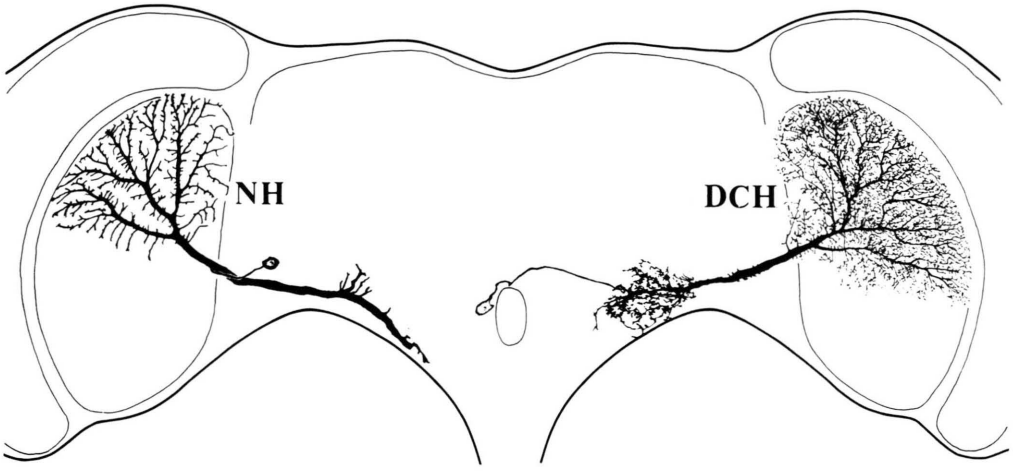

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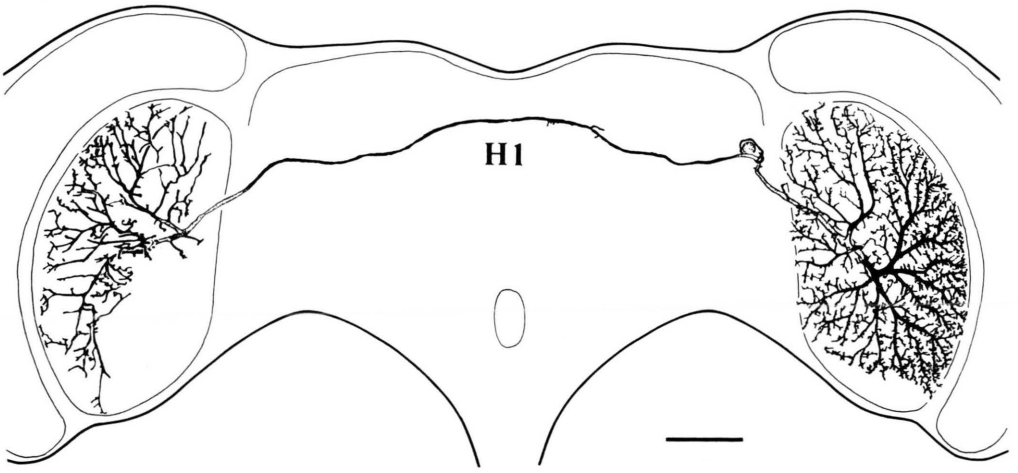

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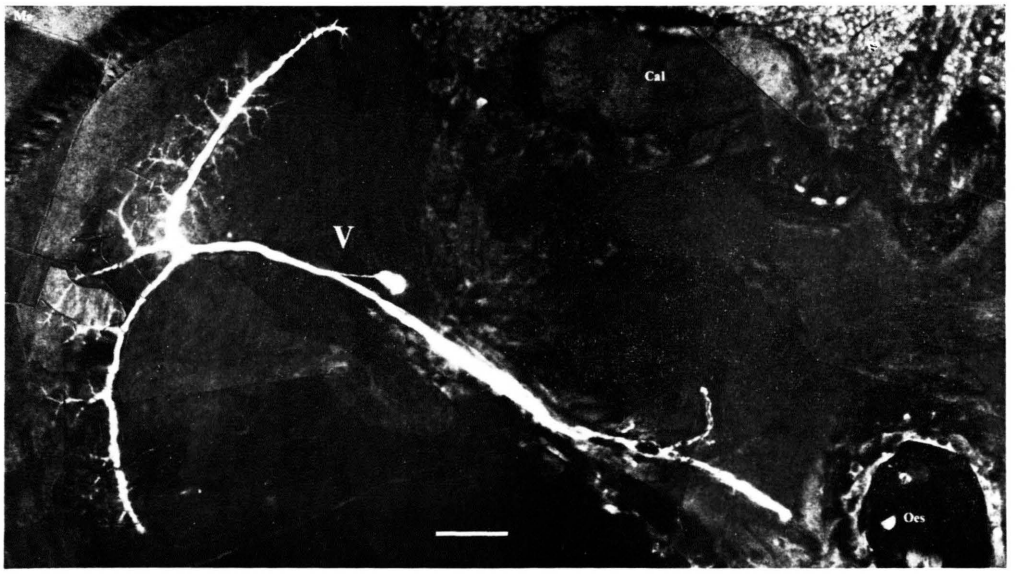

c

Fig. 1 a—c. Motion sensitive neurons of the lobula plate of *Calliphora erythrocephala* stained by intracellular injection of procion yellow. The tracings (a, b) were obtained from 12 μm frontal sections and show the injected cells drawn into a semi-schematic vertical elevation of the brain (lateral structures, indicated by thin lines: lobula plate and medulla; oval structure in the center: oesophagus). a. The *north horizontal cell* (NH) and the *dorsal centrifugal horizontal cell* (DCH). b. The heterolateral Hl-Cell (dendritic arborisation: right lobula plate; telodendron: left lobula plate). c. Photographic montage of a type A *vertical cell* of the left lobula splate. The montage is from technical reasons uncomplete. Most of the fine dendritic side branches in the lobula plate, especially those, emerging from the ventral main dendrite are not shown here. Me = medulla, Cal = calyx, Oes = oesophagus. Calibration markers: 100 μm (b), 50 μm (c).

Functional Characterization and Anatomical Identification of Motion Sensitive Neurons in the Lobula plate of the Blowfly *Calliphora erythrocephala*

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8 classes of homo- and heterolateral wide field neurons of the Lobula plate of *Calliphora erythrocephala* were investigated electrophysiologically and identified by intracellular injection of procion yellow. All recorded neurons were motion-sensitive. Some of the homolateral neurons respond only with graded potentials to visual stimulation; all heterolateral elements generate spike potentials. Connections between identified neurons were investigated by means of simultaneous double recordings. The described neurons are compared with units known from earlier extracellular studies.

Motion sensitive neurons in the optic lobes and midbrain of flies, which are assumed to be functionally involved in optomotor reactions have been described by several authors (Bishop *et al.* [1], Mc Cann, Dill [2], McCann, Foster [3]). Whereas the functional properties of some of these neurons have been studied in great detail, their anatomical identification was not resolved, since extracellular recording techniques were used in the former investigations. Histological localizations of the recording sites (Bishop, Keehn [4], McCann, Dill [2]) indicated, however, that some motion-sensitive wide field neurons are located in the 3rd optic neuropil, which, in Diptera, consists of two separate regions, lobula and lobula plate. Like the 1st and 2nd optic neuropils, these are composed of retinotopically arranged periodic columns of interneurons, each representing a defined optical axis in the visual field of the ipsilateral compound eye. In the lobula plate, different types of wide-field elements exist. These neurons connect specific arrays of columns with the perioesophageal region of the posterior protocerebrum ("posterior slope"), from which descending neurons originate. Other wide field elements connect both lobula plates directly (Strausfeld [5, 6]). Two systems of lobula plate "giant" elements, the horizontal and vertical cells, have been described anatomically by Pierantoni [7]. Recently, Dvorak *et al.* [8] reported first results on recordings and dye-injections in the horizontal cells of various species of Diptera.

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This short note summarizes electrophysiological and anatomical results on extra- and intercellulary recorded, and stained, wide-field neurons of the lobula plate of the blowfly *Calliphora erythrocephala*.

The experiments were carried out on female animals (age: 8–15 days) from the stock of the laboratory. Standard recording-, intracelluar staining- (Procion Yellow, M4RAN), and histology-techniques were used. Tracings of procion-filled neurons were obtained from 12 μm frontal sections. Reconstructions of the stained cells shown here for demonstration are graphically projected onto a frontal elevation of the brain. The stimulus consisted of a moving grating in front of each eye (Field: $50^\circ \times 70^\circ$, field center: 50° lateral from the frontal head axis in the equatorial plane of the eye, average luminance (incandescent source): 70 cd/m^2, spatial wavelength of the grating: 18°, contrast: 0.8, contrast frequenccy: 1.6 cycles/sec). The gratings were aligned with respect tot he head axes of the animal and were independently controllable in time and direction of motion.

In the following, the physiological and anatomical properties of the investigated neurons will be first described separately, suceeded by a discussion of their connections and functional roles.

Identified Cells

1. The H-Cells (Fig. 1 a)[+]

The *horizontal cells* (H-cells) form a class of three large homolateral elements, which have been topographically labelled by Pierantoni [7] as *north-*, *equatorial-* and *south-horizontal cells* (NH, EH, SH). The dendrites of each cell extend over approximately one third of the anterior lobula plate surface, showing a clear density gradient: the highest dendritic density is found in the distal region of the lobula plate, which represents the frontal visual field of the ipsilateral eye *, **. The axons of the three H-cells project to the ipsilateral posterior slope, where they converge to form a tight, shightly twisted bundle. From each cell a few short collaterals emerge near the axon terminals. The cell bodies are located in

[+] Figs 1 a–c see Plate on page 628 b.

* An outline of the retinotopic projections in the optic neuropils is given by Strausfeld [6]. In short, the distal part of the lobula plate (nearest the medulla) represents the frontal visual field of the ipsilateral eye, the proximal part (nearest the central protocerebrum) represents the lateral visual field.

** The term ipsilateral is defined here as that half of the protocerebrum, which contains either the mentioned cell or its dendritic part; motion presented in the visual field of the ipsilateral or contralateral eye will be termed as ipsilateral or contralateral motion.

the posterior rind of the optic pedunculus. All three H-cells were investigated and stained. A photographic montage of an EH-cell is published in Strausfeld [5]. The reconstruction of a procion-injected NH-cell, whose dendrites invest the dorsal part of the lobula plate surface, is shown in Fig. 1 a (left side). Recordings from H-cells showed typical, stimulation-dependent patterns of synaptic activity (Fig. 2 lower trace) and small, spike-like potentials superimposed

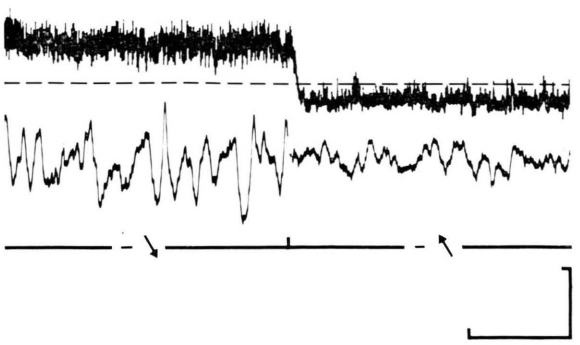

Fig. 2. Intracellular recordings from a north horizontal cell (NH), stimulated with ipsilateral backward motion (−↘) and forward motion (−↖). The upper trace (dc-coupled; calibration: 20 mV/1 sec) illustrates the dc-shifts of the membrane potential elicited by motion in the preferred (−↘) and the reverse direction (−↖). The broken line indicates the level of the resting potential (−55 mV). The lower trace (ac-coupled; calibration: 10 mV/50 msec) shows parts of the upper trace in higher time resolution illustrating the characteristic stimulation-dependent patterns of synaptic activity recorded from horizontal cells.

on DC-shifts of the membrane potential. Full-sized, regenerative action potentials were not observed. No significant physiological differences could be found between recordings from the dendrites and the axons. It is assumed that graded potentials are the normal responses of H-cells to visual stimulation. All recorded H-cells showed similar responses when stimulated with moving gratings: ipsilateral horizontal backward motion leads to a massive depolarization, forward motion to a hyperpolarization of the cell membrane (see Fig. 2, upper trace). Contralateral forward motion elicits highfrequency spike-like potentials which possibly are passive backspread of local spikes in the axon-collaterals. Hence, horizontal cells show binocular responses and are activated by ipsilateral backward and contralateral forward motion. No significant responses to vertical motions were found.

2. The CH-Cells (Fig. 1 a)

A second class of wide field elements at the anterior lobula plate surface consists of two cells which will be termed *dorsal* and *ventral centrifugal horizontal cell* (DCH, VCH). The main arborisation of each cell, which gives rise to a dense population of typical presynaptic swellings, nearly homogeneously invests one half of the dorsoventral extent of the lobula plate (see Fig. 1 a). The axons project to the ipsilateral posterior slope. Here, a second densely-spined arborisation overlaps the axons and axonal collaterals of the H-cells. The cell-body fiber of the CH-cells originates in this area and tranverses the central protocerebrum in a medio-frontal direction. The cell bodies reside near the oesophageal foramen in the frontal rind of the protocerebrum. Accordings to anatomical criteria this cell type is clearly centrifugal.

No spike-activity was recorded from CH-cells; ipsps can be elicited selectively by contralateral backward motion, while at least two types of high frequency epsps can be induced by motion in the opposite direction. In spite of no, or hardly detectable, synaptic noise, ipsilateral stimulation elicits clear graded dc-shifts of the membrane potential. The cells are depolarized by ipsilateral backward motion and hyperpolarized by ipsilateral forward motion. They do not respond to vertical motions.

The specific combination of inhibitory and excitatory ipsi-and contralateral inputs of the CH-cell results in an enhanced responsiveness to horizontal rotatory motions: maximal depolarization, *i. e.* activation is achieved by combined contralateral forward and ipsilateral backward motion.

3. The H1-Cell (Fig. 1 b)

The H1-cell is a unique lobula plate element, whose dendrites (right side, Fig. 1 b) cover almost the whole anterior lobula-plate surface. As in the horizontal cells, the highest dendritic density is found in the distal region of the lobula plate. The main branches of the dendrites converge in the internal chiasma to form the axon, which passes the lobula without any collaterals in a dorsofrontal direction. After crossing the central midbrain near its frontal surface, the axon projects – again without any side branching – through the contralateral lobula. The telodendritic endings (left side, Fig. 1 b) are located in close proximity to the H- and CH-cells in a superficial, anterior layer of the contralateral lobula plate.

As may be expected from the dimensions of the axon (diameter: 5 μm, length: 1200 μm) this cell functions with spike-transmission (velocity: about 1 m/sec; measured by simultaneous double recording from the dendritic and telodendritic region of the cell).

The H1-cell responds selectively to ipsilateral horizontal forward motion; motion in the reverse direction causes inhibition.

4. The H2-Cell

The H2-cell is a second directionally-selective spike-transmitting heterolateral neuron with the same preferred direction as the H1-cell. Only parts of the axon in the posterior slope could be stained. It is assumed that this element is identical to a neuron filled by Hengstenberg (personal communication), which has a dendritic arborisation in the dorsal part of the lobula plate and whose axon terminal in the contralateral posterior slope lies in close apposition to the endings of the contralateral H-cells and the dendritic domains of the CH-cells.

5. The H3-Cell

The complicated structure of the heterolateral wide field H3-cell shows a main arborisation area in the proximal half of the lobula plate. Three smaller branching areas occur in the ipsiaterial ventrolateral protocerebrum, beneath the ipsilateral pedunculus of the mushroom body and beneath the contralateral pedunculus. Branches of the latter arborisation extend ventrally so as to overlap the dendrites of the contralateral CH-cells. This was directly demonstrated by a double staining of an ipsilateral DCH and a H3-cell from the contralateral lobula plate. The presumed dendritic arborisation of the H3-cell in the lobula plate is located near its anterior surface; some of its ventral branches turn backwards and reach the level of V-cell dendrites (see below) at the posterior surface of the lobula plate. The spike-transmitting H3-cell is the only heterolateral motion-sensitive element so far found in the lobula plate, which responds specifically to ipsilateral backward motion. Again, this cell is inhibited by motion in the reverse direction.

6. The V-Cells (Fig. 1 c)

The *vertical cells* (V-cells) represent a class of nine serially arranged T-shaped large elements located near the posterior surface of the lobula plate neuropil (Pierantoni [7]). Their axons project in an almost straight and coherent bundle to the ipsilateral posterior slope and end near the oesophagus with few short side-branches. In the lobula plate the axon of each cell divides near the equatorial midline into a dorsal and ventral main branch which project vertically to the upper and lower margins of the neuropil. Most of the fine dendritic side branches emerging from the two main trunks run in a lateral direction. Anatomically, this class of cells can be subdivided into three groups. The dorsal main branches of the first three cells in the distal region of the lobula plate are nearly straight (type A), those of the next two cells are curved medially (type B). The dorsal branches of the last four cells are curved in a lateral direction (type C). Fig. 1 c shows the photographic montage of a type A vertical cell. Signals recorded from vertical cells show a strong similarity to those recorded from horizontal cells *i. e.* characteristic patterns of synaptic activity and graded changes of the membrane potential; single spikes are only found occasionally, their generation does not seem to be stimulus dependent. Vertical cells respond only to ipsilateral stimulation. Recordings from type A vertical cells revealed selective sensitivity to ipsilateral downward motion. Under this stimulation, synaptic activity of considerable amplitude is superimposed on a depolarizing DC-shift of the membrane potential. Motion in the reverse direction leads to suppression of the synaptic activity and hyperpolarisation of the cell. Type B verticals showed a reduced sensitivity to vertical motion and respond with a graded depolarizing potential to ipsilateral backward movement. No responses to visual stimulation could be recorded from type C verticals; it is assumed that their receptive fields lay outside the stimulated area of the eyes.

4. The V1-Cell

The very fine beaded branches of the heterolateral V1-cell spread over the posterior surface of the lobula plate in the same layer as the vertical cells. They clearly show blebs and hence are interpreted as presynaptic. The dendritic arborisation of the cell surrounds the terminals of the vertical cells in the opposite posterior slope. Here, the short cell-body fiber originates; the cell body is located in the posterior rind of the protocerebrum. The axon passes just above the oesophagus through the posterior protocerebrum and enters into the ventral half of the lobula plate.

The V1-cell generates spike potentials and is strongly excited by ipsilateral downward motion (Fig. 3). Interstingly, a reduced but still significant

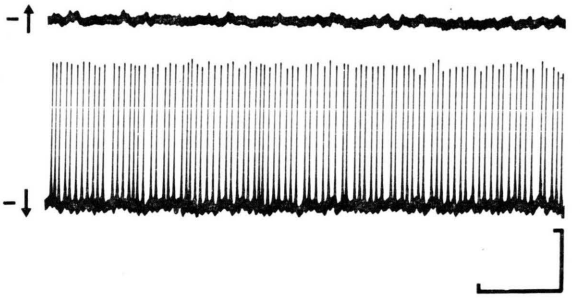

Fig. 3. Intracellular recordings from the axon of a V1-Cell. The cell shows a massive response to ipsilateral downward motion (−↓), and is completely inhibited by upward motion (−↑). Calibration: 25 mV/100 msec.

response can be elicited by ipslateral, horizontal backward motion *. Ipsilateral upward motion as well as forward motion inhibit the activity of the cell. No response to contralateral stimulation was found.

8. The V2-Cell

The V2-cell is a unique heterolateral element, whose dendrites are located between the layers of the horizontal and the vertical cells in the middle of the lobula plate. The axon crosses the posterior protocerebrum quite superficially *via* the posterior optic tract. Like the dendrites, the contralateral telodendritic arborisation of the cell is located in a medial layer of the lobula plate.

The cell generates spike potentials as do the other described heterolateral elements, and responds to ipsilateral upward motion. A comparably strong response could be elicited by ipsilateral forward motion *. Motion in the reverse direction causes inhibition; responses to contralateral stimulation could not be observed.

Interactions between the Identified Cells

1. Simultaneous extracellular double recordings from the axons of both H1-cells show that the H1-cells inhibit each other mutually and that the inhibitory interaction must take place in the lobula plate.

2. Simultaneous intra-/extracellular recordings from CH- and H1-cells show that the H1-cell is presynaptic to the contralateral CH-cells. Since the interaction between these cells can only take place in the lobula plate (see Fig. 1 a and 1 b), the telodendron of the CH-cells must be pre- and postsynaptic. Hence, the clearly defined anatomical polarity of the CH-cells is not as evident physiologically.

3. Double recordings from the posterior slope revealed the existence of at least two H2-cells, and demonstrated that these cells are also excitatory input elements to the contralateral CH-cells. From anatomical data (see description H2-cell) it must be assumed that the H2-synapses are located on the dendritic branches of the CH-cells in the posterior slope.

4. The CH-cells are inhibited by contralateral backward motion. The H3-cell is the only backward motion-sensitive element found which extends to the contralateral protocerebrum. Since its terminal arborisation overlaps the CH-cell dendrites it is highly suggestive that this cell acts as an inhibitory input element to the CH-cells, generating the described ipsps.

5. The postsynaptic potentials elicited in CH-cells under contralateral stimulation by spike transmitting heterolateral elements can be clearly recognized. On the other hand, ipsilateral stimulation of the CH-cells leads to graded potentials without any discrete synaptic potentials. This is to be expected, if their ipsilateral input elements act with graded potentials. Since the CH- and the non-spiking H-cells show the same reactions to ipsilateral stimulation and since the dendrites of the CH-cells are in close contact to the terminals of the H-cells, it is possible that both types of cells are synaptically connected in the posterior slope region.

6. Recordings of H-cells show characteristic synaptic activity under ipsilateral stimulation, in which – even in recordings from the dendrites o the H-cells – individual psps cannot be discriminated. Since few, multisynaptically-linked input elements are expected to produce discrete psps (*i. e.* psps of relatively high amplitude) in these cells, it is probable that a great number of independent input elements act synaptically on H-cells under ipsilateral stimulation. These input elements may be motion-sensitive small field elements of the lobula plate columns. The spike-like potentials recorded in H-cells under contralateral stimulation have the same mean frequency as the spikes of the H2-cells. It is possible that these cells are not only presynaptic to the CH-cells but also to the H-cells.

7. From the anatomy and the response of the V1-cell it must be assumed that this cell is postsynaptic to the vertical cells. Interestingly, this cell responds not only to downward motion but also – albeit more weakly – to horizontal backward motion. As described above, both responses were found in different types of vertical cells. Hence, the V1-cell seems to be coupled not only to a single vertical cell but to the complete group of vertical cells.

Discussion

The main functional property of the described lobula plate neurons is their directionally selective motion sensitivity. Only the long *heterolateral* elements function with regular *spike transmission*, whereas the identified *homolateral* neurons respond mainly with *graded potentials*.

The present state of knowledge allows a first comparison with the extracellular recorded motion units summarized by McCann and Foster [3]. The H1-cell is definitely identical with the well studied class II-1 unit; it is assumed that the H3-cell is the class II-3 unit, the V1-cell the class II-2 and the V2-cell the class II-4 unit. The fact, that H-, CH- and V-cells

* Since the directional selectivity of the cells was only tested with horizontal and vertical motions, it cannot be excluded, that their preferred direction is oriented obliquely between both test directions.

do not generate regular spike-activity may explain, why these elements were not discovered in extracellular studies.

In the recent paper by Dvorak *et al.*[8] different homolateral cells belonging to the horizontal system were described. All of these cells were claimed to be sensitive to contralateral stimulation; more detailed physiological data were not presented. In addition to the H- and V-cells the existence of three "mimetic" horizontal (*h*-cells) and nine small vertical (*v*-cells) as well as wide field horizontal cells (*WF*-cells) was discussed. Whereas some of the cell illustrated in that paper were in fact horizontal cells, it can be clearly seen in the shown photographs that others, interpreted by those authors as horizontal cells are doubtlessly centrifugal horizontal cells. Methylene blue stained 2 μm sagittal serial sections of the lobula plate of *Calliphora* demonstrate that only five large cell profiles exist in the anterior part of the lobula plate, which – as reconstructions from the semi-thin preparations have shown – belong to the 3 H-cells and the 2 CH-cells described here (Boschek, personal communication). Since these semi-thin preparations and the present dye-injected material show no other large profiles in this region, the existence of the proposed *h*- and *WF*-cells remains quite enigmatic. The *v*-cells discussed by the above authors were not found in the present study. These problems of anatomical identification and interpreation may only be solved by the comparison of dye-injected material with reconstructions from serial semi-thin sections and Golgi-impregnated cells of the lobula plate and midbrain.

The described motion-sensitive neurons reveal some interesting properties concerning the functional and morphological architecture of the lobula-complex of Diptera. Both neuropils of the lobula-complex receive their input from small, periodic medulla-elements, which cross the complex structured inner chiasma and terminate in the columns of the lobula and the lobula plate. The internal organization of these nueropils shows an interesting difference. In the lobula, a variety of different classes of super-periodic output-elements is found (Strausfeld[6]). The characteristic feature of the lobula plate, on the other hand, is the existence of only few, tangentially oriented wide-field elements, leading either to the contralateral lobula plate or to the dendritic regions of the descending neurons in the posterior slope. It is an attractive hypothesis, that the active process of motion detection does not take place in the lobula-complex, but in the medulla (various types of motion sensitive units in the medulla are known; DeVoe[9], Mimura[10]) and that this information, transmitted to both neuropils of the lobula-complex, is represented retino-topically in directionally selective periodic small-field elements in the single columns. The described architectonic difference between lobula and lobula plate may reflect different functions. The organisation of the lobula suggests that further complex abstractions from the incoming visual information take place in this region. The lobula plate, however, seems to serve basically as a retinotopic neural representation of motion in the ipsilateral visual field, on which a few, simple operations are performed by the wide-field elements. The function of these neurons seems to be a) the spatial integration of motion information over particular parts of the ipsilateral visual field, b) the transmission of signals to the contralateral lobula plate, c) the compilation of motion information from both eyes and d) the quick transmission of this information to the motor system *via* descending neurons. The existence of directionally-selective motion-sensitive units in the cervical connective (Hengstenberg[11]) and the direct connection of those units with lobula-plate neurons has already been demonstrated by intracellular dye-injection (Hengstenberg, personal communication) and by cobalt-diffusion experiments in the thoracic ganglion (Strausfeld[5]). Hence, the lobula plate seems to be specialized to mediate the fast and precise reactions to moving visual stimuli that are required for an effective flight and navigation control.

I would like to thank Dr. R. Hengstenberg and Dr. R. DeVoe for numerous discussions and helpful suggestions concerning intracellular recording techniques.

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