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The optic lobe of *Drosophila melanogaster*

II. Sorting of retinotopic pathways in the medulla

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Summary. We present a quantitative evaluation of Golgi-impregnated columnar neurons in the optic lobe of wild-type *Drosophila melanogaster*. This analysis reveals the overall connectivity pattern between the 10 neuropil layers of the medulla and demonstrates the existence of at least three major visual pathways. Pathway 1 connects medulla layer M10 to the lobula plate. Input layers of this pathway are M1 and M5. Pathway 2 connects M9 to shallow layers of the lobula, which in turn are tightly linked to the lobula plate. This pathway gets major input via M2. Pathways 1 and 2 receive input from retinula cells R1-6, either via the lamina monopolar cell L1 (terminating in M1 and M5) or via L2 and T1 (terminating in M2). Neurons of these pathways typically have small dendritic fields. We discuss evidence that pathways 1 and 2 may play a major role in motion detection. Pathway 3 connects M8 to deep layers of the lobula. In M8 information converges that is derived either from M3 (pathway 3a) or from M4 and M6 (pathway 3b), layers that get their major input from L3 and R8 or L4 and R7, respectively. Some neurons of pathway 3 have large dendritic fields. We suggest that they may be involved in the computation of form and colour. Possible analogies to the organization of pathways in the visual system of vertebrates are discussed.

Key words: Visual system – Optic lobe – Medulla – Retinotopic pathways – Motion detection – Colour vision – *Drosophila melanogaster* (Insecta)

Synaptic contacts can be made only between neurons that appose each other and the shape of neurons reflects this basic requirement (e.g., Miller and Jacobs 1984). In the insect brain two major types of neuropil organization can be found, glomerular and stratified. Glomerular organization prevails when the numbers of incoming and outgoing neurons differ substantially and is thus a strong indication of functional convergence (e.g., in the glomeruli of the antennal lobes or in the optic glomeruli of the central brain; Strausfeld 1989a) or divergence (e.g., in the calyces of the mushroom bodies; Schürmann 1974). Stratification of synaptic specializations is required whenever several sets of incoming fibers have to confer their topological relationship onto multiple similar-sized sets of outgoing fibers. Such is the case in the retinotopically organized visual brain regions of vertebrates (e.g., Szentágothai 1973) and invertebrates (e.g., Cajal and Sánchez 1915; Campos-Ortega and Strausfeld 1972).

In primates at least two major visual retinotopic pathways can be distinguished, a parvo- and a magnocellular pathway; the pathways are clearly discernable in the lateral geniculate nucleus where they reside in different layers. The magnocellular pathway is colour-blind and seems to be involved in movement detection, while the parvocellular pathway is involved in the perception of form and colour (review: Livingston and Hubel 1988). It has been claimed (Strausfeld 1989a; Strausfeld and Lee 1991) that in insects a similar division between parallel colour-blind movement-sensitive and colour-processing visual pathway can be made. The data of our paper support this hypothesis by demonstrating the sorting of pathways in the medulla.

Since the pioneering work of Cajal and Sánchez (1915) two basic types of nerve cells, columnar and tangential neurons, have been known to underlie the optic lobe of insects. Comprehensive accounts of these neurons are available for the Diptera *Eristalis, Calliphora, Syrphus* (Strausfeld 1970), *Musca* (Strausfeld 1971, 1976), and *Drosophila* (Fischbach and Dittrich 1989). It has been shown by these authors that not only tangential, but also columnar neurons, contribute significantly to the organization of the medulla neuropil into layers. Fischbach and Dittrich (1989) proposed that this stratified organization reflects the structural separation of retinotopically organized functional pathways. In fact, deoxyglucose activity labeling of parts of the visual field

* During the final editing of this work our friend A.P.M. Dittrich was tragically killed in an accident. Without him this and the previous work would never have been completed

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using moving stimuli shows that different layers perform different functions (Buchner et al. 1984; Bausenwein 1988). The present paper extracts information about the spatial organization of different functions from neuronal shapes in the visual system of *Drosophila melanogaster*.

Materials and methods

Golgi-impregnated material used in this study

The Golgi preparations used are those underlying the study of Fischbach and Dittrich (1989). We evaluated camera lucida drawings presented there as well as original preparations and micrographs of optic lobe neurons from adult wild-type *Drosophila melanogaster* of both sexes. For a few cell types several drawings of individual cells were compared to examine variability within members of a cell type. For all calculations the complete set of documented columnar neurons of the lamina and medulla that participate in the formation of the medulla neuropil was used.

The 59 neurons used (see Fischbach and Dittrich 1989 for details) were as follows: the long retinula fibers R7 and R8, which provide direct retinal input to the medulla; the five lamina monopolar cells L1–L5, T1, and the medulla centrifugal cells C2 and C3, which altogether connect lamina and medulla; the medulla intrinsic neurons Mi1–Mi10, as well as distal medulla neurons, Dm1, Dm2, Dm6, and Dm7; the transmedulla cells Tm1, Tm3–Tm15, Tm17–Tm22, Tm25, which exclusively link medulla and lobula; and transmedulla-Y cells, TmY1–TmY13 and T2, T3, and T4 cells. A few columnar medulla neurons do not show arborizations in the medulla (e.g., Tm23, Tm24) and were not included in our evaluation. Y cells do not connect distal with proximal medulla layers (see Fig. 2) and were, therefore, not used in the respective calculations.

Subdivision of the optic lobe into different layers

Medulla. We used the 10 subdivisions of the medulla defined in the previous study (Fischbach and Dittrich 1989).

Lobula. For the purpose of the present study we subdivided the lobula into two layers only. LoS comprises layers Lo1–Lo4, and LoD comprises Lo5 and Lo6 (Fischbach and Dittrich 1989). We are aware that this subdivision is a rather crude one, but it is useful to demonstrate differences in the projection patterns of the proximal medulla layers (Fig. 6).

Lobula plate. Although known to be functionally subdivided into four directionally movement-sensitive layers (Buchner et al. 1984), it was not subdivided for the present study.

Information about the "connectivity" between layers can be gained by an analysis of neuronal shapes

A neuronal cell type is said to connect two layers if it has specializations in either layer. The problem is to obtain a realistic estimate of the functional weighting of these specializations. An exact but impractical solution would be to count the number of cell type specific synapses inside each layer by use of the electron microscope. A more practical but less satisfying solution would be to assign a "1" or a "0" to each layer depending on whether a cell does or does not arborize there, as was done by Fischbach (1983). In the present paper we apply a more sophisticated procedure. We assume that the number of synapses formed by a certain cell in a given layer is proportional to the size of its specialization. Some support for this notion comes from electron-microscopic investigations on the dipteran lamina, where the size of a photoreceptor terminal covaries to a certain extent with the number of its presynaptic sites (Fröhlich and Meinertzhagen 1987). We thus measured the size of specializations of all described columnar cell types in each layer.

Scanning of camera lucida drawings and micrographs

Drawings of individual cells were scanned (black and white scanning) with an Abaton 300/FB Scanner. Vertical and horizontal resolution of the scanned images is 300 dpi (dots per inch), this corresponds to 137 pixels (picture elements)/mm^2. Micrographs had to be scanned in a half-tone mode, which uses a 4×4 dither matrix (which could be converted to 16 greyscales). This reduced the spatial resolution to 72 dpi vertical and horizontal. The scanned pictures were stored and analyzed with the help of a MacII computer. A disadvantage of using micrographs instead of camera lucida drawings is the poor depth resolution of conventional light microscopes, which give a focused picture of only a small part of the impregnated neuron. Furthermore, computer-aided single cell evaluation from micrographs is nearly impossible if several neurons are impregnated in the same region. We thus rely mainly on the scanning of camera lucida drawings.

All scans were compared to the original drawings in about fourfold magnification (screen resolution of a 256 greyscale monitor, 72 dpi vertical and horizontal). All details of the drawings are conserved in these high resolution scans (Fig. 1).

Calculating arborization densities, standardization, and normalization

We estimated the density and size of specializations by integrating the number of picture elements occupied by the cell at any depth of the medulla (lateral integration of optic density). The maximum density of specializations of each cell was normalized to 100%. This normalizes the maximum contribution of each cell type, which could underestimate the part played by large-field cells. However, large-field neurons are stained with a lower frequency than many small-field columnar neurons. Therefore, the number of a given cell type per optic lobe seems to be inversely correlated to the extent of its lateral arborizations. This effect may work towards equalizing the contributions of small- and large-field neuronal cell types to connectivity.

Due to the curvature and the posterior-anterior size gradient of the medulla and also due to oblique planes of sectioning, the original drawings differ somewhat in size. With the original drawings having a length of almost 70 mm, the vertical extent of the scans (the apparent thickness of the medulla) is around 750 lines and varies slightly. We therefore introduced a second standardization by reducing the length of the density profiles to 100 data points. The data obtained this way are arborization densities per 1% medulla depth. Such data can be used directly to superimpose different cell types (Figs. 2, 3) or to calculate the degree of arborization ($A_{k,i}$) for any cell type k in medulla layer i.

All neuronal standard profiles were plotted and compared to the full resolution profiles to exclude calculation artefacts. In Fig. 1 the computer-drawn shape of a scanned neuron and its assigned arborization profile are shown.

Evaluating a connectivity profile for a given layer

The contribution of all cell types (k) to the interaction of a given pair of layers (i, j) may be estimated by multiplication of the degrees of arborization:

$$I_{k,i,j} = A_{k,i} \times A_{k,j}.$$

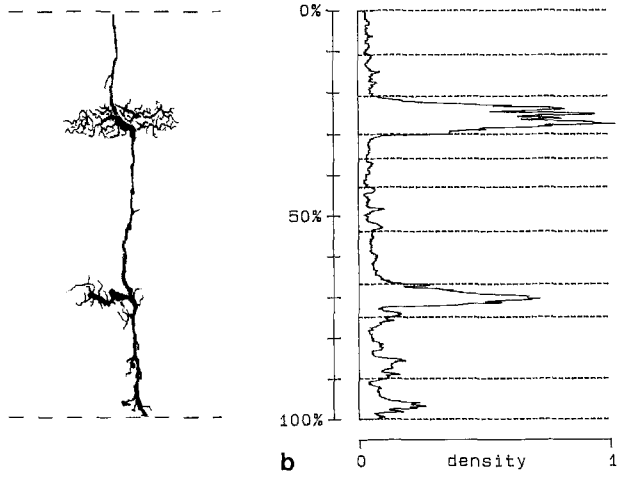

Fig. 1a, b. Computer-captured TmY11 cell (**a**) as an example for the digitizing of all columnar cell types. A drawing of a Golgi-stained cell is scanned in a 300 dpi × 300 dpi bit map to calculate a density profile of the cell's specializations (**b**). The maximum value of the profile was normalized to 100%

The absolute "connectivity factor" ($Cf_{i,j}$) between any given pair of layers i, j is then defined as the sum of all n cell type specific interactions $I_{k,i,j}$:

$$Cf_{i,j} = \sum_{k=1}^{n} I_{k,i,j}.$$

The conectivity factors are, of course, reciprocal ($Cf_{i,j} = Cf_{j,i}$).

This method of evaluating the degree of connectivity between layers is an approximation, and pre- and postsynaptic terminals will be weighted differently due to their shape differences. Nevertheless, the absolute connectivity factors $Cf_{i,j}$ are thought to reflect the potential strength of functional interactions between layers i and j (Fig. 7). The 10 medulla layers differ greatly in thickness and contain different amounts of arborizations (see Fig. 2). This leads to the complication that while in absolute terms, layer M10 has fewer connections to M1 than does M9 ($Cf_{1,10} < Cf_{1,9}$; Fig. 7), the relative strength of M1 neurons in M10 is much higher than in M9 ($Cf_{1,10}/\max(Cf_{i,10}) > Cf_{1,9}/\max Cf_{1,9}$); Fig. 8). For a given layer i the relative strength of the connectivity factor (i, j) is calculated according to

$$RCf_{i,j} = Cf_{i,j}/\max\,(Cf_{1,j}, Cf_{2,j}\ldots., Cf_{10,j})$$

The maximum function is used to normalize the absolute connectivity factor to the maximum Cf of the layer considered. Normalizing the absolute connectivity factors leads to nonreciprocal data sets, due to the maximum function. The relative strength of the connectivity factors should be more important for a functional interpretation of the connectivity of each single layer (Fig. 8).

Estimating the effect of uncertainties of the borders and number of layers

Uncertainties in the exact border position of a layer are expected to cause "cross-talk" between neighbouring layers. Such uncertainties could be due either to the effect of laterality of the medulla (e.g., the thickness of the serpentine layer M7 decreases from anterior to posterior) or to distortions of the drawings. We tried to estimate the degree of this cross-talk by evaluating only those arborizations that are positioned in the center of a layer. We obtained a slightly sharper differentiation of neighbouring layers but did not find qualitatively different connectivity patterns (data not shown). We also tried to subdivide the layers M3, M6 and M8, where some neurons have arborizations in a stratum of the layer only. Although there seems to be some specificity in these sublayers, this procedure did not reveal a higher order of organization within the overall connectivity pattern (data not shown).

In our standard procedure the relative size of the axon is part of the density profile. This may be justified because the possibility of synapses at the level of the axon cannot be excluded. To estimate the relative contribution of the axon to the connectivity factors we subtracted the axon from the density profiles. We found that this procedure also had no major effect on the pattern of the connectivity factors (data not shown).

Definition of terms used in this paper

Density profile, arborization density of neurons as a function of neuropil depth; connectivity factor (Cf), potential strength of interactions between 2 layers; relative Cf (RCf), the connectivity factor normalized to the maximum Cf of the layer studied.

Results

Superposition of the density profiles of columnar cell types

Without question, Tm and TmY cells are the most numerous cell types in the optic lobe of *Drosophila* (Fischbach and Dittrich 1989). They differ mainly in that TmY cells always send an axonal branch into the lobula plate, while most Tm cell types never do, others only very rarely. Is this difference in axonal projections correlated with differences in arborizations at the level of the medulla? To answer this we superimposed the scanned density profiles of all Tm and TmY cells and compared the resulting density distributions (Fig. 2). The result shows that Tm neurons have maximal densities in M3 and M8/M9 and minimal densities in M5, M7, and M10. TmY cells are different in that they have dense arborizations in M3, M5, and M8.

Y cells differ from TmY cells by the position of their cell body, which is behind the lobula plate, and by the extent of their arborizations inside the medulla neuropil. Their superimposed profiles (Fig. 2) show that they branch exclusively in the proximal medulla (layers M8–10).

Medulla intrinsic neurons (Mi) branch in all medulla layers, some even in the serpentine layer M7 (Fig. 2D). Most of their arborizations are housed in layers M3, M5, and M8. Their arborization pattern in the medulla is thus similar to that of TmY neurons.

Lateral extension of arborizations

The layers of the medulla differ in thickness and in the arborization characteristics of their neurons. For the most numerous neuronal class, the transmedulla neurons (Tm and TmY), we evaluated the maximum lateral size of arborization in each layer except the serpentine layer M7 (Fig. 3). These cells have rather small arborizations in layers M2, M5, M9, and M10. The largest average extension is found in layers M3, M4, and in M6 and the smallest in M10.

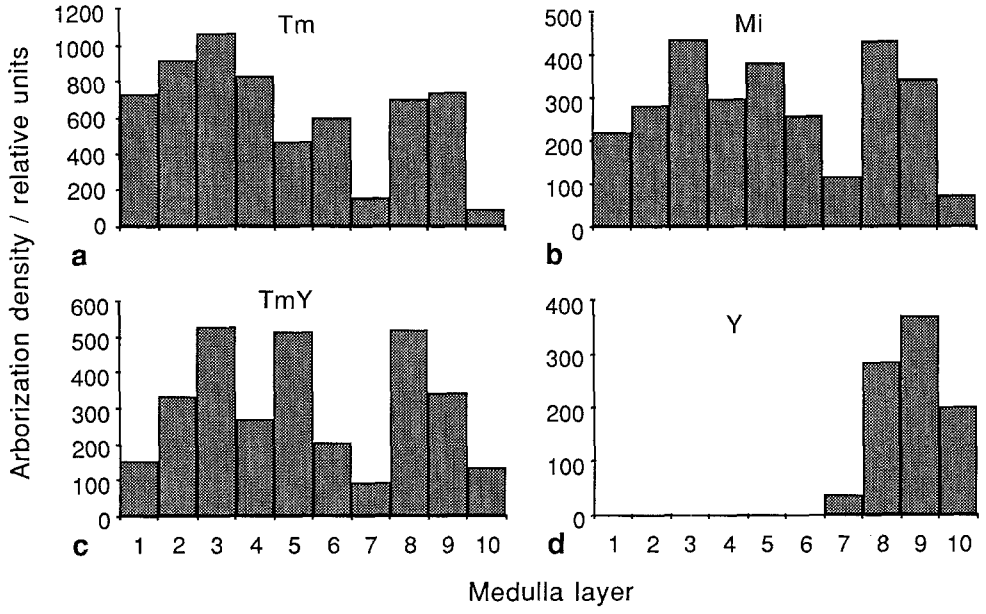

Fig. 2a–d. The summed density profiles of the cells in four major cell types. **a** The Tm cells have their maximum arborizations in M3. The densities in M8 and M9 are similar to each other. Remarkable is the relatively weak arborization density in M7 and M10. **b** The TmY cells also arborize densely in M3. In comparison to the Tm cells they are relatively weak in M1, M4 and relatively strong in M5, M8. **c** The density profile of Mi cells similar to that of TmY cells. **d** Y cells arborize only in the proximal medulla. Their arborizations are more evenly spread over the proximal medulla than those of the other classes

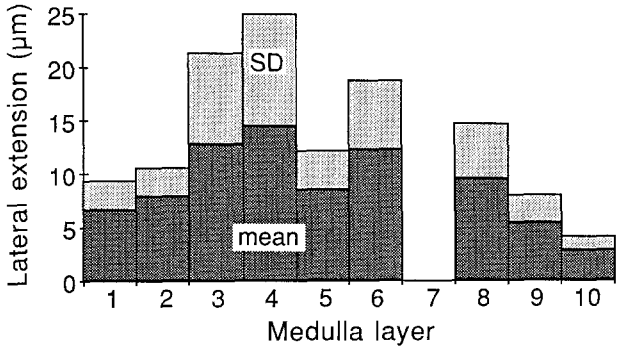

Fig. 3. The lateral extent of arborizations for transmedulla neurons (Tm and TmY). The *dark grey bars* represent the mean maximum extent of neurons in each medulla layer. The *light grey bar* adds the standard deviation. Lateral extent of arborizations in layers M1, M2, M5, M9 and M10 is less than 10 µm. The variance in M3 and M4 is rather high, demonstrating the existence of cells with large arborizations

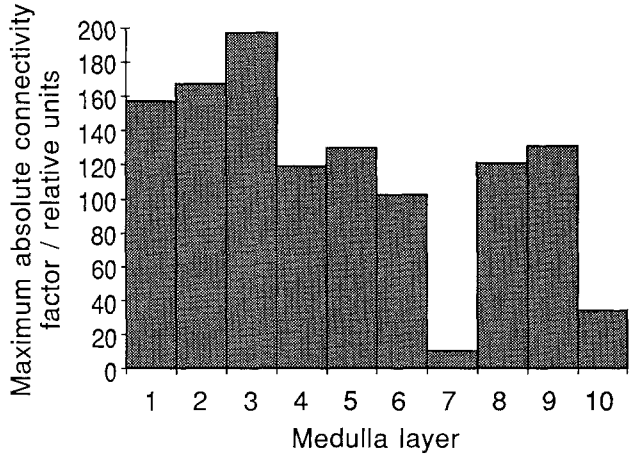

Fig. 4. Maximum connectivity factors of each medulla layer that were used to normalize the data from Fig. 7 to produce Fig. 8. They are a measure for the possible strength of interaction within each layer. Layer M7, the serpentine layer, has the lowest value. This layer divides the medulla into an inner (proximal) and outer (distal) part. It houses the large tangential medulla fibers, which come from or project to the posterior optic tract. The small contribution of the serpentine layer to connectivity means that columnar neurons rarely branch in it (we do not consider contributions of tangential neurons). M10 also seems to be poorly connected to the other medulla layers. This is partially because M10 is occupied by a hypercolumnar set of T4 cells (four cells per column), which do not branch in other layers of the medulla

The medulla layers contribute differently to intramedullary connectivity mediated by columnar neurons

The peaks of the summed connectivity factors are found in layers M2, M3, and M9 (Fig. 4). M7 (the serpentine layer) , where the axons of large tangential neurons reside, is hardly connected to other medulla layers by columnar neurons. M10 is also a special case. It is packed with several T4 neurons per column, which connect to the lobula plate and do not contribute to the connectivity with other medulla layers, and the sum of all medullar connectivity factors of M10 is, therefore, small. These differences between the medulla layers justify plots of the relative contribution of a given layer in addition to the absolute values of the connectivity factors (compare Fig. 7 to Fig. 8 and see Materials and methods).

Retina and lamina are exclusively and specifically connected to layers in the distal medulla

Superposition of all inputs from the lamina and retina to the distal medulla and calculation of the relative strength of each input type as a function of medulla depth clearly demonstrate that different layers get different input (Fig. 5). All inputs are confined to the distal medulla (layers M1 to M6).

We do not consider possible interactions in the outer optic chiasm, e.g. the long visual fibers R7 and R8 are

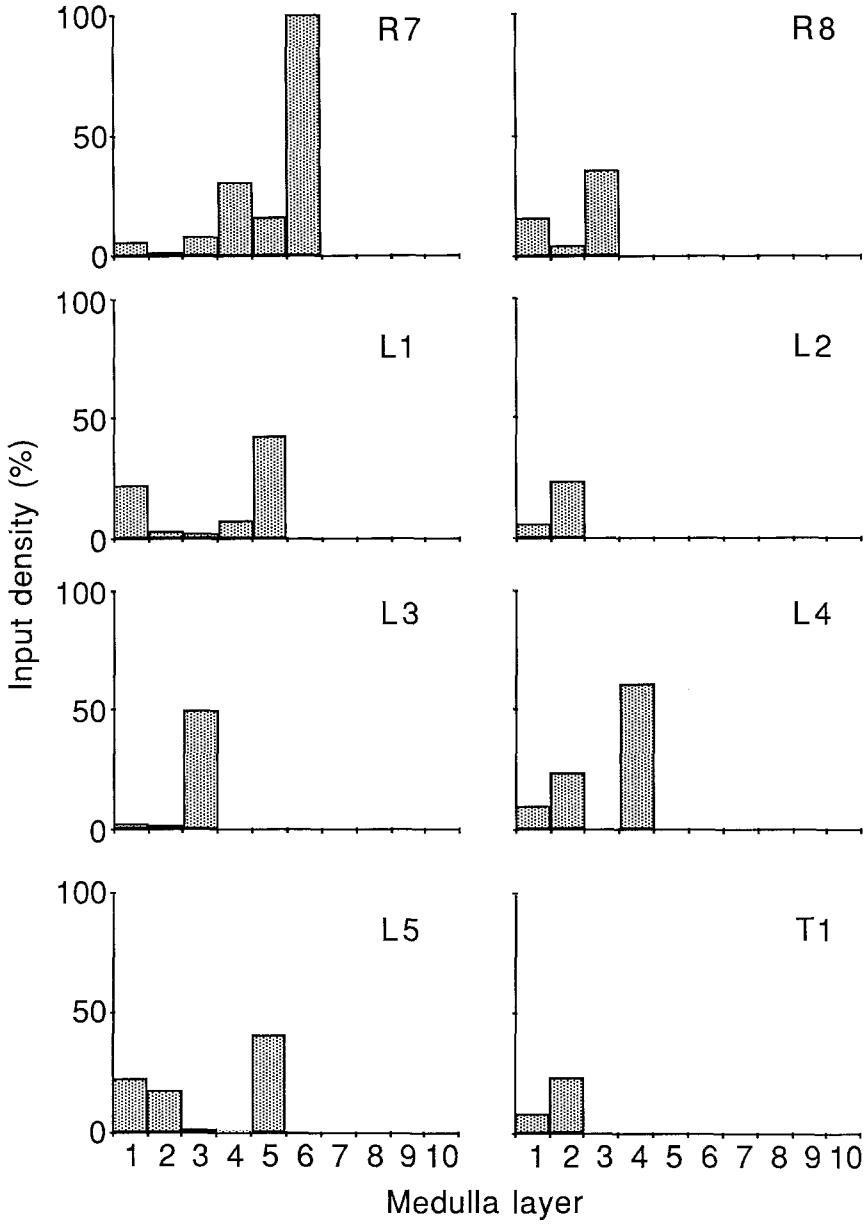

Fig. 5. The relative density of input neurons in the medulla layers (input density). The $A_{k,i}$ values for a given neuron k in layer i have been normalized relative to the sum of the $A_{k,i}$ values of all other input neurons. Lamina monopolars L1 and L5 are strong inputs to layers M1 and M5. Layer M2 is the projection stratum for T1 and L2, which have their thick arborizations spread over the entire depth of the layer. L4 and L5 monopolars also contribute strongly to the input into M2. R8 is one of the main inputs to M3. The arborization of L3 resides in a substratum of this layer. L4 provides about 60% of the input into M4. Retinula cells R7 here often possess an axonal thickening. Finally R7 provides the only input into layer M6. No lamina-derived interneuron extends that far

already thickened just before they enter the medulla neuropil (see Fig. 3A of Fischbach and Dittrich 1989). Specializations of C2 and L2 also extend into that region.

The terminals of the monopolar cells of the lamina (L1–L5), together with the long receptor cell (R7, R8) axons, define the layers of the distal medulla (Fischbach and Dittrich 1989). We have calculated the arborization profiles of these input neurons. When the same data are normalized to the sum of arborization densities of all inputs in that layer to give the relative strength of input neurons (Fig. 5), their different weighting in the medulla layers becomes even clearer, e.g., R7 is the only input neuron in M6.

Layer M1 is defined by the distal terminal of the L1 cell. It also contains parts of the terminals of other cells connecting lamina and medulla, the most prominent of which is L5. The distal specialization of L5 is, however, not confined to M1, it extends into M2. Furthermore, Meinertzhagen and O'Neil (1991) have shown that L5 neurons hardly get any input in the lamina. They may functionally be similar to medulla intrinsic neurons. L1, therefore, has to be considered to be the main input neuron into layer M1.

Layer M2 is defined by the terminal specialization of L2. It contains in addition the terminals of T1 cells and branches of L4. The position of T1 cell bodies in the medulla cortex gives these neurons the morphological appearance of centrifugal cells, however, according to a careful electron-microscopic study by Meinertzhagen and O'Neil (1991), they are pure output elements of the lamina. The L4 neurons differ from other lamina monopolars by sending collaterals into neighbouring cartridges. It is not clear whether their tiny branches in M2 contact several columns in *Drosophila*.

M3, which is a rather broad layer, is only in part occupied by L3. The R8 terminals also play an impor-

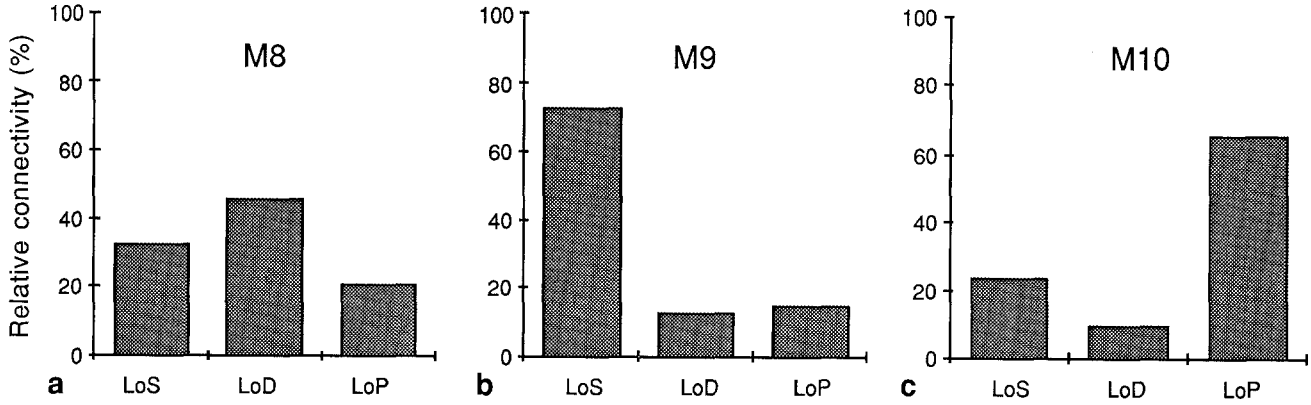

Fig. 6a–c. The relative connectivity of the output layers of the medulla to the lobula complex. **a** The cells arborizing in M8 have most of their terminal specializations in the deeper layers of the lobula (LoD). **b** About 75% of M9 cells arborize in the superficial layers of the lobula (LoS). **c** The cells arborizing in M10 show a high preference for the lobula plate

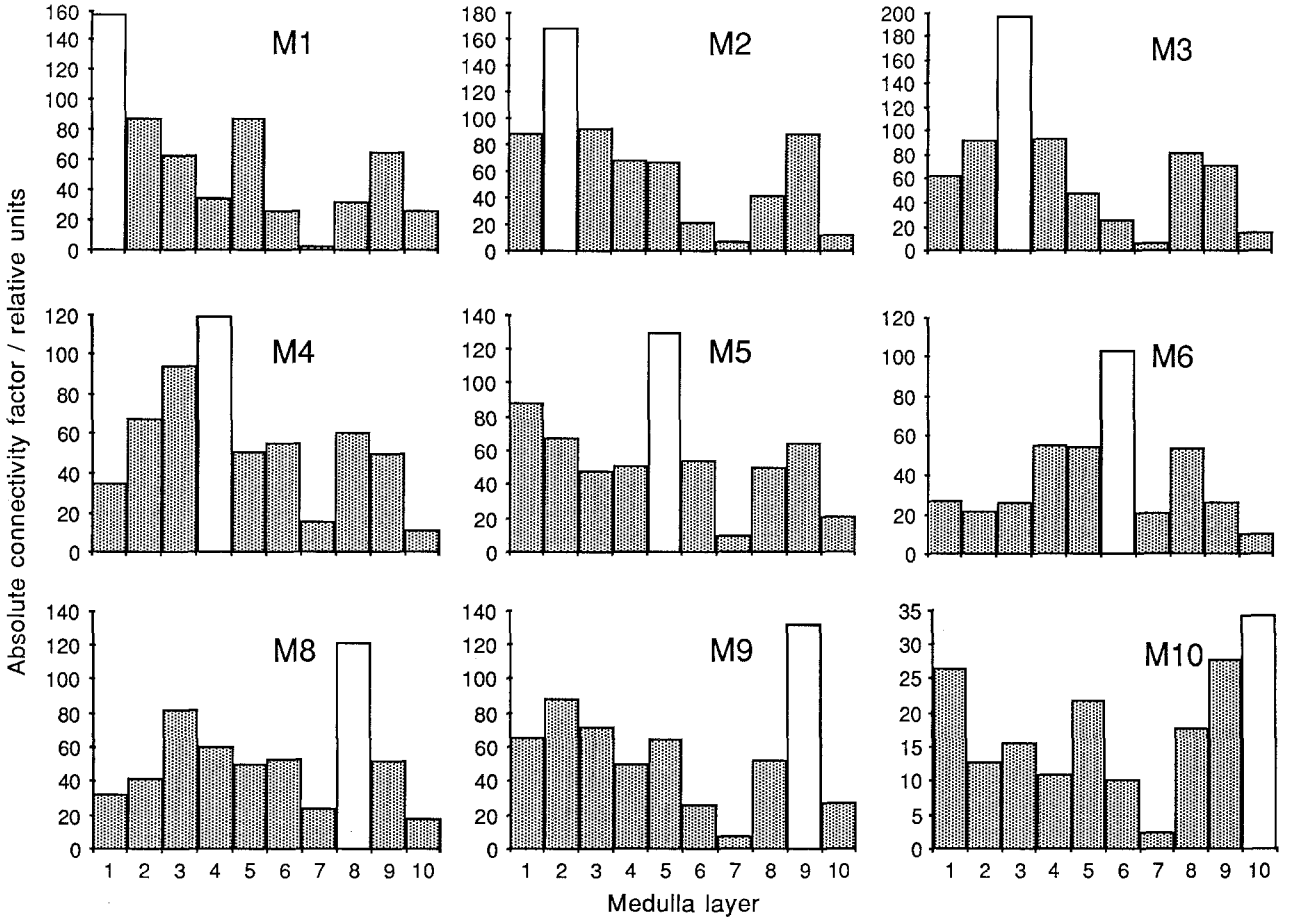

Fig. 7. The absolute connectivity factors $Cf_{i,j}$ of a given medulla layer (*white bar*) with the other nine layers (*shaded bars*). Layers are differentially connected to each other, e.g., the connectivities of M1 to M5 and of M8 to M3 are high, while those of M2 to M6 and of M8 to M1 are low

tant role in its connection pattern. M4, a smaller layer, gets mainly input from L4, while in M5 the terminal arborizations of L1 and L5 dominate. As already mentioned above, the L5 neuron may functionally be similar to a medulla intrinsic neuron. L1, therefore, has to be considered to be the main input neuron into layer M5. Finally, M6 gets direct input from the retina via the long receptor fibers of R7. No lamina monopolar neuron extends that far into the distal medulla.

The output connections of the layers of the proximal medulla are very different

While the layers in the distal medulla get specific input, the layers of the proximal medulla are preferentially connected to different parts of the lobula complex. Neurons arborizing in layer M8 tend to project deeply into the lobula (LoD, Fig. 6A), those in M9 strongly prefer shallow layers of the lobula (LoS, Fig. 6B), while neurons

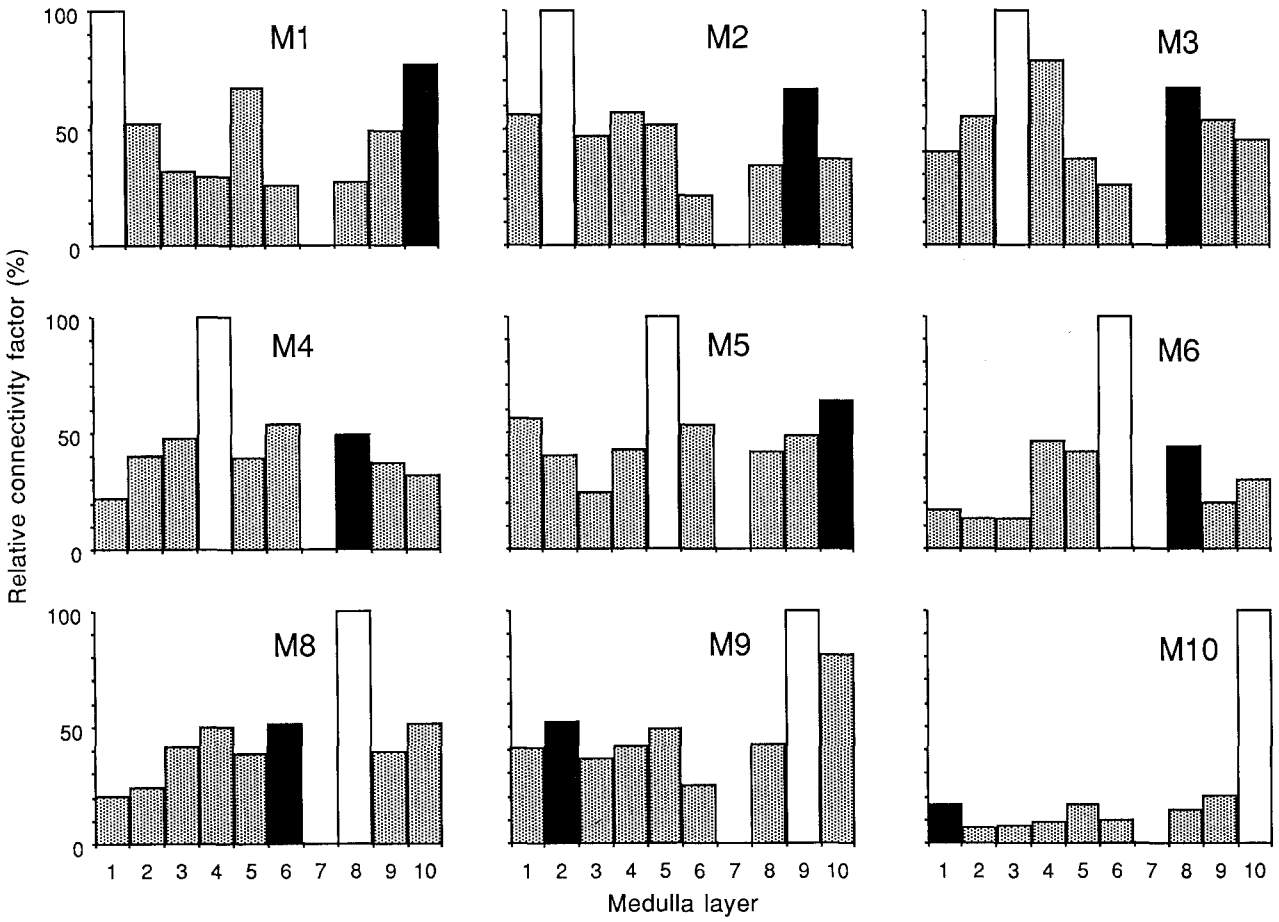

Fig. 8. The relative connectivity factors $RCf_{i,j}$ of a given medulla layer (*white bar*) with the other layers (*filled bars*). M7 has been omitted. The $RCf_{i,i}$ values (*white bar*) were used to normalize the data. The meaning of the $RCf_{i,j}$ may be understood by considering the example of M10 (*lower right*). The extremely low relative connectivity factors of M10 with the other layers mean that the contribution of neurons branching in M10 to the connectivity of those layers is very small. Only a few of the neurons in layers M1–9 branch in M10. This is also evident from Fig. 7. The *black bar* emphasizes that layer in the opposing part of the medulla (across the serpentine layer M7) in which neurons of the reference layer are most prominent. It is clearly seen that reference layers M1, M2, and M3 pick M10, M9, and M8, respectively

of M10 project into the lobula plate (Fig. 6C). The question now arises, whether the different input layers of the distal medulla are equally linked to all three layers of the proximal medulla, or whether different input layers are connected to different output layers.

The connectivity factor between different layers of the medulla

The scanned profiles of all columnar cell types of the medulla were used to characterize the relationship between the input and output layers of the medulla. We calculated the absolute and relative connectivity factors (Cf, RCf) between any given pair of layers as explained in Materials and methods. Figs. 6 and 7 give an overview of the results obtained. Table 1 lists those pairs of medulla layers that show especially strong connections (two strongest RCfs) and those that obviously do not have much to do with each other (weakest RCf).

The connectivity factors between the distal and proximal medulla layers are very different, e.g., M2 is most strongly connected to M9, while M3 favors M8, as is obvious from plots of the absolute Cf values (Fig. 7) and their relative strengths (Fig. 8).

Connections of M1 and M5 to M10

L1 cells form a bistratified terminal in the distal medulla with specializations in M1 and M5. Medulla neurons interacting with L1 may preferentially branch in these two layers. Figs. 7, 8 show that M1 and M5 are more strongly connected to each other than with any other layer of the distal medulla. Neurons strongly connecting M1 and M5 are: L1, Mi1, C2 and C3. C2 and C3 are centrifugal neurons of the medulla presynaptic to synaptic dyads of L2 and either L1, L3, a receptor terminal or an amacrine process in the lamina. Mi1 is one of the most frequently impregnated neurons in Golgi preparations of the optic lobe and seems to occur in each column (Fischbach and Dittrich 1989).

Neurons with specializations in M10 also arborize in M1 and M5 (Figs. 7, 8). Among these neurons are

Table 1. Medulla layers with high and low relative connectivity

Strong connections	Rare connections
M1 : M5, M10[a]	M1 : M6
M2 : M4, M9	M2 : M6
M3 : M4, M8	M3 : M6
M4 : M6, M8	M4 : M1
M5 : M1, M10	M5 : M3
M6 : M4, M8	M6 : M2/M3
M8 : M6, M10	M8 : M1
M9 : M2, M10	M9 : M6
M10: M1, M9	M10: M2

[a] M1: M5, M10 means that neurons branching in M1 have a strong tendency to branch in M5 and M10. This relationship is not completely reciprocal; a portion of those neurons branching in M5 do not branch in M1

Mi1, TmY8, C2, and C3. The strong connection of M10 to the lobula plate (Fig. 6C) is mainly due to the T4 neurons, which channel information down into the different layers of the lobula plate.

Connections of M2 to M9

Figs. 7, 8 show that layer M2 is preferentially connected to M9, and Fig. 6B stresses the very close relation of M9 to LoS. We, therefore, believe that M2, M9, and LoS form a functional pathway. Typical neurons connecting M2 and M9 to LoS are Tm1, Tm2, Tm4, Tm6, Tm14, and TmY12. LoS has a very close relationship to the lobula plate, e.g., via the T5 cells, which are very similar to T4 cells in shape. T5 cells terminate in the lobula plate at four different levels, which is suggestive of their multiple occurrence in a single visual column (Fischbach and Dittrich 1989).

Connections of M3 and of M4, M6 to M8

Layers M3 and M6 are both strongly connected to M8, although they are not strongly connected to each other (Fig. 8). M4 also shows connections with M8. M3 and M4 are the layers that house the terminal specializations of the two types of R8 cells (Fischbach and Dittrich 1989). The connection between M3 and M8 is especially interesting, because of M8's connections to M6, which contains the terminal specializations of R7.

These data suggest that the peripherally separated R8 and R7 channels converge in M8, which in turn connects to LoD (Fig. 6A). A neuron that reflects this pathway is Tm19. Dm4 selectively connects M4 to M6, and Tm7, Tm8, and Tm22 are neurons that arborize in M4, M6, and M8.

Neurons shared by pathways 3a and 3b

Although the absolute and relative connectivity of M3 to M6 is surprisingly low (Figs. 7, 8), one neuron type, Tm5, arborizes in M3, M6, and M8. This neuron is very frequently impregnated in Golgi preparations and seems to occur at least one per column. Other neurons connecting layer M3 and M6 are TmY10 and TmY11.

Discussion

We are convinced that a detailed analysis of neuronal shapes yields much information about functional pathways in the optic lobes of *Drosophila*. Although the existence of synapses between neurons cannot be proven without an electron-microscopical analysis, it is possible to exclude the existence of synapses between neurons that do not come close to each other. Furthermore, the probability that two neurons synapse with each other is higher if they have rich arborizations in the same region of the neuropil. Thus, the stratified arborizations of lamina monopolar neurons in the medulla most probably represent the wiring of lamina monopolar neurons to different sets of postsynaptic neurons. The branching of lamina monopolar neurons is reflected in the branching pattern of medulla and transmedullary neurons as has been demonstrated in this paper. Functional aspects of these structurally separate pathways are now being studied by 2-deoxyglucose activity labeling (B. Bausenwein and K.-F. Fischbach, in preparation).

Two major channels for motion detection?

The output layer M10 is strongly connected to layers M1 and M5, e.g., via the medulla intrinsic neuron Mi1. These layers subserve pathway 1 (the L1 channel, see Fig. 9). The cells involved in this pathway are small-field neurons, which are confined to a single or a few columns and thus have the potential for preserving high spatial resolution. The hypercolumnar T4 cell as the major relay of pathway 1 to the lobula plate exists in several subtypes in each column (projecting into different directional motion sensitive strata of the lobula plate). Only T4 neurons project exclusively to the lobula plate; TmY and Y cells also project to the lobula. Activity labeling experiments (Buchner et al. 1984; Bausenwein 1988) demonstrate that all four layers of the lobula plate get columnar input. The set of T4 neurons probably gets input from the Mi1 cell, which seems to be postsynaptic to L1 in M1 and M5.

According to Strausfeld (1984, 1989b) part of the T4 input to the lobula plate in *Drosophila* and *Calliphora* is indirectly derived from L2. Our Golgi analysis gave no indication of a significant contribution of small-field retinotopic neurons that simultaneously arborize in M2 and in M10. The M2 connections differ from those of M1 and M5. L2 terminals in M2 are closely associated with Tm neurons projecting via M9 to the superficial half of the lobula, which in turn is connected to the lobula plate via the T5 and TlP cells (Fischbach and Dittrich 1989). This reflects the existence of a second pathway, the L2 pathway (Fig. 10). It may be concluded that the L1 and L2 pathways converge at the level of

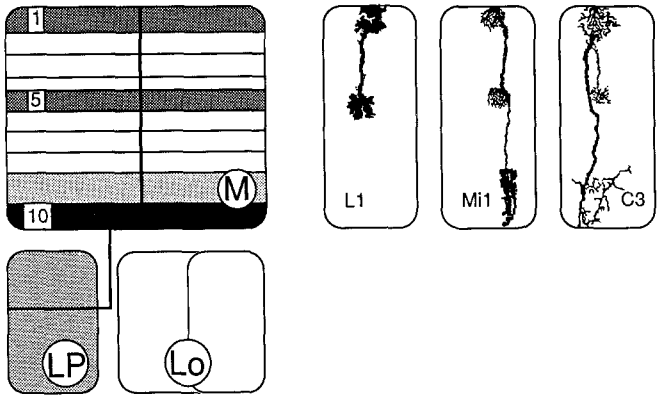

Fig. 9. *Left* Schematic view of layers of the medulla (*M*), and the lobula plate (*LP*) involved in pathway 1. The medulla output layer M10 (*black*) is preferentially connected to layers M1 and M5 (*grey layers*), which get their main input from L1. Pathway 1 may therefore tentatively be called the L1 pathway. M10 is also strongly connected to the lobula plate (*LP*). The light grey of layer M9 indicates that neurons of pathways 1 also often arborize in layer M9, where they may interact with neurons of pathway 2. Three possible neuronal members of pathway 1 are shown on the *right* (*L1, Mi1, C3*). *Lo* Lobula

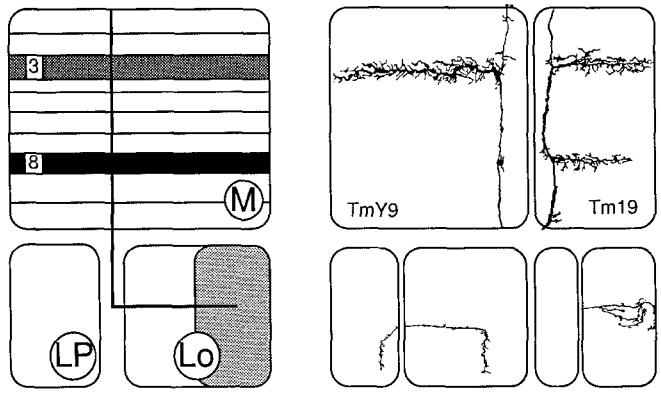

Fig. 11. Schematic view of layers of the medulla and the lobula involved in the hypothetical pathway 3a. This pathway is constituted by cells arborizing in M3 (*gey*) and M8 (*black*) that project into deep layers of the lobula (Lo, *gey*). In M3 L3 and R8 terminals are the dominant input. Pathway 3a may thus tentatively be called the L3/R8-pathway. Tm19 and TmY9 are shown on the *right*. These are extreme examples for the wide arborizations of neurons in pathway 3a

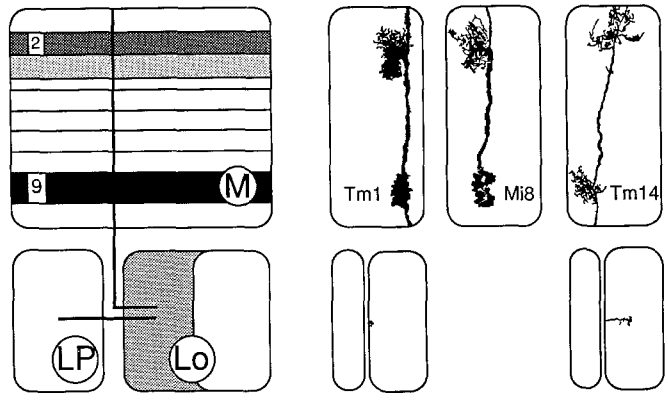

Fig. 10. Schematic view of layers of the medulla and the lobula (*Lo*) involved in the hypothetical pathway 2. The output layer M9 (*black*), which connects to the superficial layers of the lobula (LoS, *grey*), receives its main input via medulla layer M2 (*dark grey*). Pathway 2 may therefore be tentatively called the L2 pathway. Many Tm cells fit into this scheme [two examples (Tm1 and Tm14) as well as Mi8 are shown on the right]. Quite often the cells also arborize in M3 (light grey) and sometimes in M1. LoS is then connected to the lobula plate via the hypercolumnar set of T5 cells

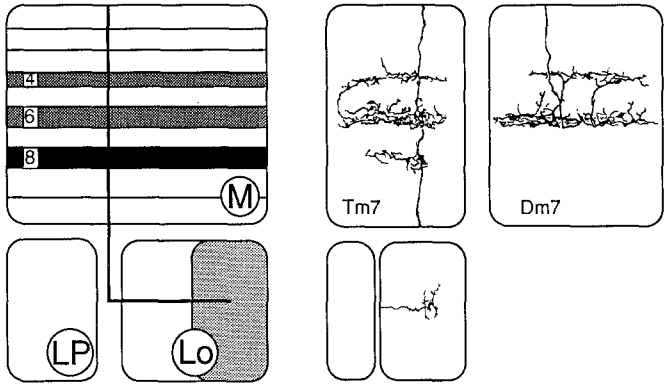

Fig. 12. Schematic view of layers of the medulla and the lobula involved in the hypothetical pathway 3b. Pathway 3b connects medulla layer M8 (*black*) to the deeper layers of the lobula (*Lo, grey*), as does pathway 3a. Most neurons of this group, however, branch also in layers M4 and M6 (*grey*), where the L4 and R7 terminals are the dominant input. It thus may tentatively be called the L4/R7 pathway. The large dendritic fields of the example cells Tm7 and Dm7 shown on the *right* are similar to those of pathway 3a

the lobula plate. This agrees with reports on *Calliphora* (see Strausfeld 1989b). Very interestingly, like their T4 counterparts, the T5 cells are hypercolumnar. As in the L1 pathway, cells in the L2 pathway are small-field neurons. In addition, both pathways may involve the same number of synapses on their routes to the lobula plate (e.g., L1-Mi1-T4 versus L2-Tm1-T5). It seems that the existence of a twin pair of large monopolar cells (LMC), L1 and L2, is not a simple duplication of channels to reduce the signal-to-noise ratio at the level of the lamina (see Laughlin 1984), as this separation is carried through the medulla into the lobula plate. We suggest that there is a functional specialization that begins with the very subtle differences in the synaptic input patterns of these two LMCs (see Meinertzhagen and O'Neil 1991; for larger flies, see Shaw 1984). Possibly, both pathways play a part in the motion detection, as they converge in the lobula plate and retain their fine-grained retinotopic relationship, just as is required for the two input channels of elementary movement detectors (Reichardt and Poggio 1976; Buchner 1976).

It is very likely that both pathways interact, not only in the lobula plate, but also in the medulla. One possible site is layer M9, since the terminal collaterals of the Mi1 neuron extend over both M9 and M10. The interaction in layer M9 is an asymmetric one, probably relaying

information from pathway 1 to pathway 2. This can be inferred from the appearance of Mi1's collaterals, which look like typical presynaptic terminals (see discussion in Fischbach and Dittrich 1989). Layers M1, M2, and M9 are also connected via several Tm neurons (e.g., Tm12, 13, 14, 25) and TmY8.

Pathway 2 does not project only to the lobula plate. In the shallow layers of the lobula it may connect to tangential as well as to columnar neurons projecting to optic foci of the midbrain.

We are confident that data from ongoing 2-deoxyglucose studies will shed further light on the cellular components of the elementary movement detection circuitry.

A separate pathway for colour coding?

The compound eye of *Drosophila* contains several classes of receptor cells, which differ in spectral sensitivity due to the expression of at least three different opsin genes (Fortini and Rubin 1990). Retinula cells R1–6 express Rh1 and display a uniform spectral sensitivity with peaks in the green and in the ultraviolet (UV) (Harris et al. 1976). Sight at low light intensities is based on these receptors (Heisenberg and Buchner 1977; Fischbach 1979), while the R7 and R8 receptors dominate fast (Hu and Stark 1977) and slow (Fischbach 1979) phototactic behaviour at higher light intensities. There are two groups of R7 and R8 cells (R7y/R8y and R7p/R8p), occurring in pairs and differing in their spectral characteristics (Kirschfeld et al. 1978). The majority group (R7y, 70%) expresses Rh4; the minority group (R7p,30%) expresses Rh3 (Fortini and Rubin 1990). Both R7 types have spectral sensitivity peaks in the UV range (review: Hardie 1986) Two types of R8 cells absorb best in the green-yellow (R8y) and blue (R8p). The spectral sensitivities of all receptors of the compound eye of *Drosophila*, therefore, provide sufficient information to allow colour vision.

Main input neurons to pathways 1 and 2 are the lamina monopolar cells L1 and L2. On the basis of their connectivity they are thought to have the same broad spectral sensitivity as R1–R6. The same spectral sensitivity has been found in motion-sensitive neurons of the lobula plate and in optomotor responses (rev: Kaiser 1975; Heisenberg and Buchner 1977; Tinbergen and Abeln 1983; Srinivasan and Guy 1990).

Colour discrimination tasks and learning paradigms showed that flies have true colour vision (*Eristalis*: Ilse 1949; *Drosophila*: Menne and Spatz 1977); their color space has been described as trichromatic (*Lucilia*: Fukushi 1989, 1990). In genetic dissection experiments *sevenless* mutants, which lack retinula cells R7, no longer show colour-specific phototactic responses (Harris et al. 1976; Heisenberg and Buchner 1977; Fischbach 1979) or learning (H.C. Spatz, personal communication). On the other hand, *outer rhabdomeres absent* mutants lacking the rhabdomeres of R1–R6 also perform poorly in these tasks (Fischbach 1979), suggesting that all types of retinal cells are involved in colour vision.

The long visual fibers of receptor cells R7 and R8 project directly into the medulla, emphasizing levels M3 (R8) and M6 (R7) (Fig. 4). Fischbach and Dittrich (1989) describe two types of R7 and R8 cells that differ slightly in projection depth. A rare form of R8 terminates in M4, while a rare form of R7 extends to the border of M7. Another important contribution to the input in M3 and M4 may be derived from R1–R6 via lamina monopolar neurons L3 and L4, respectively. Thus receptors of all spectral sensitivity types are putative inputs to pathway 3. M3, M4, and M6 are the outer medulla layers of pathway 3's subdivisions, 3a and 3b, which converge in M8 and in deep layers of the lobula (Figs. 11, 12). We speculate that, as has been suggested for muscoid Diptera (Strausfeld and Lee 1991), concerned with the processing of colour, with M8 as a possible site of trichromatic interaction.

Electrophysiological evidence on the chromatic properties of medulla neurons in flies is extremely sparse. In bees, UV-sensitive medulla neurons (Hertel 1980; Kien and Menzel 1977a, b) have some anatomical properties in common with fly neurons involved in pathways 3a and 3b. Hertel describes UV-sensitive neurons with larger visual fields that arborize in the medulla layer "directly distal" to the serpentine layer (layer M6 in *Drosophila*), and other highly UV-sensitive neurons that arborize in the layer directly proximal to the serpentine layer (thus comparable to *Drosophila's* layer M8).

The visual field sizes of neurons in pathway 1 and 2 differ from those in pathways 3a, b

Most cells of pathways 1 and 2 have very small dendritic fields (Figs. 3, 8, 9). The terminals often appear rather dense and homogeneous. Even though their relative position within a cartridge is unknown, neurons of pathway 1 and 2 are confined to a single neuroommatidium or at least to a small group of adjacent columns. Lateral flow of information mediated by tangential neurons is not considered here.

In contrast, several neurons of pathways 3a, b have broad and very fine arborizations that extend into many neighbouring columns (Figs. 3, 11, 12). Possibly, conservation of spatial resolution is not the main function of these neurons. And, although the two-dimensional nature of the camera lucida drawings loses much information about the three-dimensional shapes of the dendritic trees, microscopic observations show that some of these neurons display radial symmetry, while others do not. We also have little knowledge about how often these cells occur per column.

Functional specialization of lobula and lobula plate

Studies using genetic dissection (*Drosophila*: Bausenwein et al. 1986), laser ablation (Geiger and Nässel 1982) and microsurgery (*Calliphora*: Hausen and Wehrhahn 1983), and electrophysiological recordings (*Calliphora, Musca*: Hausen 1981; Egelhaaf 1985) show that the lobula plate is mainly concerned with the processing of directional motion information. It plays an important part in con-

trolling compensatory motions of both the head and body in flight and during walking in response to turning stimuli (see Egelhaaf et al. 1988; Hausen and Egelhaaf 1989). There is no evidence from histological or electrophysiological investigations that the lobula plate contains colour coding channels.

In contrast, the lobula of flies is relatively unknown. Anatomical studies (Strausfeld 1989b) have described four classes of neurons in the lobula of larger flies:

(1) Isomorphic assemblies (ColA cells) that project to giant neurons. Similar lobula columnar neurons (Lcn) have been described in *Drosophila* by Fischbach and Dittrich (1989).

(2) Male-specific neurons of the frontal eye region, which may be involved in tracking or pursuit. In *Drosophila* no obvious sexual dimorphism in the lobula has been described so far.

(3) Neurons that subtend the dorsal eye zone (marginal zone) and are thought to be involved in the analysis of polarized light. A special dorsal eye zone also exists in *Drosophila* (Fortini and Rubin 1990), but interneurons specific for this visual field have so far not been described.

(4) Wide-field neurons. Several neurons of this class have also been demonstrated in *Drosophila* (Fischbach and Dittrich 1989).

Our analysis has shown that the target regions of pathways 2 and 3 divide the lobula into two halves, with the presumed colour-coding pathway 3 projecting to deep layers of the lobula and pathway 2 to shallow layers. Computation of motion is separated from computation of colour and form in vertebrates also (Livingston and Hubel 1988).

The advantages of a digitized library of neuronal shapes

Even though our database is so far a two-dimensional one only, it may be sufficient to allow the study of questions that are hard to address otherwise. First, it should be possible to analyze complex patterns of sets of multiple cell types, e.g., in immunohistochemical or autoradiographic labelings, to identify or at least exclude certain cell types. For this purpose we are now expanding the database to include immunohistochemical impregnations and activity labeling autoradiograms (B. Bausenwein, in preparation). Deoxyglucose activity labeling may reveal correlates to pathways 1, 2, and pathway 3.

Using two-dimensional digitization, it may possible to compare sets of columnar neurons of different strains of *Drosophila melanogaster* (e.g., mutants with reduced visual lobes) to detect which neurons are deleted by mutation. In the past, this task has proven to be rather difficult, due to overall size changes and other distorting factors, which now may be corrected by the computer. Finally, the medulla neurons of related species may be compared. Up to now, the homology of columnar neurons between different species could be established only in a subset of cells (see discussion in Fischbach and Dittrich 1989). Comparative studies could lead to an understanding of the conserved functional features of the visual neuropil of insects (Osorio 1991).

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