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Activity labeling patterns in the medulla of *Drosophila melanogaster* caused by motion stimuli

B. Bausenwein and K.-F. Fischbach

Institut für Biologie III, Schänzlestrasse 1, W-7800 Freiburg, Federal Republic of Germany

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Summary. We quantitatively describe 2-deoxyglucose (2-DG) neuronal activity labeling patterns in the first and second visual neuropil regions of the *Drosophila* brain, the lamina and the medulla. Careful evaluation of activity patterns resulting from large-field motion stimulation shows that the stimulus-specific bands in the medulla correspond well to the layers found in a quantitative analysis of Golgi-impregnated columnar neurons. A systematic analysis of autoradiograms of different intensities reveals a hierarchy of labeling in the medulla. Under certain conditions, only neurons of the lamina are labeled. Their characteristic terminals in the medulla are used to differentiate among the involved lamina monopolar cell types. The 2-DG banding pattern in the medulla marks layers M1 and M5, the input layers of pathway p1 (the L1 pathway). Therefore, activity labeling of L1 by motion stimuli is very likely. More heavily labeled autoradiograms display activated cells also in layers M2, M9, and M10. The circuitry involved in the processing of motion information thus concentrates on pathways p1 and p2. Layers M4 and M6 of the distal medulla hardly display any label under the stimulus conditions used. The functional significance of selective activity in the medulla is discussed.

Key words: 2-Deoxyglucose – Visual system – Visual pathways – Neuronal activity labeling – Motion detection – Neuroanatomy – *Drosophila melanogaster* (Insecta)

The visual system of *Diptera* is a useful system for studying information uptake, analysis, and processing (Götz 1972; Heisenberg and Wolf 1984; Reichardt 1986). Much detailed knowledge has been accumulated, especially on the neuropils of the optic lobes, particularly the lamina and the lobula plate. The rather simple anatomical organization of the lamina has been described ultrastructurally in *Drosophila* (Meinertzhagen and O'Neil 1991) and in larger flies (Shaw 1984), and lamina function has been explored in electrophysiological studies (*Drosophila:* Coombe et al. 1989; *Musca, Calliphora:* Laughlin 1984, 1989). The giant tangentials of the lobula plate, as spatially integrating output elements, are also known in considerable detail (Hausen 1984).

The function of the medulla, which mediates all ipsilateral retinal input to lobula and lobula plate and houses the majority of visual neurons (*Drosophila:* Fischbach and Dittrich 1989; *Musca:* Strausfeld 1976, 1984), has been less extensively studied. Physiological evidence about the function of medulla neurons is sparse (DeVoe 1985). Recently the responses of identifiable medulla neurons to motion and flicker stimuli have been described in flesh flies (Gilbert et al. 1991). Anatomically, the medulla has been well studied and the stratified appearance has attracted special attention (Cajal and Sanchez 1915; Campos-Ortega and Strausfeld 1972; Strausfeld 1970, 1976; Fischbach 1983; Fischbach and Dittrich 1989). Strausfeld (1984, 1989a) introduced the idea that there are two separate channels through the medulla of flies, the so-called L1, L2 channel (thought to comprise small color-insensitive neurons) and the L3 channel (thought to comprise large color-sensitive neurons), and compared this dichotomy to magno- and parvocellular visual pathways in primates (Strausfeld 1989b). Neurons of the two channels arborize at different depths of the medulla neuropil and supply either the lobula or the lobula plate (Strausfeld and Lee 1991).

In *Drosophila,* 10 distinct layers of arborization depths have been described (Fischbach 1983; Fischbach and Dittrich 1989). A computer-based analysis of Golgi-impregnated columnar cell types of *Drosophila* has shown that at least three sets of layers are connected (Bausenwein et al. 1992), defining three retinopically organized pathways in *Drosophila*. Pathway 1 (p1) uses layers M1, M5, and M10 and is strongly connected to the lobula plate. Pathway 2 (p2) connects layers M2 and M9 and projects to the outer lobula, which then is strongly connected to the lobula plate also. Pathway 3 (p3) consists of two groups of cells mainly arborizing

Correspondence to: B. Bausenwein

in M3, or in M4 and M6, which converge in layer M8 and project to the inner lobula layers.

We here describe functional activity of the medulla neuropil using the 2-deoxyglucose (2-DG) neuronal activity labeling method. This method was adapted for use in *Drosophila* to search for directional movement sensitivity (Buchner et al. 1979). These studies demonstrated that the lobula plate is organized into directionally sensitive layers. Later studies using the 2-DG method have shown stimulus-specific activation in the lamina and medulla (Buchner et al. 1984b; Bülthoff 1986). We present here a detailed analysis of activity labeling patterns in response to large-field motion stimuli. The label distribution and localization are compared to the connectivities proposed in the computer evaluation of arborization densities (Bausenwein et al. 1992).

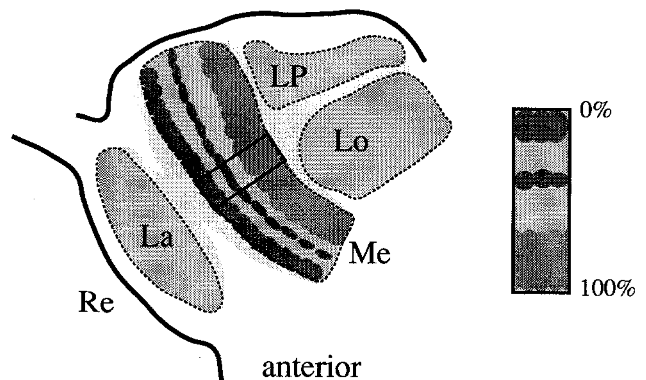


Fig. 1. Schematic representation of an autoradiogram of a horizontal section of the visual lobe of the fly. This scheme also shows the position of the medulla slice used for the quantitative description of label distribution. To evaluate the label distribution, we used only small central slices about 2–4 columns wide to diminish the effect of the overall curvature of this neuropil. They were taken at a level where the esophagus can be seen transversing the brain in full length. All autoradiograms shown in the following figures are oriented accordingly. *Inset* Illustrates the depth in the medulla (Me) with 0% being the distal surface and 100%, the proximal. *Re* Retina; *La* lamina; *LP* lobula plate; *Lo* lobula

Materials and methods

Experimental flies and 2-DG labeling

Two-day-old female flies of the *Drosophila melanogaster* strain "wild-type Berlin" were used in all experiments. The animals were isolated and prepared the afternoon before the experiment. About 5–15 min before the experimental stimulation they were injected with 50–150 nl of [5,6]^{3}H-2-deoxyfluoroglucose (New England Nuclear, NET 902 or American Radiolabeled Chemicals, ART – 105) in doses of about 20 µCi following the description of Buchner and Buchner (1983). The animals were then subjected to one of three different stimulus conditions:

(1) Rotating striped cylinders (height: 80°) of defined spatial wavelength (18°) and constant angular speed (20°/s).

(2) In the object response paradigm (Bausenwein et al. 1986) the flies were stimulated with a single rotating black stripe (width 10°, height 80°), at defined angular velocities (40°/s in the examples shown). The flies' torque was monitored and recorded.

(3) In the landing response paradigm, the flies were situated in front of a Tektronix oscilloscope (Phosphorus p31, 200 Hz frames), where two stripes (width 8°) moved from front to back (from 5° to 60°, mean angular velocity 120°/s) on either side, simulating an expansion stimulus characterizing the fly's approach towards an object. The stimulus frequency (0.3 Hz) was chosen to prevent habituation. Landing responses were observed and counted.

For each situation at least six flies were used, with the exception of the fly shown in Fig. 10. This autoradiogram is from a fly kindly provided by E. Buchner. It was stimulated with flicker on one side and motion on the other. Although this situation has not been studied in the same detail as the stimuli described above, the autoradiogram illustrates the possibility of reducing the activity patterns, to obtain a better description of the cellular basis of the label patterns.

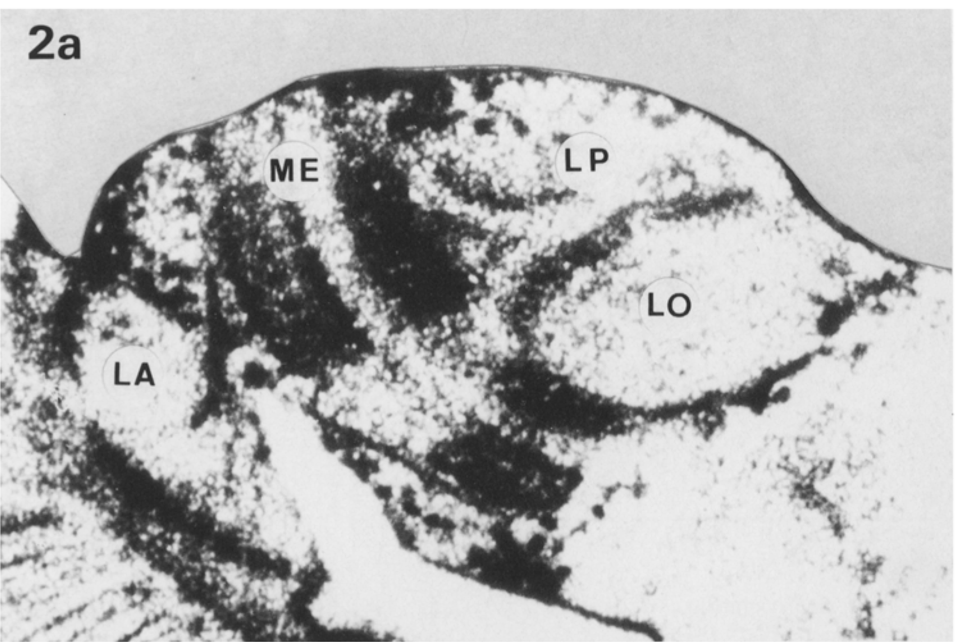
All preparations except those shown in Figs. 2 and 10 are from stationarily flying flies. Stimulus exposure in an otherwise dark room lasted between 20 and 90 min, depending on flight perseverance. All experimental animals were shock-frozen immediately after the experiment and freeze-dried according to Buchner and Buchner (1980). The Epon-embedded material was then sectioned (2.5–3.5 µm) and transferred to slides prepared as described by Buchner and Buchner (1983). They were exposed to either stripping film (Kodak AR10) or emulsion (EM-1, Amersham; L-4, Ilford) on a second slide. This "sandwich autoradiography" (Buchner and Buchner 1983) allows multiple exposures. Exposure times were between 4 and 14 days.

Scanning of autoradiograms

Prints of the autoradiograms (ARGs) were scanned with a flatbed scanner (Abaton 300/FB) using the program C-Scan. Vertical and horizontal resolution of the scanned images is 300 dpi (dots per inch), this corresponds to 137 pixels (picture elements)/mm^2. The density of the labeling was scanned in half-tone mode, which uses a 4×4 dither matrix (which thus could be converted to 17 greyscales). This reduced the spatial resolution to 75 dpi vertical and horizontal. The scanned pictures were stored and analyzed with a Macintosh II computer. The autoradiogram shown in Fig. 5 was scanned directly from the microscope to the computer using a standard black/white videocamera and a framegrabber board (Rasterops 364).

Fig. 2. Autoradiograms of the visual lobes of a stationarily walking fly stimulated for 3 h with a rotating striped drum. Intense labeling can be found in various parts of the visual lobe, as has been reported by Buchner and coworkers (Buchner et al. 1984b). The unstimulated part of the medulla corresponding to the visual field where motion was hidden behind a black screen is hardly labeled in comparison to the stimulated fields, showing that the labeling is indeed caused by selective activation of visually stimulated neurons. *LA* Lamina; *ME* medulla; *LO* lobula; *LP* lobula plate. **a** Back to front; **b** front to back

Fig. 3. The 2-deoxyglucose labeling pattern in the visual lobes of *Drosophila* stimulated with a rotating grating. This fly was stimulated during stationary flight (duration 90 min), as were all flies shown in the following figures (except Fig. 10). To avoid edge effects at stimulus borders, screens (Fig. 2) were not used. The autoradiograms reveal strong activation of neuronal elements in the medulla on both sides. In the medulla, the activation patterns are similar to those of walking flies. Apparent spatial resolution in the pattern seems to be somewhat higher (compare to Fig. 2). Whereas in the lobula plate different layers are activated depending on the direction of motion, in the medulla back-to-front and front-to-back stimulation lead to similar activation patterns. **a** Back to front; **b** front to back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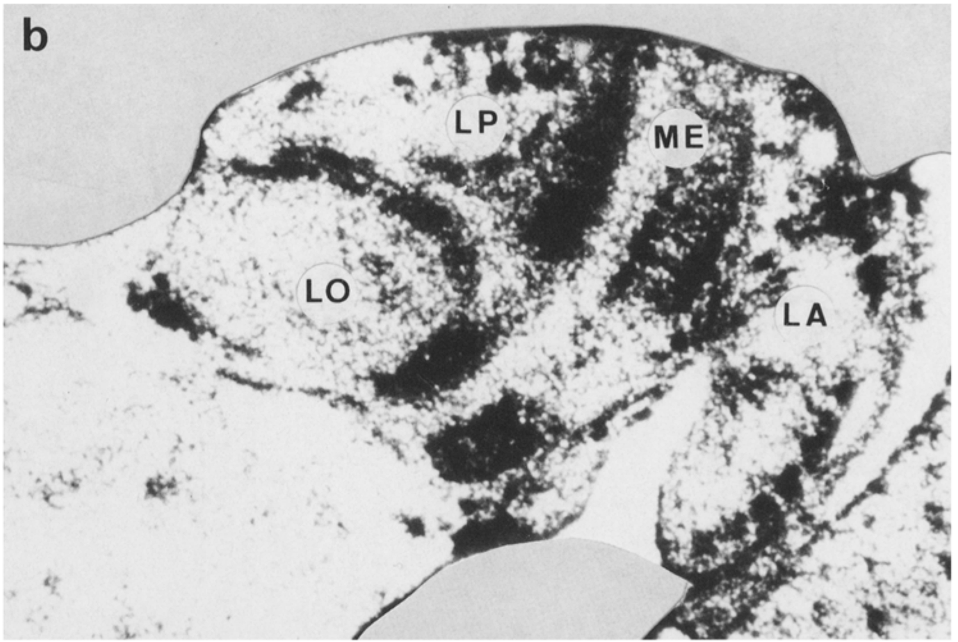
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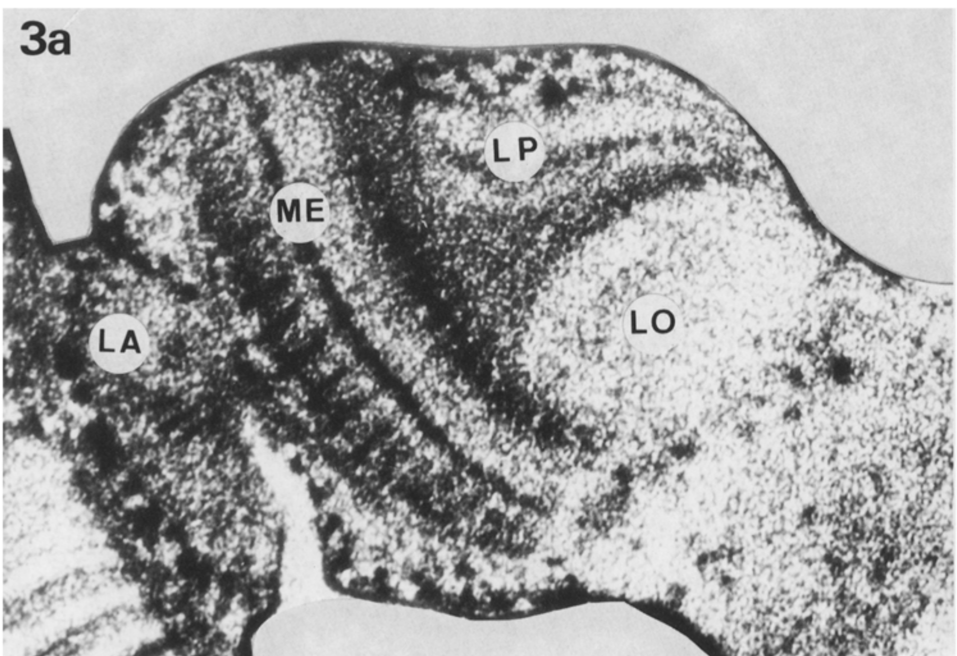
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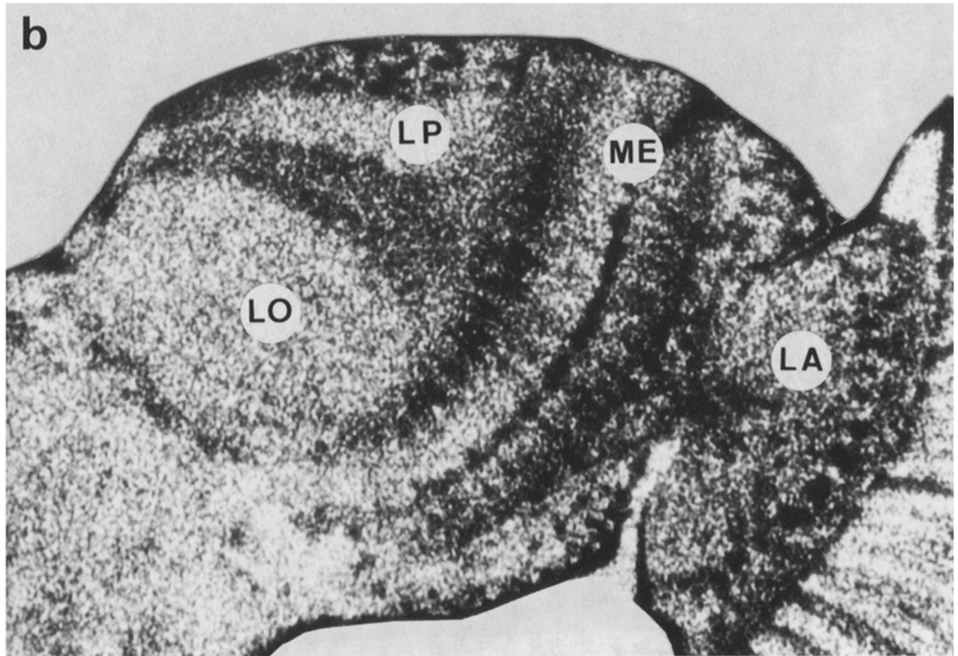
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All scans were compared to the original autoradiograms in a scale nearly 1:1 (screen resolution of the greyscale monitor 72 dpi vertical and horizontal). Most details visible in the autoradiograms were conserved in these scans.

Image analysis

The scans were viewed using the public domain programs "Image" of the National Institutes of Health, Research Services Branch, NIMH. One of us (B.B.) wrote programs to convert the raw scanner data to standard formats and to analyze sections of scanned pictures. Calculations of optical versus neuropil depths follow the description of Bausenwein et al. (1992), with 0% medulla depth specifying the distal neuropil border (pointing towards the outer chiasm), 100% to the proximal border (inner chiasm). To diminish the effects of the change in layer size from anterior to posterior and to avoid curvature distortions, we used a small central section of the medulla for the quantitative evaluation. The patches used are about 2–4 columns wide, and were taken from horizontal sections at the level of the esophagus. Maximum and minimum density values were taken to normalize the density profiles. Density profiles (Figs. 4, 7, 9, 11) represent typical samples of at least five scans, taken at three or more sections. Fig. 1 shows a schematic representation.

Neurite density profile analysis (NDPA)

The earliest reports using 2-DG activity labeling in *Drosophila* describe the differential uptake of glucose as localized in "bands" in the medulla (Buchner et al. 1979; Buchner and Buchner 1983). These bands consist of a superposition of specifically stimulated columnar cell types (Buchner et al. 1984b). If one assumes that a neuron is labeled homogeneously throughout its cell body, dendrites, and axons (and all data indicate that), the labeled layers should indicate regions where the labeled neurons are most densely represented. The 2-DG-labeled layers were compared to those defined in a study on the connectivity of columnar medulla neurons (Bausenwein et al. 1992). For comparison with single neurons we used the neurite density profiles of Golgi-impregnated cells collected in our profile database (Bausenwein et al. 1992). We term this approach neurite density profile analysis (NDPA). The 2-DG label distributions of the autoradiograms have been added to our image and profile databases.

Results

2-DG stratification highlights layers described in the analysis of Golgi-impregnated neurons

We repeated the experiments of E. Buchner and coworkers with stationarily walking and flying *Drosophila* and found stimulus-dependent labeling in the medulla as described by Buchner et al. (1984b). Quantitative evaluation of the scanned activity distribution revealed that the labeled bands resided in certain, constant depths within the medulla neuropil. Fig. 2 shows intense labeling in the optic lobe of a walking fly after stimulation with a rotating striped cylinder. The stimulus dependence is clearly demonstrated by the weak glucose uptake in the unstimulated region of the eye. In this part of the visual field the motion stimulus was hidden behind a stationary screen. Fig. 3 shows the effect of a similar stimulation, but without screens, in a flying fly. The activity pattern – at least in the medulla – does not

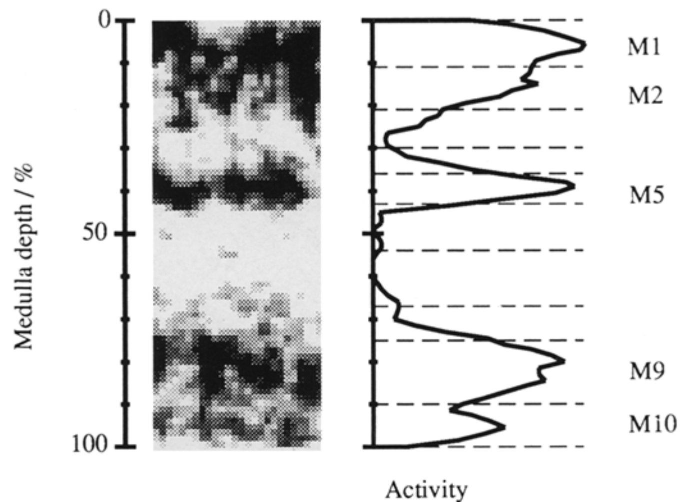

Fig. 4. Quantitative evaluation of the label distribution after large-field stimulation with rotating gratings. The diagram shows label density as a function of medulla depth. Label density is normalized to minimum and maximum values of the profiles. The label concentrates in layers M1, M2 and M5 in the outer medulla. In the inner medulla, there is a broad band of activation, including layers M9 and M10, with the maximum in layer M9 (compare with Figs. 2, 3). Activity profiles of medullae stimulated with front-to-back or back-to-front stimuli do not differ significantly

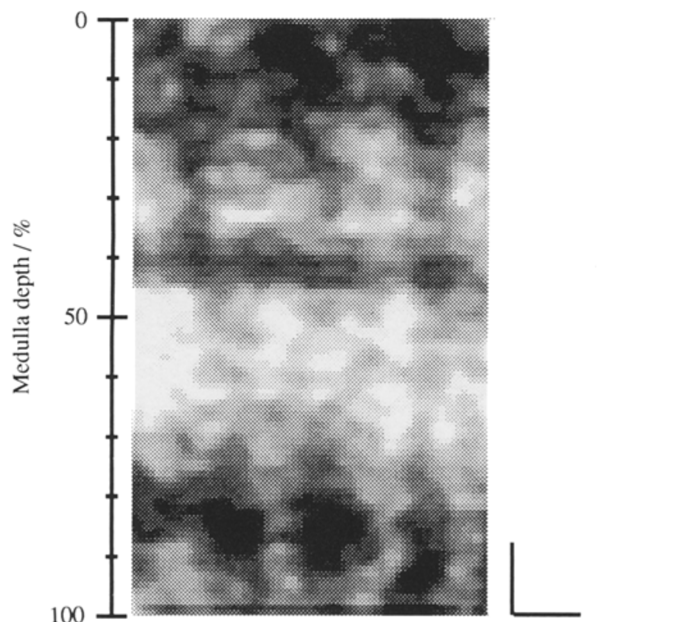

Fig. 5. A high magnification micrograph showing the label distribution of about three columns in the medulla after large-field motion stimulation. Activity maxima are not only confined to certain layers, but are laterally restricted within single columns. This can clearly be seen in layers M1, M2, M9 and M10. In layer M5 the maxima are not as clearly separated, indicating a potential site of lateral interaction. The light, unlabeled band between internal and external medulla demonstrates that tangential neurons of the medulla whose axons transverse the serpentine layer (M7) are not labeled. *Bars*: 5 μm

differ qualitatively from that of a walking fly. When we use the layers found in a Golgi study (Fischbach and Dittrich 1989) to characterize the activity distribution, the peaks of activity label fall into layers M1, M2, M5, M9, and M10 (Fig. 4). The good fit of the 2-DG

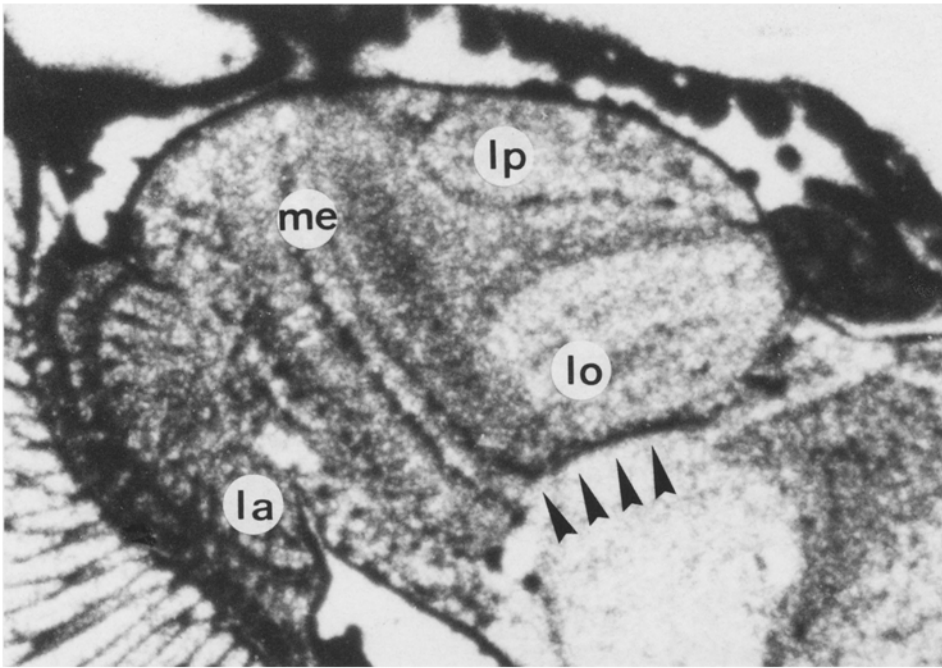

Fig. 6. The activity pattern of the optic lobe after stimulation with a rotating black stripe (side shown stimulated with back-to-front motion). The activity pattern for this stimulus differs from the one for moving gratings by an additional intensely labeled band between distal and proximal medulla (serpentine layer). Active fibers can be followed into Cucatti's bundle (*arrowheads*). The serpentine layer houses the axons of large medulla tangential fibers, whose number and arborization characteristics are not documented in detail (for example neurons, see Fischbach and Dittrich 1989)

activity pattern with the stratification in the Golgi layer system is informative. The labeled layers form two distinct subsets of interconnected layers, the p1 pathway (M1, M5 and M10) and the p2 pathway (M2, M9; Bausenwein et al. 1992). The direction of stimulus movement which determines which layers are labeled in the lobula plate, cannot be inferred from the activity pattern of different layers in the medulla, thus confirming suggestions by Buchner et al. (1984b).

The high magnification of the 2-DG pattern shown in Fig. 5 reveals that the maximum activity resides within single columns. Quite often labeled profiles appeared to connect layers M1 and M5. Sometimes profiles connecting outer and inner medulla could be detected (Fig. 5). Lateral spread of activity was not a prominent feature within the medulla under the experimental conditions described sofar. This showed that the banded appearance indeed resulted from a superposition of columnar elements and was not due to tangential elements. Most structures activated by the movement of the striped drum seem to have rather small dendritic fields, the width of single visual columns (see Fig. 5). This was also shown to be a characteristic feature of neurons in pathways p1 and p2 (Bausenwein et al. 1992).

Label distribution in the medulla is stimulus specific and does not depend on specific behavioral activities

When a rotating striped drum was the stimulus, the same layers were 2-DG-labeled irrespective of whether the flies had been walking or flying (compare Figs. 2 and 3). However, the activity pattern was sharply defined by properties of the visual motion stimulus used. Here we present some other characteristic stimulus-specific activation patterns. In experiments using a single rotating stripe we found strong activation of the M7 layer, the serpentine layer, in addition to the layers reported above (Fig. 6). The serpentine layer was labeled in both visual lobes although the stripe moved from front to back on one side and from back to front on the other. Layer M7 houses large-field tangential neurons (Fischbach and Dittrich 1989). Some of the medulla tangentials are known to transfer information via Cucatti's bundle to the posterior slope region, and others to connect the two optic lobes (Strausfeld 1970). Their dendritic and terminal arborizations reside in many layers of the medulla. The label intensity in M7 was high, and could even surpass that in layers M1 and M5 (Fig. 7). The

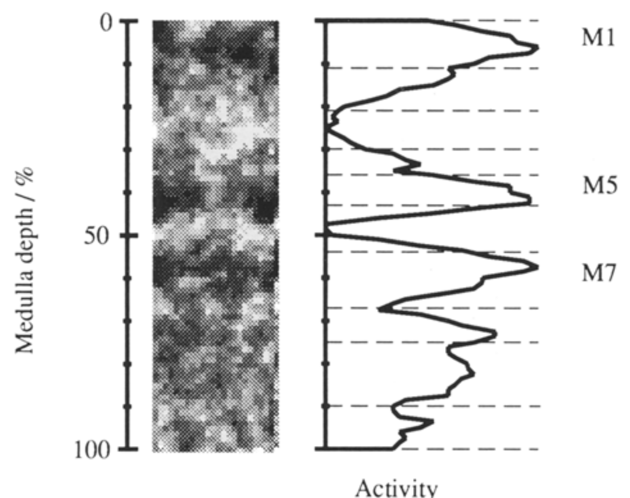

Fig. 7. Label density analysis of the activity pattern following single stripe stimulation. In the outer medulla, layers M1 and M5 are strongly labeled. M2 and inner medulla layers are labeled as well, though the label appears to be slgihtly weaker. This may be due to the reduced contrast frequency. However, there is strong additional neuronal activity in the serpentine layer (M7). Activity in this layer is among the highest activity values in the profile

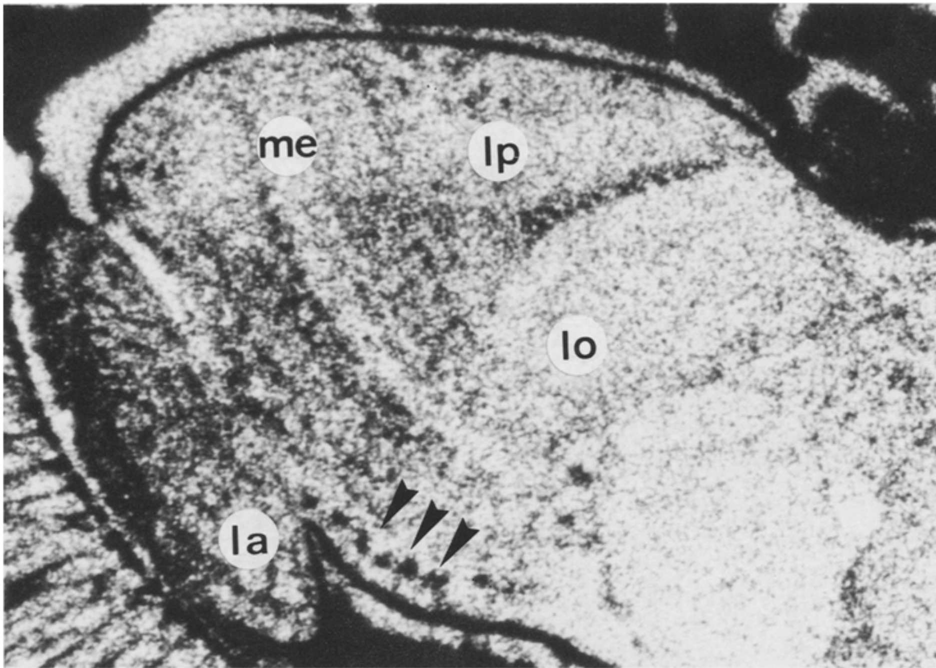

Fig. 8. Activity pattern of a fly stimulated with two stripes moving from front to back in a 60° wide area excluding the frontal visual field (15°) presented on an oscilloscope screen. The uptake of glucose is clearly stimulus specific, as has already been shown in Fig. 2. Activity patterns resulting from this stimulation can easily be discriminated from the situations described earlier. See Fig. 9 for a quantitative description of the label distribution in the medulla. In this autoradiogram (this fly shows rather low glucose uptake) only few intensely labeled cells dominate the activity pattern. See the labeled cell bodies in the medulla rind (*arrowheads*), which are distributed in a banded fashion. Their number indicates that a single columnar cell type of the medulla is labeled. A banded appearance of medulla cell bodies has also been observed in immunohistochemical stains of T1 cells (Buchner et al. 1988). Note the strong label in inner chiasm and an outer lobula layer, which is not accompanied by activity in the lobula plate. It is thus rather likely that the labeled medulla neurons in this preparation are Tm cells

simultaneous activation of layers M5 and M7 showed that there is a layer in between (M6), which was not activated by the motion stimuli used.

M3 was selectively activated during stimulation in the landing response paradigm (see Materials and methods). Again, there was no effect of the direction of motion: M3 could also be activated by back to front motion (data not shown). Unilateral presentation of moving single stripes or periodic stripe patterns also elicited this activity pattern on the stimulated side. Fig. 8 (ARG) and Fig. 9 (label intensity) show an example of the activity pattern after bilateral expansion stimuli presented on an oscilloscope. In these preparations, activity label in M2 was much weaker than after striped drum stimulation (compare Figs. 8, 9 to Figs. 3, 4). In some preparations under the same stimulus conditions, we also found activation of M8 (data not shown). The simultaneous labeling in M3 and M5 uncovered the M4 layer. We thus have directly or indirectly identified all 10 layers defined by the evaluation of Golgi material. Number and position (relative depths) of the activity bands correspond to the Golgi strata. This is strong evidence for the functional significance of the layers. All layers but M4 and M6 have been labeled under certain motion stimuli. Labeling in M1 and M5 was a general characteristic feature of the activity pattern found under all motion stimulus conditions used. In addition we found layers M2, M9, and M10 to be labeled, when

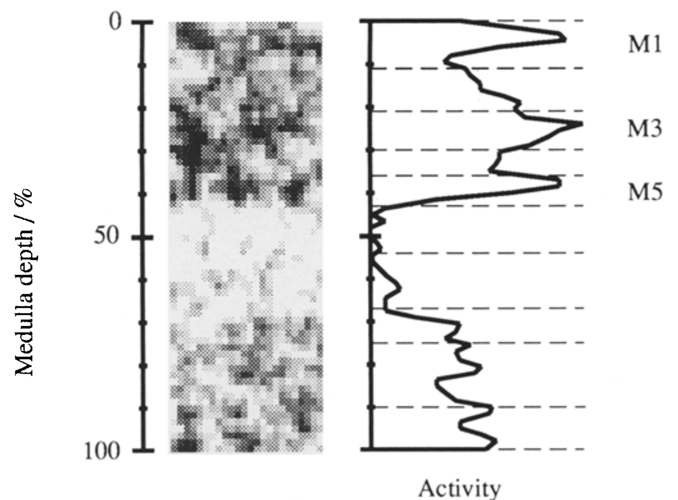

Fig. 9. Label density distribution in the medulla of a fly stimulated with the stimulus of Fig. 8. The activation of neurons arborizing in layer M3 is very prominent. Layers M2 and even M1 display much weaker intensity than in the autoradiograms shown in Figs. 2–7. M10 is also labeled. Sometimes significant label can also be detected in layer M8 (comp. Fig. 8), which concentrates in each column in a rather dense, small, blob

the flies were exposed to rotating striped drums. Also in addition to M1 and M5, we found under specific stimulus conditions labeling in M3, M7, and M8; thus neurons arborizing in these layers seem to be concerned

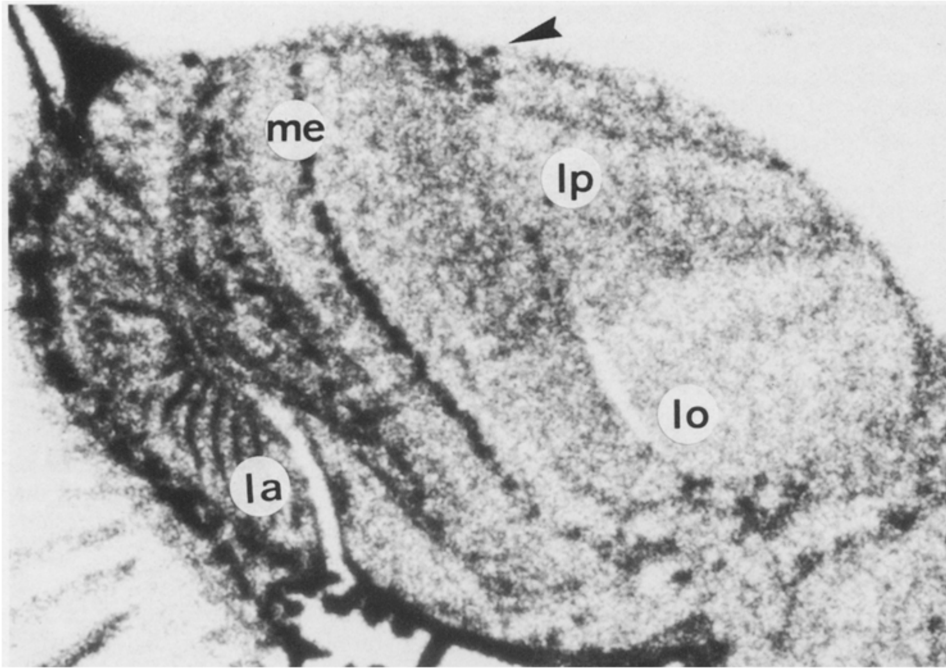

Fig. 10. Activity labeling of medulla input layers only. This fly, which was stimulated on one side with motion and on the other side with flicker, shows strong label uptake in the lamina and outer medulla (flicker side shown, preparation kindly provided by E. Buchner). The lack of label in the inner medulla is not a general feature of flicker stimuli (Buchner et al. 1984). The lobula complex is not significantly labeled. Note that this autoradiogram shows labeling in a rind region (*arrowhead*) known to house the cell bodies of four different cell types (C2, C3, T2, T3: Fischbach and Dittrich 1989). Only a subset of this cluster is labeled. This pattern can be seen on both sides of the fly

with the evaluation of specific aspects of the motion stimulus.

Evidence for the involvement of several distinct cell types

The activity pattern may be further dissected by preparations showing weaker 2-DG uptake. It is thus possible to discriminate not only between activity in the layers of pathways 1 and 2 but also between input layers of the medulla (M1–M6) and the proximal layers M8–M10. Fig. 10 shows an autoradiogram of a fly stimulated by motion on one side and flicker on the other. The activity profile (Fig. 11) in the distal medulla of both sides was very similar to that in Fig. 4 (M1, M2, M5). However,

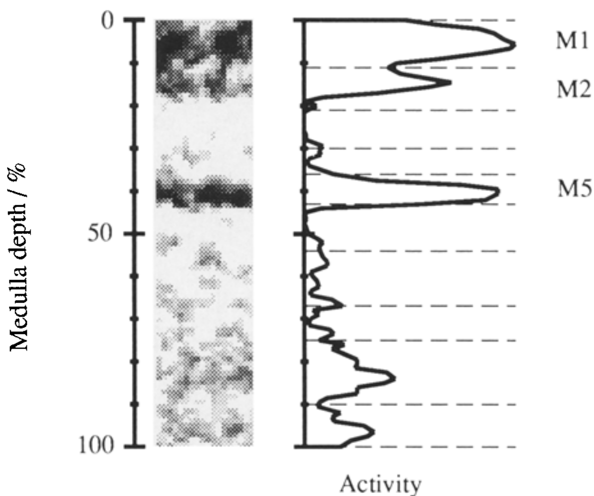

Fig. 11. Label density distribution in the medulla of the preparation shown in Fig. 10. The maximum label concentrates in medulla layers M1, M2 and M5. At least part of this label is due to lamina monopolar cells

labeling in the proximal medulla, in layers M9 and M10, was almost reduced to background activity.

In some preparations (Fig. 10) we found strong 2-DG uptake in a posterior, medial part of the medulla rind known to house the cell bodies of T2, T3, C2, and C3 cell types (Fischbach and Dittrich 1989). The density of that labeling suggests that only a subpopulation of this cluster was labelled, likely one or two cell types. The neuropil pattern was neither composed exclusively of activated T3 or T2 cells (which in the proximal medulla occupy M9 mainly) nor exclusively of C2 cells (which have their proximal specializations in layers M8 and M10).

Discussion

Does 2-deoxyglucose label whole cells?

For a discussion of the hierarchy of labeling it is crucial that 2-DG labels whole neurons not just portions of them. Several findings clearly indicated whole cell labeling:

(1) Evidence from identified cells or groups of cells. In the 2-DG-labeled H1 neuron of *Drosophila,* which has a very long axon transversing the whole brain, a rather homogeneous distribution across the axon was reported (Bausenwein et al. 1990). Buchner and coworkers (1984a) described the two labeled CH neurons of *Musca* to be easily identifiable due to the typical position of their cell bodies. Unlabeled HS and VS profiles were found to be accompanied by a corresponding number of unlabeled cell bodies (Buchner et al. 1984a).

(2) Accordance of labeling in various rind and neuropil regions of the supraesophageal ganglion. Different patterns of neuropil and fiber labeling were in marked ac-

cordance with different patterns of labeled cell bodies (data not shown). The intensity of the label in cell bodies and neuropil was not distributed gradually. Recent data using small-field stimulation show labeling of medulla cell bodies of stimulated columnar neurons only (K. Plötze, unpublished).

(3) Relative independence of the label patterns from exposure time. It was not possible to compensate for different amounts of injected glucose by varying the autoradiographic exposure times. The observed patterns changed their overall intensity, but did not display qualitative changes, i.e., reveal new bands after long exposure times.

In conclusion, there were no obvious indications of any labeling gradient within a 2-DG-labeled cell, and it is reasonable to assume that the 2-DG label was homogeneously distributed in neurons.

How then could a gradual difference in glucose uptake result in a hierarchy of labeling? Obviously we have to assume highly nonlinear transfer functions. The label intensity of different neurons was determined by the accumulated dose of 2-DG per exposed surface. Glucose uptake as a function of physiological activity or duration of the experiment may also be highly nonlinear. Also, autoradiographic emulsions have a rather steep contrast transfer function. At any rate, these nonlinearities may result in different numbers of cell types visible in autoradiograms. This fortuitous effect allows the cellular unraveling of complex label patterns using preparations with different uptake intensities.

Structural separation of retinotopically organized functional pathways in the medulla

Stratification is an important general organizational principle of visual neuropil, as has been stated by various authors (Campos-Ortega and Strausfeld 1972; Strausfeld 1989b). For the medulla of *Diptera,* stratification has led to hypotheses about the functional diversity of cells underlying these layers. Strausfeld (1989b) and Strausfeld and Lee (1991) suggested the existence of two pathways, the so-called magnocellular (m) and parvocellular (p) pathways, which they suggest are specific for motion detection and color processing, in analogy to the organization of the primate visual system. In a quantitative evaluation of the Golgi-impregnated shapes of neurons we found three preferentially connected sets of layers, housing pathways p1, p2, and p3 (Bausenwein et al. 1992). Two of these are thought to be involved in motion detection, while pathway p3 is assumed to be color sensitive.

This study now shows that motion-stimulus-selective uptake of 2-DG is strictly confined to a subset of medulla layers – the same layers inferred from anatomical studies to underlie motion detection. Moreover, the 2-DG activity patterns after stimulation with rotating gratings clearly support the concept of preferred connectivity among different layers in the medulla. The motion-induced activity maxima in layers M1, M2, M5 in the outer and M9 and M10 in the inner medulla co-map with pathways p1 and p2. The data shown indicate that the pathways predicted from the stratification pattern of the individual Golgi cell types exist as physiological circuits. Moreover, the visualization shows that these pathways differ in their physiological and probably functional properties. After stimulation with rotating striped drums, the activity label concentrates in pathway p1 and, with slightly weaker intensity, in pathway p2. The pattern of the label indicates that the main components of the activation pattern are columnar neurons. There is no evidence suggesting that lateral interactions play a major role at the level of the medulla neuropil under the stimulus conditions described, with the interesting exception of M7, which can be activated specifically with single stripe stimuli (Fig. 5). For a quantitative analysis of lateral spread of information we need to carry out experiments using motion stimuli in smaller, more narrowly defined visual fields.

The labeling of medulla tangentials is exciting, because it is intriguing to see "qualitative" differences in the activity pattern of the medulla under slightly different stimulus conditions. It is now evident that there is stimulus-pattern-specific processing peripheral to the giant neurons of the lobula plate. Up to now, the detection of small-field figures has been attributed solely to the input properties of certain tangential neurons in the lobula plate, e.g., figure-detection neurons (FD-neurons, Egelhaaf 1985). This point will be further addressed in another study. Our findings clearly demonstrate two forms of parallel processing in the medulla. Parallel processing exists not only in the topological domain (columnar multiplication) but also in the functional domain (stratification, probably serving feature extraction).

Two motion-sensitive pathways: p1 and p2

Pathways p1 and p2 together may correspond to the magnocellular (m) pathway in Strausfeld's (1989b) conceptual organization of the medulla of insects. In our computer analysis of neuronal cell shapes in *Drosophila,* there is strong evidence for a separation of these two pathways. This starts with the duplication of the input channels L1 and L2, although these cells receive very similar inputs (Meinertzhagen and O'Neil 1991). The set of medulla cells connecting the layers of p1 differs significantly from the one subserving p2. Typical neurons for p1 are the medulla intrinsic cell Mi1, and C2, C3 cells, which all arborize in M1, M5, and M10. M10 is connected to the four motion-sensitive layers of the lobula plate via T4 cells. They are supplemented by several TmY cells, which project to lobula *and* lobula plate (in contrast to transmedullary Tm cells, which project exclusively to the lobula).

Pathway p2, however, is composed of cells arborizing in layers M2 and M9. Several Tm cells of this type project to the superficial half of the lobula, which is connected to the lobula plate layers, e.g., via T5 cells, which look very similar to the T4 cells of p1. Thus, p1 and p2 may engage the same minimal number of synapses

to reach the lobula plate. Despite the clear differences in the arborization patterns of the neurons involved, the two pathways p1 and p2 may interact at several sites, and may also finally converge in the lobula plate. Outputs of p2 can also be further processed in the lobula. The exact position of the activity label in the lobula has not been evaluated yet.

Under all stimulus conditions used, labeling of M2 was not observed without label in layers M1 and M5. The stronger labeling of p1 may either indicate an intrinsic hierarchy of activity distribution or it may show that we did not employ conditions under which there is preferential activity of p2. The presumed major input cells, L1 and L2, despite minor differences in their specific connectivity in the lamina (Meinertzhagen and O'Neil 1991), are thought to behave like a twin pair (Laughlin 1984, 1989), and electrophysiological differences seem to be rather subtle (Hardie et al. 1989). The different label intensity, however, may also be due to the clear differences in the gestalt of the cells, e.g., L2 has only one terminal in the medulla (in layer M2), whereas L1 has two terminals (in M1 and M5) and the axon diameter of L1 is significantly smaller than that of L2 (Braitenberg and Hauser-Holschuh 1972), which thus has a smaller surface/volume ratio. This might result in a higher glucose consumption of L1 compared to L2 independent of electrophysiological function.

On the other hand, the differential labeling of pathway 2 is not restricted to its input cell L2. T1 is a second connection of the medulla with the lamina (presumed to be an input cell to the medulla, see Meinertzhagen and O'Neil 1991). This cell type may contribute substantially to the characteristics of this pathway. Moreover, p2 is anatomically distinct from p1 in its primary projection area, the distal lobula. Under some stimulus conditions layers in the distal lobula are strongly labeled. It is not known whether the duplication of channels p1 and p2 is caused by a clear functional separation, or whether they are only subtle adjustments of a single function. Braitenberg has speculated that L1 and L2 may serve different channels of motion detection (1972; Braitenberg and Hauser-Holschuh 1972). Though some of his models (e.g., where L1 and L2 serve directionally opposite channels for horizontal motion) very likely can be ruled out now, it is still conceivable that the two channels are involved in motion detection proper, e.g., in on or off circuits (Franceschini et al. 1989). Gilbert et al. (1991) describe motion and flicker responses of medulla neurons of *Sarcophaga*. The recorded cells differ in their response characteristics depending on the depth of their dendritic arborizations. Recorded neurons arborizing at the level of L1 tend to respond to motion with substantial depolarizations and sometimes overlaid with direction- or orientation-selective ripples, and seem to reflect complex synaptic interactions. In contrast, the responses of neurons arborizing in the stratum of the L2 terminal are characterized by periodic fluctuations with only a slight DC component. If the recorded neurons are a representative sample, these data indicate a functional separation of cells following L1 and L2 in *Sarcophaga*.

Interestingly, in the 2-DG activity patterns of *Drosophila* we do not find simultaneous activation of layers M2 and M3, which have been described to be a common terminal site of many neurons (Fischbach and Dittrich 1989). Candidates for this group of cells include Tm9, Mi4, Mi8, Mi89. These cells very likely may now be excluded from the list of neurons responsible for the activity patterns described in this paper.

In conclusion, our activity labeling experiments show that the motion detection apparatus peripheral to the lobula plate tangentials can be further analyzed using the 2-DG technique.

How many cells are activity labeled by large-field motion stimulation?

Retinula cells. Retinula cells, terminating in the medulla, are clearly not labeled under the situations tested. This is shown by the lack of label uptake in layer M3, where most R8 cells terminate, and the unlabeled layer M6, where all R7 fibers terminate. We also have evidence that R1–R6 are not intensely labeled (data not shown). Although the reason for the lack of label is not understood, it may indicate a principal difference in the glucose supply of retinula cells (in the bee drone, glucose is supplied to retinula fibers mainly from glycogen stored in glial cells: Tsacopoulos et al. 1988).

Lamina cells. Certain lamina monopolar cells (LMCs) are heavily involved in the label pattern in the distal medulla. This is especially true for label in layers M1 and M5, which may be due to labeling of L1. Activity patterns in the lamina are the subject of another publication (B. Bausenwein et al., in preparation).

Medulla cells. Which and how many medulla cells are 2-DG labeled due to their involvement in the processing of movement stimuli? An exact count of labeled cell bodies in the medulla cortex would require three-dimensional reconstruction of serial autoradiograms. Only a very small fraction of all medulla cells is visibly labeled in the experiments reported. Estimations indicate that at least three cell types per column contribute to the label pattern. This pattern may be composed of two lamina inputs, and at least one or two medulla intrinsic or transmedulla cells. In some experiments, labeled cell bodies of the C2/C3/T2/T3 cell type can be detected (Fig. 10). Unfortunately, the label pattern of the lobula, which could indicate the participation of T2 or T3 cells, does not allow us to decide which cell types are activated or what the relevant parameters for their differential activation are. They may interact with pathways p1 (C2, C3 and T2) or p2 (C3, T2 and T3) or both (Bausenwein et al. 1992).

Large-field motion stimuli label medulla outputs into both the lobula and lobula plate. The columnar patterns in the lobula plate and medulla layer M10 (Figs. 2, 3) may be due to the activity of T4 cells. Labeling of a distal lobula layer may be caused by the transmedulla neuron Tm1 or the T5 cell (Fischbach and Dittrich 1989). The highest label intensity in the inner medulla

is in layer M9, which houses the arborizations of many Tm cells, projecting into superficial layers of the lobula. The relatively weaker labeling of M10 (Fig. 3) may indicate that more cells arborize in M9. Furthermore, the main output cell of M10 (T4) exists in four subtypes in each column (Fischbach and Dittrich 1989). If these cells have selective response properties, only a fourth of all T4 cells may contribute to the labeling. We are designing experiments to test the directional sensitivity of cells in layer M10. With these more detailed studies our goal is to identify the output cells of the medulla.

Correlating label and function: selectivity versus specificity

The labeled structures obviously are involved in the motion processing pathway. However, whether the selective activity of the labeled neurons is necessary or even sufficient for motion detection has not been demonstrated yet. The involvement of lamina monopolar cells is evident. We have evidence that L1 is the most strongly labeled LMC (B. Bausenwein et al., in preparation). A convincing proof will involve functional or structural dissection experiments. It is evident that medulla layers M1, M2, M3, M5, M7, M9, and M10 can be activated under conditions used in many behavioral or electrophysiological standard paradigms. For information on the specific properties of the cells underlying the activity pattern one would first like to know whether the other layers, e.g., M4, M6, and M8 also display stimulus-dependent 2-DG uptake. Evidence from recent experiments tells us that this is the case (K. Plötze, unpublished). Preliminary data indicate not only that labeling is the specific, but also that there is functional meaning to the unlabeled layers. More experiments are needed to learn about the specific parameters responsible for the activation of a set of layers or even for the identification of the underlying individual cell types.

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