% of the task, most of which confirmed previously reported connections of the lamina input terminals\textsuperscript{10,67}, the time that had been spent made it clear that we needed a method to accelerate the proofreading, while maintaining reliability. After testing on subsets of synapses, we recruited six proofreaders and assigned each postsynaptic site at random to two of these proofreaders, using their consensus to determine the correct tracing (see Reliability). In total, this proofreading step took ~3240 person-hours to complete.

4) Anchor body refinement. Given that neurons were identified based on arbor shape (see Cell Identification), anchor bodies that extended outside the reference column had to be traced outwards sufficiently far to reconstruct a recognizable shape. To this end, each anchor body was assigned to a proofreader with the goal of reconstructing the entire shape of the neuron to which it contributed. Only those proofreaders who could reconstruct all the major branches and points of exit for five different neurons within the medulla were chosen to participate in anchor body refinement. Therefore, it appears that this task is performed close to error-free by the remaining proofreaders, although it is very slow (taking ~4500 person-hours in total). In addition, after this refinement all the reconstructed neurons were then manually inspected by an expert.
Any that looked unrealistic were identified and corrected. This took an additional ~500 person-hours of expert time.

5) Selective sparse tracings. To characterize the candidate motion circuit further, we sparsely traced connections between members of the circuit in 18 columns surrounding the reference medulla column (Fig. 4, Supplementary Table 3). In particular, we traced (a) processes of 20 Tm3 neurons postsynaptic to the 18 columnar L1 neurons, (b) processes of 19 T4 neurons postsynaptic to these 20 Tm3 neurons, and (c) processes of the same 19 T4 neurons postsynaptic to the 18 columnar Mi1 neurons. This sparse proofreading took a total of ~3000 person-hours, using five proofreaders working without duplication on different subsets of the tracing. Note that it was not necessary to trace the processes of the 18 Mi1 neurons postsynaptic to the 18 L1 neurons in the surrounding columns because the initial dense tracing showed that >99% of the L1 to Mi1 synaptic contacts were from the L1 to the Mi1 in the same column, without any spread to Mi1s in adjacent columns.

After examining the results and realizing the importance of the precise numbers of connections, to increase our confidence in the proofreading results, we had an expert (ST2) repeat all the sparse tracing independently (spending ~1500 person-hours). With this additional proofreading we were able to reduce the fraction of false negatives in this data set. In the case of postsynaptic processes of type (a), we were able to proofread 77% of the PSDs. Similarly, we were able to proofread 56% of postsynaptic processes in (b), and 57% of postsynaptic processes in (c).

In addition, to assess the direction preference of T4 neurons, we successfully traced 16 out of the 19 T4 neurons’ axons to the lobula plate (Fig. 4a, 6b). Then we annotated and determined the center of mass of the locations of T-bars in the lobula plate for each T4. Finally, we computed the relative arborization depth by dividing the center of mass distance from the outer edge of the lobula plate by the lobula plate’s thickness, within the section containing the center of mass (Fig. 6b). This tracing and annotation of synapses into and within the lobula plate was performed mostly by an expert (ST), taking a total of 100 person-hours.

**Cell Identification.** We assigned the neurons reconstructed from TEM to specific cell types by matching their shapes to those obtained from Golgi impregnations or from confocal microscopy of genetically labeled single neurons (Supplementary Table 2). An initial set of potentially identifiable reconstructions was selected by ST, and the precise matching was performed by two experts (ST and AN) working together (for a total of ~100 person-hours). The matching of cell types was facilitated by registering cells to the medulla strata, focusing on the stratum location and appearance of their arbors, and occasionally their axon calibers. For most cell types, we reconstructed multiple representatives from TEM (Supplementary Fig. 1). This provided an estimate of the inter-column variation, which also aided in making the match.
In many cases, the match between EM reconstructions, confocal images and Golgi profiles appeared unequivocal (see Supplementary Table 2). Matches that were considered acceptable by both experts and for which there was no disagreement between the experts accounted for about two-thirds of the cell types in Supplementary Table 2. All of the modular cell types used to construct Fig. 3 were in this most reliably identified group. For other reconstructions, the precise identification was more difficult. For example, difficulties in connecting the smallest processes to the cell body resulted in a reduced density of fine processes in EM, as compared with light microscopy. Further, the limited total volume in EM reconstruction resulted in the truncation of neurons with extensive lateral arborizations, such as many amacrine and tangential cells, as well as the loss of the axon terminals from neurons with projections out of the medulla, such as Tm and TmY cells (Supplementary Fig. 2). Because of these limitations the other identifications in Supplementary Table 2 remain somewhat uncertain and in several cases identify a small group of similar neurons rather than a single cell type. These identifications could be best confirmed via further expansion of the reconstruction to include a larger portion of the arbor, or the reconstruction of more examples. Nevertheless some of these less conclusive matches were considered sufficiently informative to justify inclusion in the table. Further notes on the matching of individual cell types can be found in Supplementary Table 2. Other reconstructions that were only matched to a very general class (e.g. likely tangential) are not included in Supplementary Table 2 but shown in Supplementary Figure 1. Some of these might be identified with additional information from light microscopy (for example from the relative positions of cell types).

The images of genetically labeled single neurons that we used were produced as part of the Janelia Fly Light Team Project and their generation and analysis will be described in detail elsewhere. Briefly, a “flip-out”-based approach was used to stochastically label individual neurons which were visualized together with a reference pattern (anti-Brp staining) by immunofluorescence microscopy. Images were segmented using NeuronSeparator (Myers et al., unpublished) and the rotated views shown in Supplementary Fig. 2 generated using NeuronAnnotator (Janelia Fly Light Scientific Computing Team, unpublished), a modified version of Vaa3D. The GAL4 lines that were used were R34C04 (for Mi13), R26H07 (for Mi15) and R57C10 (all other cell types).
3. Supplementary Tables

Supplementary Table 1. Results of proofreading individual postsynaptic sites, throughout the 4k x 4k central region of interest. The full table (~700 pages) includes 33641 postsynaptic sites in total, and includes all the synaptic connections identified after reconstruction. It is included as a separate file: Supplementary Table 1 – Complete Medulla Results.xlsx. The first page of this table is included here (see following page).
## Presynaptic Site Details

| Postsynaptic Sites ID | Name / Body ID | Location x | y | z | Additional Comments | Proofreading Details |
|-----------------------|----------------|------------|---|---|---------------------|---------------------|
| 1                     | 200            | 5697       | 7408 | 168 |                     | 2 reached same anchor body |
| 2                     | 200            | 5697       | 7408 | 168 |                     | Both are orphan |
| 3                     | 200            | 5697       | 7408 | 168 |                     | Both are orphan |
| 4                     | 200            | 5697       | 7408 | 168 |                     | Both are orphan |
| 5                     | R7 205         | 4240       | 5833 | 169 | L5 206              | Densely Named |
| 6                     | R7 205         | 4240       | 5833 | 169 | 204                 | Densely Named |
| 7                     | C2 445362      | 5599       | 4818 | 169 |                     | Both are orphan |
| 8                     | C2 445362      | 5599       | 4818 | 169 | L3 520639           | Densely Named |
| 9                     | C2 445362      | 5599       | 4818 | 169 |                     | Both are orphan |
| 10                    | 200            | 5637       | 7492 | 169 | L2 198              | 1 reached named body |
| 11                    | 200            | 5637       | 7492 | 169 |                     | Both are orphan |
| 12                    | 200            | 5637       | 7492 | 169 |                     | Both are orphan |
| 13                    | 200            | 5637       | 7492 | 169 | L3 491118           | 1 reached named body |
| 14                    | 200            | 5562       | 7217 | 170 |                     | Both are orphan |
| 15                    | 200            | 5562       | 7217 | 170 |                     | Both are orphan |
| 16                    | 200            | 5562       | 7217 | 170 | L5 226              | 1 reached named body |
| 17                    | C2 214         | 4201       | 5747 | 172 | M15 222             | Densely Named |
| 18                    | C2 214         | 4201       | 5747 | 172 | 204                 | Densely Named |
| 19                    | C2 214         | 4201       | 5747 | 172 | L3 208              | Densely Named |
| 20                    | C2 214         | 4201       | 5747 | 172 | L3 206              | Densely Named |
| 21                    | R7 205         | 4532       | 5956 | 173 | L3 206              | Densely Named |
| 22                    | R7 205         | 4532       | 5956 | 173 | L2 212              | Densely Named |
| 23                    | C2 214         | 4275       | 5730 | 174 | M15 222             | Densely Named |
| 24                    | C2 214         | 4275       | 5730 | 174 | L1 209              | Densely Named |
| 25                    | C2 214         | 4275       | 5730 | 174 | L3 206              | Densely Named |

Comments:

1: Matrix is iterated by postsynaptic sites. Each postsynaptic site is associated with a single presynaptic T-bar (but multiple postsynaptic sites can be associated with each T-bar). There are a total of 33641 postsynaptic sites in this table. This number is less than the total of 38465 postsynaptic bodies that we annotated within our region of interest. The remaining 4824 sites were either associated with a presynaptic T-bar that was not located within an anchor body (and, hence, were not proofread), or were simply not proofread by two independent proofreaders. In both cases, the postsynaptic sites were not included in the table.

2: Identity of the body within which the presynaptic T-bar (for the postsynaptic site of interest) is located. This is either a named body, or a large, anchor body (see Supplementary Methods).

3: Location of each annotated T-bar or postsynaptic site is provided using pixel coordinates (in the xy plane) and the section number (in the z axis).

4: Identity of the body to which the postsynaptic dendrite was traced. Either an identified neuron was found, in which case the name is provided, or a large, anchor body was identified (see Supplementary Methods). In which case the arbitrary numerical body ID was provided. This identification was provided either if both proofreaders reached the same body, or if one proofreader reached a body, and the second proofreader terminated their proofreading prior to reaching a body (and the cases were detailed in Proofreading Details). In the case where both proofreaders were unable to proofread the dendrite to a body, a "?" is included. Finally, in the case, where there is a disagreement between both proofreaders, where both proofreaders reached an anchor body or an identified body, the type of disagreement was noted in the Proofreading Details column and either a "?" was again noted, or the more confidently identified body was noted (e.g. the named body if one proofreader traced to a named body, and the other traced to an anchor body). For the purposes of constructing Fig. 3, only the two proofreader agreement results from this table were utilized.

5: Any further postprocessing necessary to obtain the results in this table is detailed in these columns.
**Supplementary Table 2.** The table contains 56 cell types, exemplified by 256 neurons reconstructed from EM, and identified by comparing their arborization with light microscopy. For every such cell type (Supplementary Fig. 1), we report the number of individual cell profiles that were reconstructed in the central seven columns, as well as the methods by which its identity has been validated. Y(es), and M(ultiple) indicate the number of potentially matching cell types as observed by Golgi (as compared with 21) or GSC, either one match in the case of Y, or greater than one match in the case of M. Cell types were classified as Medulla intrinsic neurons (Mi), Distal medulla amacrine neurons (Dm), Proximal medulla amacrine neurons (Pm), Transmedulla neurons (Tm), Transmedulla Y neurons (TmY) (with a Y shaped projection to both lobula and lobula plate), T cells, and Y cells, according to 21. Tm and TmY neurons were grouped together because they are most easily distinguished by reconstructions beyond the medulla region, information missing from our analysis. The most confident identifications, which are considered essentially unequivocal (with some minor qualifications included as footnotes), are emphasized in bold type. We also identify a subset of modular cells, as defined in the text. With a few exceptions (see footnotes), cell types were included in the modular set if five or more examples were reconstructed from the seven central columns.

(see following 2 pages)
| Retina / Lamina | EM | Golgi | GSC | Modular |
|----------------|----|-------|-----|---------|
| R7             | 7  | Y     | Y   | Y       |
| R8             | 7  | Y     | Y   | Y       |
| L1             | 7  | Y     | Y   | Y       |
| L2             | 7  | Y     | Y   | Y       |
| L3             | 7  | Y     | Y   | Y       |
| L4             | 3  | Y     | Y   | Y²      |
| L5             | 7  | Y     | Y   | Y       |
| C2             | 5  | Y     | Y   | Y       |
| C3             | 3  | Y     | Y   | Y²      |
| T1             | 1  | Y     | Y   | Y²      |
| LaWF1          | 2  | Y     | Y   | -       |

| Mi              | EM | Golgi | GSC | Modular |
|-----------------|----|-------|-----|---------|
| Mi1             | 7  | Y     | Y   | Y       |
| Mi3-like³       | 1  | Y     | Y   | -       |
| Mi4             | 7  | Y     | Y   | Y       |
| Mi9             | 7  | Y     | Y   | Y       |
| Mi10³           | 2  | Y     | Y   | -       |
| Mi13⁵           | 3  | -     | Y   | -       |
| Mi14⁵           | 1  | -     | Y   | -       |
| Mi15⁵           | 4  | -     | Y   | Y⁶      |

| Dm              | EM | Golgi | GSC | Modular |
|-----------------|----|-------|-----|---------|
| Dm1⁷            | 2  | M     | M   | -       |
| Dm2             | 5  | Y     | Y   | Y       |
| Dm3-like⁸       | 3  | Y     | M   | -⁹      |
| Dm4⁴⁰           | 2  | Y     | Y   | -       |
| Dm7-like¹¹      | 1  | Y     | Y   | -       |
| Dm8             | 5  | Y     | Y   | Y¹²     |
| Dm9⁵,13         | 1  | -     | Y   | -       |
| Dm10⁵,14        | 2  | -     | Y   | -       |

| Tm / TmY        | EM | Golgi | GSC | Modular |
|-----------------|----|-------|-----|---------|
| Tm1             | 7  | Y     | Y   | Y       |
| Tm2             | 7  | Y     | Y   | Y       |
| Tm3¹⁷           | 11 | Y     | Y   | Y (1.5)³⁸ |
| Tm4             | 7  | Y     | Y   | Y       |
| Tm5a¹⁰          | 4  | Y¹⁹   | M   | -       |
| Tm5b¹⁰          | 1  | Y¹⁹   | M   | -       |
| Tm5Y¹⁹,2⁰       | 3  | Y     | M   | -       |
| Tm6/Tm14²¹      | 4  | M     | Y   | Y⁶      |
| Tm9             | 4  | Y     | Y   | Y⁶      |
| Tm12/Tm25²²     | 1  | M     | Y   | -       |
| Tm16²³          | 2  | M     | Y   | -       |
| Tm20            | 5  | Y     | Y   | Y       |
| Tm23/Tm24²²     | 4  | M     | M   | -       |
| Tm28/TmYg²²     | 1  | M     | M   | -       |
| TmY3            | 4  | Y     | Y   | -       |
| TmY4²³          | 2  | M     | Y   | -       |
| TmY5-like²⁴     | 2  | Y     | Y   | -       |
| TmY5a²⁴         | 5  | Y     | Y   | Y       |
| TmY10           | 2  | Y     | Y   | -       |
| TmY13²³         | 2  | M     | Y   | -       |
| TmY14⁵,²⁵       | 2  | -     | Y   | -       |

| T              | EM | Golgi | GSC | Modular |
|----------------|----|-------|-----|---------|
| T2⁶⁶           | 5  | Y     | Y   | Y       |
| T2a²⁶          | 6  | Y     | Y   | Y       |
| T3             | 3  | Y     | Y   | -       |
| T4             | 29 | Y     | Y   | Y (4)²⁷ |

| Y              | EM | Golgi | GSC | Modular |
|----------------|----|-------|-----|---------|
| Y3/Y6²⁸       | 3  | M     | Y   | -       |
| Y4            | 2  | Y     | Y   | -       |

¹ Cells that are distributed one per column (syneriodic) or more than one per column (ultraperiodic; Tm3 and T4). The stoichiometry for each ultraperiodic cell is given in brackets. In most cases, the syn- or ultraperiodicity of the cell types were supported by the reconstruction of multiple representatives within the central seven columns of our reconstruction.

² Since fewer than 5 cells were reconstructed, the syneriodic nature of these cell types (assumed to be inherited from the lamina) was further confirmed using specific GAL4 drivers (lines described in (Tuthill et. al., Neuron, 2013)).

³ Layer pattern appears to differ slightly from Golgi.

⁴ Good match between EM and GSC. Golgi profile also matches well overall. However, unlike both EM and GSC, it shows most proximal branches in stratum M9 and not M10.

⁵ These cell types were not identified in the Golgi study (Fischbach and Dittrich (1989)) but are very similar to cells found by GSC (see Supplementary Figure 2).
Four neurons were reconstructed for each of these cell types within the central seven columns. This is generally less than our threshold for synperiodicity. Indeed, identification of additional cells of these types within the seven columns would solidify their inclusion in the modular set. However, this assignment is, at present, additionally supported by the convergence of the spatial distribution of their arbor, the evidence of tiling across at least a subset of the medulla columns, and some evidence from light microscopy.

EM reconstructions have limited width, providing insufficient information to reconstruct the entire cell and distinguish Dm1 from fragments of some other cell types (such as Dm6 and other similar cells seen by GSC).

GSC labeling revealed another type of Dm neuron with a slightly more distal position, which is also a plausible match for the EM reconstructed cells. The identification of the Dm3-like cells is therefore currently considered uncertain.

Light microscopy data (AN, unpublished) suggest that Dm3 is likely synperiodic. However, the other potential match for the Dm3-like cells (see 8) is not. Since the cell identification is not definitive, and the reconstructions may even possibly represent a mix of both potential matches, there is insufficient evidence to include Dm3-like in the modular set.

The Dm4 reconstructions match the GSC data well. However, both the EM reconstructions and Golgi drawings appear to show only parts of Dm4 cells since typical GSC examples are multicolaminar with prominent bouton-like terminals in M3/M4 of approx. 10 columns.

The match to the Golgi data is not certain: The Dm7 Golgi profile arborizes in M6 but our EM reconstruction arborizes in M7.

The identification of five Dm8 cells in the central seven columns suggests that the multicolaminar Dm8 is perhaps synperiodic, and, as such, it has been included in the modular set.

The Dm9 EM reconstruction matches the light data well but seems restricted to a single column while all GSC examples of this cell type are multicolaminar. This difference is most likely due to the incomplete reconstruction of fine processes connecting the Dm9 arbors in different columns. A cell type similar to Dm9 has been shown in Gao et al. (2008).

Dm10 is similar (and possibly identical) to a cell type described in Morante and Desplan (2008) as Dm1-5.

Several cell types resemble Pm1 or Pm2 when only part of the cell is reconstructed (as in the EM examples). These include Pm1 and Pm1a in Fischbach and Dittrich (1989), as well as multiple similar cells in the GSC data. These matches are therefore ambiguous and the reconstructed fragments might include multiple cell types.

More than 5 fragments of both Pm1 and Pm2 were reconstructed but because of the extended multi-columnar spread of these cells (as seen by GSC) and the possible inclusion of multiple cell types in these groups (see 15, above), neither Pm1 or Pm2 are considered to be syn- or ultraperiodic.

Some of the reconstructed examples of Tm3 might be Tm3Y, as described by Golgi. Tm3Y differs from Tm3 only in a very small lobula plate branch and is therefore indistinguishable within our EM reconstruction. However, most Tm3-like cells seen by GSC do not have a lobula plate branch.

The number of Tm3s identified within the central seven columns - 11 - indicates that Tm3s are likely ultraperiodic with approximately 1.5 cells per column.

Classification of the Tm5a and Tm5b subtypes, which were not distinguished by the Fischbach and Dittrich Golgi studies, follows Gao et al. (2008), and is based on the width of the arborization in M6. However, we note that the ascending dendrite in the EM reconstructions appears to end in M4 and does not extend to M3, as described in Gao et al., potentially due to some fine branches being missing in the EM reconstructions. GSC labeling identified many Tm5-like cells with considerable variability (which may represent more than two cell types.)

As for Tm3/Tm3Y, the presence or absence of a Y-branch cannot be determined from the EM reconstructions. GSC data include Tm5(Y)-like cells both with and without a lobula plate branch.

Fischbach and Dittrich distinguish Tm6 and Tm14 based on small differences in their layer specificity in medulla and lobula. The EM reconstructions and GSC data appear more consistent with a single Tm6/Tm14 cell type.

The three pairs: 1) Tm12 and Tm25, 2) Tm23 and Tm24 (and other similar cells seen by GSC) and 3) Tm28 and Tm9 appear to be indistinguishable based on their medulla arbors alone.

To distinguish TmY13, TmY4, and Tm16 (and TmY11 and TmY2, found only in the Golgi study), we first compared the GSC images (which include medulla, lobula and lobula plate) of these similar cells to the Golgi drawings. We then utilized the GSC images as an intermediary, identifying the best match between EM reconstructions and the GSC images, based on layer positions and shape of arbors in the medulla.

TmY5-like and TmY5a cells fasciculate together within medulla columns. Based on this feature, two “unknown TmY-1” neurons (Supplementary Fig. 1) might be variants of TmY5-like. GSC images do not clearly distinguish TmY5-like and TmY5a cells.

GSC data indicate that this new cell type, which was named TmY14, is an unusual TmY with both a lobula and lobula plate branch as well as a projection to the central brain.

Very slender arbors in distal and proximal medulla appear to be incomplete in the EM reconstruction.

Ultraperiocdic. The total number of neurons with axon branch points within our seven column region, 29, supports an estimate of approx. 4 neurons per column.

The Y3 and Y6 cell types shown in Fischbach and Dittrich differ in their lobula complex arbors but are very similar in the medulla.
**Supplementary Table 3.** Numbers of synaptic contacts for cell types involved in the candidate motion detection circuit. Numbers are given from a given cell type (for L1) or all neurons of one cell type (for Mi1 and Tm3) (rows, presynaptic) to each cell of another type (columns, postsynaptic).

|   | Tm3   |   |   |   |   |   |   |   |   |   |
|---|-------|---|---|---|---|---|---|---|---|---|
|   | 3453  | 210 | 194 | 321031 | 62 | 64633 | 3620 | 5135970 | 388678 | 346731 |
| L1 | 68    | 76  | 70  | 70   | 70 | 65   | 66   | 66   | 70   | 49  |

|   | Tm3   |   |   |   |   |   |   |   |   |   |
|---|-------|---|---|---|---|---|---|---|---|---|
|   | 44324 | 382033 | 239965 | 4331343 | 5025067 | 467463 | 967907 | 468139 | 5530404 | 5656185 |
| L1 | 53    | 60  | 54  | 60   | 44 | 55   | 70   | 49   | 34   | 34  |

| Mi1 | T4              |   |   |   |   |   |   |   |   |   |
|-----|-----------------|---|---|---|---|---|---|---|---|---|
|     | 277709 (T4-1)   | 386464 (T4-2) | 280303 (T4-3) | 2341 (T4-4) | 278848 (T4-5) | 545716 (T4-6) | 455131 (T4-7) | 545944 (T4-8) | S189 (T4-9) | 476680 (T4-10) |
| 215 | 8               | 19 | 17 | 20 | 21 | 2   | 10  | 24  | 6   | 11  |
| 446263 | 4             | 7  | 0  | 7  | 1  | 7   | 0   | 23  | 0   | 0   |
| 29565 | 26             | 0  | 15 | 1  | 8  | 16  | 4   | 0   | 0   | 0   |
| 194027 | 4             | 1  | 10 | 0  | 1  | 13  | 25  | 0   | 0   | 0   |
| 24070 | 0              | 1  | 1  | 1  | 2  | 0   | 0   | 0   | 1   | 23  |
| 170689 | 0             | 1  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 23  |
| 5809 | 1              | 1  | 10 | 19 | 0  | 6   | 23  | 2   | 0   | 0   |
| 15557812 | 0            | 0  | 0  | 0  | 0  | 0   | 1   | 0   | 0   | 0   |
| 14141500 | 2             | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   |
| 10051409 | 1             | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   |
| 455187 | 4              | 0  | 5  | 0  | 0  | 0   | 7   | 0   | 0   | 0   |
| 3912 | 0              | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   |
| 4314313 | 0            | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   |
| 4218151 | 0           | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   |
| 28650283 | 0          | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 0   |
| 5509511 | 0            | 0  | 0  | 0  | 0  | 0   | 0   | 0   | 0   | 1   |
| 30385 | 0              | 0  | 0  | 0  | 0  | 0   | 2   | 11  | 0   | 0   |
| 172527 | 0            | 0  | 0  | 0  | 0  | 0   | 3   | 0   | 0   | 0   |
| 588525 | 0            | 0  | 0  | 1  | 0  | 0   | 2   | 1   | 0   | 0   |
|     | T4     |     |     |     |     |     |     |     |     |     |     |
|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | 588435 (T4-11) | 460193 (T4-12) | 515936 (T4-13) | 586983 (T4-14) | 546035 (T4-15) | 475117 (T4-16) | 547009 (T4-17) | 547221 (T4-18) | 455135 (T4-19) |
| 215 | 29     | 3   | 2   | 1   | 7   | 8   | 5   | 2   |
| 446263 | 1     | 31  | 0   | 0   | 18  | 0   | 0   | 0   |
| 29565 | 1     | 3   | 0   | 0   | 0   | 0   | 0   | 0   |
| 194027 | 0     | 0   | 22  | 0   | 0   | 3   | 31  | 2   |
| 24070 | 15    | 0   | 6   | 0   | 0   | 31  | 13  | 2   |
| 170689 | 2     | 0   | 0   | 1   | 0   | 1   | 0   | 0   |
| 5809 | 2     | 0   | 0   | 19  | 21  | 0   | 0   | 0   |
| 15557812 | 0     | 3   | 0   | 0   | 0   | 0   | 0   | 0   |
| 141141500 | 0     | 3   | 0   | 0   | 0   | 0   | 0   | 0   |
| 10051409 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 455187 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 3912 | 0     | 0   | 5   | 0   | 0   | 5   | 0   | 2   |
| 4314313 | 0     | 0   | 12  | 0   | 0   | 2   | 4   | 5   |
| 4218151 | 0     | 0   | 0   | 0   | 0   | 1   | 0   | 0   |
| 28650283 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 555951 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 30385 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 172527 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 588525 | 0     | 6   | 0   | 2   | 7   | 0   | 0   | 0   |

|     | T4     |     |     |     |     |     |     |     |     |     |     |
|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | 277709 (T4-1) | 386464 (T4-2) | 280303 (T4-3) | 2341 (T4-4) | 278848 (T4-5) | 545716 (T4-6) | 455131 (T4-7) | 545944 (T4-8) | 5189 (T4-9) | 476680 (T4-10) |
| 3453 | 0     | 5   | 0   | 8   | 8   | 0   | 1   | 8   | 6   | 1   |
| 210 | 0     | 3   | 0   | 4   | 0   | 4   | 0   | 8   | 3   | 0   |
| 194 | 0     | 3   | 0   | 4   | 0   | 4   | 0   | 8   | 3   | 0   |
| 3211031 | 1     | 2   | 0   | 0   | 5   | 0   | 0   | 3   | 0   | 0   |
| 62 | 0     | 10  | 6   | 6   | 0   | 1   | 0   | 1   | 0   | 0   |
| 584633 | 0     | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 0   | 0   |
| 3620 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5135970 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 388678 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 346731 | 0     | 1   | 0   | 1   | 5   | 0   | 0   | 9   | 3   | 0   |
| 44324 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 382033 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 239965 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 4331343 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5025067 | 10    | 0   | 3   | 0   | 6   | 0   | 0   | 0   | 0   | 0   |
| 4674663 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 967907 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 468139 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5530404 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5656185 | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

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|       | T4-11 | T4-12 | T4-13 | T4-14 | T4-15 | T4-16 | T4-17 | T4-18 | T4-19 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| T4    | 588435| 460193| 515936| 586983| 546035| 475117| 547009| 547221| 455135|
|       | 3     | 4     | 0     | 3     | 7     | 0     | 0     | 0     | 0     |
|       | 210   | 7     | 0     | 3     | 0     | 0     | 9     | 7     | 1     |
|       | 194   | 8     | 0     | 0     | 0     | 0     | 2     | 0     | 3     |
|       | 321031| 3     | 3     | 0     | 0     | 4     | 0     | 0     | 0     |
|       | 62    | 1     | 0     | 3     | 0     | 0     | 0     | 0     | 0     |
|       | 64633 | 0     | 0     | 6     | 0     | 0     | 6     | 5     | 4     |
|       | 3620  | 0     | 6     | 0     | 1     | 8     | 0     | 0     | 0     |
|       | 5135970| 0     | 0     | 3     | 0     | 0     | 0     | 3     | 0     |
|       | 388678| 0     | 0     | 8     | 0     | 0     | 2     | 5     | 1     |
|       | 346731| 0     | 0     | 0     | 1     | 0     | 0     | 0     | 0     |
|       | 44324 | 0     | 0     | 0     | 7     | 6     | 0     | 0     | 0     |
|       | 382033| 2     | 0     | 0     | 0     | 0     | 1     | 0     | 10    |
|       | 239965| 0     | 4     | 0     | 0     | 0     | 0     | 0     | 0     |
|       | 4331343| 0    | 3     | 0     | 0     | 0     | 0     | 0     | 0     |
|       | 5025067| 0    | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
|       | 4674663| 0    | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
|       | 467907| 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     |
|       | 468139| 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
|       | 5530404| 0    | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
|       | 5656185| 0    | 0     | 2     | 0     | 0     | 0     | 0     | 0     |
7. Supplementary Data

Supplementary Data 1. This folder (‘TEM Subvolume’) contains 10 images (jpeg) through a small subsection of the medulla data set (~2.5 µm x ~1.9 µm). Within each image, we show three different versions of the same section. In the top left, we show the raw TEM grayscale image. In the top right, we show an overlay of the locations of T-bars (red circles) and PSDs (blue circles) on the TEM image. In the bottom left, we show an image of the proofread segmentation, with cells delineated by different colors (as in Fig. 2b). In a subset of the segmentation images, narrow non-colored lines are an artifact of the mapping of segmentation onto the registered images, and can be ignored.

8. Supplementary Video

Supplementary Video 1. This video shows the EM image stack of the medulla region of interest (Fig. 1c). Images from the stack are progressively removed (directed towards the deeper strata), and reconstructions of 379 neurons are added in succession, in randomly selected colors. The neurons are grouped into six classes, with text describing the class being added simultaneously with the neurons from each class: (1) Photoreceptor terminals, from the retina, and neurons from the lamina that innervate the reference column within the medulla. (2) Neurons receiving direct input from the retina and lamina neurons. (3) Neurons receiving direct input from the neurons in class (2). (4) Neurons that arborize in the reference column, but spread across multiple columns. (5) Tangential neurons, of which often only a fragment passing through the region of interest has been reconstructed. (6) Additional neurons, often of the same class as neurons in classes (1) – (5), but from adjacent columns.
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