J. exp. Biol. **148**, 255–279 (1990)
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RESPONSE CHARACTERISTICS OF FOUR WIDE-FIELD MOTION-SENSITIVE DESCENDING INTERNEURONES IN *APIS MELLIFERA*

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Accepted 21 August 1989

Summary

The anatomical projections and directional tuning of four descending interneurones sensitive to wide-field motion over the compound eyes are described. The cells are slow to adapt, resistant to habituation and their responses are dependent on the contrast frequency of the periodic patterns used as stimuli. Two of the cells ($DNIV_2$ and $DNIV_4$) are maximally stimulated by movement around the longitudinal axis of the bee (simulated roll), one ($DNII_2$) by movement around the horizontal axis (simulated pitch) and one ($DNVI_1$) by movement around the vertical axis (simulated yaw). The cells are binocular, their directional response being shaped by the interaction of the inputs from each eye. The cells which respond predominantly to roll ($DNIV_2$ and $DNIV_4$) have their arborizations restricted to the ipsilateral side of the brain and thoracic ganglia, i.e. the side which contains the cell soma. The cell responding to pitch ($DNII_2$) has its arborizations distributed bilaterally, invading similar regions of the neuropile in both sides of the brain and thoracic ganglia. The cell which responds to yaw ($DNVI_1$) has its major dendritic field in the ipsilateral side of the brain and descends into the thoracic ganglia in the contralateral side. The majority of its arborizations in the thoracic ganglia are confined to the contralateral neuropile.

Introduction

Descending interneurones (DNs) that are directionally selective to wide-field motion over the compound eyes have been reported in a number of insects, e.g. moths and butterflies (Collett and Blest, 1966; Rind, 1983*a*; Singarajah, 1988), locusts (Kien, 1974; Rowell and Reichert, 1986; Hensler, 1988) and dragonflies (Olberg, 1981*a*,*b*). They have also been briefly reported in the bee (Fletcher *et al.* 1984; Goodman *et al.* 1987). Neurones exhibiting this type of response can report course deviations to the thoracic motor centres and thus may contribute to visually mediated course control (Reichert and Rowell, 1985; Reichert *et al.* 1985).

Course deviations in a flying insect will lead to both rotational and translatory

Key words: insect vision, motion-sensitive interneurones, directional tuning, contrast frequency, honeybee brain.

image motion over the compound eyes. Examining the directional tuning of descending interneurones with stimuli which simulate rotational and translatory motion may give a more comprehensive picture of each neurone's directional properties. It is also important to know whether the velocity response characteristics of putative optomotor neurones match those of optomotor responses that have been measured in behavioural experiments. Here we give an account of the anatomy, velocity response characteristics and directional tuning (during simulated rotational and translatory motion) of four descending interneurones in *Apis mellifera*. The effect of ipsilateral and contralateral visual inputs to each neurone is also examined.

Materials and methods

Preparation

Adult worker honeybees, *Apis mellifera*, engaged in active foraging, were collected from the hive entrance for this study. After brief carbon dioxide anaesthesia and removal of the wings, the bees were placed ventral surface up into a metal holder and the thorax and abdomen secured using strips of tape and sticky wax. The neck membrane was revealed by tilting and securing the head, and the cervical connectives and prothoracic ganglion were exposed by dissection. After cutting the nerve roots from the prothoracic ganglion, the recording site was stabilized by inserting a strip of Parafilm beneath the cervical connectives and waxing the strip to the thoracic cuticle. This procedure allowed extracellular recordings to be made for up to 1 h.

Electrophysiology

The recording electrodes, drawn from thin-walled filamented glass capillaries (Clark Electromedical GC100 TF-10; o.d. 1.0 mm, i.d. 0.7 mm), were filled with 3 mol l^{-1} cobaltous chloride solution and had tip resistances of between 20 and 40 MΩ when in contact with the tissue. They were inserted into the cervical connectives slightly anterior to the prothoracic ganglion. No subthreshold neural activity could be detected when recording from this site. Glass indifferent electrodes were drawn from thick-walled glass and filled with bee saline (47 mmol l^{-1} NaCl, 27 mmol l^{-1} KCl, 18 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$; Florkin and Jeuniaux, 1964). These were positioned in the exposed thorax just posterior to the prothoracic ganglion. Signals were amplified (WPI M-707), displayed on a storage oscilloscope (Tektronix 5111N) and stored on tape (Racal Store 4).

Stimulation

The bee was stimulated by the movement of either a large, square-wave grating of black and white stripes attached to the outer surface of a drum (100 mm in diameter and 110 mm in length) or by two gratings which formed flat belts rotating about vertical axles 110 mm apart (Fig. 1). The drum-mounted grating could b

positioned frontally, stimulating the frontolateral region of both eyes, or laterally, stimulating one eye. It was positioned such that the mid-point of its axis of symmetry was aligned with either the longitudinal axis (LA) or horizontal axis (HA) of the head, with the surface of the eye 30 mm from the grating. The belt-mounted gratings were positioned laterally, one on each side of the head, with the centre of the grating patterns aligned with the HA of the head. Each lateral belt was positioned 30 mm from its respective eye. The drum subtended 78° at the eye and each lateral belt subtended 122°. Each lateral belt stimulus was viewed only by the eye adjacent to it. The bee could be stimulated either by the drum-mounted grating or by one or both of the lateral belt gratings, but not simultaneously by all three. Frontal or lateral patterns not in use were swung out of the test area.

The gratings mounted on the drum or the belt could each be moved back and forth through the visual field of the insect, driven by reversible d.c.-servomotors whose speed was held constant by an electronic feedback mechanism. The speed of movement of these gratings could be varied from 5 to 1150° s^{-1}, as subtended at the eye. Between each presentation of the moving patterns, the drum-mounted grating could be turned about either the LA or HA of the head and the belt-mounted gratings about the HA, using stepper motors. The drum was turned in steps of 20° and the lateral belts in steps of 22.5°. The LA or HA was always aligned with the centre of the stimulating pattern, regardless of the direction in which the pattern was moving. The direction, velocity and angle of presentation of the patterns were controlled by a microcomputer (BBC B), *via* interfacing units, allowing a programmed sequence of directional stimuli to be presented to the bee. The timing of each event was controlled by a quartz clock-driven pulse generator (Digitimer 3290) (Patterson and Pomfrett, 1988).

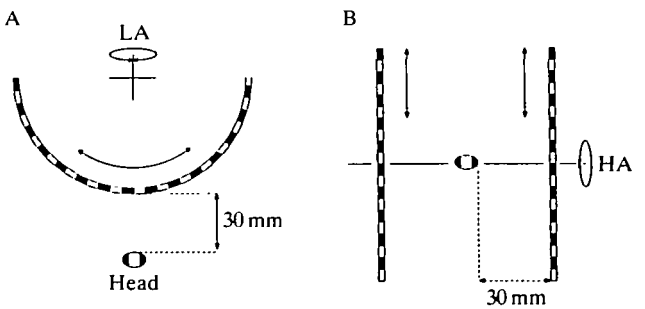

Fig. 1. (A) Visual stimulation of the bee was provided by a striped pattern attached to the outer surface of a drum 100 mm in diameter which could be moved in either direction. The drum could be positioned frontally or laterally (stimulating one eye only) and could be turned through 180° about the longitudinal axis (LA) or horizontal axis (HA) of the bee's head in steps of 20°. (B) Stimulation could also be provided by two striped patterns attached to non-slip, motor-driven belts, placed one on each side of the head. Each pattern could be moved forwards or backwards and could be turned through 180° about the HA of the bee's head in steps of 22.5°. The bee's head was positioned 30 mm from the drum and lateral belt stimuli.

Stimulus regimes

The directional tuning of the cells was tested by stimulating in the following ways. (a) Rotational motion was simulated by moving the lateral gratings simultaneously in opposite directions. (b) Translational motion was simulated by moving the lateral belts simultaneously in the same direction. (c) The response to monocular stimulation was examined by moving each lateral grating independently. (d) The response to frontal binocular stimulation was examined by moving the drum stimulus through the anterior visual field. The stimulus sequence consisted of: 1 s of grating motion in one direction, an interstimulus interval of 1 s, followed by 1 s of motion in the reverse direction. The gratings were then automatically turned into a new position and, after a 1 s interval, the stimulus sequence was repeated until the gratings had been presented at predetermined angles throughout 360°.

The velocity response characteristics were examined using either the drum-mounted or belt-mounted gratings. The stripes were aligned perpendicular to the mean preferred direction of each cell and different grating speeds were presented non-systematically. The bee was presented with patterns which had spatial wavelengths of 36°, 19° or 15°, as measured at the centre of each grating.

Data analysis

Signals were analysed on-line, in addition to being stored on tape, making use of an interface (Versatile Laboratory Aid: Data Harvest Ltd) between the amplifier and the microcomputer. The interface counted spikes during each experiment and transferred data to the microcomputer which was programmed to associate each incoming data block with the appropriate stimulus presentation. The response frequency was calculated and displayed as a polar plot of spike frequency against the angle of stimulus presentation (for further details of polar plot presentation see legend of Fig. 12). The response frequency was calculated as the number of spikes in the 1 s period of motion minus the spontaneous activity of the cell. The spontaneous activity was averaged from a 5 s period of recording at the beginning and end of each experimental test.

The preferred direction of motion for those cells whose excitatory response was polarized in one overall direction was obtained by calculating the mean vector angle (MVA) of the circular histogram (Batschelet, 1981). A correction for 20° sample grouping was applied to the data. The MVA was used to compare the directional preferences of the same cell in different preparations or to examine the directional preference of a single cell at intervals during extended stimulation. Statistical significance of directedness was determined by Rayleigh's test (Batschelet, 1981). Each cell showed characteristic differences in the size of the response to binocular and monocular stimulation. To test if these differences were significant (e.g. if the response to simulated roll was greater than the response to simulated translatory motion) the total excitatory response obtained during binocular and monocular stimulation in each preparation was averaged and compared using a two-tailed *t*-test.

Anatomical investigation

Cobalt chloride was injected into a neurone at the end of an experiment by passing a square-wave current of between 1×10^{-8} and 5×10^{-8} A at 2 Hz through the recording electrode for a period of 5 min. After 30 min of diffusion time the brain and thoracic ganglia were removed under bee saline. The tissue was placed in ammonium sulphide (0.5 % by volume in saline) for 5 min and fixed in FAA (12 ml of 40 % formalin, 80 ml of 100 % ethanol, 8 ml of acetic acid) for 1 h. The intact ganglia were then intensified (Bacon and Altman, 1977). Intensified cells were drawn in whole-mounted preparations and viewed from the dorsal aspect before embedding in Araldite and sectioning at 25 μm intervals. Whole-mount and sectioned preparations were drawn using a *camera lucida* attachment on a Zeiss photomicroscope.

Sections were viewed using Nomarski differential interference contrast, which revealed the axon in cross-section and many of the longitudinal tracts. The drawings presented (Figs 2B, 3B, 4B and 5B) show the most prominent tracts in order to orientate the reader. The nomenclature for the tracts was based on that adopted for the locust thoracic ganglia (Tyrer and Gregory, 1982) and the bee prothoracic ganglion (Rehder, 1988).

Results

Anatomy

The brain arborizations of 12 descending neurones (DNs) sensitive to wide-field motion have been briefly described in the bee and the cells classified into six groups (groups DNI to DNVI) on the basis of similarities in their morphology within the brain (Goodman *et al.* 1987). In this paper we give the first detailed description of the morphology of four cells from three of these groups within the brain and the thoracic ganglia. We have followed the usage of Strausfeld and Bacon (1983) and Strausfeld *et al.* (1984) in regarding all neuropile adjoining the optic pedunculi as deutocerebral. Throughout this account the half of the brain in which the cell soma lies is termed ipsilateral and the compound eye on that side is termed the ipsilateral eye.

$DNIV_2$ and $DNIV_4$

Cells $DNIV_2$ and $DNIV_4$ belong to a group of cells whose arborizations are confined to the ipsilateral half of the brain and thoracic ganglia. The two cells arborize in the posterior deutocerebrum ($DNIV_4$ lying more anterior than $DNIV_2$) with their somata lying adjacent in the lateral deutocerebral rind (Figs 2A,C, 3A,C). They both send fine branches into the ocellar tract, lying closely opposed to the large axons of the ocellar L and L_D neurones. $DNIV_2$ gives off a number of blebbed collaterals in the tritocerebrum and in the suboesophageal ganglion that extend ipsilaterally into the ventrolateral neuropile (Fig. 2A,C). Both cells descend through the ventral connective and prothoracic and fused mesometathoracic ganglia within the median dorsal tract (MDT) (Figs 2B, 3B). The cells have

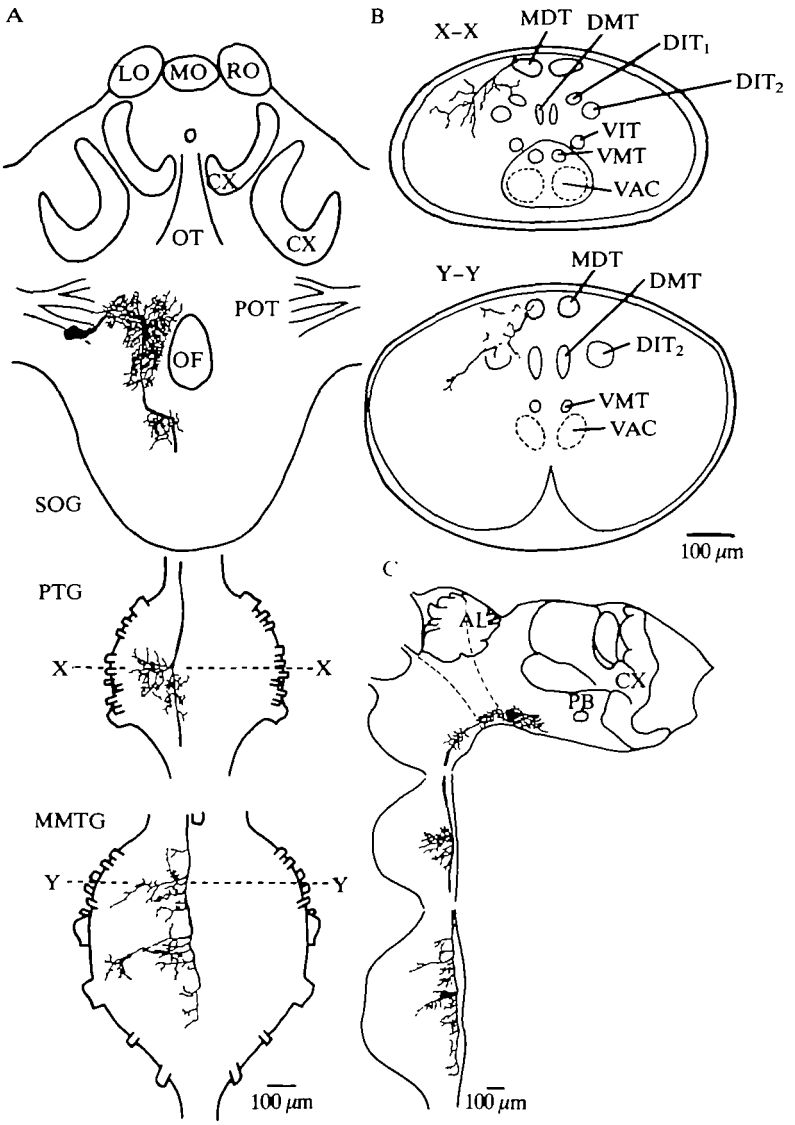

Fig. 2. The projections of cell $DNIV_2$ within the brain and thoracic ganglia, drawn from (A) the dorsal and (C) the lateral aspects. The arborizations of $DNIV_2$ are confined to the ipsilateral half of the brain and thoracic ganglia. (B) Drawings of 25 μm sections taken at X–X and Y–Y through the prothoracic and mesothoracic ganglia showing the position of the axon within the median dorsal tract (MDT) and the extent to which the thoracic neuropile is invaded by the branches of $DNIV_2$. AL, antennal lobe; CX, calyx; LO, MO, RO, left, median and right ocelli; OF, oesophageal foramen; OT, ocellar tracts; PB, protocerebral bridge; POT, posterior optic tracts I, II and the posterior optic commissure; SOG, suboesophageal ganglion; PTG, prothoracic ganglion; MMTG, mesometathoracic ganglia; DIT_1 and DIT_2, dorsal intermediate tract 1 and 2; DMT, dorsal median tract; VIT, ventral intermediate tract; VMT, ventral median tract; VAC, ventral association centre; area enclosed by dashed lines in C indicates the oesophageal foramen.

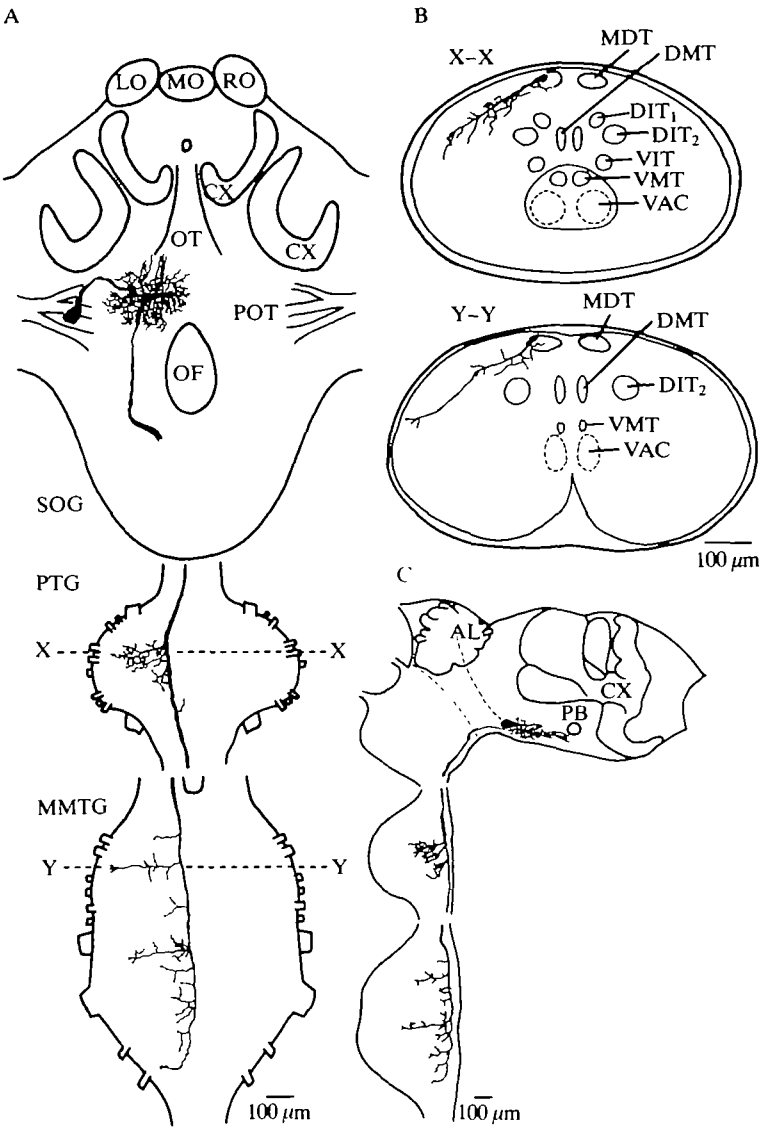

Fig. 3. The projections of cell $DNIV_4$ within the brain and thoracic ganglia drawn from the dorsal (A) and lateral (C) aspects. The arborizations of the cell are confined to the ipsilateral half of the brain and thoracic ganglia. Fine branches extend into the base of the ocellar tract. (B) Drawing of 25 μm sections through the prothoracic and mesothoracic ganglia taken at X–X and Y–Y and showing the axon travelling in the MDT. Abbreviations as in Fig. 2.

very similar arborization patterns within the ganglia. In each cell, one conspicuously large branch extends laterally and ventrally in the prothoracic ganglion, terminating in many blebbed dendrites (Figs 2A, 3A). Numerous branches are given off in the mesometathoracic ganglion, including a large branch in each of the

mesothoracic and metathoracic segments which extends as far as the lateral wall of the ganglion. The cells terminate in the neuropile of the first two abdominal ganglia which are fused to the posterior region of the mesometathoracic ganglion (Markl, 1966).

$DNII_2$

Cell $DNII_2$, in contrast to $DNIV_2$ and $DNIV_4$, branches extensively in both halves of the brain, suboesophageal ganglion and prothoracic and mesometathoracic ganglia. The trunk of its dendritic tree arches above the oesophageal foramen and there are extensive arborizations in the median posterior deutocerebral neuropile on both sides, extending into the lateral deutocerebrum towards the base of the lobula in the ipsilateral side of the brain (Fig. 4A,C). The soma lies adjacent to the lateral edge of the protocerebral bridge. Above the oesophageal foramen, the cell extends fine dendrites anteriorly into both sides of the ocellar tract. Large blebbed collaterals which arborize in both halves of the dorsal neuropile of the tritocerebrum and suboesophageal ganglion are also given off by the axon as it descends into the ipsilateral cervical connective.

The axon of $DNII_2$, like those of $DNIV_2$ and $DNIV_4$, descends through the thoracic ganglia in the MDT (Fig. 4B). However, unlike $DNIV_2$ and $DNIV_4$, this cell shows an extensive and strikingly symmetrical pattern of branching in the prothoracic and mesometathoracic ganglia (Fig. 4A). In the mesometathoracic ganglion one large branch leaves the axon in the region where the metathoracic segment begins and crosses into the contralateral MDT, where it travels through the metathoracic segment, terminating in the neuropile of the first two abdominal ganglia. The main axon of this cell extends into the abdominal ventral connective for at least another 50 μm.

$DNVI_1$

Cell $DNVI_1$ is one of two morphologically similar descending interneurones that have their soma in the suboesophageal ganglion (Goodman *et al.* 1987). The major arborizations of this cell lie within the ipsilateral posterior deutocerebrum (Fig. 5A), but as the axon crosses beneath the gut it sends a collateral into the contralateral posterior deutocerebrum where it arborizes to produce a small subfield. The cell also gives rise to a small ipsilateral dendritic field in the dorsal neuropile of the suboesophageal ganglion. The axon of $DNVI_1$ descends contralaterally into the thoracic ganglia and distributes its dendrites in the medial region of the contralateral thoracic ganglia. The axon travels in the thoracic ganglia in the dorsal intermediate tract (DIT). The DIT can be separated into four different tracts, DIT_{1-4} (Rehder, 1988), and $DNVI_1$ runs in DIT_1 (Fig. 5B). In the prothoracic ganglion, branches arising from the axon project into the dorsolateral neuropile (Fig. 5A,C). A number of branches project medially but only one crosses over into the other half of the ganglion. The axon was traced into the fused mesometathoracic ganglion for about 400 μm and some branches were observed. We were unable to trace it further into the ganglion, which may be partly becaus

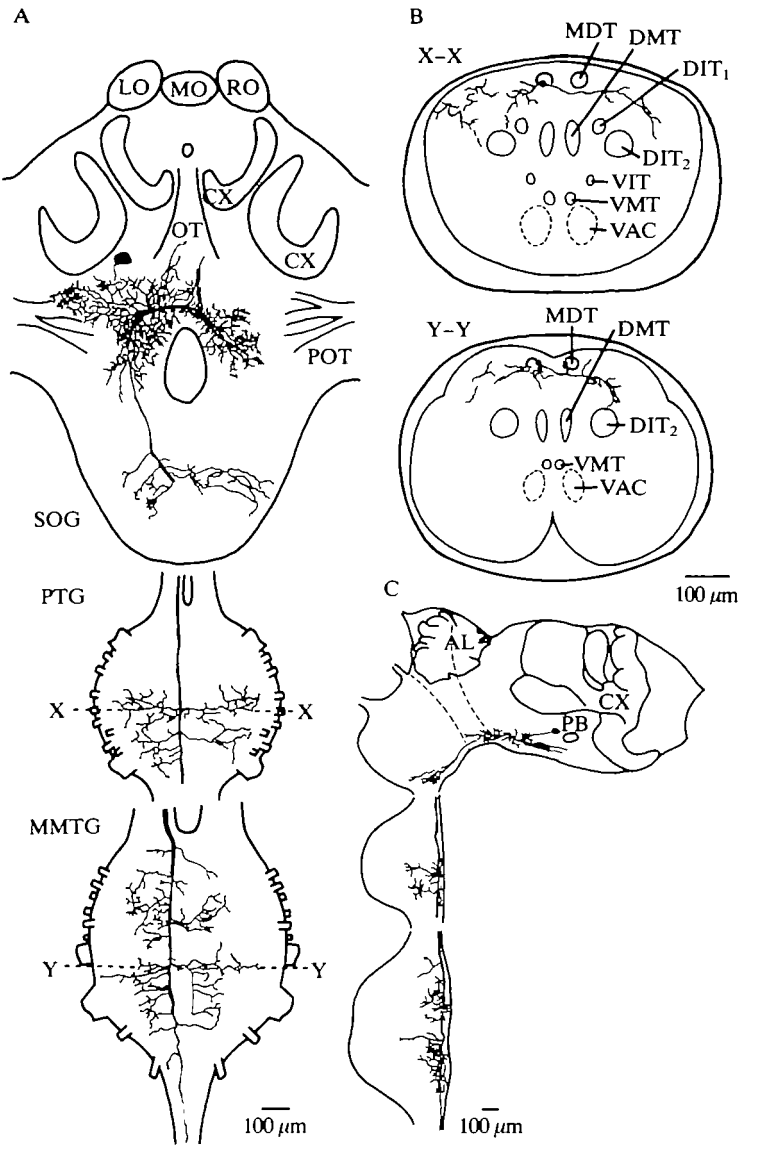

Fig. 4. The projections of cell $DNII_2$ within the brain and thoracic ganglia viewed from (A) the dorsal and (C) the lateral aspects. This cell branches extensively on both sides of the brain and suboesophageal ganglion. It sends fine branches up into the ocellar tracts. It invades similar regions in both halves of the thoracic ganglia. The axon continues into the abdominal ventral connective. (B) Sections through the prothoracic and metathoracic ganglion, at X–X and Y–Y, show the axon descending in the MDT. Large-diameter collaterals are sent out into each half of the thoracic neuropile. Abbreviations as in Fig. 2.

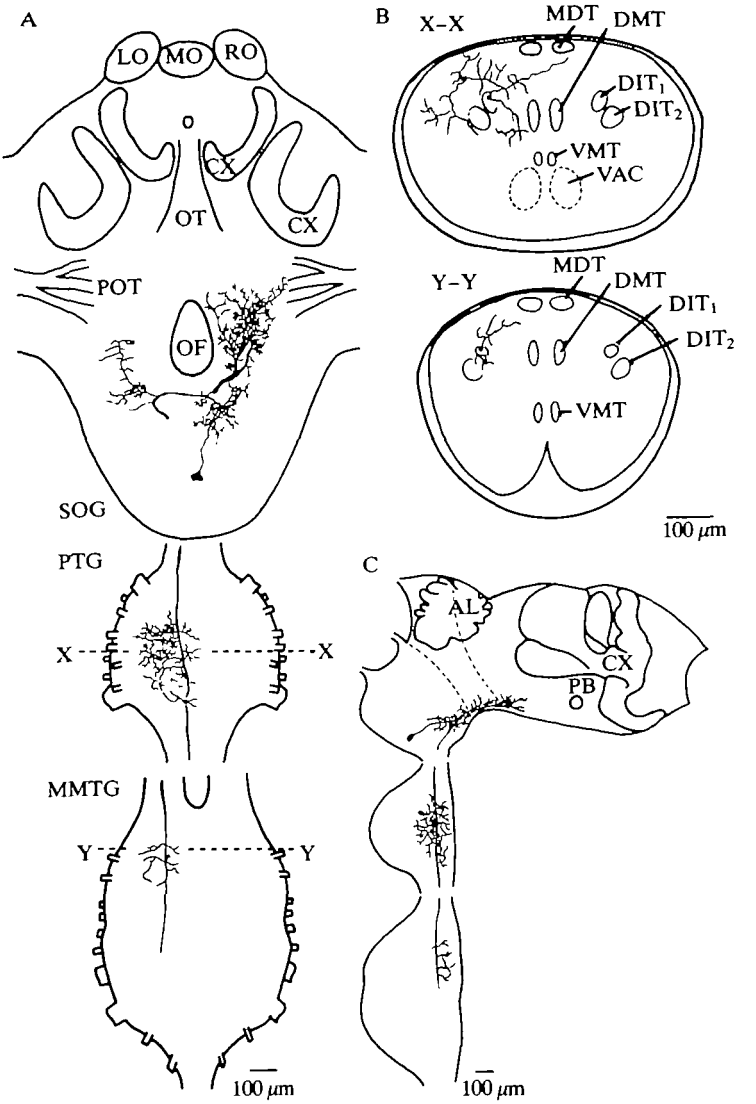

Fig. 5. The projections of cell $DNVI_1$ within the brain and prothoracic ganglion together with a partial fill in the mesometathoracic ganglion, viewed from (A) the dorsal and (B) the lateral aspects. The soma lies in the suboesophageal ganglion. The cell arborizes extensively in the ipsilateral half of the brain and suboesophageal ganglion. It crosses the brain and sends collaterals into the contralateral side of the brain before descending in the contralateral connective. Its arborizations are restricted mainly to the ipsilateral side of the thoracic ganglia. (B) Sections through the prothoracic and mesothoracic ganglia, at X–X and Y–Y, show that the axon descends in DIT_1. Abbreviations as in Fig. 2.

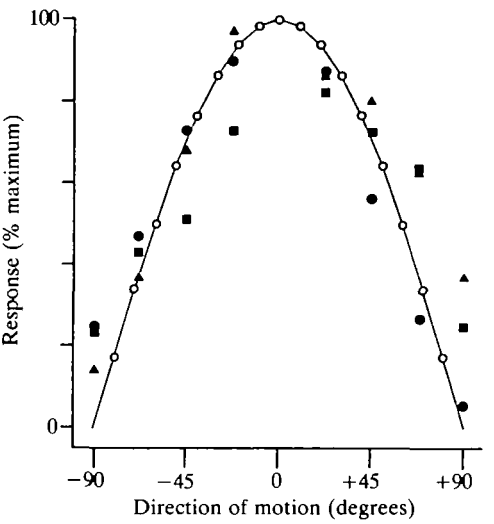

Fig. 6. Motion-induced responses of $DNIV_2$ (●), $DNIV_4$ (▲) and $DNVI_1$ (■) as a function of the direction of motion. In this figure 0° indicates the preferred direction of the cells. The response curves approximate to a cosine function (○). Each point is derived from 2–5 tests in three cells. The values are normalized with respect to the maximal response in each cell.

the axon of this cell is smaller in diameter (around 4 μm) than are the three cells previously described (whose diameters range from 7 to 12 μm).

General response characteristics of the four neurones

Each of the four cells described gives a directionally sensitive response to the motion of wide-field square-wave gratings, the spike frequency of the response depending on the angle at which the gratings are presented. All the cells give a broadly tuned response, usually being excited by motion throughout at least 90°, with response curves approximating cosine functions (Fig. 6). Movement in the non-preferred or null direction may result in inhibition of the cell's spontaneous activity. There is some variation in the level of a particular cell's response when recorded in different bees, but the overall preferred direction of the cell remains constant from preparation to preparation and within the same preparation over periods of up to 30 min (Fig. 7).

The excitatory response of $DNII_2$, $DNIV_2$ and $DNVI_1$ to movement of square-wave gratings in their preferred directions is dependent on the contrast frequency (CF) of the pattern. The CF response function of the fourth cell, $DNIV_4$, was not fully characterized. In $DNII_2$, $DNIV_2$ and $DNVI_1$ the spike rate increases nearly linearly when plotted against logCF up to a value of 8–11 Hz. Further increases in the CF lead to a decrease in the spike rate (Fig. 8). The graphs presented in Fig. 8 were constructed from measurements made in the first 1.0 s of stimulation. To establish if these represent the non-adapted response, CF response functions

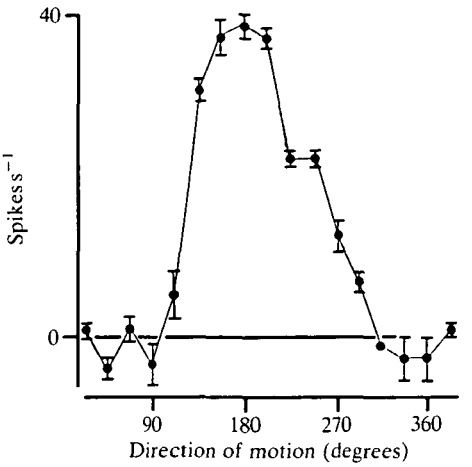

Fig. 7. The directional tuning of the cells does not alter significantly ($P<0.01$) over periods of up to 30 min, as illustrated for $DNIV_2$. In this figure, 180° represents downward motion over the ipsilateral eye. Values indicate the mean of five responses in one cell, taken at intervals during a 30 min period. Bars indicate ±S.D.

derived from the first 1.0 s were compared with those derived from the instantaneous response (i.e. the response in the first 0.1 s). There was no significant difference between the CF response function measured by either method (*t*-test, $P<0.05$) (Fig. 9). With the patterns used, the shape of the CF response function is independent of the spatial wavelength of the periodic pattern. This can be seen by comparing the CF response function derived when using a 19° and a 36° spatial wavelength pattern (compare Fig. 8A,B with D,E). However, the spatial period does affect the absolute spike rate at any given CF. For example, in $DNII_2$ the response induced by moving the stimulus at 9 Hz, with the spatial wavelength of the grating set at 19°, is only 80% of the response induced when the grating wavelength is 36° (Fig. 8A,D).

The shape of the motion-induced response, as a function of the direction of motion, is unaffected by changing the velocity of the stimulating pattern. To examine this the directional tuning of the cells was tested with the pattern motion set at 90°s^{-1} (6 Hz), 180°s^{-1} (12 Hz) or 300°s^{-1} (20 Hz). The absolute response magnitudes vary as the velocity of the pattern is altered, in accordance with the CF-dependency of the cells, but there is no change in the mean preferred direction or the shape of the response curve (Fig. 10). Fig. 8A–E shows that there is a

Fig. 8. Contrast frequency responses of $DNII_2$ (A), $DNVI_1$ (B) and $DNIV_2$ (C) determined with a spatial period of 36°. Contrast frequency responses of $DNII_2$ (D) and $DNVI_1$ (E) at a 19° spatial period. Closed circles represent the response induced by motion in the preferred direction and open circles the response to motion in the null direction. The dashed line represents the mean spontaneous activity of the cells. All points were calculated from responses in seven cells and are normalized to allow comparison. Bars are ±S.D.

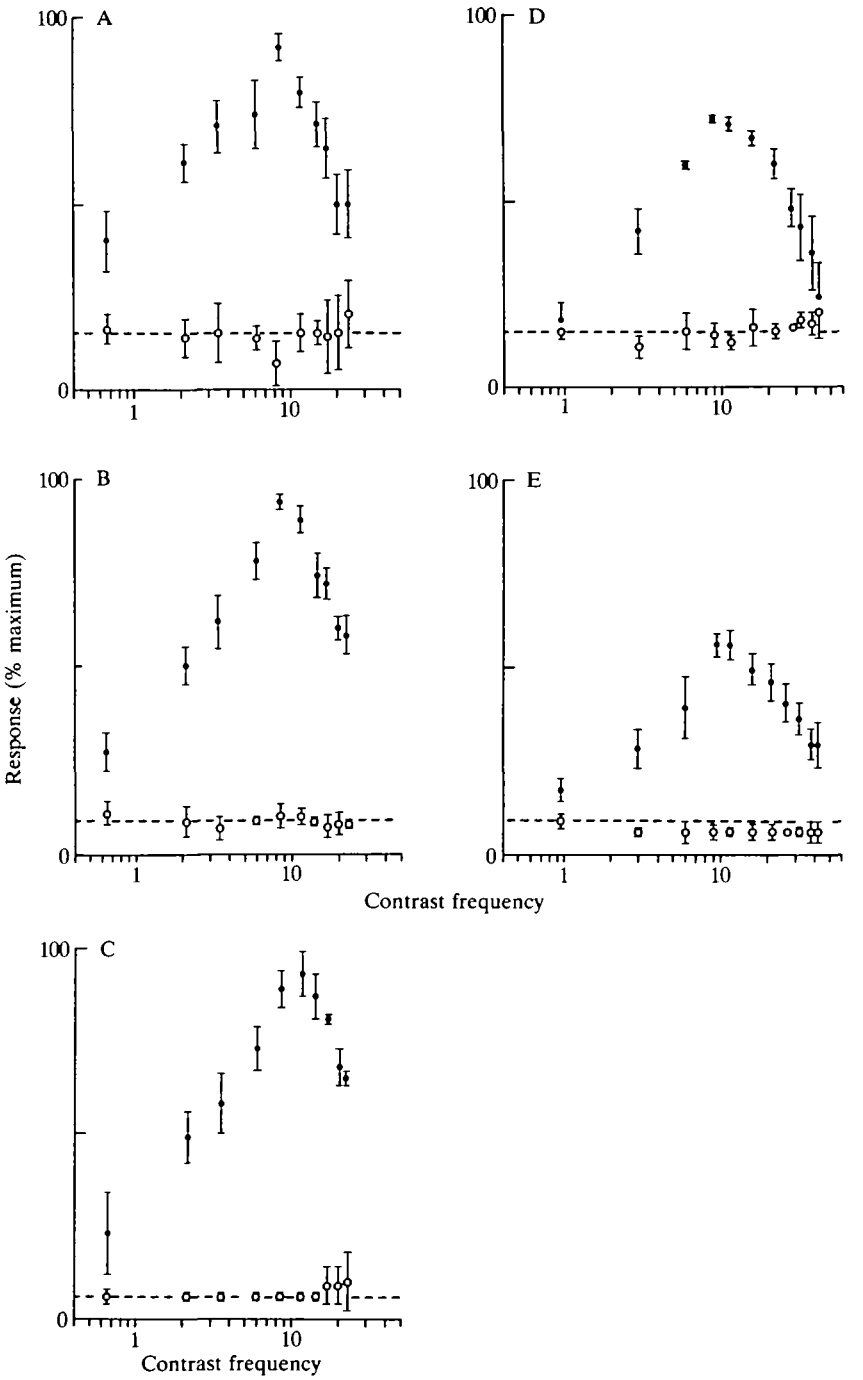
A
B
C
D
E
100
0
1
10
Response (% maximum)
Contrast frequency
Contrast frequency

Fig. 8

marked separation between the response to motion in the preferred and null directions over the range of CFs tested.

Continuous motion in each cell's preferred direction results in a rapid reduction in firing rate over the first few seconds, followed by a slower decline over a period of about 6–9 s when tested at 9 Hz CF (Fig. 11A–D). After the initial decline in

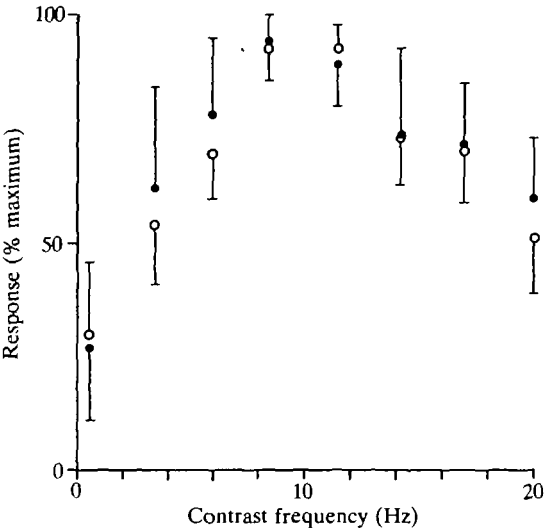

Fig. 9. Contrast frequency responses of $DNVI_1$ determined in two ways. The solid circles were derived from the spike rate in the first second of stimulation and the open circles from the instantaneous responses of the cells (i.e. first 0.1 s). Bars indicate ±S.D.

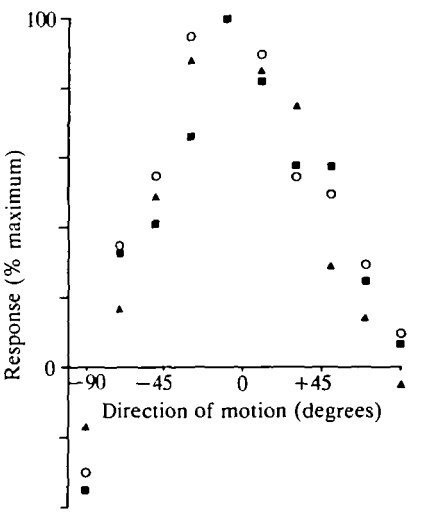

Fig. 10. The directional selectivity of cell $DNII_2$ at $90°s^{-1}$ (6 Hz, ○), $180°s^{-1}$ (12 Hz, ●) and $300°s^{-1}$ (20 Hz, ▲). Each point represents the mean of three responses in one identified cell and is normalized to allow comparison. 0° represents upward vertical motion.

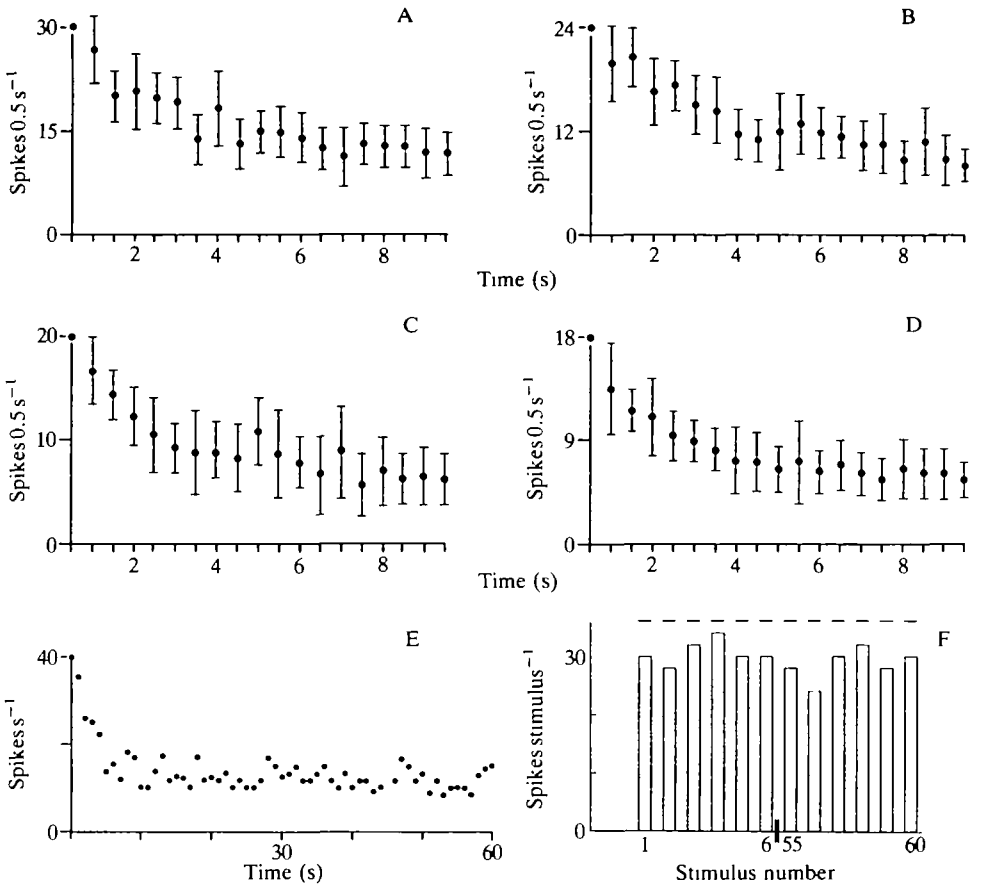

Fig. 11. The response of cells $DNII_2$ (A), $DNVI_1$ (B), $DNIV_2$ (C) and $DNIV_4$ (D) to continuous motion of the striped pattern in their preferred directions at 9 Hz. Each point is derived from the mean of three responses in one cell. Bars represent ±S.D. During the first few seconds the firing rate falls until a steady state is reached which persists for at least 60 s (E). All the cells respond consistently to repeated 1 s presentations of pattern motion at 1 s intervals. This is illustrated for $DNIV_2$ over a 60 s period (F). Top bars indicate periods of motion.

the rate of spike production a steady-state response level is attained. This has been shown to persist for periods of up to 60 s, which is the longest period tested (Fig. 11E). The cells are resistant to habituation, responding with a consistent level of excitation to successive 1 s presentations of grating motion in the preferred diection at 1 s intervals over a period of 60 s (Fig. 11F).

Directional tuning of the four cells

Two cells directionally selective for motion about the longitudinal axis ($DNIV_2$ and $DNIV_4$)

Both $DNIV_2$ and $DNIV_4$ give a large, significantly directional, excitatory

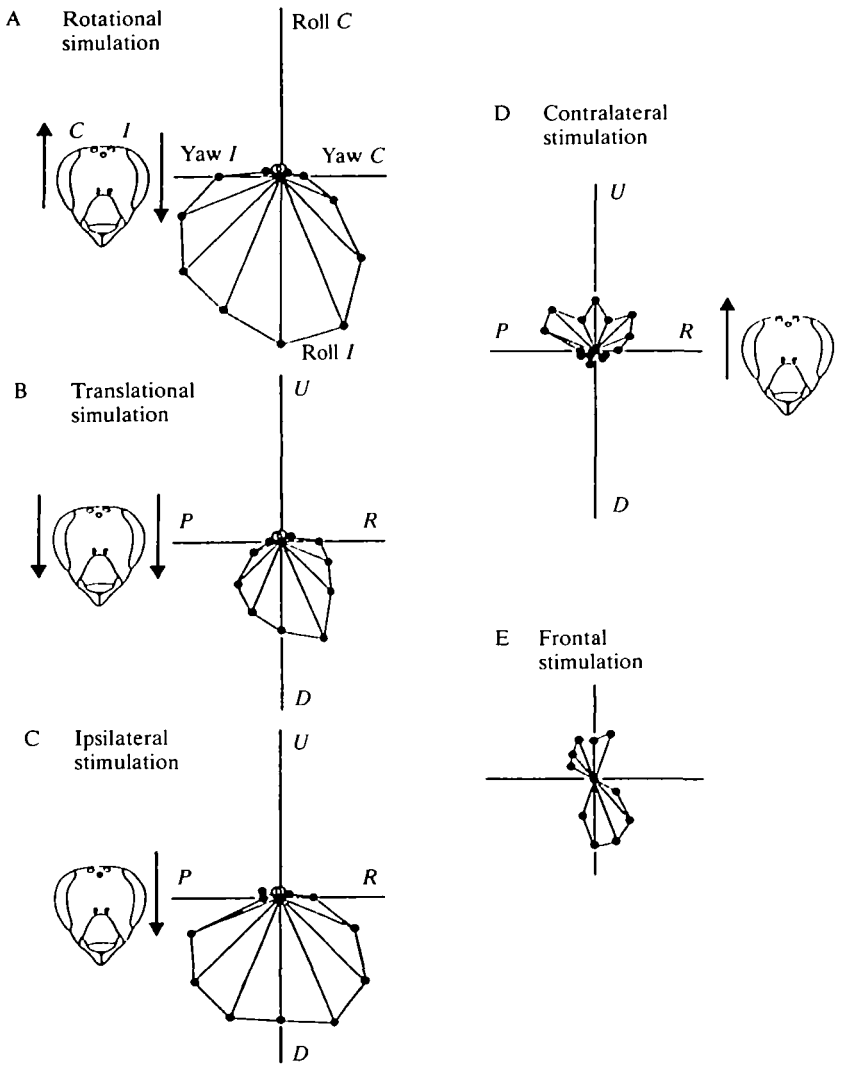

Fig. 12

response when the lateral gratings move simultaneously in a downward direction over the ipsilateral eye and in an upward direction over the contralateral eye (Rayleigh's test, $P<0.01$) (Figs 12A, 13A). Movement of the gratings in the reverse direction results in some inhibition of the spontaneous activity in $DNIV_2$ and in $DNIV_4$ when there is a resting discharge in that cell.

Monocular stimulation reveals a directional preference for vertically downward motion over the ipsilateral eye and upward motion over the contralateral eye (Figs 12C,D, 13C,D). Stimulation of the contralateral eye in all the preparations examined elicits a significantly smaller response than that obtained by motion in the downward, or preferred, direction of the ipsilateral eye (t-test, $P<0.01$). In $DNIV_4$ the response to motion over the ipsilateral eye alone is not significantly different from the response induced by simulated roll, i.e. when downward motio

Fig. 12. Directional tuning of $DNIV_2$. (A) Response to motion of the lateral gratings in opposite directions, simulating rotational motion. Roll *I*, downward pattern motion over the ipsilateral and upward motion over the contralateral eye; roll *C*, downward motion over contralateral and upward over ipsilateral eye; yaw *I*, progressive (i.e. front to back) motion over ipsilateral and regressive over contralateral eye; yaw *C*, progressive motion over contralateral and regressive over ipsilateral eye. (B) Response to motion when both lateral gratings move in the same direction, simulating translational motion, (C) monocular stimulation of the ipsilateral eye and (D) monocular stimulation of the contralateral eye. *P*, progressive motion of the gratings; *R*, regressive motion; *U*, upward motion; *D*, downward motion. Arrows indicate the form of stimulation, a single arrow indicates monocular stimulation of the eye against which it is shown. Two arrows indicate binocular stimulation and show whether the patterns are moved in the same or in opposite directions. (E) Response to frontal stimulation, presented as if the bee were looking out of the page. *I* indicates the ipsilateral eye (i.e. the eye on the side of the brain in which the cell soma lies) and *C* the contralateral eye. All points are normalized with respect to the maximal response and are means derived from measurements in five preparations. (●) number of spikes above, and (○) number of spikes below, the spontaneous activity level.

over the ipsilateral eye is accompanied by upward motion over the contralateral eye. Upward motion over the ipsilateral eye results in some inhibition of background activity in $DNIV_2$ and $DNIV_4$.

Simultaneous movement of the grating in the same direction over the lateral region of both eyes elicits a response to downward motion in $DNIV_2$ (Fig. 12B). This response to simulated translatory motion is significantly directional for each cell examined (Rayleigh's test, $P<0.01$) and is significantly smaller than the response to simulated rotational motion in the preferred direction or to monocular stimulation of the ipsilateral eye (*t*-test, $P<0.01$). $DNIV_4$ gives a small, but significantly directional, response to downward movements (Fig. 13B). Both cells give a small bidirectional response to frontal binocular stimulation, being excited by both upward and downward motion (Figs 12E, 13E).

A cell that is directionally selective for vertical motion over the frontal region of the compound eyes ($DNII_2$)

$DNII_2$ gives a large, significantly directional, response to upward vertical motion of a grating over the frontal region of both compound eyes. The background activity of the cell is inhibited by downward motion. All forms of lateral stimulation, monocular, simulated rotational or translatory motion yield only a very small response in comparison to that obtained by frontal stimulation (Fig. 14).

A cell directionally selective for horizontal motion ($DNVI_1$)

The most effective stimulus to $DNVI_1$ is one which combines horizontal progressive motion over the eye ipsilateral to the soma with horizontal regressive motion over the contralateral eye. This simulated rotational motion elicits a large excitatory response from the cell (Fig. 15A). Motion in the opposite direction, i.e.

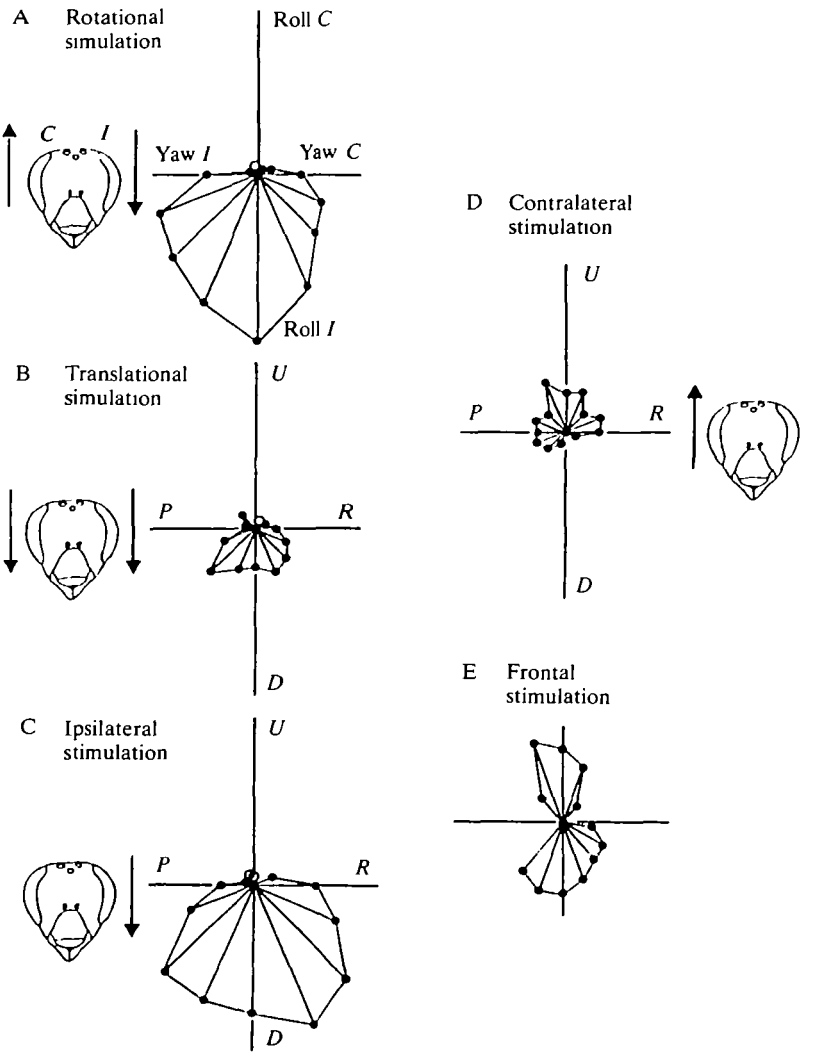

Fig. 13. Polar plots showing the directional tuning of $DNIV_4$. A and B represent the responses to lateral binocular stimulation and C and D show the responses to monocular stimulation. Each point is normalized to the maximal response and is derived from measurements in four separate preparations. (E) The response to frontal stimulation (mean of two cells). Abbreviations as in Fig. 12.

horizontal regressive motion over the ipsilateral eye combined with progressive motion over the contralateral eye, inhibits the background activity of the cell. In the example of $DNVI_1$ illustrated in Fig. 6 the cell is maximally excited by progressive motion over the right eye and regressive motion over the left. Stimulation of the frontal region of both eyes elicits a similar response: excitation when the direction of motion is from the contralateral eye to the ipsilateral eye, and inhibition when motion is in the opposite direction (Fig. 15E).

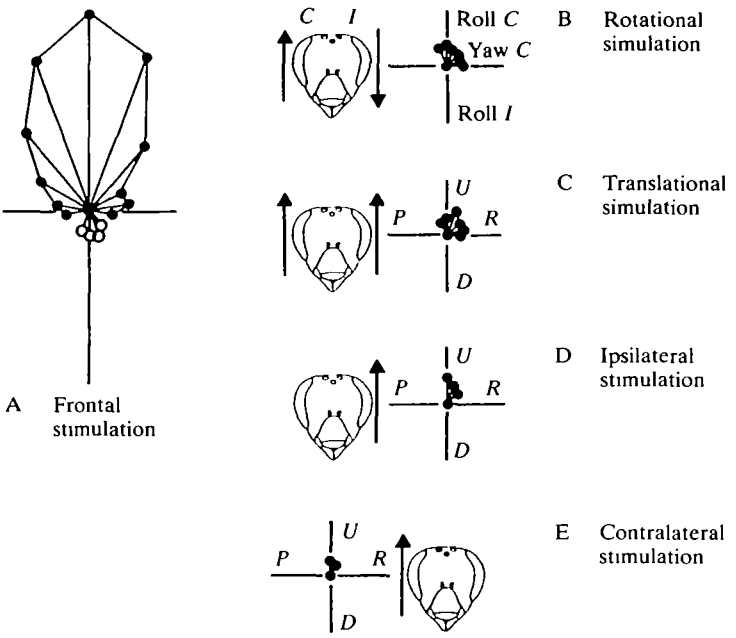

Fig. 14. Polar plots showing the directional tuning of the cell $DNII_2$. Upward motion over the frontal region of the two eyes (A) elicits a large response with inhibition shown to motion in the null direction. Lateral stimulation, whether binocular (B, C) or monocular (D, E) elicits only a small response. All points are normalized to the maximum value and represent the mean responses from three preparations. Abbreviations as in Fig. 12.

Monocular stimulation by the laterally situated gratings reveals a large excitatory response to progressive motion over the ipsilateral eye and a significantly smaller – but highly directionally selective – response to regressive motion over the contralateral eye (t-test, $P<0.01$). Both eyes show a slight inhibition of background activity in response to motion in the opposite direction (Fig. 15C,D). Simulated translatory motion elicits a response to horizontal progressive motion over the two eyes (Fig. 15B). The absolute spike frequency of this response is not significantly different from the response obtained by monocular stimulation of the ipsilateral eye but is significantly smaller than the response to simulated yaw (t-test, $P<0.01$). This would suggest that the contralateral eye has little inhibitory influence over the cell when it is stimulated in its null direction but it does have an excitatory input to the cell when stimulated in its preferred direction.

Discussion

Tuning characteristics

The directional tuning of the four cells described is such that they are maximally stimulated by movement of the visual panorama around the longitudinal ($DNIV_2$

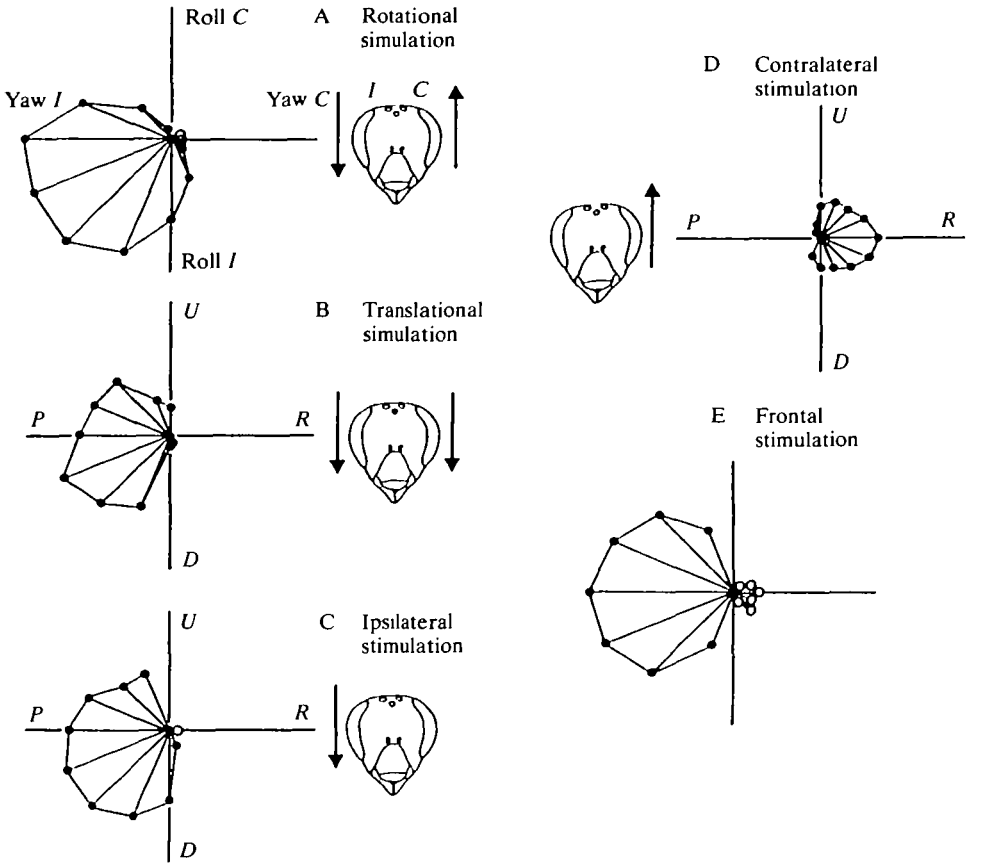

Fig. 15. Polar plots showing the directional tuning of cell $DNVI_1$. The optimum stimulus for $DNVI_1$ is a combination of progressive pattern motion over the ipsilateral eye and regressive motion over the contralateral eye, either over the lateral regions of the eye (A) or over the frontal regions (E). A smaller response is given to progressive motion of the patterns over both eyes (B). Monocular stimulation elicits a response to progressive motion over the ipsilateral eye (C) and a small response to regressive motion over the contralateral eye (D). All points are normalized to the maximal response and represent averages from three preparations. Abbreviations as in Fig. 12.

and $DNIV_4$), vertical ($DNVI_1$) or horizontal ($DNII_2$) axes of the bee. The overall response of each cell appears to be shaped by interactions between the inputs from each compound eye. For example, in $DNIV_2$ an excitatory response to rotation about the longitudinal axis is achieved by combining a maximum sensitivity to downward motion over the lateral region of the ipsilateral eye with sensitivity to upward motion over the lateral region of the contralateral eye. The ipsilateral eye provides the major input to the cell. The response to simulated rotational motion, however, is significantly larger than that to stimulation of the ipsilateral eye alone. Similarly, in $DNVI_1$ an excitatory response to horizontal progressive motion over the ipsilateral eye, combined with an excitatory response to horizontal regressive

motion over the contralateral eye, results in a significantly larger response to motion about the bee's vertical axis than that to motion over the ipsilateral eye alone.

The effect of inhibitory interactions can also be seen: for example, the large response obtained in $DNIV_4$ during monocular stimulation of the ipsilateral eye is significantly reduced when the contralateral eye is stimulated simultaneously by motion in the same direction. This is presumably due to inhibitory inputs that arise from the contralateral eye which, under these circumstances, is being stimulated in the non-preferred direction. Similarly, there is no response to simultaneous upward motion over the lateral regions of both compound eyes in $DNIV_2$ or $DNIV_4$, although the cell responds to motion in this direction when the contralateral eye is stimulated alone. Movement in the null direction (upward) over the ipsilateral eye thus appears sufficient to inhibit the cell's response to motion in the preferred (upward) direction of the contralateral eye.

The function of bee DNs

Various characteristics of the DNs – their directionally sensitive response to wide-field motion, contrast frequency (CF)-dependent response, resistance to habituation and slow rate of adaptation – suggest that they may be involved in course-correction mechanisms in *Apis*. Without further information regarding their action on target neurones we cannot assign them a specific role, but consideration of their individual response characteristics and morphologies allows us to suggest possible functions for further investigation. The observation that cell $DNII_2$ responds to upward motion over the frontal regions of both compound eyes but yields only a very small response to lateral stimulation suggests that it may signal downward pitch to the thorax. During forward flight there is a frontally directed nodal point at which the flow-field over the eyes has a minimum velocity (Collett and King, 1975). Pitch may be more readily detected at this frontal nodal point than in the lateral regions of the eyes, where the image velocity is greatest. Behavioural studies on *Drosophila* show that movement of the visual surround over the frontal regions of the eyes is the most effective stimulus for evoking optomotor pitch responses (Blondeau and Heisenberg, 1982).

The correction of pitching motion requires symmetrical changes to the motor system. The pair of $DNII_2$ neurones distribute their information symmetrically to both halves of the thoracic ganglia, invading the same areas of neuropile in both sides. The DNM neurone of the locust, which has been shown to respond specifically to pitching deviations, has a similar bilateral distribution of branches within the thoracic ganglia (Griss and Rowell, 1986; Rowell and Reichert, 1986). Since course deviations made by an insect will rarely be perfectly symmetrical, the presence of a pair of cells, each sending information to both halves of the ganglia, means that signals derived from both sides can be compared by the thoracic interneurone/motor neurone pool. In this way, banking, for example, could be distinguished from rotation about the horizontal axis.

Correction of roll deviation requires an asymmetrical change in muscular

activity on either side of the body (Srinivasan, 1977) and, while not necessarily implying that their effects are entirely restricted to one half of the ganglion, it is of interest that the arborizations of $DNIV_2$ and $DNIV_4$ are confined to the ipsilateral half of the thoracic ganglia. Both cells are also excited by downward translatory motion, suggesting that they could signal lift. If the thoracic neurone pool monitors the activity of the two pairs of neurones on each side of the ganglia it is possible that $DNIV_2$ and $DNIV_4$ may be involved in both roll and lift correction, although the available evidence from *Drosophila* suggests that lift/thrust control mechanisms are separate from those involving optomotor stabilization (Heisenberg *et al.* 1978; Heisenberg and Wolf, 1984).

$DNVI_1$ is maximally excited by a simulated turn about the insect's dorsoventral axis, responding to progressive motion over the eye contralateral to the side of the body in which the axon descends and regressive motion over the other eye. Its directional properties suggest that it could have a role in compensatory correctional movements of the head or whole body in the yawing plane. The correction of yawing deviations requires an asymmetrical change in muscular activity on each side of the body (Götz *et al.* 1979). $DNVI_1$ crosses the brain and descends in the connective on the inside of a simulated turn, its arborizations being restricted mainly to the contralateral half of the prothoracic ganglion. This suggests that $DNVI_1$'s effect may be to alter muscular activity on the inside of the involuntary turn in such a way as to increase power output. This is in contrast to the yaw-sensitive descending neurone (D1) of *Manduca sexta*, which has similar directional tuning properties to $DNVI_1$ but descends ipsilaterally (Rind, 1983*a*). D1 is believed to induce wing movements that would cause braking on the ipsilateral side of the body, i.e. on the outside of the turn (Rind, 1983*b*). During its orientation flights the bee builds up its visual landmark memory by making voluntary sideways translatory movements which involve the careful control of yaw so that it remains in a fixed orientation with respect to the visual panorama (Wehner, 1981). It is possible that $DNVI_1$, which also responds strongly to horizontal motion across the frontal visual field, might participate in the control of such voluntary movements.

From examination of the horizontal head-turning movements of a tethered bee in the centre of a striped drum, it has been concluded that the output elements controlling compensatory head movements would be excited by progressive motion over the lateral posterior region of the eye on the outside of a turn and regressive motion over the frontal region of the eye on the inside of a turn (Moore and Rankin, 1982). $DNVI_1$ is excited by progressive motion over the eye on the outside of a turn and regressive motion over the eye on the inside. However, we are not yet able to say whether there is a similar partitioning of the regions of the compound eyes which have inputs to this cell.

To assess further the functional role of the cells, their contrast frequency (CF) response characteristics should be compared with those of optomotor responses observed in the bee under similar stimulation conditions. The optomotor yaw response of *Apis* is dependent on the CF of moving periodic gratings, the response

being maximal at values between 8 and 10 Hz (Künze, 1961). The optomotor pitch and roll responses have not been characterized in *Apis*. Despite differences in pattern presentation (the bees in Künze's experiments viewed the striped gratings through slits in a stationary glass screen) the CF sensitivity of the optomotor yaw response is similar to the response characteristics of cell $DNVI_1$, which is specifically tuned to detect deviations in the yawing plane (i.e. both peak at 8–10 Hz). However, the reported dependence on CF of horizontally tuned wide-field motion-sensitive lobula units is displaced towards lower CFs, peaking at between 2 and 3 Hz (Kaiser and Bishop, 1970).

The rate of adaptation is more rapid at higher CFs in the H1 neurone of the fly (Maddess and Laughlin, 1985). Thus the CF sensitivity of a cell, determined as the spike rate at the end of a period of motion, will be affected by CF-dependent adaptation, particularly at high frequencies. Adaptation can significantly shift the peak response towards lower CFs. Indeed, the response of the H1 neurone peaks at 8–10 Hz when the sensitivity is measured from the instantaneous (non-adapted) response, but peaks at around 2 Hz if measured in the final 100 ms of a 6.7 s period of continuous motion (Maddess and Laughlin, 1985). It is particularly important to measure the CF sensitivity of neurones in the unbiased (non-adapted) state when comparing CF response curves derived electrophysiologically with those obtained from optomotor behaviour. The responses obtained from $DNII_2$, $DNIV_2$ and $DNVI_1$ were measured over the first 1.0 s, which gave CF response curves that were not significantly different from those derived from the instantaneous response (i.e. the response in the first 0.1 s). However, the CF response curves derived by Kaiser and Bishop (1970) were calculated by averaging the spike frequency in the last 20 s of a 30 s period of pattern motion. If the CF-dependent rates of adaptation of the lobula units in the bee are similar to those of the lobula plate neurones in the fly, this may explain the difference between the peak values reported by Kaiser and Bishop (1970) and those reported in this paper and by Künze (1961).

The descending neurones (DNs) of the bee have highly tuned responses to visual stimulation of the compound eyes. The directional tuning and CF-dependent characteristics are similar to those described for lobula units of the bee (Kaiser and Bishop, 1970; DeVoe *et al.* 1982; Hertel and Maronde, 1987) and fly (Hausen, 1981; Maddess and Laughlin, 1985). This suggests that visual information derived from the compound eyes and carried in the DNs is essentially the same as that carried in lobula units. However, studies on the properties of DNs in the dragonfly and locust suggest that DNs involved in course control receive information from more than one sensory channel (Olberg, 1981*b*; Reichert *et al.* 1985; Rowell and Reichert, 1986). The DNs of the dragonfly and locust integrate inputs from compound eyes, ocelli and wind-sensitive hairs in such a way that an optimal response is given to specific displacements in space. Two of the bee descending neurones, those which may be implicated in the control of pitch ($DNII_2$) and yaw ($DNVI_1$), are excited by wind over the head (M. R. Ibbotson, unpublished data), although it is not known if input from this sensory channel

affects the directional tuning of the cells. The effect of appropriate ocellar stimulation would also seem to be worth exploring in the bee, since three of the DNs have spined arborizations within some part of the neuropile occupied by the extensive branching of the L, S and L_D neurones across the posterior slope and in the perioesophageal region. It is also interesting to note that the three vertically sensitive descending neurones send fine branches up into the ocellar tracts, suggesting that there may be quite complex interactions between DNs and ocellar neurones.

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