IDENTIFICATION OF DIRECTIONALLY SELECTIVE MOTION-DETECTING NEURONES IN THE LOCUST LOBULA AND THEIR SYNAPTIC CONNECTIONS WITH AN IDENTIFIED DESCENDING NEURONE

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Summary

The anatomy and physiology of two directionally selective motion-detecting neurones in the locust are described. Both neurones had dendrites in the lobula, and projected to the ipsilateral protocerebrum. Their cell bodies were located on the posterio-dorsal junction of the optic lobe with the protocerebrum. The neurones were sensitive to horizontal motion of a visual stimulus. One neurone, LDSMD(F), had a preferred direction forwards over the ipsilateral eye, and a null direction backwards. The other neurone, LDSMD(B), had a preferred direction backwards over the ipsilateral eye.

1. Motion in the preferred direction caused EPSPs and spikes in the LDSMD neurones. Motion in the null direction resulted in IPSPs.

2. Both excitatory and inhibitory inputs were derived from the ipsilateral eye.

3. The DSMD neurones responded to velocities of movement up to and beyond $270°s^{-1}$.

4. The response of both LDSMD neurones showed no evidence of adaptation during maintained apparent or real movement.

5. There was a delay of 60–80 ms between a single step of apparent movement, in either the preferred or the null direction, and the start of the response.

6. There was a monosynaptic, excitatory connection between the LDSMD(B) neurone and the protocerebral, descending DSMD neurone (PDDSMD) identified in the preceding paper (Rind, 1990). At resting membrane potential, a single presynaptic spike did not give rise to a spike in the postsynaptic neurone.

Introduction

The processes in the visual system which underlie the detection of movement and form the basis of directional selectivity are of widespread significance. At the behavioural level, direction-selective motion detection can be described by essentially the same models in insects and man (Hassenstein and Reichardt, 1956; van Santen and Sperling, 1984, 1985).

It has not yet been possible to look in detail, during optomotor stimulation, at directionally selective motion-detecting (DSMD) neurones which are synaptically linked. Grzywacz and Koch (1987) have highlighted the need for more experimental evidence of the transformations made along the visual pathway at synapses between DSMD neurones, because their theoretical predictions show that neither of the biophysical processes thought to underlie directionally selective motion detection – shunting inhibition and summation followed by a threshold (Torre and Poggio, 1978) – are adequate on their own to describe the motion-detecting process observed at a behavioural level. In the vertebrate visual system, it is likely that direction selectivity, as described by the Reichardt model, is neither produced at one level nor by a single process. Rather, it is probably established in some form and then refined gradually. For example, in the turtle retina, the velocity preferences of directionally selective ganglion cells are sharper than those recorded from amacrine cells (DeVoe *et al.* 1985).

Extracellular responses have been recorded from directionally selective neurones in the locust and in the grasshopper lobula (Northrop and Guignon, 1970; Kien, 1974*a,b*). The anatomy and the output connections of these lobula, directionally selective neurones are unknown, although Kien (1974*a,b*, 1977) noted similarities in the direction-selective responses of the lobula neurones to those of descending neurones to the same stimuli. Kien (1974*a,b*, 1977) hypothesized that the lobula neurones were presynaptic to the descending neurones and that they underlay the optomotor head-turning response of the locust. Subsequently, other descending neurones with axons in the connectives have been shown to be responsive to motion over the compound eyes and to discriminate between opposing directions of movement. These neurones are often multimodal, responding to stimuli from combinations of compound eyes, ocelli, head hairs and neck hairs, and have been termed deviation detectors (Gris and Rowell, 1986; Rowell and Reichert, 1986; Hensler, 1988). The input neurones which confer a directional response to motion over the compound eyes on such deviation detectors are not known. One descending direction-selective motion-detecting neurone PDDSMD has been characterized physiologically and anatomically (Rind, 1990). This neurone was not found to be multimodal.

The present paper describes two DSMDs at a more distal level, in the lobula (third optic neuropile), and establishes that one of these lobula neurones excites PDDSMD monosynaptically. These lobula DSMD neurones are both sensitive to horizontal motion, but they have opposite preferred directions.

Materials and methods

Preparation

Adult *Locusta migratoria* were purchased from Animal Magic, Brighton. The locust was dissected as described by Rind (1987) and the ocellar nerves were cut. The locust was mounted dorsal side up. While the locust viewed a stimulus,

intracellular recordings were made from the neurones in the third optic neuropile (the lobula) and in the protocerebrum.

Stimuli

Two arrays of small, green rectangular LEDs, each subtending $5° \times 8°$ at the eye, were used to produce apparent movement of a striped pattern. Each array consisted of 18 LEDs arranged in an arc, centred on the eye. The two arrays were placed and controlled independently of each other. In most experiments, each array was aligned horizontally over an $8° \times 90°$ arc in the equatorial–dorsal region of the eye. The arrays could also be aligned vertically, to test the sensitivity of a neurone to vertical apparent movement.

Within each array, the LEDs were controlled to produce apparent movement of a striped stimulus, with each LED within the arc representing a stripe. At any one time, six LEDs within each 19-LED array were illuminated simultaneously, with each pair of illuminated LEDs separated by two unilluminated LEDs. Apparent movement consisted of sequential illumination of the six neighbouring LEDs. The sequential illumination of the LEDs was recorded as a stepping trace, each step indicating extinction of six LEDs and simultaneous illumination of their neighbours. The sequential illumination or extinction proceeded from left to right for eight sequences and then reversed for eight. In all the figures, an upward step indicates apparent movement in the anticlockwise direction (backwards over the left eye and forwards over the right one), and a downward step indicates apparent movement in the clockwise direction. Apparent movement, such as that produced by LED illumination, has been found to be effective in exciting motion-detecting neurones in the fly and locust (Pick and Buchner, 1979; Osorio, 1986; Rind, 1987).

Movement of a vertically striped drum (period $6°$ at the eye) was also used to confirm the response to real movement. The locust viewed the stripe moving through an $18° \times 12°$ window in a white card. To minimize edge effects, the white card was made of the same material as the white on the striped drum, and was placed 0.5 cm in front of the drum.

In all experiments, the locust had become adapted to a darkened room during daytime. Light levels were approximately equivalent to $3.64–10.92 \, mW \, cm^{-2}$, measured using a calibrated silicon solar cell (R.S. Components) to which a diffusing screen had been fitted. The cell was calibrated using a KIPP solarimeter which was itself calibrated at the National Physical Laboratory.

Recording and identification techniques

Intracellular recordings were made using glass capillary microelectrodes filled with a saturated solution of hexamminecobaltic chloride. Electrodes had d.c. resistances of $60–100 \, M\Omega$ and were connected to amplifiers incorporating bridge circuits. A virtual ground amplifier was used to monitor current injections. All neurones characterized physiologically were subsequently stained using 500 ms, 10 nA pulses every second, for 1 h. The brains were fixed in 10 % formaldehyde buffered to pH 7. Stained neurones were intensified and later drawn in a whole-

mount of the brain and optic lobe. Some preparations were then embedded in resin (Spurr) and 5 μm sections cut horizontally through the optic lobe and sagittally through the brain. All figures show intracellular recordings from neurones with processes in the left optic lobe or the left protocerebrum. In the course of the experiments, 36 neurones were characterized and stained, all sensitive to movement in the horizontal plane: six of each directional selectivity in the optic lobe (12 in all) and 24 directionally selective neurones with cell bodies in the protocerebrum.

Results

Responses of the neurones to movements of a visual stimulus

Two types of neurone were characterized both by their responses to movements and anatomically. Neurones were routinely stained after recordings had been made. All recordings were made from processes of the neurones either in the lobula or in the stalk which connects the optic lobe to the protocerebrum. The neurones responded most vigorously to movements in the horizontal plane. In the absence of movement they had a resting discharge of 5–20 spikes s^{-1} (Fig. 1A, C). Movement in one direction (preferred) caused an increase in excitation, and movement in the opposite direction (null) caused inhibitory potentials (IPSPs). The IPSPs were reduced in amplitude when the neurone was hyperpolarized by injection of negative current (Fig. 1D). Neurones were found with preferred direction both forwards (Fig. 1B downward stepping) and backwards (Fig. 1C, D upward stepping) over the ipsilateral eye. The two directional selectivities could be found in a single preparation. On 12 occasions, directionally selective neurones were stained completely, six of each preferred direction. Neurones with the same preferred direction showed a consistent morphology in the lobula, optic stalk and protocerebrum (for example, see Figs 2, 5, 12). In these experiments neurones of one directional selectivity probably represent a unique neurone. Neurones with a preferred direction forwards over the ipsilateral eye were termed lobula LDSMD(F), and those with a preferred direction backwards over the ipsilateral eye were termed LDSMD(B).

Morphology of the LDSMD(F) neurone

The LDSMD(F) cells arborized within the lobula and projected from the lobula into the ipsilateral protocerebrum. The cell body lay on the boundary between the optic lobe and the protocerebrum. In the following description, the projections of the neurones within the brain are described relative to the body axes of the locust (anterior/posterior, dorsal/ventral, proximal/distal).

Fine dendritic processes (<1 μm) arborized within the neuropile of the lobula (Figs 2 and 3). When the lobula was viewed in horizontal section these processes occupied a restricted region of 150 μm×100 μm×50 μm within the anterior, proximal lobula (Fig. 3). The processes of a lightly stained LGMD2 neurone

arborized near the distal surface of the lobula (Fig. 3A, C) and showed no overlap with the processes of the LDSMD(F) neurone (Fig. 3B).

At higher magnifications under the light microscope (Fig. 3C, D), fine processes could be seen extending into the lobula neuropile from the dendritic branches. From the lobula, an axon projected into the protocerebrum, and gave rise to
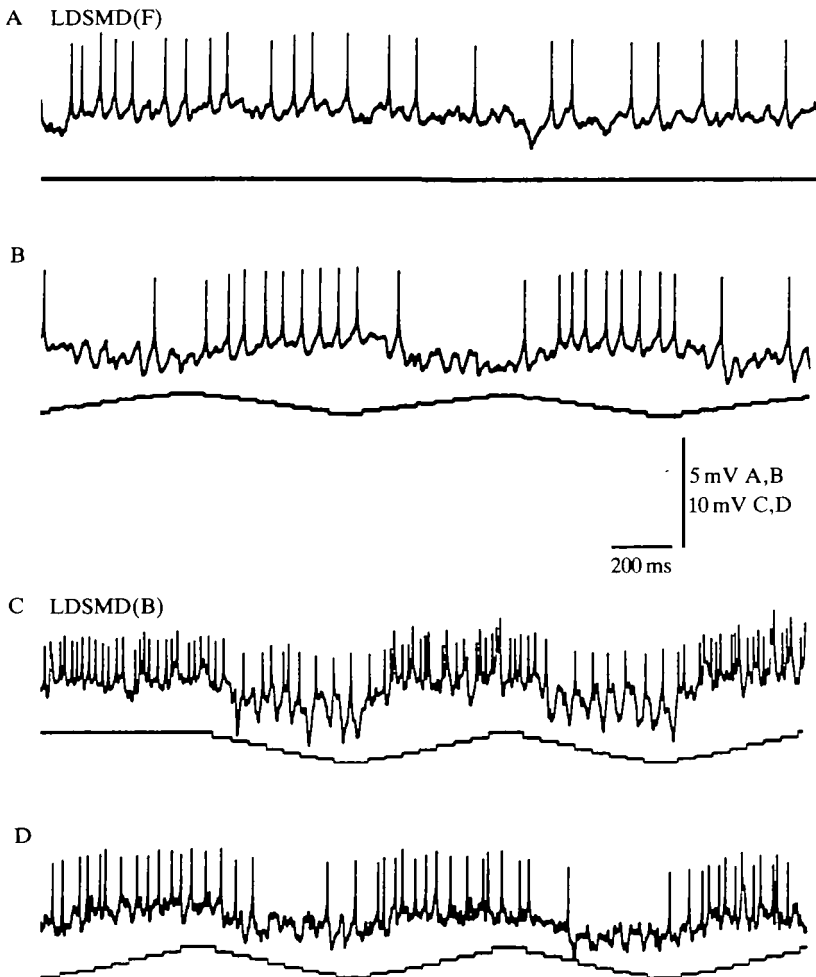

Fig. 1. Response to horizontal apparent movement recorded in separate experiments from two neurones of opposite directional selectivities. Responses were from the dendritic processes of the neurones each in the left lobula. Each neurone was subsequently stained, and its morphology revealed. Responses of a neurone with preferred direction forwards over the ipsilateral eye: (A) in the absence of apparent movement; and (B) to oscillating direction of apparent movement to the right (downward stepping) and to the left (upward stepping). (C, D) Responses of a second neurone which had a preferred direction backwards over the ipsilateral eye: (C) in the absence of apparent movement followed by oscillating directions of apparent movement; (D) to oscillating direction of apparent movement, when the neurone has been hyperpolarized with 5 nA of negative injected current.

LDSMD(F)

A

Fig. 4

Fig. 3 →

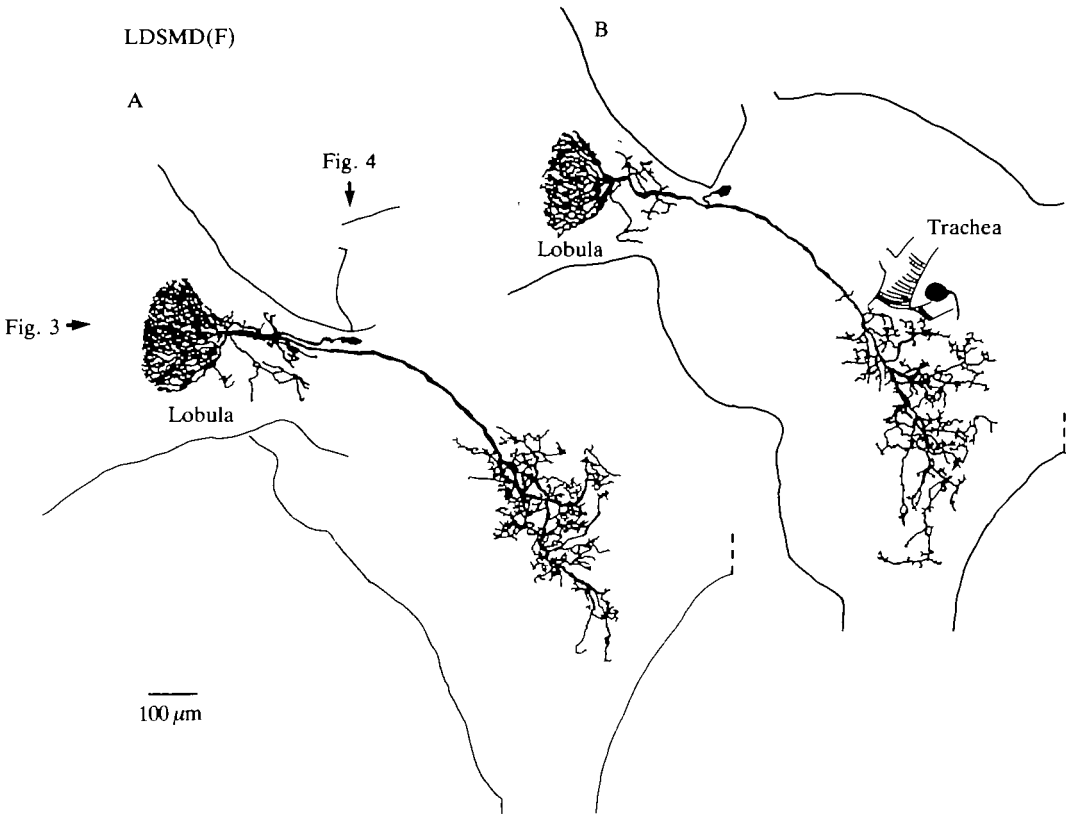Lobula

Lobula

B

Trachea

100 μm

Fig. 2. (A, B) Morphology of two stained LDSMD(F) neurones with preferred
direction was forwards over the ipsilateral eye. The neurones have been drawn from a
whole-mount of the brain, viewed from behind. A dotted line delineates the extent of
the lobula neuropile, and a broken line indicates the midline of the brain. Arrows
indicate positions of sections photographed in Figs 3 and 4.

several long (up to $150 \, \mu m$) narrow ($1 \, \mu m$ in diameter) branches which ran in the
neuropile of the optic stalk (see Figs 2, 3A). A slender neurite ($3-5 \, \mu m$ in
diameter) left the axon $100-150 \, \mu m$ proximal to the lobula and ran to the
$15 \, \mu m \times 30 \, \mu m$ cell body located dorsally on the posterior surface of the junction of
the protocerebrum with the optic lobe. In the optic stalk, the neurite and the axon
of the LDSMD(F) neurone ran in a dorsal tract. Fig. 4 shows a sagittal section
through the brain at the level of the LDSMD(F) neurone cell body. The cell body
lay in a group of 6–8 cell bodies of similar size, shape and nuclear staining, as seen
in $5 \, \mu m$ sections stained with Toluidine Blue. The tract of axons containing the
LGMD1 axon (Rind, 1987) is marked with an arrowhead in Fig. 4. After giving
rise to the primary neurite, the axon projected unbranched for $250 \, \mu m$ into the
neuropile of the posterior slope of the protocerebrum. The neurone branched in
the protocerebrum and extended processes laterally towards the midline of the
brain. It projected in a consistent overall pattern into the tritocerebrum (Fig. 2A,
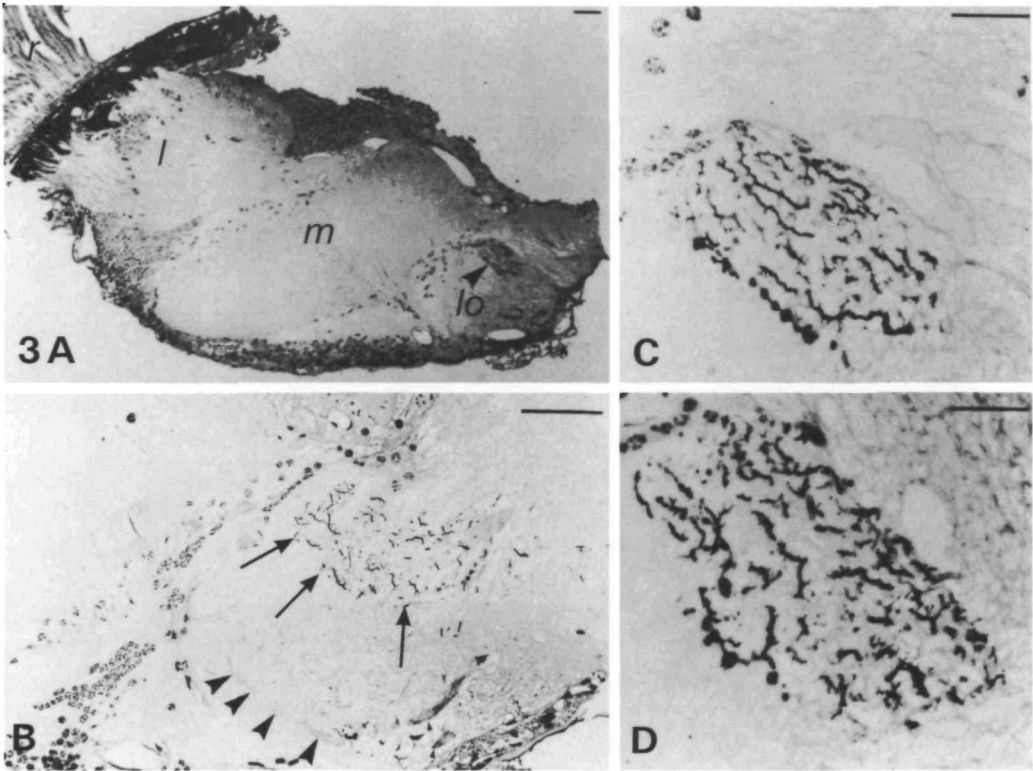
Fig. 3. Fine detail of the structure and position of a DSMD(F) neurone in the lobula.
(A) Horizontal section through an optic lobe containing a stained DSMD neurone
(arrowhead) with a preferred direction forwards over the ipsilateral eye. Scale bar,
100 μm. (B) Detailed view of the lobula, seen in horizontal section at a more ventral
level than in A. The processes of the darkly stained LDSMD(F) neurone (arrowed)
and a lightly stained LGMD2 neurone (arrowheads, lobula giant motion detector 2;
Rind, 1987) are both visible in the lobula. Scale bar, 100 μm. (C, D) Fine structural
detail of the LDSMD(F) neurone dendrites within the lobula. The section shown in C
is a magnified portion of that seen in A. The section shown in A is 15 μm more dorsal
than that in D. *l*, lamina; *lo*, lobula; *m*, medulla; *r*, retina. Scale bars, 50 μm.

B). The extent and overall shape of the six neurones stained in different
preparations were very similar, but the detailed shape showed some variations
among neurones. For example, the process which projects into the tritocerebrum
could arise directly from the main axon (Fig. 2B) or from a large secondary
process (Fig. 2A).

Morphology of the LDSMD(B) neurone

LDSMD(B) had a dense mesh of dendrites in a restricted area of the proximal,
anterior lobula neuropile, co-extensive with the lobula projections of the
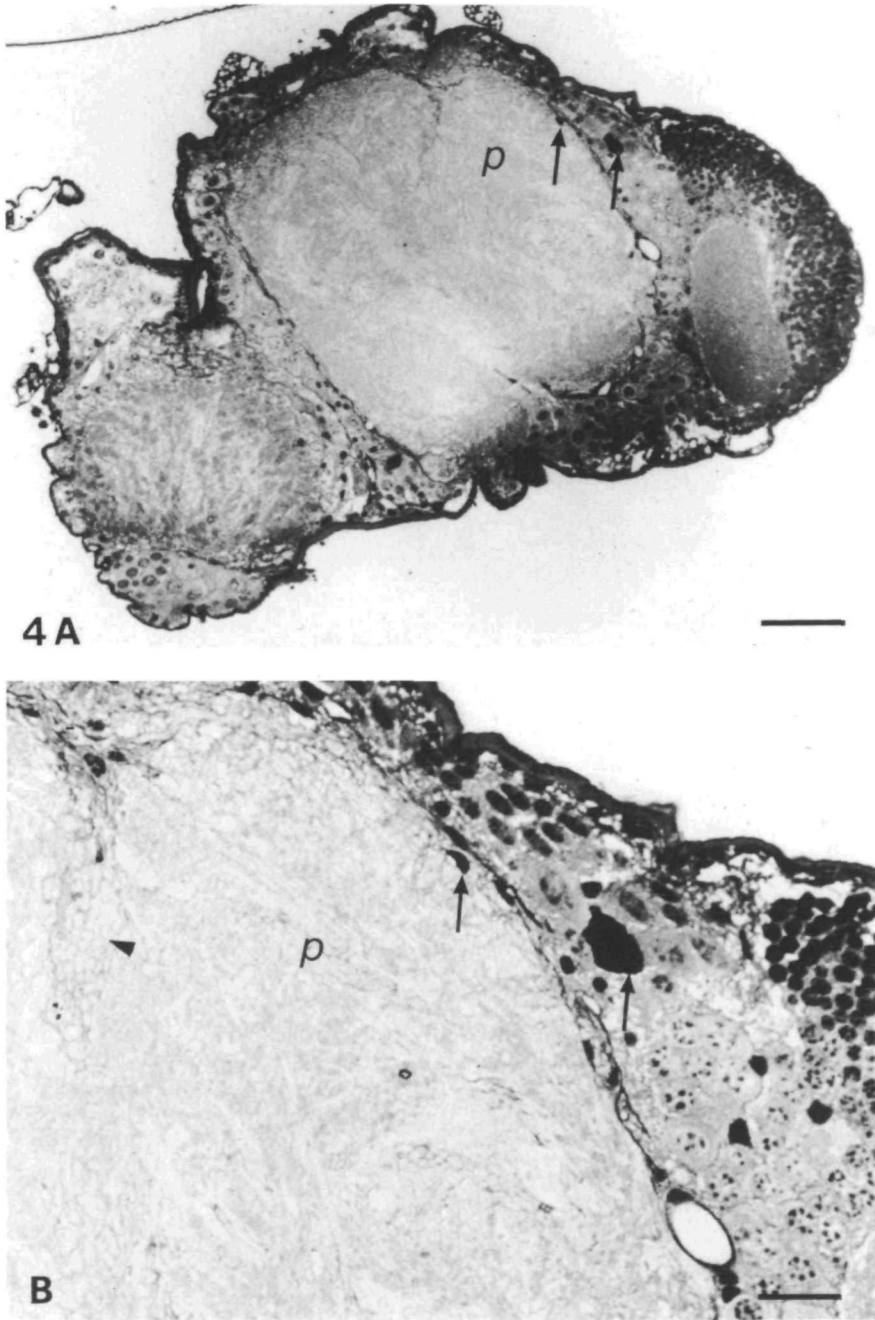LDSMD(F) neurone, but with a reduced dorsoventral extent (Figs 5, 12). The

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Fig. 4. A stained LDSMD(F) neurone in the brain cut in sagittal section at the level of its cell body. (A) Section showing the cell body and axon position in the protocerebrum. Scale bar, 100 μm. (B) Detail of A. Note the cluster of similarly sized and shaped cell bodies around the cell body of the stained LDSMD(F) neurone. The cell body and axon of LDSMD(F) are arrowed. The axon tract containing the axon of the LGMD1 is indicated by an arrowhead. p, protocerebrum. Scale bar, 50 μm.
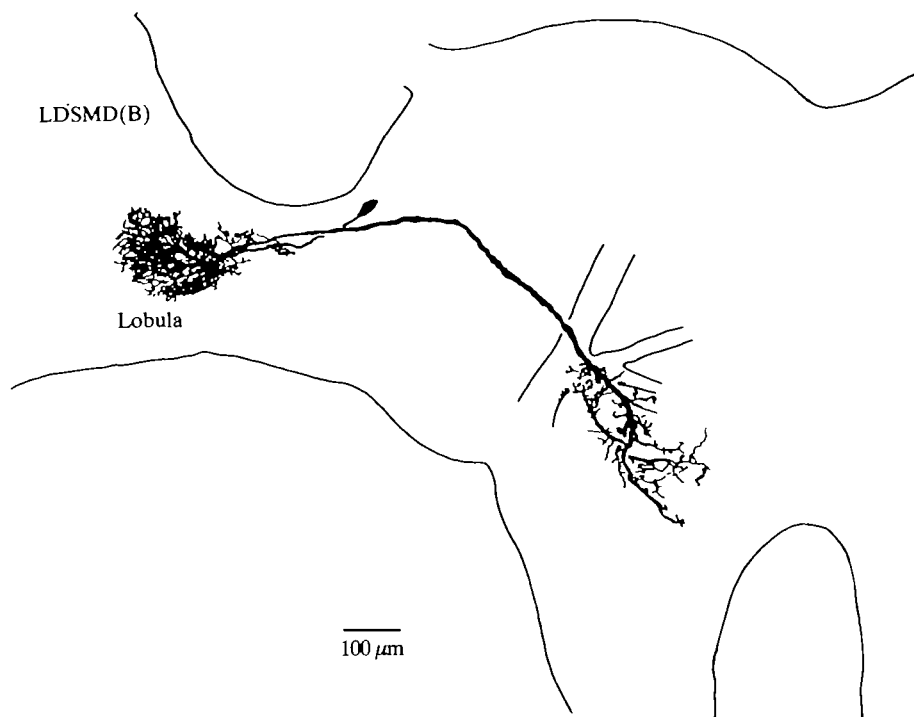
Fig. 5. Morphology of a stained LDSMD(F) neurone with a preferred direction backwards over the ipsilateral eye. The neurone has been drawn from a whole-mount of the brain viewed from behind. A dotted line delineates the extent of the lobula neuropile.

neurite arose at the proximal border of the lobula and projected to a 15 μm×30 μm cell body. The cell body was located at the posterior, dorsal border between the optic lobe and the protocerebrum. The main axon of the LDSMD(B) neurone narrowed to 3–5 μm in diameter in the optic stalk before projecting unbranched into the protocerebrum. The axon expanded to up to 15 μm in diameter as it passed beneath a conspicuous tracheal trunk penetrating the posterior, medial slope of the protocerebrum (see Figs 2, 5). At this level, the axon produced secondary processes. These processes extended laterally and ventrally, but did not extend into the tritocerebrum (see Figs 5, 12).

The directional response to movement

Neurones of both preferred directions, LDSMD(F) and LDSMD(B), gave a clear directional response to apparent movement at velocities up to and beyond 270°s^{-1}, with 18 apparent movements per second [LDSMD(F), Fig. 6A]. The response to movement over both eyes in either the excitatory or the inhibitory direction (Fig. 6B) showed no difference when compared with the response to movement over the ipsilateral eye alone (Fig. 6C). Monocular stimulation in another LDSMD(F) neurone, in which the IPSPs in response to movement in the
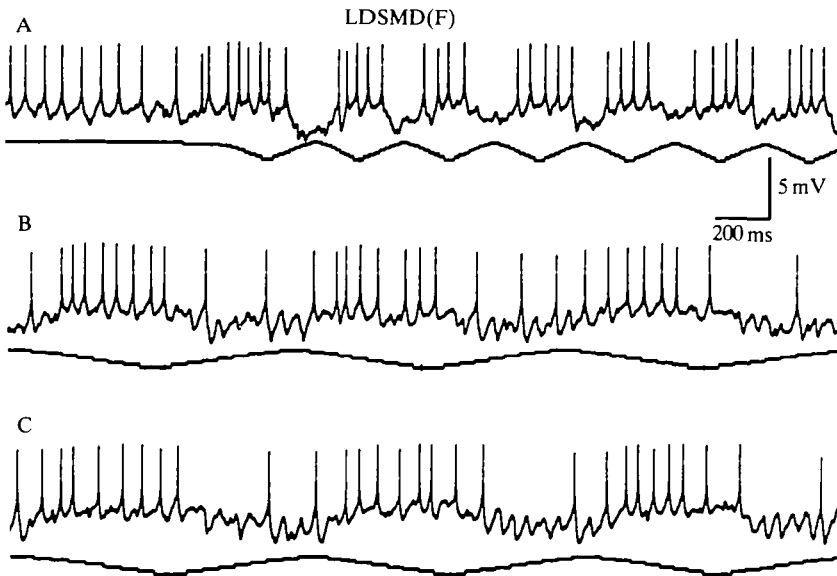
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Fig. 6. Response of a LDSMD(F) neurone to different velocities of apparent
movement and a demonstration that input is derived from the ipsilateral eye only.
(A) In the absence of apparent movement, apparent motion was commenced at a
velocity of $270°s^{-1}$. (B) Response to apparent movement at $90°s^{-1}$ over both eyes;
(C) response to apparent movement at $90°s^{-1}$ over the ipsilateral eye only.

null direction were accentuated throughout the experiment by depolarizing the
neurone with injected positive current, revealed input to LDSMD(F) in response
to each apparent motion step. The response to apparent motion over the
contralateral eye was no different from activity in the neurone in the absence of
movement (Fig. 7A, Di,ii; compare Fig. 7C). When apparent motion occurred
over the ipsilateral eye alone, large IPSPs in the DSMD neurone occurred during
apparent motion in the null direction (Fig. 7B, Ei) and EPSPs were revealed in
response to motion in the preferred direction (Fig. 7B, Eii). A spike occurred
following an IPSP in response to movement in the null direction, and may have
been due to rebound excitation (Fig. 7B, Ei). The above experiments show that
both the excitatory and the inhibitory components of the response to motion were
derived from inputs from the ipsilateral eye. In this preparation, the excitatory
response in the preferred direction was greatly attenuated. In experiments where
recordings were made from the LDSMD neurones for over 1 h with electrodes
filled with hexamminecobaltic chloride, the spiking excitatory response to move-
ment in the preferred direction was lost. However, signal averaging revealed the
presence of the underlying IPSP (Figs 7Ei, 8A) and EPSPs (Figs 7Eii, 8B) in
response to movement. This abolition of spiking by hexamminecobaltic chloride
enabled the time course of the PSPs underlying the direction-selective response of
these lobula neurones to be studied.

The response of both identified LDSMD neurones to individual steps of

apparent movement of a multi-stripe stimulus are shown in Figs 8 and 9. Signal averaging reveals the delay and time course of the response to apparent movement (Fig. 8). Following a single step of apparent movement, the delay in neurone LDSMD(F) before either an IPSP or an EPSP was initiated ranged between 60 and 80 ms. From Fig. 8 The IPSP had a duration of 60 ms with a time-to-peak of 30 ms and a time-to-decay-to-36 %-of-peak of 15 ms, the EPSP had a duration of
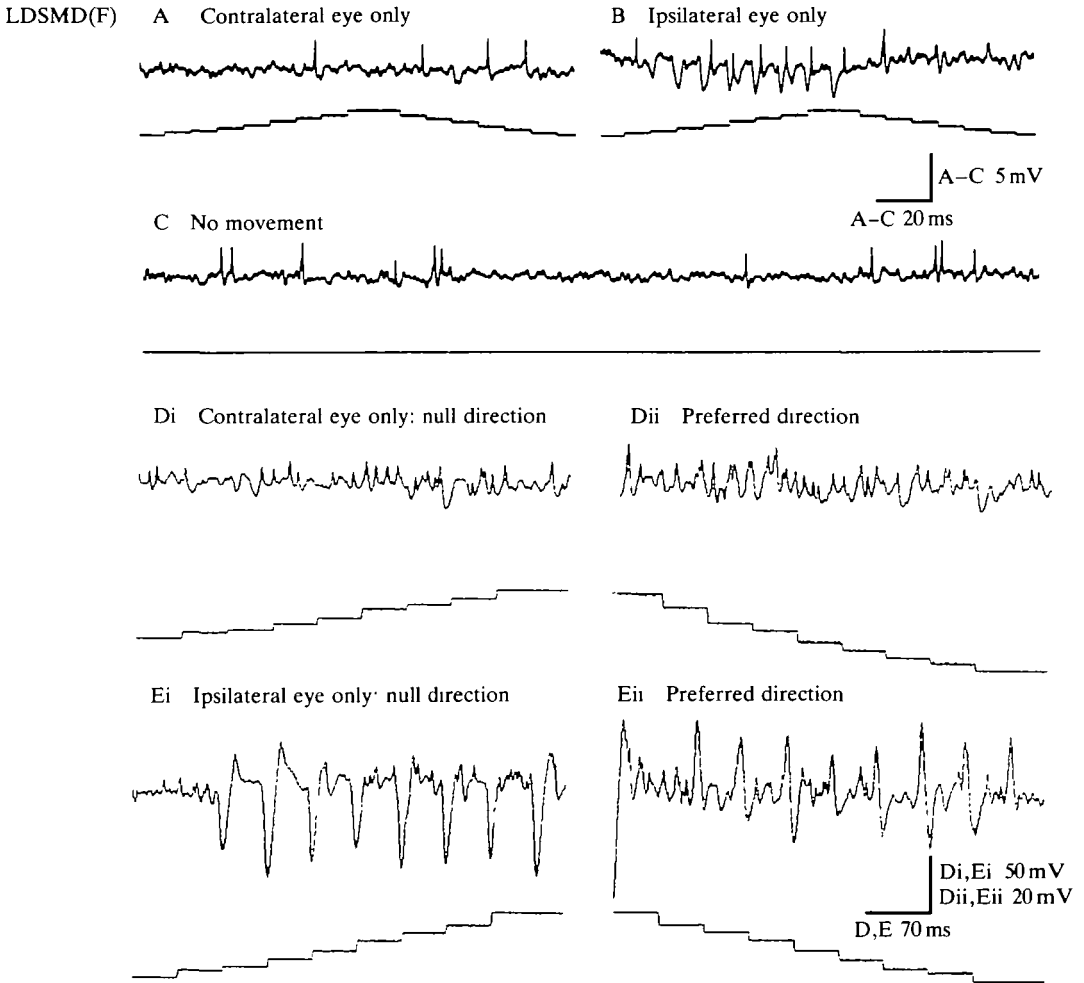

Fig. 7. The PSPs seen in response to apparent movement are derived from inputs from the ipsilateral eye. Recording from the dendrites in the left lobula of a LDSMD(F) neurone during monocular stimulation. (A) Apparent movement over the contralateral (right) eye only, movement in the null then the preferred direction. (B) Ipsilateral eye only, movement in the null then the preferred directions. (C) In the absence of movement. (D) Averaged response (eight sweeps) to eight steps of apparent movement over the contralateral eye in (i) the null direction and (ii) the preferred direction. (E) Averaged response (eight sweeps) to eight steps of apparent movement over the ipsilateral eye in (i) the null direction and (ii) the preferred direction.
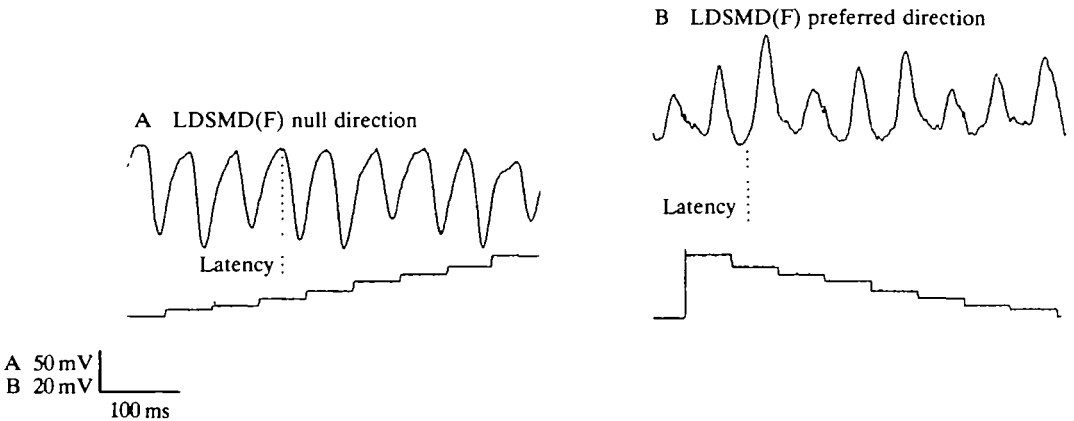
Fig. 8. Latency and time course of the response to individual apparent multi-stripe movements in (A) the null direction and (B) the preferred direction. The response was recorded intracellularly from a lobula DSMD(F) neurone. Thirty-two sweeps have been averaged. The first two IPSP/EPSPs occur in response to previous unshown apparent motion steps.

50 ms with a rise time of 25 ms and a time-to-decay-to-36 %-of-peak of 18 ms. In a single neurone, the delay following an apparent motion step was the same irrespective of whether the step was in the preferred or the null direction (compare Fig. 9A, C and 9B, D). Similarly, there was no consistent difference in the delay when it was compared in the preferred direction or null direction between neurones with opposite preferred directions (compare Fig. 9A–E with 9F–H). There was no consistent difference in the delay, even when this comparison was made at the same velocity of movement (Fig. 9E, H). The delay could not always be precisely measured, as the responses of the LDSMD neurone, which had been depolarized by injection of 1–5 nA of positive current, appeared as discrete potentials even during real, low-velocity continuous movement (compare Fig. 11A and 11B).

No evidence of adaptation was seen in the response of an LDSMD neurone to continuous apparent or real motion either in the preferred or the null directions (Figs 10, 11A; Table 1). In the experiment shown in Fig. 10, the neurone gave a clear directional response to apparent movement of $120°s^{-1}$ [LDSMD(F) Fig. 10A]. There was no evidence of adaptation during maintained movement in

Fig. 9. Latency and time course of the response to individual apparent multi-stripe movements in the null and the preferred directions. The response was recorded intracellularly from two lobula DSMD neurones LDSMD(F) and LDMD(B) with opposite preferred directions. Response of a LDSMD(F) neurone with a preferred direction forwards over the ipsilateral eye to (A, B) two movements in the preferred direction; and (C, D, E) movements in the null direction. Response of a LDSMD(B) neurone with a preferred direction backwards over the ipsilateral eye to (F, G) movements in the preferred direction and movements in the null direction. In H three successive traces have been aligned. All records are on the same time scale.

LDSMD(F)   LDSMD(B)

A   Preferred   F   Preferred

Latency   Latency

B   Preferred   G   Preferred

Latency   Latency

C   Null   H   Null

Latency

D   Null

Latency

E   Null

Latency   Latency
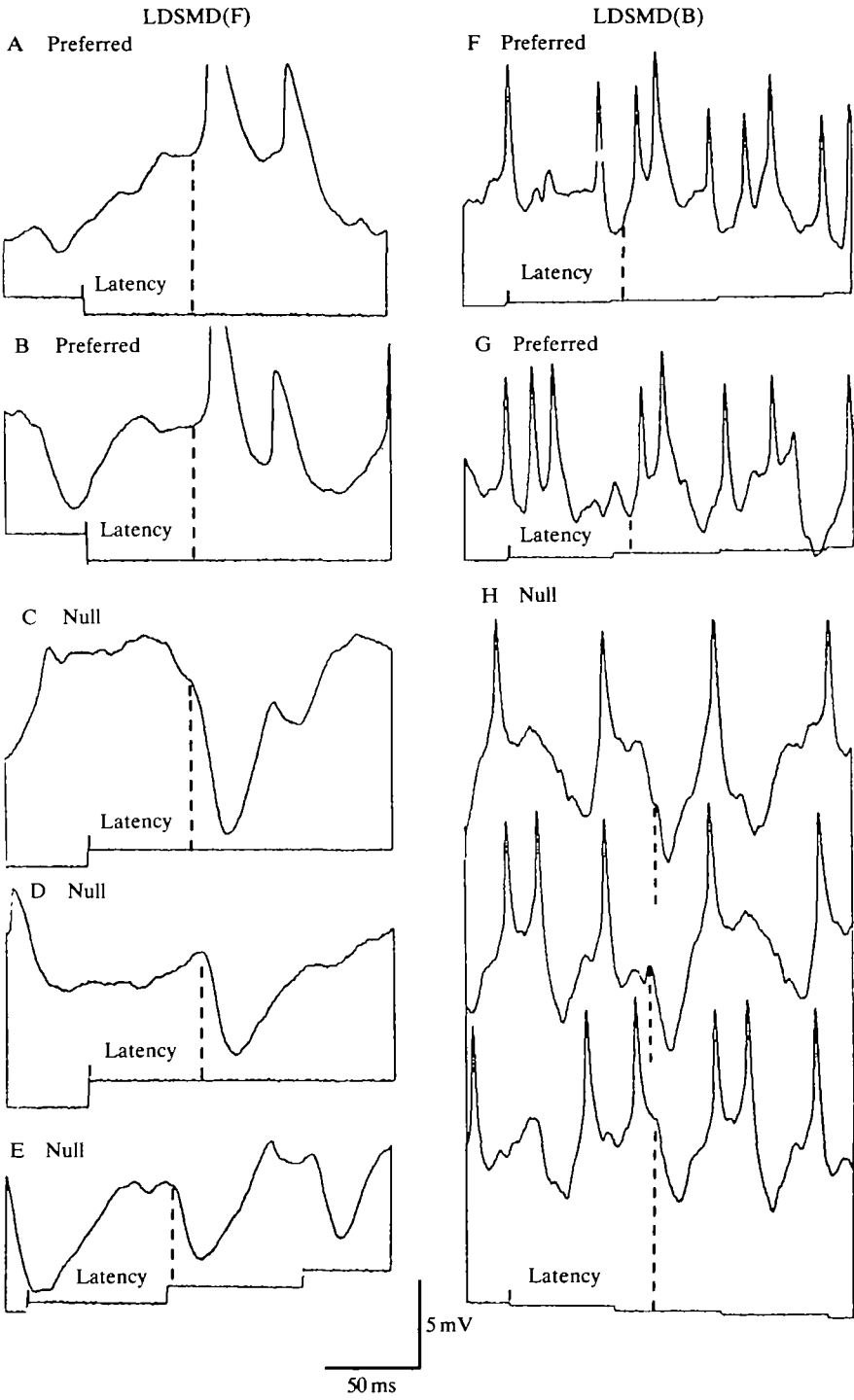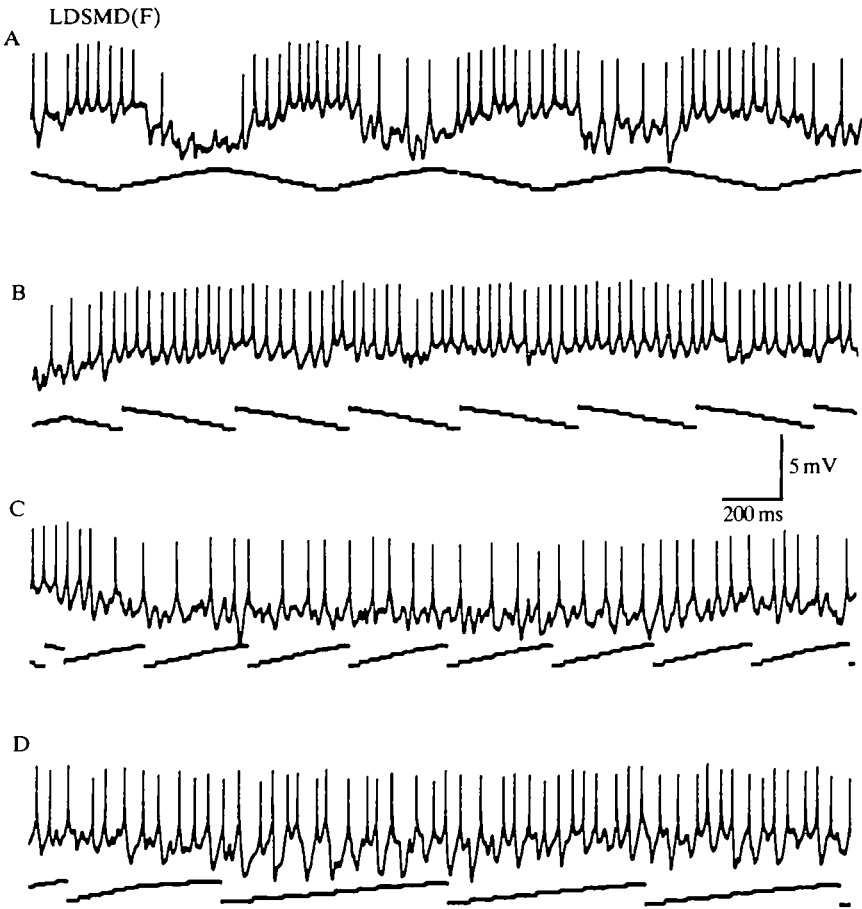
5 mV

50 ms

Fig. 9

Fig. 10. Lack of adaptation of the response of the LDSMD(F) neurone to maintained apparent motion in the preferred or the null directions. (A) Response of the neurone to oscillating directions of apparent movement at $120°s^{-1}$; (B) response to continuous apparent motion in the preferred direction at $120°s^{-1}$; (C) response to continuous apparent motion in the null direction at $130°s^{-1}$; (D) the response to slowing the velocity of apparent motion, discrete IPSPs are revealed.

either the preferred or the null directions. The response of the neurone to the first 2.6s of the motion in either the excitatory or the null direction is shown in Fig. 10B, C. The response of this neurone to apparent movement is expressed quantitatively in Table 1. This analysis further demonstrates that the response of the LDSMD(F) neurone does not adapt to continuous stimulation.

Output connections made by the LDSMD neurones

A DSMD neurone, termed PDDSMD, with a cell body and dendrites in the ipsilateral protocerebrum and an axon in the ipsilateral nerve cord, has been identified (Rind, 1990). The PDDSMD neurone has a preferred direction
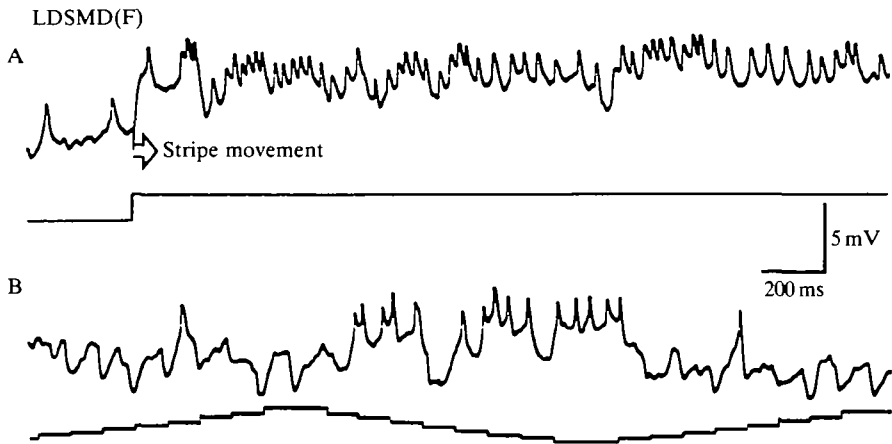
LDSMD(F)

Fig. 11. Response to real and apparent movement compared. (A) Response of a lobula LDSMD(F) neurone in the left lobula to real movement of a striped drum forwards ($24° s^{-1}$ at 4 Hz) over the ipsilateral eye. (B) Response of the same neurone to oscillating directions of apparent motion ($50° s^{-1}$ at 3 Hz). The preferred direction is forwards over the ipsilateral eye.

Table 1. *Quantitative evidence for a lack of adaptation*

Block number	Number of spikes	
	Null direction	Preferred direction
1	5	10
2	6	10
3	5	9
4	5	10
5	5	9
6	5	10
7	6	10
8	5	10
	Mean 5.25	Mean 9.75
	s^2 0.188	s^2 0.188

The number of spikes was counted in eight successive 400-ms blocks during the first 3 s of an apparent movement stimulus.

The LDSMD(F) neurone was responding to apparent movement in either the null or the preferred direction at $130° s^{-1}$. The response of this neurone to continuous apparent movement is also shown in Fig. 10.

The mean spike number and the variance (s^2) of each response from this mean was calculated. If adaptation were occurring there should be a large variance of the spike numbers about the mean. This was not found to be the case.
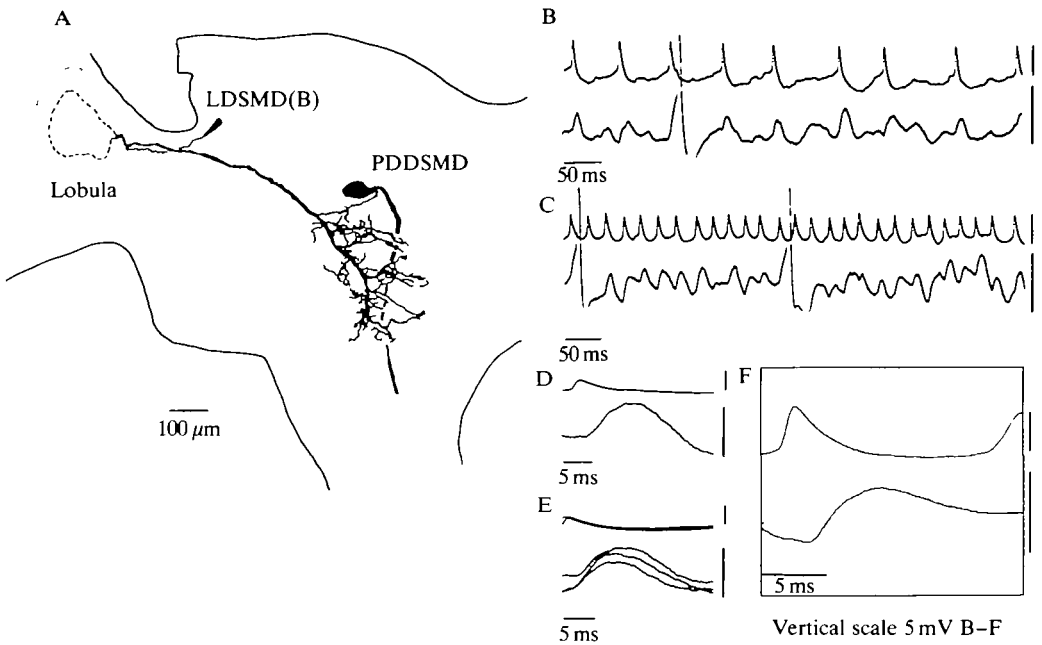
Fig. 12. Characterization of the monosynaptic excitatory connection between the DLSMD(B) neurone and a descending DSMD neurone with its cell body in the protocerebrum (PDDSMD). All records were made in the absence of apparent motion. Spikes in the lobula DSMD neurone occur in response to depolarization of the neurone by current injection (5–7 nA). In each record (B–D) the top trace is a recording from the dendrites of the DSMD neurone in the lobula and the bottom trace is a recording from the cell body of the descending DSMD neurone in the protocerebrum. (A) The DSMD neurones with the same preferred direction (backwards over the ipsilateral eye) stained in the same preparation. One neurone arborizing in the lobula, the other with an axon in the connective. Both were drawn from a whole-mounted brain viewed from behind. (B) Postsynaptic potentials (PSPs) in the descending protocerebral DSMD neurone follow each spike in the lobula DSMD neurone. Spikes arise from these potentials. (C) These EPSPs in the descending DSMD neurone consistently follow spikes in the lobula DSMD neurone up to rates of 70 Hz, the highest rate observed in the lobula DSMD neurone. (D) The time course of a single EPSP in the descending DSMD neurone following a spike in the lobula DSMD neurone. (E) Three superimposed sweeps triggered from a spike in the lobula DSMD neurone to show a consistent, short latency to the EPSP in the descending DSMD neurone. (F) Signal-averaged response (eight sweeps) showing the 2 ms synaptic delay.

backwards over the ipsilateral eye and forwards over the contralateral eye. In the protocerebrum, there was anatomical overlap between the axonal branches of both the LDSMD neurones and the dendrites of PDDSMD. Connections between the ipsilateral LDSMD neurones and PDDSMD were sought. On four occasions, recordings were made from pairs of ipsilateral neurones, one lobula, and one descending DSMD neurone. On each occasion both neurones were stained. On three of these occasions both neurones had the same preferred directions

(Fig. 12). Fig. 12 illustrates the characterization of one such pair of DSMD neurones and their connections. The stained neurones are outlined in Fig. 12A. At resting membrane potential, a spike in the LDSMD(B) neurone consistently produced a single 1–3 mV potential in the descending PDSMD neurone (Fig. 12B–F). The size of the excitatory potentials depended on the membrane potential of the descending DSMD neurone. They were accentuated by hyperpolarizing the neurone with injected negative current, suggesting they are mediated by a conductance increase. The EPSPs were observed to follow spikes in the DLSMD(B) neurone up to the highest spike frequencies produced by the LDSMD neurone (70 Hz, Fig. 12C). In Fig. 12D, a single EPSP is shown (in PDDSMD) following a spike in the lobula DSMD(B) neurone. In Fig. 12E, three superimposed sweeps are shown triggered from a spike in the lobula DSMD neurone, and the average of eight such records is shown in Fig. 12F. The EPSPs had a consistent 2 ms latency measured from the initial inflection of the spike to the first recordable inflection of the EPSP (Fig. 12D–F). The measurements were taken between a recording site at the base of the dendritic tree of the DSMD neurone in the lobula and the cell body of the descending DSMD neurone in the protocerebrum. This short latency is consistent with a monosynaptic connection between the neurones. The LGMD–DCMD synapse has a latency of 1.7 ms when measured from equivalent points (the dendritic fan of the LGMD and the cell body of the DCMD: Rind, 1984). In a single preparation, good recordings were made from a DSMD neurone in the lobula with a preferred direction forwards over the eye [LDSMD(F)] and an ipsilateral descending neurone with the opposite preferred direction over the same eye. In this preparation, no PSPs followed a spike in the lobula neurone, even after depolarization of the descending DSMD neurone to accentuate any IPSPs.

Functional characterization of the direct synaptic connection between DSMD neurones

Fig. 13 shows examples of recordings from two identified, synaptically linked, DSMD neurones: LDSMD(B) projecting from the lobula to the protocerebrum and PDDSMD projecting from the protocerebrum to the thoracic nervous system. The recordings were made during apparent movement in either the preferred direction (backwards over the ipsilateral eye, forwards over the contralateral eye) or the null direction (forwards over the ipsilateral eye, backwards over the contralateral eye).

Two speeds of movement are shown in the preferred direction and one in the null direction for this pair of neurones. The presynaptic neurone had been hyperpolarized by 5 nA in Fig. 13C and F, which enabled the PSPs to be seen more clearly (compare Fig. 13E and F).

Although LDSMD(B) has been shown to excite PDDSMD monosynaptically (Figs 12, 13), it was not possible to hyperpolarize LDSMD(B) sufficiently to stop it spiking in response to apparent movement. Such an experiment would have demonstrated the importance of the connection between LDSMD(B) and
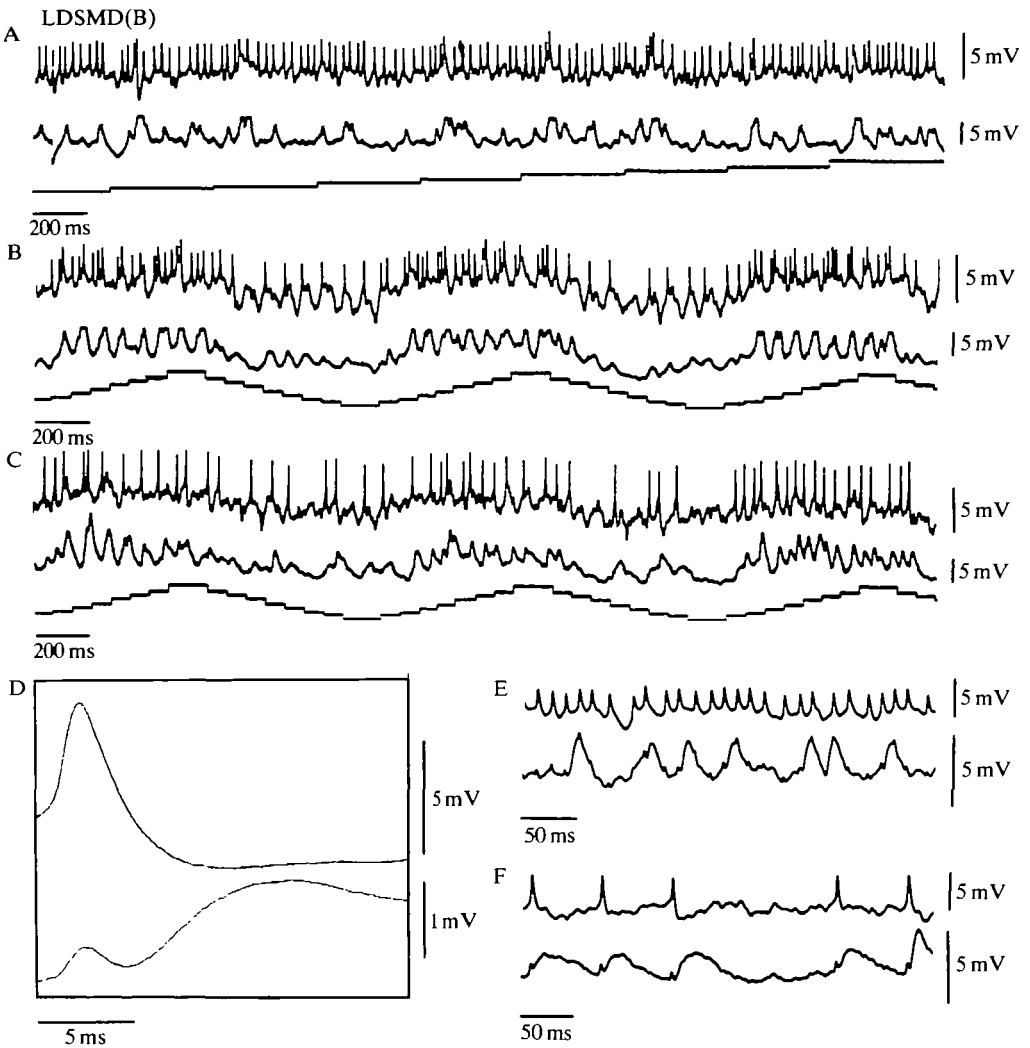
PDDSMD, compared with inputs that PDDSMD receives from other DSMD neurones.

Discussion

In the locust lobula, DSMD neurones with opposite preferred directions have been characterized for the first time. Both detect horizontal motion, one neurone, LDSMD(F), has a preferred direction forwards over the ipsilateral eye and the other, LDSMD(B), has a preferred direction backwards over the ipsilateral eye. LDSMD(B) was found to excite an identified protocerebral descending direction-selective movement detector (PDDSMD) monosynaptically (Rind, 1990).

Fig. 13. Functional characterization of the monosynaptic connection between DSMD neurones. Recordings were made from the dendrites of the LDSMD(B) neurone in the lobula (top trace) and from the cell body of the PDDSMD neurone in the protocerebrum (middle trace A–C, bottom trace D–F). The responses are from two synaptically linked DSMD neurones on the left of the animal. (A) Response to apparent movements in the preferred direction (towards the left over both eyes). (B) Response to apparent motion alternating between the preferred and the null directions at a velocity of $75°s^{-1}$. In A and B the response of the postsynaptic descending DSMD neurone has been clipped by the tape recorder during the recording. (C) Response to alternating directions of apparent motion during which the presynaptic neurone was hyperpolarized by injection of 5 nA of negative current. In the LDSMD(F) neurone the spikes produced in response to apparent movement in the null direction are no longer synchronized with each apparent movement by rebound excitation following the IPSP. (D) A signal-averaged trace (average of eight sweeps) triggered from a spike in the presynaptic neurone in the absence of apparent movement. The first inflection of the EPSP in the descending PDSMD neurone is obscured by an artefact produced by signal coupling between the two electrodes. (E) A continuous recording from the two DSMD neurones in the absence of apparent movement. At this level of presynaptic spiking the EPSPs following a spike in the lobula DSMD(F) neurone are very difficult to resolve. (F) A continuous recording from the two DSMD neurones at the same gain as in E during apparent movement in the null direction at $75°s^{-1}$. The presynaptic neurone has been hyperpolarized by 5 nA to reduce its level of activity further. The EPSPs are clearly discernible following each spike in the lobula DSMD neurone.

Comparative anatomy and characterization of inputs to lobula DSMD neurones

The lobula arborizations of LDSMD(F) and LDSMD(B) were co-extensive. This contrasts with horizontally sensitive DSMD neurones in the dipteran lobula (HSE, HSS, HSN), which partition the lobula into three marginally overlapping anatomical regions (Hausen, 1982*a*). In the locust, LDSMD neurones occupy a restricted area of anterior lobula close to the stalk of the optic lobe, whereas in the fly the HSE, HSS and HSN neurones arborize in the anterior-most surface of the lobula plate. The small size of the locust horizontally sensitive LDSMD neurones compared with the dipteran neurones further emphasizes the apparent lack of structural homology between them. LDSMD(F) and LDSMD(B) are homo-lateral, that is they do not cross the mid-line of the brain, but project to the ipsilateral protocerebrum. In addition, they receive their inputs from the ipsilateral eye only. In the dipteran lobula plate, some neurones sensitive to horizontal movement cross the midline of the brain, and others arborize only in the ipsilateral protocerebrum (homolateral). All the homolateral neurones (HSE, HSS, HSN and CH) have the same preferred direction: backwards over the ipsilateral eye. In addition to these ipsilateral inputs, they all (except HSS) also receive inputs from the contralateral eye (Hausen, 1982*a*). In the locust, the LDSMD neurones receive EPSPs and produce spikes in response to horizontal movement in the preferred direction over the ipsilateral eye. In response to movement in the null direction over the ipsilateral eye, locust LDSMD neurones receive discrete IPSPs which hyperpolarize them and reduce the resting spike rate.

The discrete nature of the IPSPs, even during real movement, suggests that they result from the activity of a small number of input neurones. In contrast, dipteran lobula plate H neurones receive their ipsilateral eye input from a large number of presynaptic neurones, so it is not possible to resolve individual IPSPs and EPSPs from ipsilateral eye stimulation (Hausen, 1982a). The IPSPs which occur in the locust LDSMD neurones of both directional selectivities in response to movement in the null direction sharpened the directional selectivity of the LDSMD neurones' response.

There is no evidence for adaptation in the response of the LDSMD neurones to continuous real or apparent motion in either the preferred or the null direction. A lack of adaptation has also been observed in the response of the postsynaptic PDDSMD neurone (Rind, 1990). Adaptation to continuous motion has been shown to occur in the response of the H DSMD neurones (HSE, HSS, HSN and H1) in the dipteran lobula plate (Hausen 1982b). It has been suggested that adaptation increases the neurones' ability to signal changes in temporal frequency (Maddess and Laughlin, 1985).

The LDSMD(B)-PDDSMD synapse

The LDSMD(B) neurone with a preferred direction backwards over the eye monosynaptically excites the ipsilateral PDDSMD neurone. The 2–3 mV amplitude EPSPs follow a spike in the lobula DSMD with a short latency (<2 ms from initiation of the presynaptic spike to initiation of the PSP). A latency of this order is consistent with a monosynaptic connection, because it includes conduction times both from the dendritic processes of the DSMD in the lobula to the terminals in the protocerebrum and from the synapse to the cell body of the descending DSMD neurone. A latency of 1.8 ms was found across the synapse between similar sites in the larger, and therefore more rapidly conducting, LGMD1–DCMD neurones (Rind, 1984). The operation of the connection which LDSMD(B) makes with the descending neurone PDDSMD (Rind, 1990) contrasts with the operation of the connection which the non-directionally selective neurone LGMD1 makes with the DCMD (Rind, 1984). At the connection between LGMD1 and DCMD, a spike in the presynaptic neurone invariably gives rise to a spike in the postsynaptic neurone, even at frequencies up to 300 Hz. The reliability of this synapse as a relay originally led to the suggestion that LGMD1 made an electrical synapse with the DCMD (O'Shea and Rowell, 1975). In the directionally selective neurone PDDSMD, the threshold for spiking was reached, in the experiments reported here, only by summation of EPSPs, so that single spikes in the presynaptic LDSMD(B) were not followed one-for-one by spikes in the PDDSMD. This allows more integration of inputs by the postsynaptic neurone, and also enhances the selectivity of the directional response: only spikes at the rates seen during the response to motion in the preferred direction led to spikes in the postsynaptic neurone. This phenomenon is under further investigation. The above results suggest that horizontal directional selectivity may first appear in the response of medullary neurones to motion, but the selectivity of the response is progressively

sharpened both by neurones in the lobula and at the synapses they make with identified descending neurones such as PDDSMD. The behaviourally monitored direction-selective motion-detecting response reflects this refinement at the various stages in the motion-detection pathway. Torre and Poggio (1978) proposed two alternative biophysical models to describe the interactions underlying the direction-selective motion-detecting responses of single neurones in the visual system. However, at the single-cell level, neither of these models produces all the features of the behavioural response (Grzywacz and Koch, 1987). Grzywacz and Koch (1987) suggested that the emergence of the direction-selective motion-detecting response, described phenomenologically by Reichardt (see Reichardt, 1987, for a review), occurs in the visual system at the level at which neurones are integrating information from large numbers of direction-selective cells. The directionally selective pathway into the locust lobula, then to the protocerebrum, and from there down the nerve cord would be amenable to such an analysis, particularly when the contribution of the synapse between the two DSMD neurones in the direction-selective pathway can be identified.

Organization of inputs to descending neurones

The PDDSMD neurone receives inputs from both eyes: the preferred direction is backwards over the ipsilateral eye and forwards over the contralateral eye. LDSMD(B) excites PDDSMD monosynaptically during backward movement over the ipsilateral eye, but there is no anatomical overlap between LDSMD(F) from the contralateral eye and PDDSMD. The role of LDSMD(F) in shaping the response of descending DSMD neurones to horizontal motion is not yet known. One possibility is that the two neurones mutally inhibit one another. No descending DSMD neurones with a preferred direction forwards over the ipsilateral eye and backwards over the contralateral one have been found in this or previous studies (Kien, 1974a,b, 1977; Rind, 1987, 1990). Kien (1974a,b, 1977) recorded from DSMD neurones in the circumoesophageal connectives, and the optic stalk and concluded that they underlay the optomotor response of the locust to horizontal motion. Although PDDSMD and presynaptic LDSMD(B) share features with the neurones described by Kien, it is not possible to state with certainty that they are synonymous with them, or that they underlie the optomotor response of the locust to horizontal movement. Neurones in the locust optic lobe also contribute to deviation detection by three identified descending neurones (Simmons, 1980; Reichert *et al.* 1985; Gris and Rowell, 1986; Rowell and Reichert, 1986; Hensler, 1988). Three of these deviation detectors are excited by horizontal motion over the compound eyes. Two, DNI and PI(2)5, are excited by movement towards the ipsilateral side; the other, DNC, is excited by movement towards the contralateral side and thus has the same directional preference as LDSMD(F). Thus, neurones like LDSMD(F) and LDSMD(B) may contribute to the responses in the horizontal plane (yaw) of deviation-sensitive neurones which integrate the sensory information from compound eyes, ocelli, wind hairs on the head and neck receptors.

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