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Deoxyglucose mapping of nervous activity induced in *Drosophila* brain by visual movement

I. Wildtype

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Summary. Local metabolic activity was mapped in the brain of *Drosophila* by the radioactive deoxyglucose technique. The distribution of label in serial autoradiographs allows us to draw the following conclusions concerning neuronal processing of visual movement information in the brain of *Drosophila*.

1. The visual stimuli used (homogeneous flicker, moving gratings, reversing contrast gratings) cause only a small increase in metabolic activity in the first visual neuropil (lamina).

2. In the second visual neuropil (medulla) at least four layers respond to visual movement and reversing contrast gratings by increased metabolic activity; homogeneous flicker is less effective.

3. With the current autoradiographic resolution (2–3 μm) no directional selectivity can be detected in the medulla.

4. In the lobula, the anterior neuromere of the third visual neuropil, movement-specific activity is observed in three layers, two of which are more strongly labelled by ipsilateral front-to-back than by back-to-front movement.

5. In its posterior counterpart, the lobula plate, four movement-sensitive layers can be identified in which label accumulation specifically depends on the direction of the movement: Ipsilateral front-to-back movement labels a superficial anterior layer, back-to-front movement labels an inner anterior layer, upward movement labels an inner posterior layer and downward movement labels a superficial posterior layer.

6. A considerable portion of the stimulus-enhanced labelling of medulla and lobula complex is restricted to those columns which connect to the stimulated ommatidia. This retinotopic distribution of label suggests the involvement of movement-sensitive small-field neurons.

7. Certain axonal profiles connecting the lobula plate and the lateral posterior protocerebrum are labelled by ipsilateral front-to-back movement. Presumably different structures in the same region are labelled by ipsilateral downward movement. Conspicuously labelled foci and commissures in the central brain cannot yet be associated with a particular stimulus.

The results are discussed in the light of present anatomical and physiological knowledge of the visual movement detection system of flies.

Introduction

The visual movement detection system of dipterans has been intensively investigated by behavioural, anatomical, physiological and genetic techniques with the prospect of eventually unravelling a neuronal information processing system of an intermediate degree of complexity between, for example, a molluscan ganglion and the mammalian cortex. Recent reviews on the various approaches to the system are available (Reichardt and Poggio 1976; Poggio and Reichardt 1976; Götz 1983; Buchner 1984; Heisenberg and Wolf 1984; Strausfeld 1976, 1984; Fischbach 1983; Hengstenberg 1982; Hausen 1984; cf. Bülthoff and Buchner (in preparation) for comments and references on the genetic approach). Behavioural input-output analysis has led to a theoretical understanding of the interactions essential for movement detection and to a detailed scheme of 'elementary movement detectors' which must be assumed to connect to the ommatidial array of the compound eyes (rev.

Abbreviation: DG deoxyglucose

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Buchner 1984). Neuroanatomy has described the shape and branching pattern of a large number of interneurons in the optic lobes and has classified them as 'columnar' or 'tangential' according to their morphological architecture. Since for each ommatidium in the compound eye there is one column in each visual neuropil, most columnar neurons may be assumed to be involved in centripetal or centrifugal transmission of local signals from the visual surround, whereas tangential neurons are appropriate for integration of information from local interneurons over extended portions of the visual field (rev. Strausfeld 1984). Electrophysiology, combined with anatomical identification through dye injection, has provided substantial information on early visual processing in the first visual neuropil (lamina) and on sets of interneurons in the posterior neuromere of the third visual neuropil (lobula plate). These interneurons seem to be responsible for the integration of visual movement information over the entire visual field of both eyes and for relaying this information to neurons descending to the motor centres of the thorax. Between the lamina and the lobula plate there is, however, a highly complex neuropil, the medulla. In spite of intensive efforts, electrophysiology has so far failed to characterize the anatomically identified interneurons of the medulla and to localize the site of movement detection (elementary movement detectors). In fact, the small diameter of most medullary neurons may defy routine electrophysiological probing. Alternative methods to obtain functional information on nerve cells have therefore been sought. One such method has been widely applied in recent years, mainly to monitor regional functional activity in the vertebrate brain (rev. Sokoloff 1982). The technique makes use of the increased metabolic demand of physiologically active neurons. As glucose is the major fuel of the brains of both vertebrates and invertebrates (Wegener 1981), the level of local glucose consumption is a measure of neuronal activity. The technique which was developed by Sokoloff et al. (1977) is based on the finding that 2-deoxy-D-glucose is transported like glucose across the blood-brain barrier and the neuronal cytoplasmic membrane, and is phosphorylated by hexokinase at a similar rate as glucose. While glucose-6-phosphate is broken down to CO_2 and H_2O which rapidly diffuse across cell boundaries and cannot easily be localized, deoxyglucose-6-phosphate is not further metabolized and accumulates intracellularly at a rate indicative of the cell's metabolic activity. By the use of radioactively labelled deoxyglucose, the distribution of metabolic activity in the brain can thus be made visible in autoradiographs of histological sections, provided that post-mortem diffusion of the labelled compounds is prevented.

This 'autoradiographic deoxyglucose technique' for functional metabolic mapping has been adapted to provide the high spatial resolution required for its application in the brains of small insects (Buchner and Buchner 1980, 1983). Early results on movement-induced labelling in the medulla have been reported (Buchner et al. 1979). Here we use the improved technique to localize movement-specific and direction-specific metabolic activity throughout the brain of wildtype *Drosophila melanogaster*. Metabolic mapping in a number of visual mutants will be reported separately (Bülthoff and Buchner, in prep.).

Methods

A detailed description of the deoxyglucose (DG) technique for insects has been published (Buchner and Buchner 1980, 1983). Therefore only a brief outline will be given here. For qualitative functional mapping the choice of several experimental parameters is probably not critical as long as they lie within a certain range (see below).

Female *Drosophila melanogaster* (wildtype strain 'Berlin', 3 to 10 days old) were used in the experiments. Each fly was attached by the thorax with dental cement 'Scutan' (ESPE) to a small colour-coded plastic stick for easy handling and identification. The head was fixed to the thorax so that a well-defined visual stimulus could be presented. In some cases the animals were deprived of food and water prior to the experiment since low blood glucose levels have been reported to increase DG uptake in vertebrates (Young and Deutsch 1980). Usually we covered the legs and antennae of the flies with 'Scutan' about 1–2 h before the experiment in order to reduce non-visual sensory input to the brain. Tritiated deoxyglucose or fluorodeoxyglucose at high specific activity (16–40 Ci/mmole) was obtained from Amersham or New England Nuclear in aqueous solution and concentrated 200–1000 fold by freeze-drying and immediate reconstitution to yield 10–50 μCi in a 50 nl droplet. This droplet was either fed to the animal from a microliter syringe or injected through a broken-off micropipette into the hemocoel of the abdomen. Incubation, usually in the presence of a well defined visual stimulus, was for between 45 min and 8.5 h. Since autoradiographic grain density and contrast varied considerably from animal to animal even under identical experimental conditions, it was difficult to optimize the procedure. We have, however, reliably obtained satisfactory results with injection of 20 μCi/fly and incubation for 4 h. At the end of the incubation period the flies were shock-frozen by immersion in melting nitrogen ('slush') and freeze-dried at −90 °C for 7 days. To date, this is the only technique by which we can prevent post-mortem diffusion of the (water-soluble) labelled substances. We have not been able to retain stimulus-specific label in *Drosophila* by brief aqueous fixation in glutaraldehyde and OsO_4 solutions (Buchner and Buchner 1980). Periodate-lysine-paraformadelhyde fixative has been proposed for the fixation of sugars and has been used successfully for deoxyglucose mapping in vertebrates (Durham et al. 1981). Although substantial amounts of label were retained with this method in the cell bodies of 6 injected flies, we see no traces of stimulus-induced label in the neuropil. Dehydration

Table 1

Autoradiograph shown in Fig.:	3a	3b	5a, 5b	11	6a	6b	7a	8	7b	4	10
Experimental parameter											
Food and water deprivation (h)	10	–	12	22	–	–	5	3	–	12	5
Antennae covered (c), free (f)	c	f	c	f	c	c	c	c	c	f	c
Legs immobilized (i), free (f)	i	f	i	f	f	f	i	f	i	f	i
Amount of DG, fluoro-DG (F) (μCi)	40	F20	30	20	50	20	20	50	20	40	20
Application, fed (f), injected (i)	f	i	f	f	i	i	i	i	i	f	i
Incubation period (h)	5	1.25	5	8.5	4	4	0.75	0.75	4.25	5.5	1
Exposure period (days)	10	12	10	5	10	10	10	10	14	10	10

of the unfixed tissue by acetone at +4 °C, as suggested by Sejnowski et al. (1980), also caused loss of all stimulus-induced label. Freeze substitution at −70 °C in acetone retained the gross distribution of label, but the autoradiographs appeared to be less sharp, and the method in our hands was more capricious (Bülthoff and Rodrigues, unpublished results).

The freeze-dried flies were fixed in OsO_4 vapour for 1 h and embedded in 'EPON' (Shell) under mild vacuum. After polymerization, semi-thin (2–3 μm) sections were cut on dry glass knives. Dry contact autoradiography was achieved by a 'sandwiching' technique which has been described in detail by Buchner and Buchner (1983). It provides the intimate contact between the section and the autoradiographic film (Kodak AR 10) required for high spatial resolution, and saves the sections for reexposure, staining, reembedding etc. as well as for precise localization of metabolic activity by simultaneous viewing of section and corresponding autoradiograph in a two-stage comparison microscope (Zeiss). It should be noted that due to decay statistics and psychophysical peculiarities of human vision, small structures labelled only 20–50% more strongly than the surrounding tissue are very difficult to detect in autoradiographs unless the background label has an optical density in the range ≥ 1 OD unit (90% absorbance). Obviously, saturation of the film, on the other hand, must be avoided. To achieve optimal optical density, exposure times usually were 10–12 days, but 3 days and 40 days were in some cases more appropriate. Due to latent image fading, low concentration of label in the tissue cannot be fully compensated by long exposure. The detailed treatment of the preparations whose autoradiographs are shown in Figs. 3–8, 10, 11 has been listed in Table 1.

Visual stimulation. Quantitative determination of glucose uptake as used in the vertebrate deoxyglucose technique (Sokoloff et al. 1977) is not feasible in small insects. Comparison of individual preparations subjected to different stimulus regimes therefore is often inconclusive. However, even small differences in evoked metabolic activity can be reliably detected without resorting to large numbers of preparations and statistical analysis, if the stimuli to be compared, can be incorporated into a single experiment. Due to the bilateral symmetry of the visual system the stimulus set-up of Fig. 1 is appropriate for a highly sensitive comparison between flicker- and movement-evoked metabolic activity. Any asymmetry in the distribution of label can be safely related to movement-specific nervous activity if the light signals in individual receptors on both eyes are equal. In this case the two stimuli differ only with respect to the phase relation of the signals in neighbouring receptors, zero phase shift being characteristic of flicker, non-zero phase shift constituting the essential feature of movement. Such a stimulus configuration was realized by presenting to the right eye a moving sine-wave grating (spatial wavelength $\lambda = 20°$, speed $w = 26°/s$

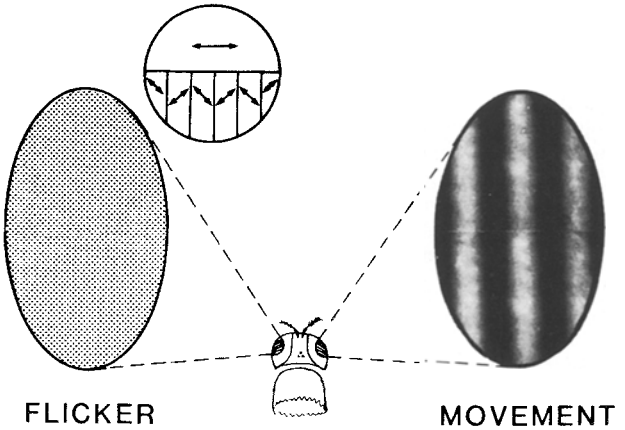

Fig. 1. Stimulus paradigm for the identification of movement-specific nervous activity. The left eye observes a homogeneously illuminated disk of 70° diameter modulated at 1.3 Hz (flicker). The moving sine-wave grating presented to the right eye is of the same size, the rest of the visual surround remains dark. Luminance, modulation depth and frequency, contrast, pattern wavelength and speed have been adjusted such that the individual receptors on both eyes receive identical sinusoidal light signals. Any difference in metabolic activity between the two brain hemispheres therefore reflects movement-specific nervous activity. The inset shows the polarizer used for the compound stimulus for flicker and reversing contrast gratings: In the lower half of the visual field the homogeneous polarizer is replaced by stripes of polarizing foil with their dichroic orientations (double-head arrows) alternating at right angles. The light signals transmitted from neighbouring stripes through the rotating analyzer are thus 180° out of phase as required for a reversing contrast grating

contrast frequency $w/\lambda = 1.3$ Hz, mean luminance $\bar{I} = 1500$ cd/m^2, modulation depth $\Delta I/\bar{I} = 0.4$). The flicker stimulus on the left eye was generated by a stationary polarizer, a rotating analyzer and a depolarizer ('Lyot'-type). Mean luminance as well as modulation frequency and depth were adjusted to give the same values as for the movement stimulus by adding to the main light beam which was modulated at 1.3 Hz between 0 and 1,200 cd/m^2 a constant beam of 900 cd/m^2. For comparison of flicker, reversing contrast gratings and movement, the polarizer consisted of a pattern composed of polarizing foil of 3 different optical axes as indicated in the inset to Fig. 1. The upper half of the left eye observed a homogeneous hemidisk of flickering light, whereas the lower half looked at a pattern of flickering stripes with the special property that the flicker signals from neighbouring stripes were 180° out of phase.

The visual field in these experiments was restricted to two

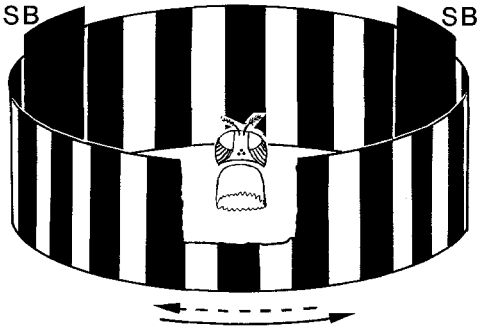

Fig. 2. Stimulus situation for the identification of direction-sensitive movement-specific activity. The fly observes a striped cylinder of 70° vertical extension rotating at constant speed and direction. A zone of 45° width on each eye is screened from the movement by two stationary black bars (*SB*) allowing for comparison of stimulated and non-stimulated tissue in horizontal sections. The stimuli for the two eyes differ only by their direction of movement (front-to-back vs back-to-front). Thus any difference in stimulus-related labelling between the two brain hemispheres reflects direction-specific nervous activity

circular disks of 70° diameter. This aids the identification of stimulus-induced nervous activity in autoradiographs of the retinotopically organized visual neuropil.

In the stimulus situation of Fig. 2 we again make use of the bilateral symmetry of the visual system: The stimuli on the two eyes are identical except that on one eye the bars move from back to front and on the other from front to back. Any left-right differences in the labelling must therefore be due to nervous activity specific not just for movement but for its direction. By testing both clockwise and counterclockwise motion, asymmetries not related to the stimulus can be eliminated. Stimulus parameters in Fig. 2 were: spatial wavelength $\lambda = 18$–$26°$ (square wave), speed $w = 23.4 - 33.8°/s$, contrast frequency $w/\lambda = 1.3$ Hz, mean luminance $\bar{I} = 700$ cd/m^2, modulation depth $\Delta I/\bar{I} = 1$. Again a certain portion of the visual field of each eye was occluded from the movement stimulus by two stationary vertical black bars of 45° width to aid identification of stimulus-induced nervous activity.

In most preparations the antennae were covered with dental cement 'Scutan' in order to reduce olfactory and mechanosensory input to the brain (cf. Buchner and Buchner 1980). Some flies were allowed to walk stationarily on a styrofoam ball supported by a gentle stream of air, while the legs of others were fixed to the thorax. Details on the experimental conditions are listed in Table 1. The various parameters did not seem to qualitatively influence the labelling pattern in the visual neuropil, they may, however, modify central brain activity.

Results

Any moving object or pattern will necessarily stimulate on-off units as well as movement-specific cells. In the first experiment we therefore compare metabolic activity in movement-stimulated, flicker-stimulated and unstimulated tissue (Fig. 1). Figure 3b shows a contact autoradiograph of a horizontal section through the head of a fly stimulated

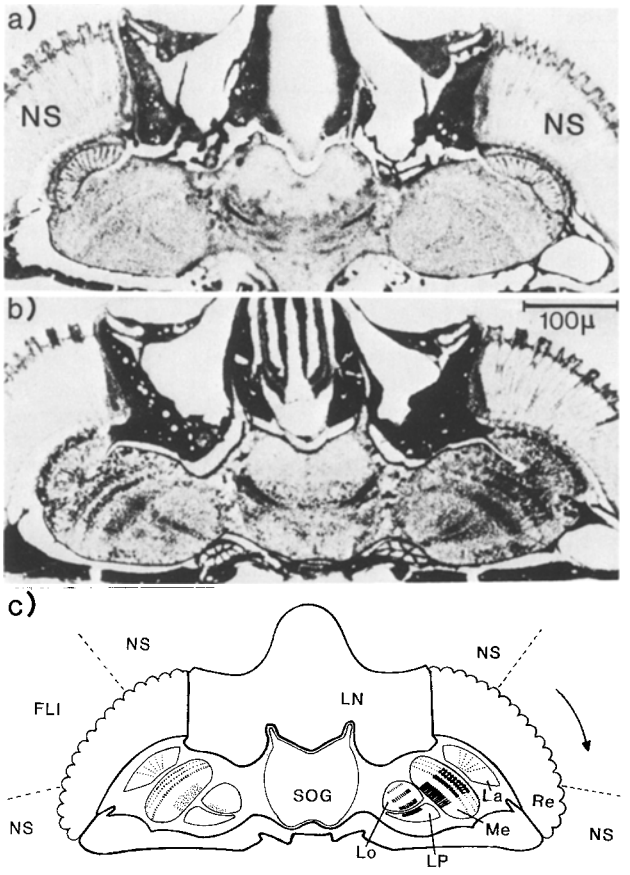

Fig. 3a–c. ^{3}H-deoxyglucose autoradiographs of horizontal sections through 2 *Drosophila* heads. During incubation (period between injection of ^{3}H-DG and freeze-fixation) the control fly (**a**) was in complete darkness. The fly in **b** was incubated under visual stimulation in the paradigm of Fig. 1. Labelling in visual neuropil connected to non-stimulated (*NS*), flicker-stimulated (*FLI*) and horizontal movement-stimulated (curved arrow) retina can readily be compared. **c** Schematic localization of labelled structures with respect to the neuropil outlines (*Re* retina; *La* lamina; *Me* medulla; *Lo* lobula; *LP* lobula plate; *LN* labellar nerve; *SOG* suboesophageal ganglion)

with a moving pattern (right eye) and a homogeneous flicker (left eye). The schematic diagram in Fig. 3c summarizes the consistent stimulus-related features of autoradiographs from 10 flies stimulated in this way, and assigns the various labelled regions to the underlying brain structures of the sections. (Note that in contact autoradiography, the sections are separated from the film prior to development and are not included in the figures.)

In the *retina,* no difference in labelling can be detected between stimulated and unstimulated ommatidia. Only very little label actually is found in the photoreceptor cells while most retinal label accumulates in Semper cells and primary and secondary pigment cells. In the *lamina,* both flicker and movement cause a slight increase in metabolic ac-

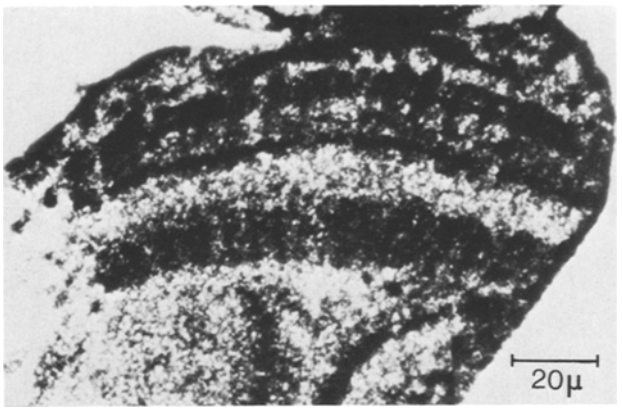

Fig. 4. Autoradiograph at high magnification of the medulla (horizontal section) of a fly stimulated by horizontal movement (Fig. 2). Note columnar arrangement of the label in all four movement-specific layers

tivity with no obvious difference between the two stimuli. The stimulus-induced labelling in the lamina may not be clearly visible in Fig. 3b, but it is repeatedly observed in most of the 46 visually stimulated preparations studied in the present work (cf. also Figs. 6–8, 10, 11). Labelling of the cellular cortex of the lamina is heavy with occasional indications of reduced activity below non-stimulated retina.

The most peripheral structures that exhibit pronounced movement-specific labelling are four tangential layers in the *medulla* (right hemispheres Figs. 3b and 5b). The three layers distal to the serpentine layer show a clear periodicity (bead-like appearance) which reflects the columnar organization of the medulla neuropil. In the broad proximal layer this periodicity is only faintly visible (Fig. 4). The increase in labelling detectable in the stimulated region of the left medulla of Fig. 3b (flicker stimulus) is variable but always smaller than the movement effect. (The possibility of interhemispheral interactions is discussed below.) A considerable portion of the stimulus-specific label is clearly restricted to that part of the medulla which connects to the stimulated ommatidia. This important feature is emphasized even more in the autoradiograph of a frontal section from another fly (Fig. 5b). In this experiment an obliquely oriented stationary black bar shielded a 18° wide strip of the retina from the movement stimulus. The unstimulated strip is visible in the labelled zone of the medulla. Again, the flicker stimulus on the left eye results only in weakly increased labelling. By comparison with autoradiographs from unstimulated control preparations (Figs. 3a, 5a) it is clear that besides this local, retinotopically arranged label we also find a general increase in metabolic

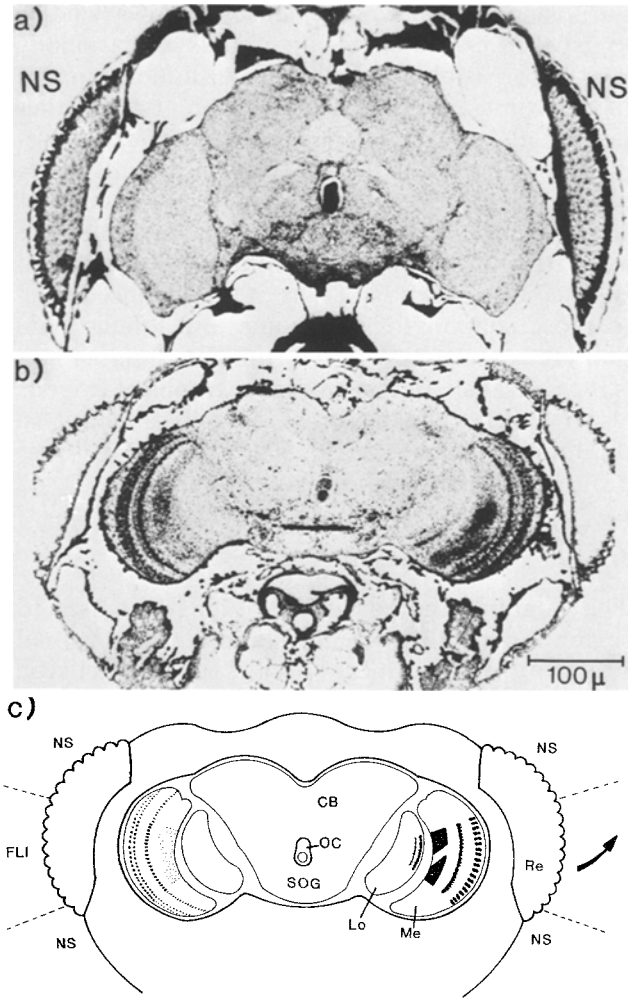

Fig. 5a–c. Autoradiographs of frontal sections through 2 *Drosophila* heads. The control fly (**a**) was in complete darkness. The fly in **b** was incubated in the paradigm of Fig. 1 which was slightly modified by the addition of a stationary black bar of 18° width attached obliquely across the pattern moving in front of the right eye. Differences in labelling between medulla neuropil connected to non-stimulated (*NS*) or flicker-stimulated (*FLI*) vs horizontal movement-stimulated (curved arrow) retina (*Re*) are apparent. The stationary black bar in front of the moving pattern shows up as a zone of reduced labelling within the heavily labelled proximal layer of the medulla (*Me*) in **b**. **c** Schematic localization of the stimulus-induced label with respect to the neuropil outlines. (*Lo* lobula; *CB* central brain; *SOG* suboesophageal ganglion; *OC* oesophageal canal)

activity throughout the brain. This increase is particularly pronounced in the optic lobes and the four layers of the medulla described above. The cellular cortex of the medulla exhibits isolated spots of heavy labelling which are likely to correspond to individual cell bodies. Whether the localization of these cell bodies is related to the position of the stimulus on the retina or not is not clear.

In the *lobula,* a tangential layer extending along

the posterior surface of the neuropil is always heavily labelled by a movement stimulus. Occasionally it is also visible, though much fainter, on the flicker-stimulated side. In addition, two further tangential layers are recognizable in most movement-stimulated lobulae. In the *lobula plate,* flicker has no recognizable effect. A single tangential layer is, however, labelled on the movement-stimulated side. Its fronto-caudal position will be described below. The restriction of part of the enhanced label to those columns of the lobula and lobula plate that are associated with the stimulated ommatidia is recognizable in most autoradiographs, it extends also into the cellular cortex caudally adjacent to the lobula plate (Figs. 3b, 6b, 7b), but it is less pronounced than in the medulla neuropil.

Direction-specific glucose uptake

The stimulus configuration of Fig. 2 was used to search for metabolic manifestations of directional selectivity of movement-specific nervous activity. Any stimulus-related asymmetry in the labelling pattern reflects directional selectivity of the underlying nervous components. At first sight, autoradiographs of horizontal sections of flies subjected to horizontal movement show no striking bilateral asymmetry, except for a small number of heavily labelled profiles in the lateral posterior protocerebrum which are labelled only on the side stimulated by front-to-back movement (Fig. 6a, arrowhead). However, careful observation of numerous autoradiographs from 9 clockwise and 11 counterclockwise stimulated flies so far has revealed two further direction-specific effects: The two anterior layers of the three movement-sensitive lobula layers described above are more heavily labelled on the front-to-back stimulated side. This difference is rather variable and has therefore not been quantified. A qualitative difference, however, is found in the lobula plate: The position of the movement-sensitive layer within this neuromere depends on the direction of the movement. With horizontal counterclockwise stimulation (Fig. 6a) label accumulates along the frontal surface of the lobula plate neuropil of the left hemisphere, while in the right lobula plate a layer below the frontal surface is labelled. More easily observed than the position of the labelled layers is the presence of the unlabelled superficial layer on the back-to-front stimulated side, viz. on the right in Fig. 6a and on the left in the clockwise stimulated preparation of Fig. 6b.

Note also in these autoradiographs the reduced labelling of a central zone in all three visual neuropil regions, a zone that connects to those ommatidia which were shielded from the visual stimulus by the stationary black stripe (Fig. 2). This again demonstrates the retinotopic distribution of part of the stimulus-induced increase in labelling throughout the visual neuropil.

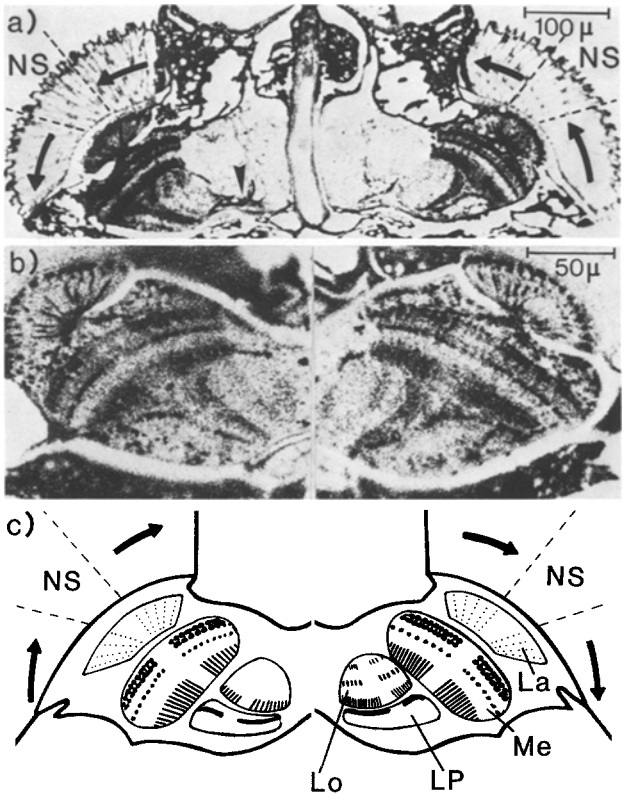

Fig. 6a–c. Autoradiographs of horizontal sections through the heads of 2 *Drosophila* which were stimulated in the rotating striped drum (Fig. 2) with horizontal counterclockwise (**a**) and clockwise (**b**) movement (curved arrows). The reduced labelling of tissue connected to non-stimulated (*NS*) retina is particularly clear in the autoradiographs in **b** which shows the optic lobes at higher magnification. Direction-sensitive labelling is found in the lobula (enhanced labelling of two anterior layers on front-to-back stimulated side), in the lobula plate (position of labelled layer) and in the lateral posterior protocerebrum where profiles are labelled (arrowhead in **a**) which may belong to cells homologous to the 'CH'-cells of *Calliphora.* **c** Schematic illustration of stimulus-induced label within the visual neuropil. *La* lamina; *Lo* lobula; *LP* lobula plate; *Me* medulla

Similar experiments have been performed for vertical movement by mounting the fly with its body axis aligned along the axis of the rotating striped cylinder. This stimulus situation simulates visual movement perceived by a freely flying animal during rotations around its body axis (roll). In the equatorial plane of the fly's visual space a zone of 70° horizontal extension is stimulated on each eye with vertically moving horizontal stripes. The frontal and caudal eye regions remain

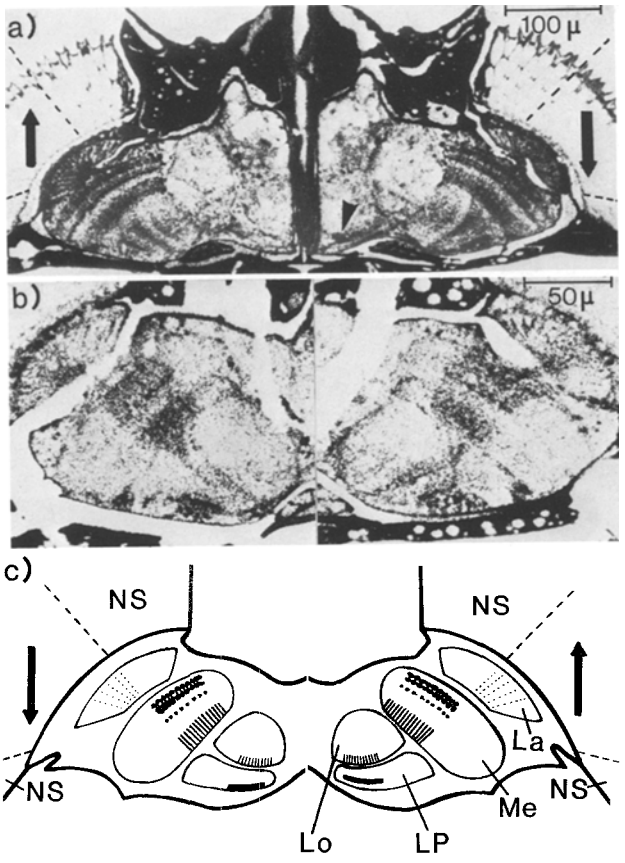

Fig. 7a–c. Autoradiographs of horizontal sections from 2 *Drosophila* which were stimulated with vertical movement (straight arrows) by orienting the flies in the rotating striped drum (Fig. 2) with their body axis aligned to the drum axis. Stripes moved upward on the left eye and downward on the right eye in **a**, and vice versa in **b**. The drum stimulated a lateral zone of 70° visual angle, frontal and caudal visual fields were not stimulated (*NS*). The retinotopic distribution of label is obvious when the inversion of retinotopic order due to the chiasm between lamina and medulla is taken into account. Direction-specific label accumulates in the lateral posterior protocerebrum (arrowhead in **a**). In addition, the position of the labelled layer of the lobula plate depends on the direction of the movement. **c** Schematic illustration of the stimulus-induced label within the visual neuropil. *La* lamina; *Lo* lobula; *LP* lobula plate; *Me* medulla

unstimulated. Autoradiographs of corresponding preparations are shown in Figs. 7a, b and 8. The distribution of label differs from that expected for a horizontally moving stimulus at the level of the lobula plate: Again a single labelled layer can be recognized in each lobula plate neuropil, occupying, however, the caudal surface for downward movement (right side in Fig. 7a, left side in b, c) and an inner zone for upward movement (left side in a, right side in b, c). In the autoradiograph of a ventral section from a third preparation the position of the two layers is more clearly recognizable (Fig. 8, arrowheads). In the lateral posterior protocerebrum we also find some asymmetric distribution of label which accumulates on the downward stimulated side only (arrowhead in Fig. 7a). These characteristics of the distribution of label were observed in 13 different preparations subjected to vertical movement stimulation.

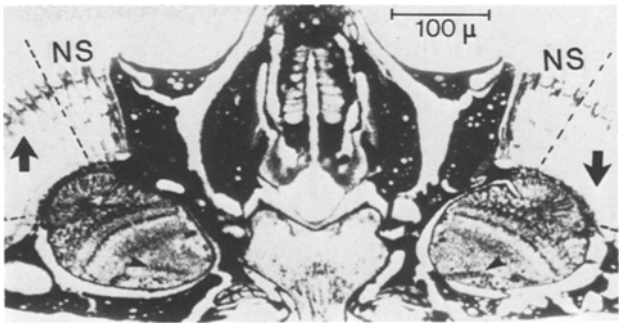

Fig. 8. Autoradiograph of a ventral horizontal section of a fly stimulated by vertical movement in the same way as the preparation of Fig. 7a (left upward, right downward). The direction-specific difference in the position of the labelled layers in the lobula plate (arrowheads) is clearly displayed

Reversing contrast gratings, habituation, wide-field interactions

In a small number of flies the distribution of label after stimulation by reversing contrast gratings was tested since these patterns have been used previously in electrophysiological experiments on movement-sensitive fly interneurons (Srinivasan and Dvorak 1980). In the present experiments the right eye was exposed to front-to-back movement as before, and the left eye was stimulated by the compound flicker stimulus (see Methods). In the three successful experiments we found an intermediate density of label in the left medulla in that region which was stimulated with reversing contrast gratings, i.e. labelling was stronger than in the flicker-stimulated region but weaker than in the movement-stimulated right medulla. In order to illustrate these effects in a semi-quantitative manner we have measured by microdensitometry the autoradiographic grain density over movement-, flicker-, reversing contrast grating- and non-stimulated neuropil of the proximal medulla. For each of five consecutive sections of one preparation the unstimulated control absorption was subtracted from the stimulated values and the difference expressed in percent of the movement effect. Mean and standard error of the mean were computed for the five sections and are shown in Fig. 9. Note that the nonlinearity of the film in the absorbance range optimal for high spatial resolution tends to attenuate the differences in Fig. 9.

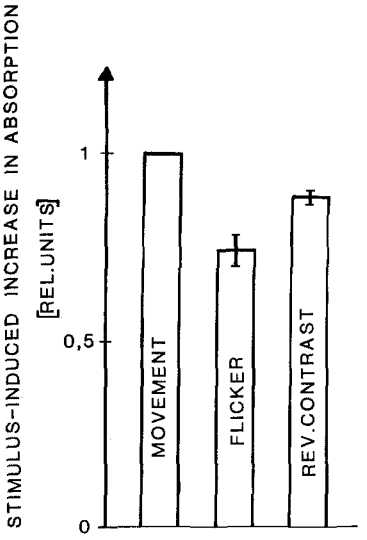

Fig. 9. Microphotometric evaluation of relative stimulus-induced increase of labelling in the proximal medulla for tissue stimulated by movement, flicker and reversing contrast gratings

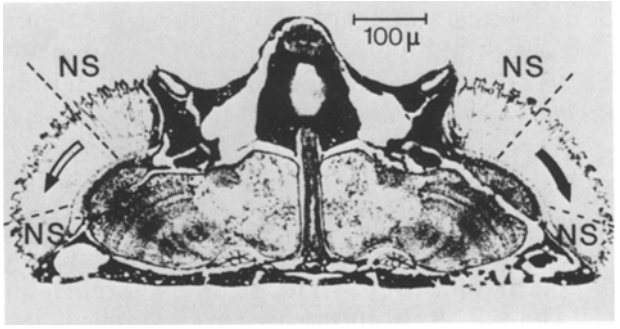

Fig. 10. Autoradiograph of a horizontal section demonstrating pronounced movement-induced labelling in a fly stimulated in the set-up of Fig. 1 with non-uniform movement. The flickering disk had been replaced by a second grating moving front-to-back as on the right but at sinusoidally modulated speed (open arrow). *NS*, non-stimulated retina

Labelling in the lobula complex under these stimulus conditions was too weak to be interpreted.

In an experiment with a second moving grating replacing the flicker stimulus on the left eye, we were interested to see if variable speed of the pattern might increase glucose uptake. This would be expected if habituation, which might reduce activity under long-time stimulation, was less effective for movement of variable compared with constant speed. The corresponding autoradiographs, one of which is shown in Fig. 10, display no systematic left-right difference and indicate therefore that either habituation of the movement-related activity in the visual neuropil is metabolically ineffective or that this habituation is not prevented by movement with sinusoidally modulated speed. A further possibility, however, would be that the dishabituating stimulus on one eye exerts its effect on the activity in both hemispheres. The latter interpretation might explain the particularly strong labelling of the stimulated regions of medulla and lobula complex in this preparation in view of the short stimulation period used (1 h).

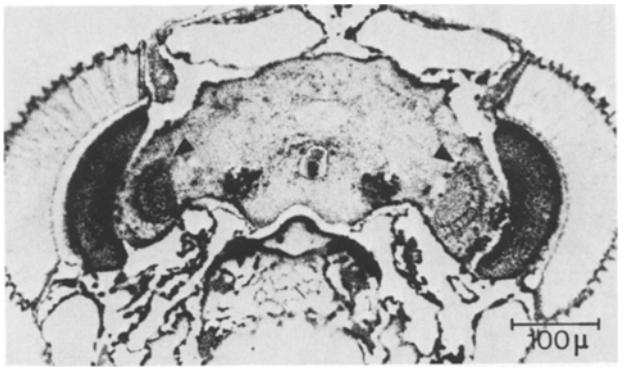

Fig. 11. Autoradiograph of a section cutting tangentially through labelled layers in the distal medulla of the preparation of Fig. 5b. The layers connected to the non-stimulated retina adjacent to the flicker-stimulated zone (left) are more heavily labelled than the corresponding layers adjacent to the movement-stimulated zone (right) (arrowheads)

Finally, a further characteristic of the labelling pattern observed consistently under the present binocular stimulation in restricted visual fields, is displayed in the autoradiograph of Fig. 11. It shows metabolic activity in an anterior frontal section of the preparation of Fig. 5b which had been stimulated with flicker on the left and with movement on the right eye. In the distal layers of the medulla (arrowheads) which in Fig. 11 are cut approximately tangentially *outside* the stimulated neuropil region, one observes a peculiar difference in labelling with clearly a higher radioactivity outside the flicker-stimulated region (left) than outside the movement-stimulated region (right). This effect may be interpreted e.g. as a manifestation of lateral inhibitory interactions reducing metabolic activity outside the highly active movement-stimulated neuropil (right) but less so outside the flicker-stimulated neuropil of low activity (left). Alternatively, excitatory bilateral interactions might produce the same effect, if, e.g., activated cells in the central right medulla would activate peripheral neuropil in the left medulla. Support for such a complementary activation is obtained from a monocularly stimulated preparation (not shown). This preparation, as well as those stimulated with flicker on one eye, indicates, however, that the *qualitative* features of the labelling described in the present work do not depend on binocular interactions but rather are governed by the stimulus on the ipsilateral eye.

Discussion

In this study we have described the distribution of visually induced metabolic activity in the optic lobes of the *Drosophila* brain with special reference to movement specificity and directional selectivity. In the following paragraphs we will try to relate these results to present day knowledge on structure, single-cell activity and over-all function at the successive stages of visual information processing in flies as revealed by neuroanatomy, electrophysiology and behavioural input-output analysis, respectively. Note that most experiments on visual movement detection utilizing the latter two techniques have been performed under essentially the same stimulus conditions, i.e. moving gratings presented to an immobilized animal (open-loop stimulation). Aspects of genetic manipulation and its manifestation in deoxyglucose autoradiographs will be discussed in another paper (Bülthoff and Buchner in prep.).

Retina. The lack of stimulus-induced labelling by deoxyglucose in the retina is interesting for two reasons: Since dipteran retinae are known to cover their energy requirements by the metabolic breakdown of carbohydrates, our negative deoxyglucose results would be compatible with the assumption that the metabolic pathway for glucose breakdown in the retina was different from that in other neuronal compartments. Diffusion barriers probably do not play a major role since (^{3}H)-3-O-methylglucose under the same conditions labels photoreceptors, pigment cells, nerve cell bodies and neuropil to a similar extent (Buchner and Buchner 1983). A route via glycogen, which is abundant in the pigment cells, is suggested by measurements of glycogen turnover in the drone retina (Evequoz et al. 1983). The second point concerns the interpretation of unlabelled cells. Our autoradiographs demonstrate that the nervous system may contain cells which are refractory to the DG method when other regions of the same preparations show strong stimulus-induced labelling. Thus unlabelled cells are not necessarily electrically inactive. Another example will be discussed below.

Lamina. This neuropil is almost as regularly structured as the compound eye. For each ommatidium there is one column comprising 5 monopolar cells which send their axons to the corresponding column of the medulla, and a small number of centrifugal and intrinsic cell processes (rev. Strausfeld 1976; Fischbach 1983). Each column is entirely encapsuled by glia cells (and has therefore been termed 'cartridge'). Intracellular recordings from the two large monopolar cells L1 and L2 have revealed functional similarities with certain types of bipolar cells in the vertebrate retina and suggest that the lamina is preoccupied with spatial and temporal pre-processing of the photoreceptor signals (adaptation, gain control, contrast enhancement) (rev. Laughlin 1984). As yet, the functional differentiation of the five monopolar cells is unclear. The structural properties of the L1 and L2 neurons have speculatively been associated with the two different channels (filters) of the 'correlation model' for movement detection (Braitenberg and Hauser-Holschuh 1972). Electrophysiology, however, has so far not revealed any significant differences in the response characteristics of the two cells. Although the weak increase in labelling by DG of stimulated lamina cannot yet be assigned to particular cells, it is compatible with the decrease in membrane resistance (and the concomitant increase in ionic pumping) observed for L1 and L2 cells during hyperpolarization under visual stimulation (Zimmerman 1978). Autoradiographs of tangential sections through the lamina also indicate that the label is associated with neuronal cartridge elements rather than with the surrounding glia. One would expect a honeycomb-like distribution of the label if the uptake was mainly by the glia cells, but this was found only in some unstimulated control preparations. Finally, the similarity of movement- and flicker-stimulated laminae in our autoradiographs is consistent with the failure to record movement-specific responses in this neuropil (rev. Laughlin 1984).

Medulla. A functional discussion of identified neurons in this largest and most complex neuromere of the visual system is not yet possible. While presumably the majority of cell types in this ganglion has been identified structurally (Strausfeld 1976; Fischbach 1983), no information on their physiology is available. In spite of great efforts stable recordings with dye injections sufficient for comparison with Golgi-stained cells have not been achieved. Unfortunately, in its present state the DG method cannot fill this gap either. Due to the poor structural preservation of freeze-dried neuropil and the complexity of the tissue, attempts to associate the label with Golgi-stained cells would be premature. Possibly, if cell-specific stains are found and can be applied to the sections after autoradiography, or if major components of the labelled structures can be eliminated by genetic techniques (cf. Fischbach 1983), further progress may be possible. Yet the picture given by the spo-

radic recordings of flicker-sensitive, movement-sensitive and direction-sensitive units in the medulla of *Calliphora* (Mimura 1972; DeVoe and Ockleford 1976; DeVoe 1980) is substantially supplemented by the pronounced movement-specific increase in metabolic activity found in four layers, including essentially the entire neuropil proximal to the serpentine layer. The retinotopic distribution of label indicates that movement-sensitive small-field neurons contribute substantially to the labelling. Thus we speculate that the neuronal correlates of the 'elementary movement detectors' might be found here. These detectors are defined as directionally selective interactions between pairs of photoreceptors. At least four types of elementary movement detectors with different orientations in the hexagonal array of the compound eye have been postulated to contribute to behavioral movement responses of *Drosophila* (Buchner 1976; Götz and Buchner 1978). However, in the DG autoradiographs of the medulla we have not been able to detect any directionally selective label. This finding can be interpreted by two alternative models: (1) Neurons branching in the medulla represent the cellular correlates of the elementary movement detectors but each detector comprises two cells with opposite directional preference and similar structure occupying the same tangential layers of the medulla such that they cannot be differentiated in our light microscope autoradiographs. (2) The directionally selective neuronal interactions take place in the lobula complex, while the medulla is engaged in the temporal and spatial filtering which must be assumed to precede these interactions. In order to explain with model 2 the difference in labelling under movement and homogeneous flicker stimulation one would have to assume that this spatial filtering includes some kind of lateral inhibition that suppresses responses to luminance changes which occur synchronously over extended regions of the visual field. The structural correlate of each elementary movement detector might in this case be a particular synaptic configuration rather than an individual neuron. Such a movement-detecting synaptic complex could, for example, be composed of two closely adjacent presynaptic terminals from non-homologous columnar neurons of the medulla and a postsynaptic membrane patch on one of the directionally selective interneurons of the lobula plate. By computer simulation such a configuration has been shown to be sufficient for the generation of directionally selective movement-specific signals (Torre and Poggio 1978).

The experiment comparing metabolic activation by movement, flicker and reversing contrast gratings was designed to discriminate between these two models. In the first model postulating directionally sensitive movement detector cells in the medulla, reversing contrast gratings should be as ineffective as the flicker stimulus. However, a human observer perceives along the edges of the bars of the reversing contrast gratings a pronounced illusion of oscillating movement, and flies respond to this kind of illusory movement in a similar fashion as to real movement (Bülthoff and Götz 1979). Thus the intermediate level of labelling in the reversing contrast experiment might correspond to nervous activity elicited in both antagonistic cells of a detector by the illusory oscillation.

On the other hand, if the labelled cells in the medulla are pure flicker detectors inhibited by synchronous modulation, reversing contrast gratings should be as effective as a movement stimulus. The fact that this is not the case (Fig. 9) supports the notion of direction-specific movement detection in the medulla, i.e. the first model.

Lobula complex. No electrophysiological data have so far been published about cellular activity in the lobula. The Golgi work (Strausfeld 1976; Fischbach 1983) again conveys a picture of such complexity that an association of the three labelled layers with individual cell types seems premature. The stronger labelling of the two anterior layers under front-to-back compared with back-to-front movement stimulation may be speculatively linked to object detection which has been proposed to be based on movement detection mechanisms with such a front-to-back vs. back-to-front difference (Reichardt 1973; Wehrhahn and Hausen 1980; revs. Buchner 1984; Heisenberg and Wolf 1984). Obviously this question has to be investigated by the DG method using appropriate visual stimuli. The retinotopic distribution of part of the stimulus-induced label again points to movement-sensitive columnar neurons with small visual fields.

The lobula plate, on the other hand, has long been known to be heavily involved in large-field movement detection. In earlier anatomical work it was speculated that morphological differences between cells running along the frontal and the caudal surface of the lobula plate might correspond to a functional specialization for horizontal and vertical movement detection, respectively (Pierantoni 1976). This conjecture has been substantiated by electrophysiological work combined with dye injection carried out in several laboratories. As reviewed recently by Hausen (1984, in preparation) more than twenty different direction-

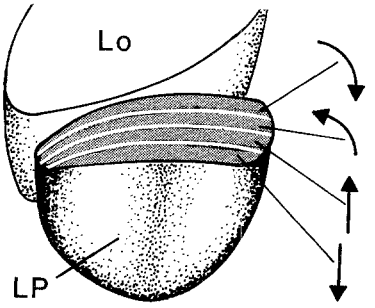

Fig. 12. Schematic representation of the four direction-sensitive movement-specific layers of the lobula plate (*LP*) as revealed by deoxyglucose autoradiographs. The horizontal cut through the right lobula plate exposes the four layers which are selectively activated one at a time by ipsilateral front-to-back (↷), back-to-front (↶), upward (↑) or downward (↓) movement (in anterior-posterior order). *Lo* lobula

ally selective movement-sensitive interneurons with large visual fields have been characterized and identified in *Calliphora,* and their arborizations were found to subdivide the lobula plate into an anterior hemi-plate of cells responsive to horizontal movement and a posterior hemi-plate of cells responsive to vertical movement. The present results with *Drosophila* demonstrate a further subdivision into four layers with specificity for front-to-back, back-to-front, upward and downward movement (in anterior-posterior order, see Fig. 12). Indeed, all arborizations of functionally identified cells in *Calliphora* seem to fit into this scheme except for two cells (V1 and H3). Even cells with different preferred directions in different parts of their visual field may fit this rule: The large dorsal dendrite of, for example, the VS8 neuron branches in the 'horizontal' hemi-plate and responds to front-to-back movement, whereas the ventral dendrite branches in the 'vertical' hemi-plate and responds to downward movement (Hengstenberg et al. 1982, 1983). It is speculated that the actual synapsing may take place in the 'correct' layer, even for the V1 and H3 cells as the branching of these cells has been investigated so far only by light microscopy which cannot unambiguously localize synaptic contacts. However, we cannot exclude that neighbouring layers might overlap to some extent and thereby accommodate cells in the 'wrong' order.

For the lobula plate, the Golgi studies provide a less complex picture on branching patterns of columnar elements and thus enable one to speculatively associate the retinotopically distributed label in the four layers with the arborizations of two classes of columnar neurones which connect the proximal medulla and the posterior lobula to the lobula plate (T4 and T5 cells, respectively; Strausfeld 1976, 1984; Fischbach 1983. Inherent in this conjecture would be the assumption that the 'medial' subclasses (T4m, T5m) occupying the medial zone of the lobula plate (as opposed to the 'horizontal' (T4h, T5h) and the 'vertical' (T4v, T5v) subclasses which arborize in the anterior and posterior zones, respectively), can be further subdivided such that four subclasses might exist for each cell type. As the arborizations of T4 or T5 cells in the medulla and lobula are indistinguishable for all subclasses, this interpretation would be consistent with the observed lack of direction-specific label in the corresponding layers. A hypothesis assigning the direction-sensitive *interactions* to the lobula plate can be reconciled with the four layers of retinotopically distributed label only by assuming that four sets of yet unidentified small-field output neurons of the lobula plate have their dendritic branches in these layers and are activated by the appropriate movement. Since no bundles of such output fibres leaving the lobula plate are observed in the autoradiographs, we favour the first model and speculate that the T4 and/or T5 cells might in fact be directionally sensitive small-field units (elementary movement detector neurons). The actual mechanism of movement detection might still conform to the synaptic model proposed by Torre and Poggio (1978). As pointed out above, we hope that cell-specific staining and genetic techniques might help to clarify the picture.

The heavily labelled profiles connecting the front-to-back stimulated lobula plate to the lateral posterior protocerebrum (Fig. 6a, arrowhead) might at first sight be suspected to be axons of the giant 'Horizontal System' (HS)-cells of the lobula plate. However, in all preparations where we can identify the large dendritic branches of these cells on the frontal surface of the lobula plate, they show less label than the surrounding tissue irrespective of the visual stimulus applied. The problem of identifying deoxyglucose-labelled and -unlabelled giant interneurons of the lobula plate has recently been investigated in *Musca* where the cells are large enough to be recognized after computer-aided reconstruction from serial autoradiographs. It was demonstrated that the 3 HS cells take up very little label, while the profiles heavily labelled in the front-to-back stimulated hemisphere belong to the two 'Centrifugal Horizontal' (CH)-cells (Buchner et al. 1984). These experiments also showed that the various CH-cell components identifiable in light microscopic autoradiographs, including the larger dendritic and telodendritic branches, the axons, the neurites and the cell bod-

ies were all heavily labelled. Since giant axons should require much less metabolic energy per unit volume than fine dendritic or telodendritic branches at the same electrical activity (Schwartz et al. 1979), we assume that the deoxyglucose is taken up and phosphorylated mainly in the dendritic and/or telodendritic regions and that intracellular diffusion of the labelled deoxyglucose-6-phosphate causes the uniform distribution of label throughout all cell components. The conjecture that label confined to stimulated neuropil regions stems from small-field neurons relies on this assumption and would not be valid if stimulated and non-stimulated dendrites of a single large-field neuron could exhibit differential labelling. Assuming homology between *Drosophila* and *Musca* or *Calliphora* we therefore propose that the profiles labelled in the lateral posterior protocerebrum of *Drosophila* (arrowhead Fig. 6a) and some fraction of the label accumulating in the front-to-back stimulated anterior-most layer of the lobula plate belong to axons and telodendritic arborizations, respectively, of cells homologous to the CH-cells of *Calliphora* and *Musca*.

Central brain. Distinct differences in metabolic labelling of central brain structures can be detected between visually stimulated preparations and flies that were deprived of any sensory input. These differences cannot yet be uniquely associated with a particular stimulus component since nervous activity related to the intrinsic state of the brain, whether it is 'asleep', 'awake', 'aroused' etc., might well contribute to these differences. Clearly, the 'central state' may be different in stimulated and deprived flies. For each stimulus component a carefully designed set of control experiments will have to be carried out in order to eliminate these ambiguities.

Conclusion

The deoxyglucose technique has proven to be highly valuable for the localization of movement-specific and directionally selective nervous activity in the brain of *Drosophila,* and for the first time opens the possibility of directly investigating nervous activity in brains of *Drosophila* mutants (Bülthoff and Buchner, in prep.) which so far have only been characterized by neuroanatomy and behavioural input-output analysis (revs. Fischbach 1983; Heisenberg and Wolf 1984). The close correspondence of most deoxyglucose results with available electrophysiological data from large flies on the one hand demonstrates that metabolic mapping in flies is physiologically meaningful and, on the other hand, shows that the well investigated anatomical homology between *Drosophila* and *Calliphora* or *Musca* is paralleled by a functional homology of similar rigidity (at least as far as visual movement detection is concerned). The deoxyglucose results support a model postulating the existence of direction-specific movement-sensitive small-field neurons (the cellular correlates of 'elementary movement detectors') in the medulla and indicate that apart from medulla and lobula complex no other centres in the head ganglia are heavily involved in large-field visual movement detection. This latter conclusion cannot, however, be extended to individual cells, in view of the dramatic difference in deoxyglucose labelling of cells apparently very similar in electrical activity (CH- and HS-cells) (Buchner et al. 1984). The physiological basis for this apparent discrepancy between electrical and metabolic activity is presently under investigation.

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