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R. Kern

Visual position stabilization in the hummingbird hawk moth, *Macroglossum stellatarum L.* II. Electrophysiological analysis of neurons sensitive to wide-field image motion

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Abstract Response properties of neurons in the cervical connectives of the hummingbird hawk moth, *Macroglossum stellatarum* L., were determined. All neurons described in this account respond directionally selectively to motion in large parts of the visual field of either eye. They respond maximally to bilateral stimulation, preferring either motion as induced on the eyes during translatory movements of the animal or when it turns around one of its body axes. Cells most sensitive to rotational motion either respond best to rotation of the patterns around the vertical axis of the animal or around its longitudinal body axis. Neurons most sensitive to translational pattern motion respond best to either simulated translations of the animal along its vertical or along an oblique axis. Most types of neurons respond tonically and do not habituate. The sensitivity to motion stimuli is not evenly distributed within the receptive field of any investigated neuron. Part of these neurons might play a role in visual position and course stabilization.

Key words Vision · Position stabilization · Optomotor neurons · Hawk moth · Insect

Abbreviations *NPBM* non-preferred bilateral motion · *PBM* preferred bilateral motion · *RH-cells* neurons that respond best to horizontal rotational motion · *RPM* rotational pattern motion · *RV-cells* neurons that respond best to vertical rotational motion · *TO-cells* neurons that respond best to translational motion along an oblique axis · *TPM* translational pattern motion · *TV-cells* neurons that respond best to translational motion along a vertical axis

R. Kern
Lehrstuhl für Biokybernetik, Universität Tübingen,
Auf der Morgenstelle 28, D-72076 Tübingen, Germany

Present address:
Lehrstuhl für Neurobiologie,
Fakultät für Biologie, Universität Bielefeld, Postfach 10 01 31,
D-33501 Bielefeld, Germany
Fax: +49 (0)521/106 6038
e-mail: roland.kern@biologie.uni-bielefeld.de

Introduction

The diurnal hawk moth, *Macroglossum stellatarum* L., sucks nectar from flowers while hovering in front of them (Knoll 1922). This peculiar feeding behaviour, which is reminiscent of hummingbirds, requires the ability of the animal to compensate for disturbances of its position relative to the flower in order to keep the proboscis in contact with the nectary. It has been shown in laboratory studies on freely flying animals that they control their position mainly by exploiting visual cues (Pfaff and Varjú 1991; Farina et al. 1994, 1995; Kern and Varjú 1998). Mechanical cues derived via the proboscis were demonstrated to play only a minor role (Zhou 1991). In a companion paper (Kern and Varjú 1998), the systems controlling the stabilizing optomotor responses have been analysed by simulating the visual consequences of disturbances experienced by *Macroglossum* in natural situations. The control systems mediating translatory and rotatory compensatory optomotor responses were shown to be largely independent of each other. The system controlling translational responses is more sensitive in fronto-lateral regions of the visual field than in lateral ones. The opposite is true for the rotational system. The sensitivity of the translational system does not change along the vertical extent of the visual field, whereas the rotational system is much more sensitive to motion in dorsal than in ventral parts of the visual field. These characteristic features of the optomotor system have been interpreted as adaptations to the optic flow *Macroglossum* might encounter in its natural habitat when hovering in front of flowers.

The present paper is a first attempt to relate the specific visual orientation behaviour of *Macroglossum* when hovering in front of flowers, to the properties of visual interneurons which might act as neural filters for

specific types of optic flow. The visual system of *Macroglossum*, which evaluates the optic flow and mediates the stabilizing optomotor responses, is organized basically as described for other insects. The retina of each superpositon eye is subserved by an optic lobe. Each optic lobe consists of three neuropils: the lamina, the medulla, and the lobula complex. The lobula complex is further subdivided into the lobula and the lobula plate. In the first two neuropils visual information is processed mainly by local, i.e. small-field elements receiving visual information from only small areas in the visual field. In the lobula complex a relatively small number of so-called tangential cells spatially pool the output of local elements by their extended dendritic arborizations (Strausfeld 1970; Strausfeld and Blest 1970; Wicklein 1994). Wide-field elements like the tangential cells in the fly lobula plate (reviews: Hausen 1981; Hausen and Egelhaaf 1989) and those in the medulla of moths (Milde 1993) and butterflies (Ibbotson et al. 1991) are thought to play a major role in computing motion information for optomotor course control. Eventually, the output information of the lobula complex is passed by descending neurons through the cervical connectives into the thoracic ganglia. Motor neuropils in the thoracic ganglia control the flight motor.

Neurons responding specifically to optic flow induced by translation or rotation of the animal have been found in several insect species at the level of the descending neurons (fly: Gronenberg and Strausfeld 1990; Borst 1991; dragonfly: Olberg 1981a,b; hawk moth: Rind 1983; locust: Rind 1990a, review: Rowell 1989; bee: review: Bidwell and Goodman 1993). The present experiments were performed on neurons in the cervical connectives of *Macroglossum*. The experiments were aimed at: (1) finding neurons that are sensitive to translational or rotational image motion, (2) determining the specificity of such cells for either of the corresponding types of image flow, and (3) investigating to what extent the sensitivity of the neurons changes within the visual field.

Materials and methods

Preparation

The experiments were carried out with adult specimen of both sexes of the hummingbird hawk moth, *M. stellatarum*. Their age ranged between 2 days and 3 weeks. The animals were taken from the stock of the institute (for breeding and keeping of animals see Farina et al. 1994; Kern 1994). They were demobilized by cold (5 min at 4 °C) and, after removal of the legs, slid into a narrow plastic tube so that the wings were immobilized but the head and the prothorax were still accessible. The scales covering the ventral thorax and the ventral part of the head were carefully removed with a fine brush and the thorax was then waxed to the tube. With the animal ventral side up, the tube was attached to a metal plate screwed to a holder equipped with a ball joint. By pulling the head backward the neck was stretched in order to gain access to the ventral neck membrane. To keep the head in this orientation, the proboscis was fixed with wax at the tip of the metal plate. The cervical connectives were exposed by dissecting the prothorax and the neck membrane. Muscles ventral to the cervical connectives were removed or cut, thus preventing movements of the tissue near the recording site. In order to stabilize the connectives during recording, they were lifted above the tissue by means of a tiny steel hook. Vaseline was filled between head and thorax on both sides forming a trough which was filled with ringer solution (NaCl 150 mmol·l^{-1}, $CaCl_2$ 3 mmol·l^{-1}, KCl 3 mmol·l^{-1}, TES 10 mmol·l^{-1}, saccharose 25 mmol·l^{-1}; pH 6.9) in order to prevent the connectives from desiccation. Animals prepared in this way allowed recording for several hours at temperatures between 18 °C and 22 °C.

Recordings

Recording electrodes were pulled (P-80 Brown Flaming Micropipette Puller, Sutter Instruments) from filamented glass capillaries (Science Products GmbH). Tips were filled with a 2% Neurobiotin (Vector Laboratories) solution, shafts were filled with a 1 mol·l^{-1} KCl solution. Electrode resistance was between 30 MOhm and 80 MOhm. Neurobiotin was used to morphologically characterize the neurons recorded from. However, for unknown reasons, stainings were not good enough for reconstruction of the cells. Recording electrodes were inserted into the left cervical connective approximately half way between the brain and the prothoracic ganglion. Indifferent electrodes were pulled from the same glass as the recording electrodes and were filled with Ringer solution. Their tips were broken and they were positioned in the haemolymph near the recording site. Signals were amplified (Cyto 701, World Precision Instruments), displayed on a storage digital oscilloscope and transferred to a PC-housed (AT386, 33 MHz) Data Acquisition Processor (DAP 1200e, Microstar Laboratories) for further processing.

Stimulation

The animal was stimulated by moving black-and-white square-wave gratings, generated on two computer monitors (PC AT386, program written in TurboPascal). The monitors (NEC MultiSync 3FG, frame repetition rate set to 60 Hz) were placed in a dark room. The screens were oriented vertically and subtended an angle of 70°. In order to shield the recording electrode from the radiation of the monitors, grounded screen wire with a mesh width of 0.15 cm was attached to the monitors in front of the screens. A stereomicroscope was used to adjust the horizontal plane of the head parallel to the horizontal plane of the holder to which the animal was attached. The holder was mounted between the monitors such that the longitudinal axis of the head coincided with the bisector of the angle between the monitors. In the vertical the holder was adjusted so that the connection line between the centres of the two screens ran through the centres of both eyes. The largest unilateral patterns extended over a visual angle of 90° in the horizontal and 82° in the vertical. The orientation of the gratings could be changed in steps of 45°. Pattern motion was always perpendicular to the orientation of the stripes. The size of the patterns and their location on the screens could be varied in order to stimulate small parts of the eyes selectively. The wavelength of the pattern was 2 cm in most experiments. Since the patterns were presented on flat screens, the angular width of pattern periods was not constant. In some experiments patterns of two periods were presented, restricted to either anterior, medial or posterior areas of the screens. In these experiments, the wavelength (in cm) was adjusted according to the azimuthal position of the pattern such that the angular horizontal extent of the two periods was kept constant at 24°. The patterns were moved at a temporal frequency of 2.5 Hz except when the dynamical properties were analysed. Pattern contrast was 98%, mean luminance 20 cd m^{-2}.

The following coordinate system is used to specify the pattern location within the visual field of the animal. In azimuth, 0° is in front of the animal, and 90° is lateral to the right (azimuth values will be given only for the right monitor screen; they are mirror-

symmetrical for the left one). In elevation, 0° corresponds to the height of the horizontal midlines of the screens, positive angles correspond to locations above, negative ones to those below the midlines.

Terminology

Since all recordings were performed in the left connective, pattern motion on the left of the animal will be referred to as ipsilateral, pattern movements on the right as contralateral stimulation. Accordingly, the left eye of the animal is ipsilateral, and the right eye is contralateral.

In order to specify the types and directions of pattern motion on the screens, the following terminology will be used: (1) the direction of unilateral pattern motion that leads to the largest response of a neuron will be termed *preferred direction*, (2) unilateral motion in the opposite direction, usually decreasing the firing rate of a neuron most strongly, will be called *null direction*, (3) bilateral motion will be termed *preferred bilateral motion* (PBM) if the patterns in front of both eyes move in the respective preferred directions, (4) bilateral motion will be named *non-preferred bilateral motion* (NPBM) if pattern motion in the visual field of one eye is in the preferred direction and pattern motion in front of the other eye in the null direction. Thus, two types of NPBM are feasible (5) *Rotational pattern motion* (RPM) around the vertical body axis of the animal means that the patterns move back to front on one screen and front to back on the other one. RPM around the longitudinal body axis is achieved by moving the patterns upward on one screen and downward on the other. RPM around other axes is defined accordingly. RPM simulates the flow field an animal experiences when turning around one of its body axes. (6) *Translational pattern motion* (TPM) along the longitudinal axis of the animal means that the patterns on both screens move front to back or back to front; TPM along the vertical body axis means that both patterns move upward or downward. TPM along other axes is defined accordingly. TPM simulates the flow field an animal experiences when translating along one of its body axis.

The main stimulus program (written in TurboPascal) was subdivided into three units that were presented in a fixed order:

1. Determination of the directional tuning curves of the neuron by means of unilateral pattern motion. The patterns ranged from 28° to 117° in azimuth and from −41° to +41° in elevation ('whole-screen patterns'). Eight directions of motion, each separated by 45°, were tested. For each direction of motion, the eyes were stimulated in turns. The sequence of movement directions as well as the order of stimulation of both eyes were randomized. The whole-screen patterns were displayed on both screens. One of the patterns was stationary.
2. Determination of the PBM. The sequence of TPM and RPM was randomized. The movement direction of the whole-screen patterns on both screens usually was restricted to the preferred and null directions determined for unilateral stimulation.
3. Determination of sensitivity gradients within the receptive field. Patterns of reduced size were moved within limited areas on the screens. The screens were subdivided either along the vertical or the horizontal axis. Along the vertical axis the screens were divided into two parts. Patterns covering the upper (lower) half of the screens ranged from 28° to 117° in azimuth and +41° (−41°) to 0° in elevation. Along the horizontal axis the screens were divided into three parts. The patterns covered the anterior, medial or posterior parts and ranged from +41° to −41° in elevation. The edges of the three pattern segments along the horizontal axis were located at 33° and 57° ('anterior pattern'), at 62° and 86° ('medial pattern'), and at 90° and 114° ('posterior pattern'). The sequence of presentation of the pattern segments was randomized.

Not all cells could be tested with each stimulation unit. The temporal sequence of stimulation in each unit was: 1.5 s no motion; 1 s pattern motion; 3 s no motion; 1 s opposite pattern motion; 1.5 s no motion.

Data analysis

Signals were processed by the Data Acquisition Processor in either of the two following ways: (1) spikes were detected by a software algorithm (written in C by K. Bartsch) and the time of their occurrence stored, and (2) the analog voltage signal was converted into digital numbers and stored (sampling rate 5 kHz). Further processing of data was done using Quattro Pro (Borland) or programs written in TurboPascal. The response amplitudes referred to in the Results were calculated as the number of spikes during the 1-s motion period minus the mean spike frequency during the 1.5-s period prior to the onset of motion. Each stimulus was presented at least four times; the spike frequencies calculated for single sweeps were averaged.

Results

General response characteristics of the neurons

A total of 58 motion-sensitive, direction-selective neurons were recorded in the left connective and could be characterized in sufficient detail. They responded best to specific bilateral motion stimuli, namely either to RPM or TPM. They were grouped into four major classes on the basis of their directional selectivity.

All neurons responded to pattern movement with a change in spike activity rather than with graded changes of the membrane potential. Spike amplitudes (up to 30 mV) of a given neuron were rather constant but differed amongst individual cells even of a particular cell type. The resting spike activity was below 10 spikes $\cdot$ s^{-1} in most cells. Some neurons showed no resting discharge at all. All cells responded to motion in the visual fields of both eyes. The maximal responses to unilateral stimulation of the right and the left eye frequently differed from each other. The maximum spike rate of a cell rarely exceeded 25 spikes $\cdot$ s^{-1} during unilateral stimulation in the preferred direction and 70 spikes $\cdot$ s^{-1} during PBM. After the pattern stopped moving in the preferred direction, spontaneously active cells tended to decrease their firing rate below the resting level for some time. After cessation of motion in a direction that decreased the firing rate, cells tended to increase their spike activity for some time above the resting level as determined before stimulation had started (Fig. 1a). Receptive field sizes were not determined in detail. All cell types responded best to motion of whole-screen patterns. The dependence of the response on temporal frequency was tested for only three cells of two different cell classes. They responded best at temporal frequencies of 2–4 Hz (Fig. 1b).

Unilateral and bilateral directional selectivity

When stimulated unilaterally, cells were excited most strongly by motion along the horizontal (Fig. 2a,b), the vertical (Fig. 2c–f) or an oblique axis tilted by 45° against the horizontal (Fig. 2g,h). The preferred directions of motion along the best axis of a given cell are

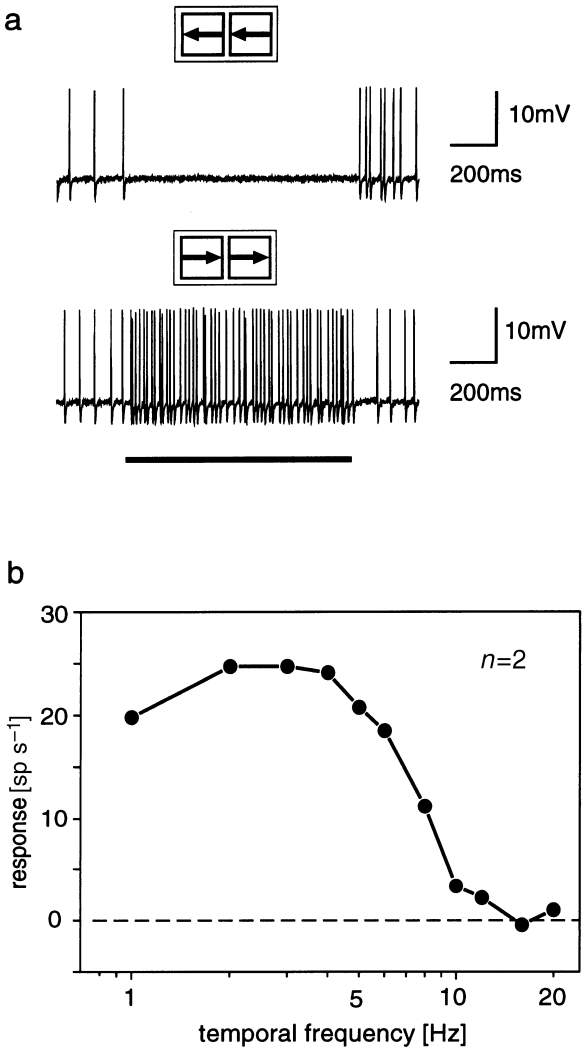

Fig. 1a Sample record of the response of a RH1-cell in the left cervical connective to horizontal rotational pattern motion. *Upper trace*: null direction motion in front of both eyes; *lower trace*: preferred direction motion in front of both eyes. *Horizontal bar* indicates 1-s period of pattern motion. **b** Dependence of the response of RH2-cells on temporal frequency. The neurons were stimulated by unilateral motion in the preferred direction in front of the contralateral eye. The sequence of stimuli with different frequencies was randomized. Note logarithmic scale on abscissa. n = number of cells

either different (Fig. 2a–d) or the same (Fig. 2e–h) for the ipsilateral and contralateral eye, respectively. The response amplitudes decrease with increasing deviation of the movement directions from the cell's preferred directions. The response amplitude is minimal during motion in the null direction and, in spontaneously active cells, may be below the resting activity before the onset of motion. In part of the cells the maximal responses to motion in the preferred direction were about the same for the ipsi- and the contralateral eye, whereas in other cells the maximal responses were considerably different for the two eyes.

In accordance with the preferred directions of unilateral motion in front of either eye cells were grouped into R- and T-cells. The preferred directions of motion in front of the ipsilateral and contralateral eye are opposite in R-cells (Fig. 2a–d) and the same in T-cells (Fig. 2e–h). From the preferred directions of unilateral motion one can predict a given cell's type of PBM. The predicted PBMs coincide well with those obtained when stimulating cells bilaterally: R-cells respond best to rotation of the stimulus pattern about one of the body axes. They were subdivided into a class responding best to RPM around the vertical body axis, i.e. to horizontal motion (RH-cells; Fig. 2a,b), and a class responding best to RPM around the longitudinal axis, i.e. to vertical motion (RV-cells; Fig. 2c,d). T-cells are most sensitive to TPM either along the vertical axis of the animal (TV-cells; Fig. 2e,f) or along an oblique axis that runs from the lower front to the upper back of the visual field of either eye (TO-cells; Fig. 2g,h). According to the preferred directions of motion in front of either eye these four cell classes were further subdivided into two subclasses each (see Fig. 2a–h).

A more detailed characterization of the cell classes concentrated on: (1) the quantitative differences between the responses to unilateral and bilateral stimulation, (2) the specificity of the cells for either translational or rotational stimulation, (3) the dynamical properties, and (4) the spatial sensitivity distribution of the cells.

What are the quantitative differences between the responses to unilateral and bilateral pattern motion?

In order to answer this question, the responses to PBM and to NPBM were compared to the linear sum of the responses induced by the corresponding unilateral stimuli ('predicted response'). Note that two types of NPBM are feasible (see Materials and methods). The predicted response was either larger, approximately equal to, or smaller than the measured response. Each of the three cases is examplified in Fig. 3.

RV1- (data not shown, Kern 1994) and RV2-cells (two leftmost columns in Fig. 3) responded to PBM about twice as strongly as predicted on the basis of the responses to unilateral stimulation. In RH-cells, whose response amplitudes to PBM are in the same range as in RV-cells, the measured values were also larger than the predicted ones but differed much less, i.e. on average by about 25% only (data not shown, Kern 1994). In TO1- (two centre columns in Fig. 3) and TO2-cells (data not shown, Kern 1994) no differences were found between the measured and the predicted responses to PBM. Again, the measured responses to PBM are in a range comparable to that in RV-cells. In contrast to all other cell classes, the measured responses of TV-cells to their PBM are weaker than predicted on the basis of the responses to unilateral stimulation. In TV1-cells the predicted response is about one-third larger than the measured one which lies in the range of 150 spikes $\cdot$ s^{-1} (data not shown, Kern 1994). Also TV2-cells responded about 30% less than predicted (two rightmost columns in Fig. 3).

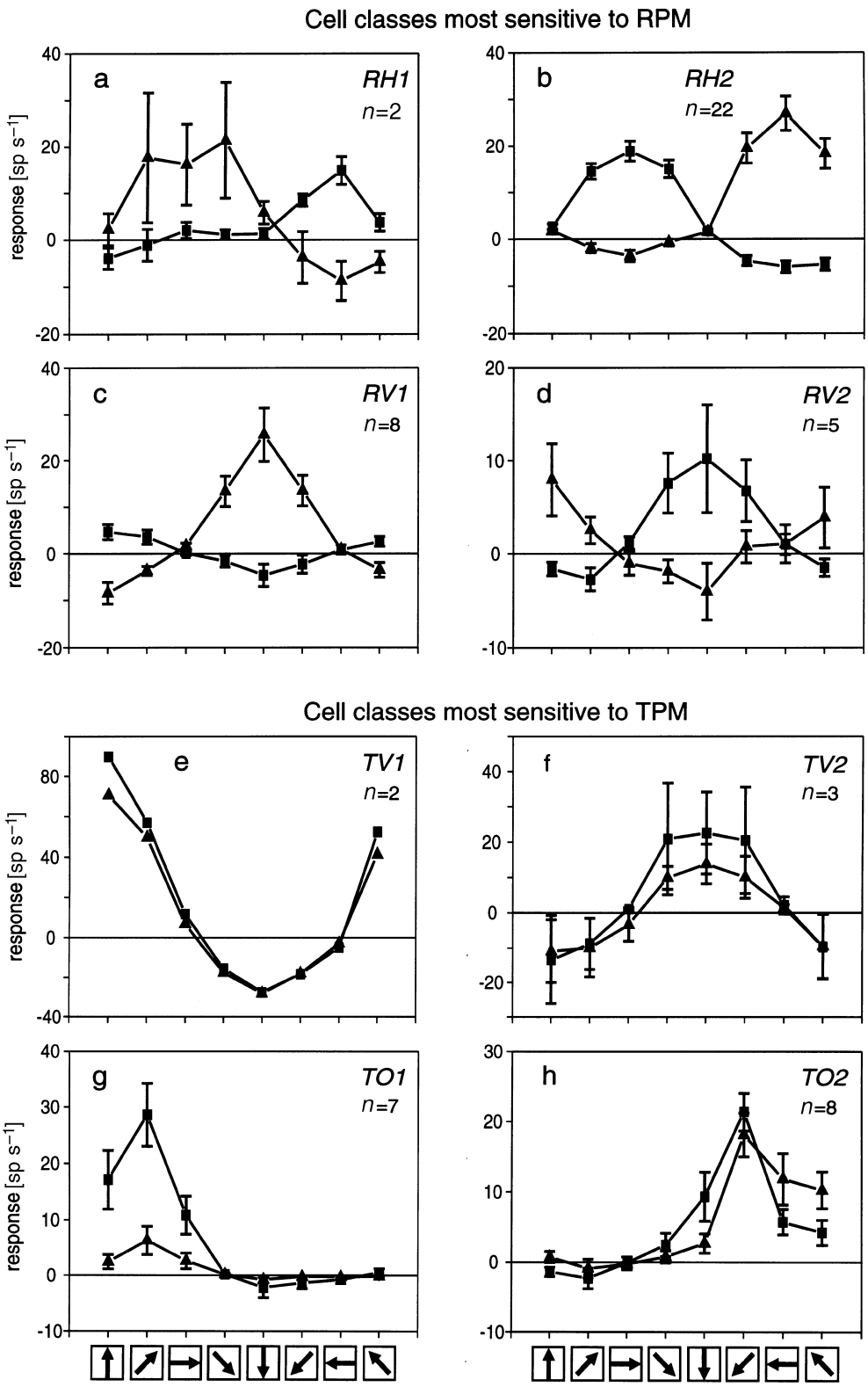

Fig. 2a–h Direction selectivity for unilateral pattern motion in front of the ipsilateral (*squares*) and contralateral eye (*triangles*), respectively. Direction of motion on the monitor screens was varied in steps of 45° as indicated by *boxes with arrows* along the abscissae of Figs. **g** and **h**. *Arrows* pointing to the right (left) represent front-to-back (back-to-front) pattern motion. Error bars: SEM. n = number of cells. **a** RH1-cells respond best to back-to-front (front-to-back) motion in the visual field of the ipsilateral (contralateral) eye. **b** RH2-cells respond best to front-to-back (back-to-front) motion in the visual field of the ipsilateral (contralateral) eye. **c** RV1-cells respond best to ipsilateral upward and contralateral downward motion. **d** RV2-cells respond best to ipsilateral downward and contralateral upward motion. **e** The preferred direction of TV1-cells is upward in the visual field of both eyes. **f** The preferred direction of TV2-cells is downward in front of both eyes. **g** TO1-cells respond best to motion along an oblique axis that runs from the lower front to the upper rear visual field of both eyes. **h** TO2-cells respond best to motion along the same axis but in the opposite direction

In all cell classes the measured responses to NPBM are, on average, smaller than or equal to the predicted ones (data not shown, Kern 1994). Hence, it can be concluded that in some cases the inhibitory effect of null direction motion in front of one of the eyes during NPBM was stronger than predicted from the responses to unilateral stimulation. This observation might be due to the fact that even with a strong inhibition during unilateral motion the spike rate cannot decrease below zero, resulting in an underestimation of the inhibitory

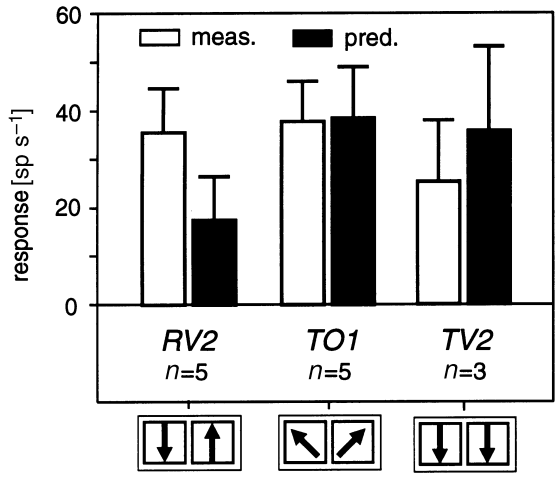

Fig. 3 Measured (*white columns*) and predicted responses (*black columns*) to preferred bilateral pattern motion of RV2-, TO1- and TV2-cells. The predicted values were calculated as the linear sum of the responses to the corresponding unilateral stimuli. Stimulus conditions as shown by the *pictograms* along the abscissa. The *two boxes* represent the monitor screens; *arrows* indicate the direction of motion as seen by the moth. For instance, the leftmost pictogram indicates downward motion on the left and upward motion on the right screen. Error bars: SEM. n = number of cells. RV2-cells respond much stronger to their preferred bilateral motion than predicted. TO1-cells respond to their preferred bilateral motion as strongly as predicted, whereas the measured response is smaller than predicted in TV2-cells

strength in cells with a low spontaneous activity. Note that during NPBM only one eye was stimulated by motion in the null direction while the other eye was stimulated by preferred direction motion.

How specifically do the cells respond to either translational or rotational stimulation?

The neurons characterized in the present study do not only respond to their PBM but also to NPBM, i.e. cells responding best to TPM also respond to RPM and vice versa. In order to quantify how specifically a cell responds to its PBM the firing rates during stimulation with PBM and NPBM have been compared by calculating a 'Specificity-Index (SI)'. The SIs were derived by dividing the differences between the responses to PBM and NPBM by the response to PBM. This was done separately for both types of NPBM (Table 1). Large SIs characterize highly specific cells. SIs larger than 1.0 indicate that the spike rate during NPBM was below the resting firing rate. Small SIs indicate that the responses to NPBM and PBM are similar. All SIs but one given in Table 1 are larger than 0.5, indicating that responses to PBM are at least twice as large as responses to NPBM. Hence, all cell classes, with the exception of TO1-cells, are rather specific to their respective PBM. In TO1-cells the response to NPBM with preferred direction motion in front of the ipsilateral eye and null direction motion in front of the contralateral eye, is only slightly weaker than the response to PBM, where ipsilateral and contralateral eye are stimulated by preferred direction motion. This finding can be attributed to the predominance of the ipsilateral eye which seems to mainly govern the response to bilateral stimulation (Fig. 2g).

Table 1 Specificity index (SI) of cell classes to their preferred bilateral motion. The index is defined as the difference between the responses to preferred bilateral motion and non-preferred bilateral motion divided by the response to preferred bilateral motion. Both feasible directions of non-preferred bilateral motion are considered separately: SI 1 = pattern motion in the preferred direction in the ipsilateral and in the null direction in the contralateral visual field; SI 2 = vice versa

Cell class	SI 1	SI 2
RH1	1.08	0.56
RH2	0.75	0.93
RV1	1.13	0.78
RV2	0.96	1.00
TV1	0.73	1.07
TV2	0.79	0.95
TO1	0.22	1.00
TO2	0.59	0.95

Time-course of the responses

For all cell classes the responses to both PBM and the corresponding unilateral stimuli had the same time-course. The neurons of most classes responded more or less tonically to motion stimuli, i.e. the spike rate decreased by less than 20% of its initial value during the 1-s period of pattern motion. Only TO1-cells responded phasically with a fast rise of the spike rate within the first 100 ms and a subsequent rapid decrease within the next 200–300 ms to response rates of less than 30% of the respective maximum value. Moreover, the response amplitude of TO1-cells continuously decreased in subsequent sweeps of the standard stimulus program; some of the TO1-cells completely ceased firing. After a pause of a few seconds and, likewise, after changing the size or the orientation of the pattern, the response recurred. Habituation was never observed in other cell classes (Kern 1994).

Sensitivity distribution within the receptive field

In order to find potential sensitivity gradients within the receptive fields of the neurons, the screens were subdivided either along their vertical or horizontal axis. The cells were stimulated by their PBM with the exception of TO-cells which were stimulated by *horizontal* TPM in order to render the obtained sensitivity distribution comparable to that attained in the behavioural experiments for translational stimulation (Kern and Varjú 1998). Not all cells recorded from could be tested with respect to their spatial sensitivity distribution.

A sensitivity gradient along the vertical extent of the visual field has been found in most cell classes. RH2-,

RV1-, and RV2-cells responded more strongly to motion in the upper than in the lower halves of the screens (Fig. 4a–c). In a few cells of these classes the preference for the upper half was only weak. In contrast, TO1-cells responded best to motion in the lower halves of the screens (Fig. 4d). None of the TO2-cells preferred motion in one-half of the screens over motion in the other (Fig. 4e). One of the two TV1-cells tested was most sensitive to motion in the lower halves of the screens but the second one had no preference (data not shown, Kern 1994). Only one RH1- and one TV2-cell could be tested with respect to their sensitivity distribution. Both cells responded best to patterns moving in the upper halves of the screens (data not shown, Kern 1994). Note, that in most cell classes the sum of the responses to motion either in the upper or lower halves of the screens is larger than the response to whole-screen pattern motion.

The sensitivity gradient along the horizontal extent of the visual field could be determined only in RH- and TO-cells. On average, RH2-cells were more sensitive to PBM in the posterior than in the anterior parts of the screens (Fig. 4a). Note, however, that in four RH2-cells virtually no difference was found. The latter also holds for the single RH1-cell tested (data not shown, Kern 1994). The TO1-cells were more sensitive to horizontal TPM in the anterior rather than in the posterior parts of the screens (Fig. 4d). In contrast, no consistent preference was found in the TO2-cells (Fig. 4e).

Discussion

Motion-sensitive neurons in the cervical connectives of the European hawk moth, *M. stellatarum* L., were characterized. They are directionally selective for wide-field motion stimuli in front of both eyes and, thus, presumably play an eminent role in optomotor behaviour. The cells have been classified on the basis of physiological criteria such as their preferred direction of unilateral and bilateral pattern motion, respectively. These criteria serve to distinguish the cells into distinct classes. Nevertheless, it cannot be excluded that each class can be subdivided further according to anatomical criteria or according to their functional properties when tested with more sophisticated stimuli than those employed in the present study. This qualification does not affect the conclusions drawn here.

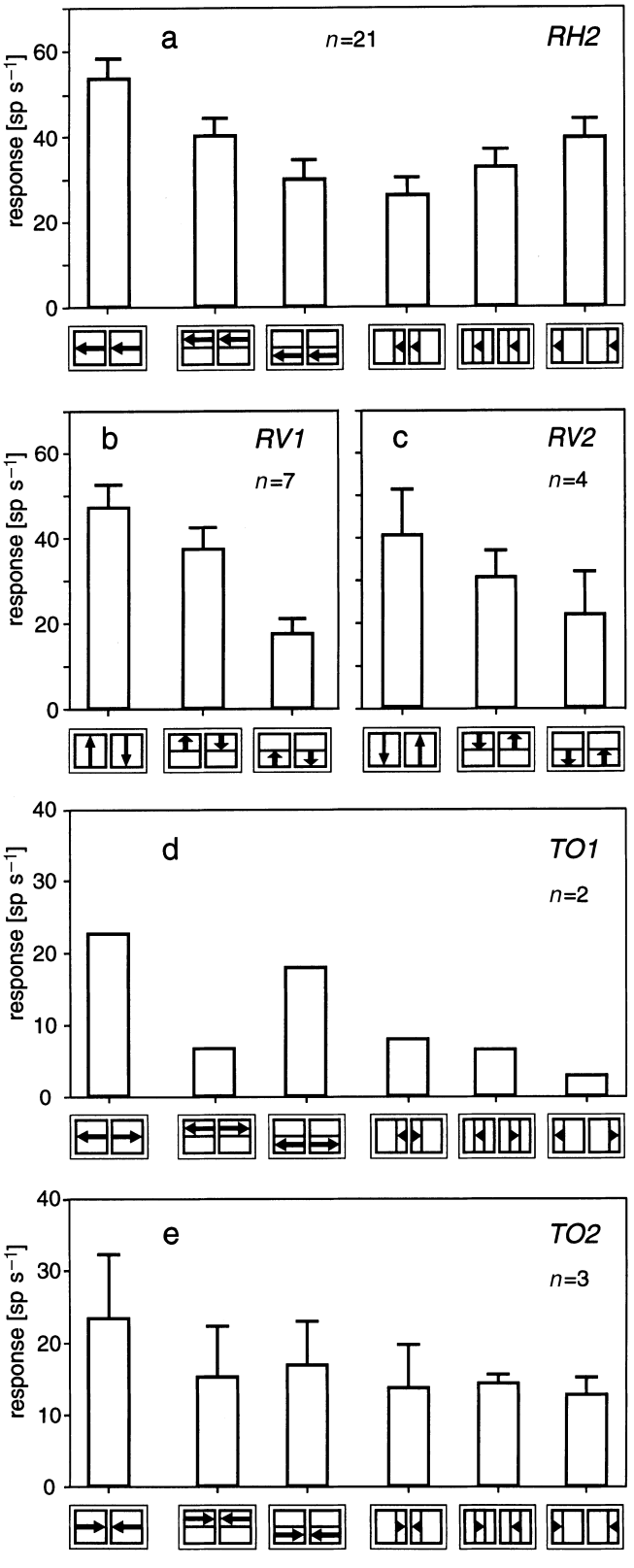

Fig. 4a–e Motion sensitivity of five cell classes in different regions of their receptive field. *Pictograms* indicate size and location of the pattern areas on the screens and the direction of motion. Error bars: SEM. n = number of cells. RH2- and RV-cells were tested with their respective preferred bilateral pattern motion, TO-cells were tested with horizontal translational motion. **a** RH2-cells pattern motion in the upper halves of the screens elicits stronger responses than pattern motion in the lower halves. The sensitivity to motion in the posterior parts of the screens is higher than the sensitivity to motion in the anterior parts. **b, c** Pattern motion in the upper halves of the screens elicits stronger responses than pattern motion in the lower halves in both RV1- and RV2-cells. **d** TO1-cells pattern motion in the lower halves of the screens elicits stronger responses than pattern motion in the upper halves. The cells are more sensitive to motion in anterior than in posterior parts of the screens. **e** TO2-cells the responses to all partial stimuli are about the same

All cells recorded from have large receptive fields and receive input from both eyes. In accordance with their sensitivities to unilateral stimulation, the recorded cells respond best to: (1) TPM either along the vertical body axis (TV-cells), (2) along an oblique axis (TO-cells), (3) RPM either around the vertical body axis, and thus to horizontal pattern motion (RH-cells), or (4) RPM around the longitudinal body axis, and thus to vertical pattern motion (RV-cells). The cells of most classes respond tonically. The classes differ with respect to their sensitivity to stimulation in different parts of the visual field.

Comparison to neural filters for optic flow in other species

Direction-selective neurons sensitive to wide-field motion have been characterized in a variety of insects and can be found at various levels of the visual pathway. Neurons that receive input from one eye only are predominantely found in the optic lobes where they pool the output of many local motion-sensitive elements with their extended dendritic arborizations (locust: Kien 1974; Rind 1990b; fly review: Hausen and Egelhaaf 1989; hawk moth: Collett 1971; Milde 1993; butterfly: Ibbotson et al. 1991). Monocular cells have been found also in the lobula plate of *Macroglossum* (Wicklein 1994). Although neurons which receive information from both eyes and are sensitive to TPM or RPM can be found in the optic lobes of various species (e.g. fly: Krapp and Hengstenberg 1996; reviews: Hausen 1981; Hausen and Egelhaaf 1989; bee: DeVoe et al. 1982; hawk moth: Collett and Blest 1966) they predominate at subsequent processing levels. Most descending neurons that have been described so far respond best to simulated rotations of the animal. There are only few reports on neurons most sensitive to simulated translation (Borst 1991; Ibbotson 1991a; Baader et al. 1992). The specificity of these cells can vary widely, ranging from cells that respond exclusively to bilateral front-to-back motion (Borst 1991) to cells which have only a relatively low specificity for translational or rotational motion (Ibbotson and Goodman 1990; Ibbotson 1991b). The specificities of the neurons found in the cervical connectives of *Macroglossum* are well within this range. It should be emphasized that neurons exploiting the visual information from both eyes in order to attain a high specificity for RPM or TPM are not only found in flying insects. Other animals with extended visual fields, such as the pigeon, possess bilateral motion-sensitive cells which have been demonstrated to be selectively responsive to translational or rotational visual flow about the vertical or longitudinal axis of the animal (Wylie and Frost 1990).

Descending neurons with preferred directions approximately along the horizontal or vertical axes of the two eyes have been found in various insect species (fly: Gronenberg and Strausfeld 1990; dragonfly: Olberg 1981a,b; bee: Goodman et al. 1987; Ibbotson and Goodman 1990; Ibbotson 1991a,b; moth: Rind 1983; locust: Baader et al. 1992). In addition, cells have been described that respond best to motion in oblique directions (Goodman et al. 1987). With respect to their directional selectivity the latter cells are similar to the TO-cells in *Macroglossum*. All the cells mentioned above are characterized by a single direction sensitivity peak. However, other descending neurons as well as neurons in the lobula complex of some species have been reported to possess two sensitivity peaks for the direction of motion (fly: Eckert 1982; Hengstenberg 1982; bee: Goodman et al. 1987; moth: Milde 1989; Fischer et al. 1990; Persel and Milde 1993; Wicklein 1994). In the present study a cell with double-peaked directional selectivity has been recorded only once (not illustrated).

Interaction of motion signals originating from different regions of the visual field

All cells characterized here respond to motion in large parts of the visual field of both eyes. Since in the peripheral visual system motion information is computed locally by many retinotopically organized elements (reviews: Hausen 1993; Egelhaaf and Borst 1993a), the local motion information has to be pooled in some way peripheral to the descending neurons. How do the local motion signals interact within the visual field of one eye and how are the motion signals originating from both eyes combined? In *Macroglossum* the local motion signals obviously are not linearly summated, since the responses of the recorded neurons do not increase linearly with increasing pattern size but approach a saturation level. This feature is reminiscent of neurons in the medulla of a butterfly (Ibbotson et al. 1991), in the lobula plate of a fly (Hausen 1982; Hengstenberg 1982; Egelhaaf 1985; Haag et al. 1992), and in the ventral nerve cord of a dragonfly (Olberg 1981a). With respect to the interaction of the signals from both eyes, the response to bilateral motion may be achieved by linear superposition of the signals followed by a saturation non-linearity which affects the signals in particular during strong stimulation. This kind of interaction has been found in the visual system of bees (Ibbotson 1991b) and is reminiscent of TV- and TO-cells in *Macroglossum*. On the other hand, the signals from both eyes may interact resulting in either a weak or a very strong enhancement (Borst 1991). A weak enhancement has been observed for the responses of RV- and RH-cells.

Sensitivity distribution along the vertical and horizontal extent of the eye

As in descending neurons of other species (Kien 1974; Olberg 1981a; Rind 1983; Ibbotson and Goodman 1990) the sensitivity distribution within the receptive fields of the neurons recorded in the present study is not homo-

geneous. RH- and RV-cells are most sensitive to stimuli in an area slightly above the equator of the eyes. RH2-cells respond more strongly if lateral rather than fronto-lateral regions of the eyes are stimulated. Neurons tuned to TPM are less uniform with respect to the sensitivity along the vertical and horizontal extent of the visual field than cells tuned to RPM. While the response of TO2-cells to horizontal TPM in all parts of the screens is almost the same, TO1-cells respond most strongly to stimuli located in the ventral halves and in the anterior and medial parts of the screens.

In *Macroglossum* regional specializations are already found at the level of the retina. Photoreceptors in the superposition eyes are organized to form local acute zones providing high visual acuity: one zone is located frontally, and slightly ventrally. Another zone is located along the equator of the eye (Bartsch and Warrant 1994). The retinal locations of these acute zones match the best-sensitivity areas of some of the neurons recorded from in the present study. However, the distribution of photoreceptor density cannot be the major reason for the sensitivity distribution within the receptive field of motion-sensitive neurons. Otherwise, RH2- and TO1-cells, for instance, should not have opposing sensitivity gradients along the horizontal extent of the visual field. Likewise, as demonstrated in behavioural experiments, the sensitivity gradient of the optomotor system along the horizontal extent of the eye is opposite for translational and rotational horizontal motion, respectively (Kern and Varjú 1998).

Possible function of the neurons

Direction-selective neurons sensitive to wide-field motion, such as those in the cervical connectives of *Macroglossum*, are usually regarded as being involved in optomotor course control (for reviews see: Hausen 1981; Wehner 1981; Collett et al. 1993). However, only in a few studies could optomotor behaviour be directly related to the properties of neurons (locust: Hensler and Rowell 1990; Hensler 1992; review: Rowell 1989; fly reviews: Hausen 1993; Egelhaaf and Borst 1993b).

In *Macroglossum*, neurons sensitive to wide-field motion may be involved in stabilizing the position of the animal when it hovers in front of a flower. The analysis of the optomotor behaviour in *Macroglossum* (Pfaff and Varjú 1991; Kern 1994; Farina et al. 1994, 1995; Kern and Varjú 1998) has been confined so far to position stabilization in the horizontal plane by compensatory translational and rotational movements. Hence, it is not possible to discuss the functional role of TV- and RV-cells in an experimentally well-established framework. TV-cells might be involved in lift or pitch control and RV-cells in stabilization against rotation of the animal around its longitudinal body axis.

The TO-cells appear to be well suited to mediate translational optomotor responses. They respond to unilateral pattern movement in a horizontal direction as well as to TPM along the longitudinal axis. In the companion paper (Kern and Varjú 1998) it was shown that the hummingbird hawk moth responds to this type of translational pattern motion by flying back-and-forth. Therefore, TO-cells might be involved in the control of the distance of the moth to a flower. Unfortunately, the translational optomotor system of *Macroglossum* has not been characterized at the behavioural level with respect to motion along oblique axes to which TO-cells respond best. In addition, it is unknown how the head and, thus, the eyes of the animal are oriented with respect to the horizontal plane during optomotor responses. The suggestion that TO-cells might be involved in the control of the distance to the flower is corroborated by the observation that TO-cells not only respond to motion of wide-field patterns on either side of the animal but also to small hand-held probes in the fronto-ventral region of the visual field (cardboard disk, diameter 2 cm). Whereas TO1-cells increase their firing rate during motion of the probe towards the animal, TO2-cells increase their firing rate during motion away from the animal. Unfortunately, this response could not be analysed systematically owing to methodological limitations. Note that in free flight *Macroglossum* responds to an approaching, respectively, retreating dummy flower (diameter 2 cm) by moving back- and-forth (Pfaff and Varjú 1991; Farina et al. 1994).

Although it is tempting to conclude that TO-cells are elements of a system controlling the distance to flowers while the animal is feeding on them, this conclusion cannot be drawn without qualifications. If the sensitivity gradients within the receptive field of both the TO1- and TO2-cells are compared to the sensitivity gradients determined in the behavioural experiments (Introduction; Kern and Varjú 1998), some discrepancies are obvious. First, TO1-cells seem to be more sensitive in ventral areas of the visual field, whereas stimulation of the dorsal and ventral areas, respectively, elicits behavioural responses of about the same strength. This aspect of the behavioural response is in agreement with the sensitivity distribution of TO2-cells along the vertical extent of the visual field. On the other hand, stronger responses are elicited in TO1-cells when the stimuli are located in fronto-lateral rather than lateral regions of the visual field, a finding that matches the behavioural results. In contrast, TO2-cells are equally sensitive to motion along the horizontal axis in all parts of the receptive field that could be tested. In conclusion, the response properties of TO1- or TO2-cells on their own coincide only partly with the behaviourally determined response properties of the translational system. However, combinations of the activities of both cell classes are conceivable that match those features.

In the lobula plate of *Macroglossum* monocular neurons have been found that are most sensitive to motion along an oblique axis (Wicklein 1994). Part of these cells from both halves of the brain might represent the input elements of the binocular TO-cells found in the cervical connectives. In contrast to other cells charac-

terized in the same study, these lobula plate neurons are reported not to respond to approaching or retreating cardboard disks of approximately 2 cm diameter (Wicklein 1994). If these lobula plate cells are the input elements of the TO-cells, the response of the latter to approaching and retreating hand-held probes must be a result of binocular interactions.

RH-cells are highly specific for the visual consequences of rotations of the animal around its vertical body axis. Moreover, the sensitivity distribution within their receptive fields matches those determined for the behavioural responses to RPM (Introduction; Kern and Varjú 1998). Thus, these cells might be involved in stabilizing the position of the animal against rotations about the vertical axis. However, one qualification has to be made: under some stimulus conditions the response of RH-cells to PBM is enhanced compared to the prediction based on the responses to unilateral stimulation. Such an enhancement has not been observed in the behavioural response to RPM. Enhancement of the cells' response to a composite stimulus is found only when the responses to the partial stimuli and thus the predicted response are comparatively weak (Kern 1994). Therefore, it is suggested that, if RH-cells are involved in mediating the behavioural responses to RPM, then the firing rate of RH-cells should always have been high when the animals responded to the stimuli in the behavioural experiments.

Although a final assessment of the functional role of the motion-sensitive neurons in the cervical connectives of *Macroglossum* is not yet possible, at least the RH-cells are likely to play a decisive role in mediating compensatory responses of the animal to rotational disturbances.

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