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MOTION-SENSITIVE CELLS: PUTATIVE LARVAL NEURONES INCORPORATED INTO THE OPTIC LOBE OF THE ADULT SWALLOWTAIL BUTTERFLY

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Summary

Intracellular recordings were made from neurones with large somata situated at the anteromedial edge of the medulla of the swallowtail butterfly *Papilio xuthus*; the neurones were then filled with Lucifer Yellow. These cells are putative larval visual interneurones incorporated into the adult optic lobe of the butterfly. There are four classes of motion-sensitive neurones. Two have a dendritic arborization in the dorsal half of the medulla and project an axon to the medial protocerebrum or the contralateral medulla. They respond to vertical downward motion with a strong burst of action potentials and their background activities are inhibited by motion in the opposite direction. Variations in position of the dendritic fields suggest that each group of neurones forms a coherent set of cells detecting vertical motion in the dorsal half of the visual field of the eye. The third class of neurones connects the lobula plate to the midbrain and is preferentially sensitive to vertical upward motion. The fourth class of neurones has a dendritic arborization in the lobula. These neurones are tonically excited by a moving grating irrespective of the stimulus orientation and movement direction. The presence of motion-sensitive medulla neurones suggests that the detection of local motion is completed in the distal medulla.

Introduction

During metamorphosis, holometabolous insects replace their larval simple eyes with adult compound eyes. The compound eye is produced by the eye imaginal disk in the epidermis and its optic lobe develops from the optic lobe anlagen in the brain (Nordlander and Edwards, 1969*a*,*b*; Edwards, 1969; Meinertzhagen, 1973). Neurogenesis in the optic lobe anlagen begins in the larva and accelerates after pupation to generate a large imaginal optic lobe at the end of the pupal period. Although the rapid growth of the imaginal optic lobe is mostly due to the production and differentiation of new imaginal neurones during the course of adult development, the optic lobe receives innervation from many neurones in the central region of the brain (e.g. Strausfeld, 1976). Immunocytochemical studies on the postembryonic development of the optic lobe revealed that many central neurones innervating the optic lobe originated from larval

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neurones that persisted throughout metamorphosis and were incorporated in the imaginal brain (Breidbach, 1990; Ohlsson and Nässel, 1987; Nässel *et al.* 1987; Ohlsson *et al.* 1989).

Recent studies following the fate of larval visual interneurones during metamorphosis of the swallowtail butterfly *Papilio xuthus* (Ichikawa, 1994*a*) and the moth *Manduca sexta* (Homberg and Hildebrand, 1994) have revealed that a particular population of neurones in the optic lobe is also of larval origin. The functional properties of the larval visual interneurones have been extensively examined in the butterfly, and colour-specific responses suggest that these neurones may mostly be dedicated to colour vision in the larva (Ichikawa, 1986, 1990, 1991). Most visual interneurones in the second larval optic neuropile can be followed throughout metamorphosis since they have large somata located in the anteromedial edge of the imaginal medulla (Ichikawa, 1994*a*). Following pupation, many larval neurones lose their larval processes and differentiate new processes that enter tangentially into the developing imaginal medulla to play a new role in visual processing.

In the present study, I have examined the anatomical and physiological profiles of neurones with a large soma in the same region as the persistent larval neurones. Most of the putatively persistent larval neurones are strongly sensitive to the motion of grating patterns. Functional roles of the motion-sensitive neurones in the optomotor flight control mechanisms are discussed.

Materials and methods

Swallowtail butterflies *Papilio xuthus* of the summer form used in the present study were caught in the wild or obtained from a laboratory culture. The legs of the butterfly were removed and the animal was mounted, dorsal surface up, to a Lucite holder. The neck was fitted into a fork-like support and the head was immobilized with beeswax so that the longitudinal axis of a compound eye was placed in the vertical plane. The optic lobe was exposed by removing the dorsal portion of the head capsule between the compound eyes. The head cavity was connected to a small chamber that was placed behind the head and filled with physiological saline (NaCl, 140 mmol l^{-1}; KCl, 5 mmol l^{-1}; $CaC1_2$, 2.4 mmol l^{-1}; $MgC1_2$, 1.3 mmol l^{-1}; glucose, 10 mmol l^{-1}). The tip of a glass pipette containing 1 % Pronase was placed on the left optic lobe for 10–20 s and part of the neural sheath covering the dorsal surface of the medulla was removed with a sharpened tungsten needle to facilitate penetration of a microelectrode.

A glass pipette microelectrode filled with 10 % Lucifer Yellow CH was inserted into the somata region of the persistent larval visual interneurones in the anterior medulla (Ichikawa, 1994*a*). An indifferent electrode was placed in the saline bath. Intracellular responses of neurones were amplified in the conventional manner and recorded simultaneously on magnetic tape and a chart recorder. After recording, Lucifer Yellow was ionophoretically injected into the neurone by passing a d.c. hyperpolarizing current of 3–5 nA for 5–15 min. The preparation was maintained at room temperature (19–23 ˚C) for 1 h. The brain in the head capsule was fixed with 4 % paraformaldehyde in 0.15 mol l^{-1} sodium phosphate buffer (pH 7.4) for 30 min, then dissected out and fixed in the same fixative for another 30 min. After washing in the buffer, the tissue was

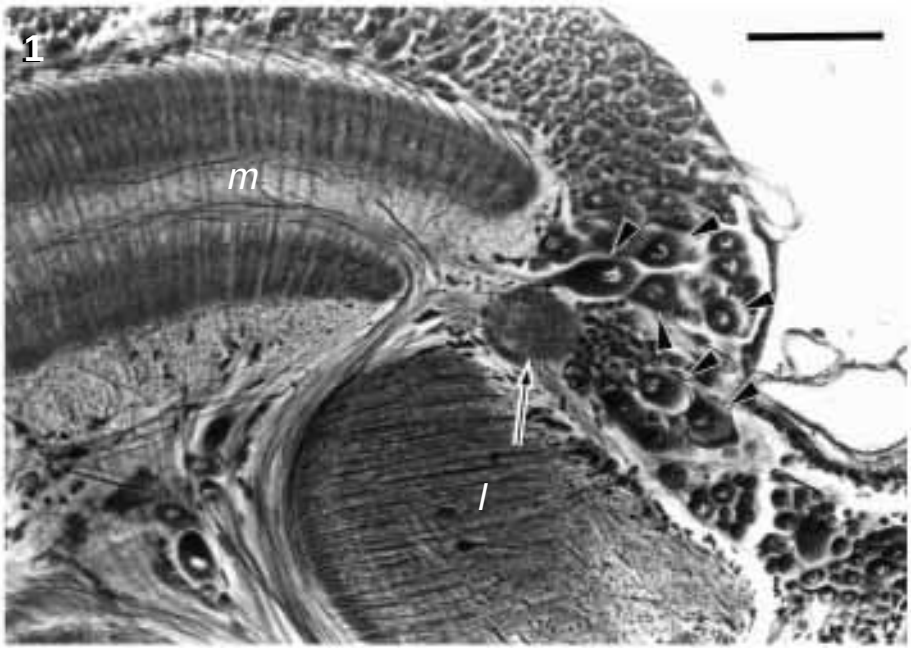

Fig. 1. Horizontal section of the anterior medulla, cut slightly above the dorso-ventral equator of the medulla. *m*, medulla; *l*, lobula; arrow, accessory medulla; arrowheads, large somata of putatively persistent larval neurones. Scale bar, 100 μm.

dehydrated and cleared with methyl salicylate. The brain was viewed, drawn and photographed using a Nikon fluorescent photomicroscope.

A 500 W xenon arc lamp was used for illumination of the eye or projection of moving patterns. Each compound eye was illuminated *via* a bundle of optical fibres (1.5 mm in diameter), one end of which was placed 1 mm from the lateral surface of the eye. White light from the lamp was focused on the other end of the light guide. The duration of illumination was controlled by a mechanical shutter interposed between the lamp and the light guide. The intensity of the stimulus at the eye was $0.3\,W\,m^{-1}$. The image of a vertical or a horizontal grating was projected onto a tangent screen made of tracing paper (5 cm×5 cm) and placed 2 cm in front of the head or 2 cm to the side of the left eye. The grating patterns had 80 % contrast modulation and a 10° spatial period at the closest distance to the eye. The mean brightness of the screen was $4000\,cd\,m^{-2}$. The vertical (or horizontal) grating was moved horizontally (or vertically) by a stepping motor equipped with a reduction gear system. Movement of grating patterns was monitored by a small photocell mounted on the corner of the screen.

Results

There are 30–40 large somata (30–40 μm in diameter) as well as many somata of medium size (20–30 μm) around the accessory medulla in the anterior medulla (Fig. 1). Most of the 90 neurones examined in the present study had a soma within the larger size range. They discharged impulses in the dark and responded to illumination with an

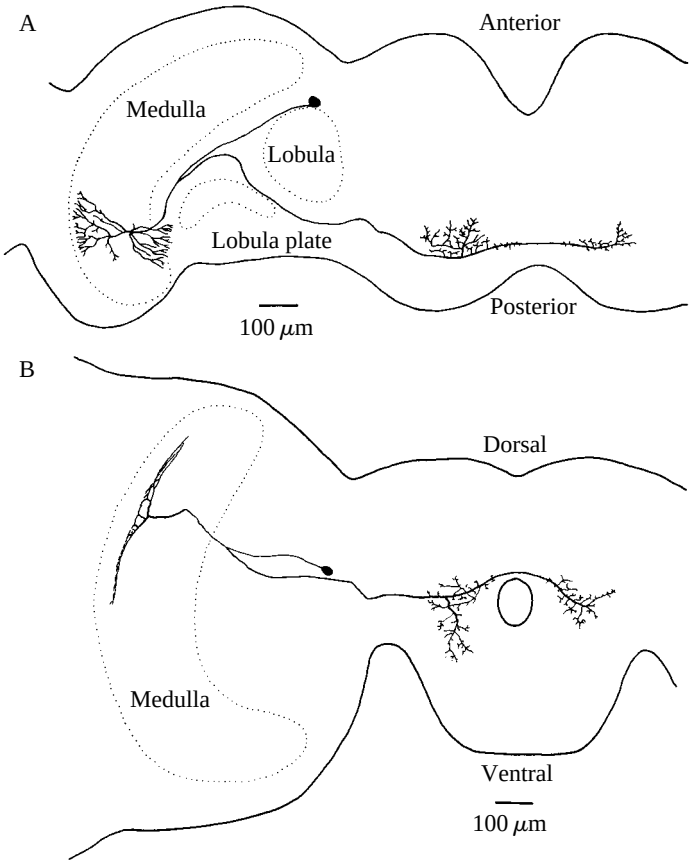

Fig. 2. *Camera lucida* drawings showing dorsal (A) and frontal (B) views of an Mp neurone. The neurone is characterized by a primary neurite projecting to an optic tract proximal to the medulla neuropile and by an axon terminal in the midbrain.

increase or decrease in the rate of discharge, associated with a slow depolarization or hyperpolarization of the membrane. The majority were characterized by sensitivity to moving grating patterns. Motion-sensitive neurones were divided into four groups (or types) according to their anatomical and physiological profiles. Two groups of neurones have a dendritic arborization in the medulla and the other two have an arborization in the lobula or the lobula plate.

Response and structural profiles of motion-sensitive neurones

Medulla neurones

Two groups of neurones with dendritic branches in the medulla showed similar responses to vertical movement of a grating pattern, but they were easily distinguished by their different terminal sites.

The first group of neurones (N=28) was termed Mp neurones. An Mp neurone projects a primary neurite to an optic tract between the medulla and the lobula complex and enters the synaptic layer of the medulla to ramify within the dorsal area of the neuropile (Fig. 2).

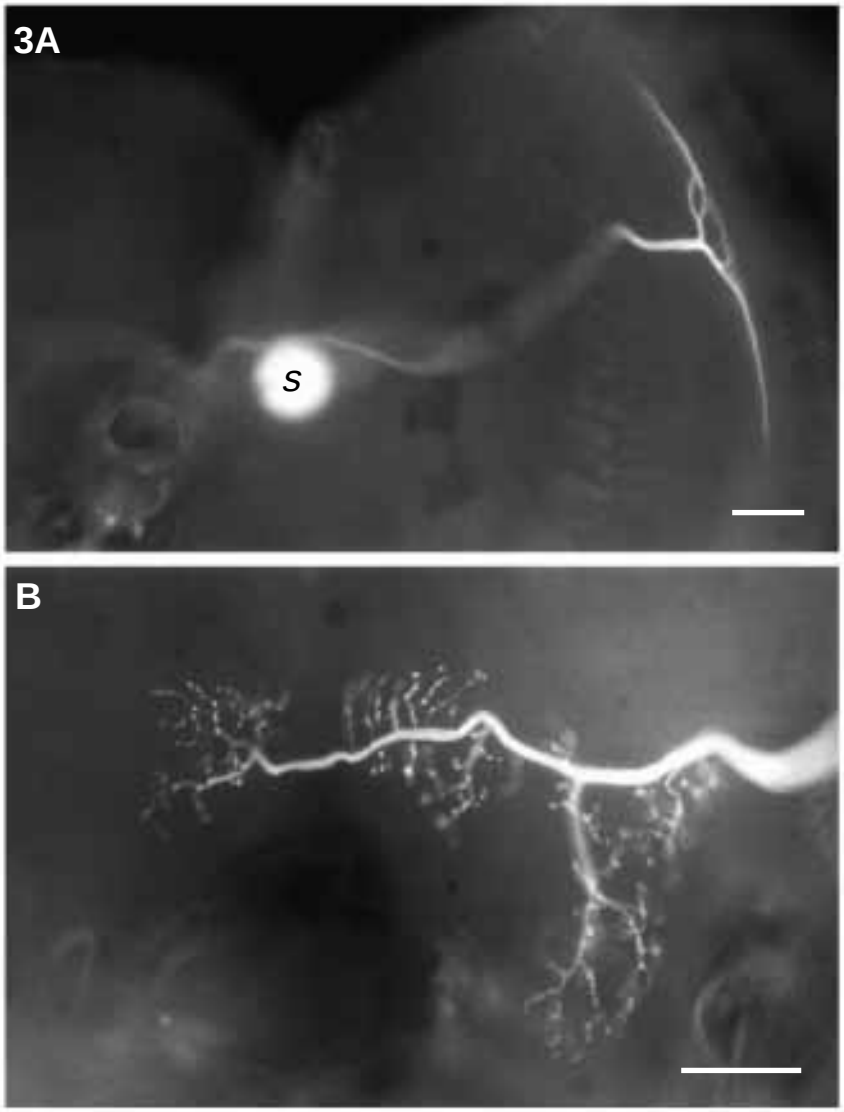

Fig. 3. Frontal views of an Mp neurone stained with Lucifer Yellow. (A) Dendritic branches in the medulla. *s*, soma (out of focus). (B) Varicose axon terminals in the midbrain. Scale bars, 100 μm.

The dendritic branches are smooth in appearance and occupy a narrow band of medulla along the dorso-ventral (vertical) axis of the brain (Fig. 3A). The dendritic field spans the entire extent of the dorsal medulla above the equator, except for the most dorsal portion of the medulla, and extends over about 20 columns of neuropile in a horizontal plane. The axon of the cell descends through the inner optic chiasma and enters the posterior optic tract to project varicose terminal branches in the medial protocerebrum around the oesophageal foramen (Figs 2, 3B).

Particular Mp neurones occupy particular territories in the retinotopic array of the

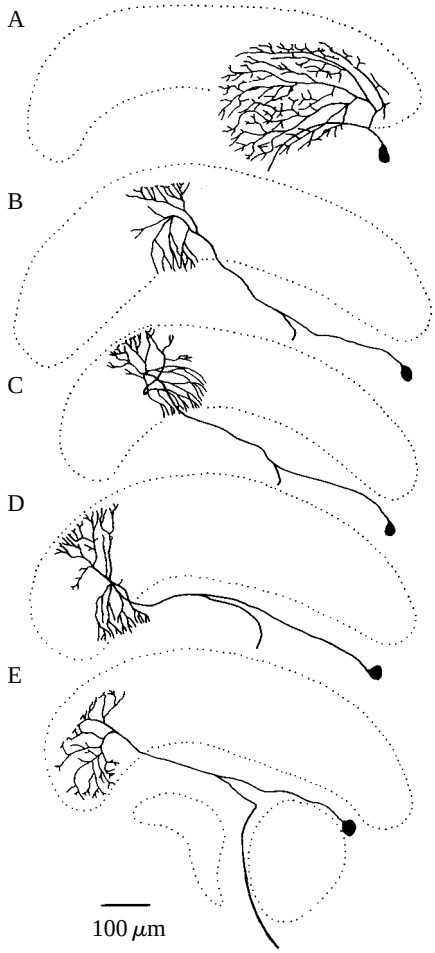

Fig. 4. *Camera lucida* drawings showing dorsal views of Mp neurones with different dendritic fields in the medulla.

dorsal half of the medulla (Fig. 4). Except for the neurone that has an elliptical dendritic field at the anterior region of the neuropile corresponding to the posterior visual field of the eye (Fig. 4A), all these neurones usually have a dendritic field looking like a vertically oriented stripe. The dendrites of each neurone extend over 15–20 % of the width of the medulla in the horizontal direction. Because periodic shifts in the position of the dendritic field can be seen in a group of Mp neurones (Fig. 4B–E), the Mp neurones may consist of a coherent set of cells that, together, cover the dorsal half of the visual field of the eye. There was no neurone that had a dendritic field below the equator of the medulla.

Mp neurones discharged impulses at 50–70 Hz in the dark and showed an increase in their rate of discharge in response to illumination of the ipsilateral (left) eye (Fig. 5A). The impulse discharge rate increased as the intensity of the flash of light increased, until it reached saturation at a level that was 50–70 % of the maximal response induced by a strong motion stimulus. The slight increase in the discharge rate during illumination of the

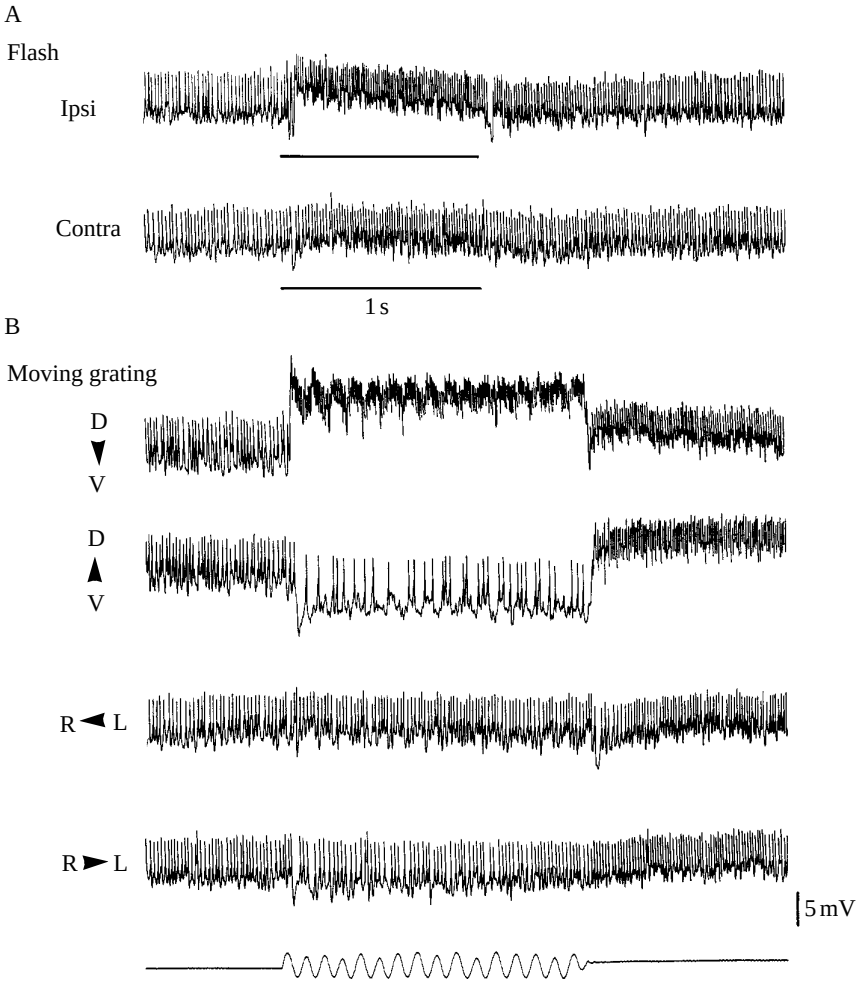

Fig. 5. (A) Intracellular responses of an Mp neurone to a 1 s flash of white light (horizontal bar) to the ipsilateral (Ipsi) or the contralateral eye (Contra). (B) Responses of the unit to vertical and horizontal movement of grating patterns projected on a tangent screen placed in front of the head. D, dorsal; V, ventral; R, right; L, left. The oscillating signal on the bottom trace is the light–dark cycles of the grating pattern monitored by a photocell mounted on the screen. The same notation is used in Figs 8, 10 and 12.

contralateral eye might be due to stray light, because the cells exhibited no significant response to contralateral stimuli when the stimulus intensity was decreased by 1 log unit. The maintained discharge of the neurone was increased slightly by prolonged (background) illumination. The neurone was strongly excited by vertical downward movement of the horizontal grating, whereas it was inhibited by upward movement of the grating (Fig. 5B). The maximal response of Mp neurones induced by vertical motion was always stronger than the saturated response evoked by stationary stimuli. There was often a small ripple or oscillation in the depolarizing and hyperpolarizing membrane potentials in time with the light–dark cycle (contrast frequency) of the moving grating (Fig. 5B). Horizontal motion of

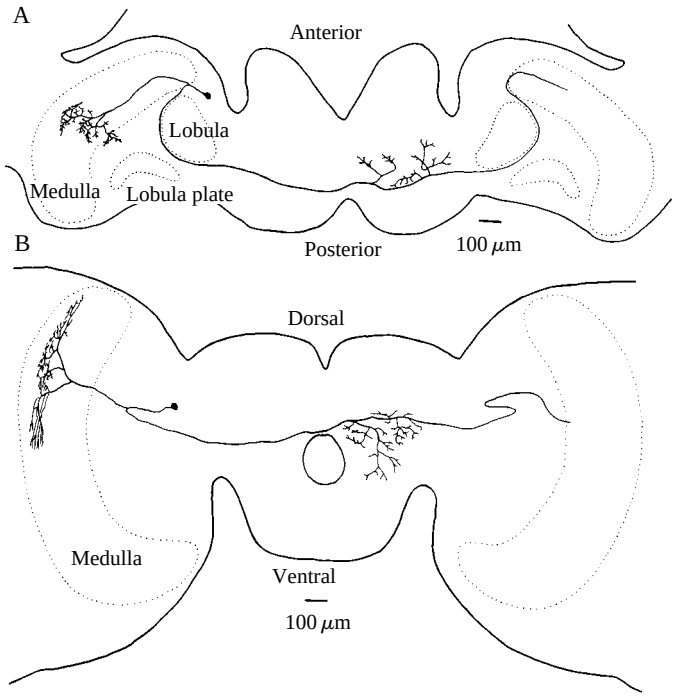

Fig. 6. *Camera lucida* drawings showing dorsal (A) and frontal (B) views of an Mm neurone. The neurone is characterized by a primary neurite projecting directly into the medulla neuropile and an axon terminal invading the contralateral medulla.

the vertical grating usually elicited a weak excitation or inhibition in the impulse discharge activity of the neurone. The response profile indicates that the 'preferred direction' of the cell does not coincide with the longitudinal axis of the compound eye but may be very close to its longitudinal axis. The change in the impulse discharge rate induced by horizontal motion of a grating pattern showed some variability, but was usually less than one-third of the maximal change in the discharge rate induced by vertical motion.

The second group of medulla neurones (N=16) were termed Mm neurones. These neurones project a primary neurite into the middle (serpentine) layer of the neuropile and extend dendritic branches dorso-ventrally in the dorsal area of the medulla neuropile (Fig. 6). An axon runs down the valley between the lobula and the lobula plate, passes the posterior optic tract and enters the contralateral optic lobe. The axon in the contralateral optic lobe ascends through the inner optic chiasma and turns anteriorly and then laterally to invade the medulla neuropile. The complete structure of the axon terminal in the medulla could not be determined because the dye fill was incomplete. Axonal branches are seen in a contralateral region of the medial protocerebrum. Most neurones also have a stripe-like dendritic field localized to a particular area of the upper half of the medulla, though a few neurones innervating the anterior area of the neuropile have an elliptical field (Fig. 7). The cell that has a dendritic arborization in the anterior area of the medulla (Fig. 7A) projects an axon deep into the contralateral medulla neuropile, suggesting that

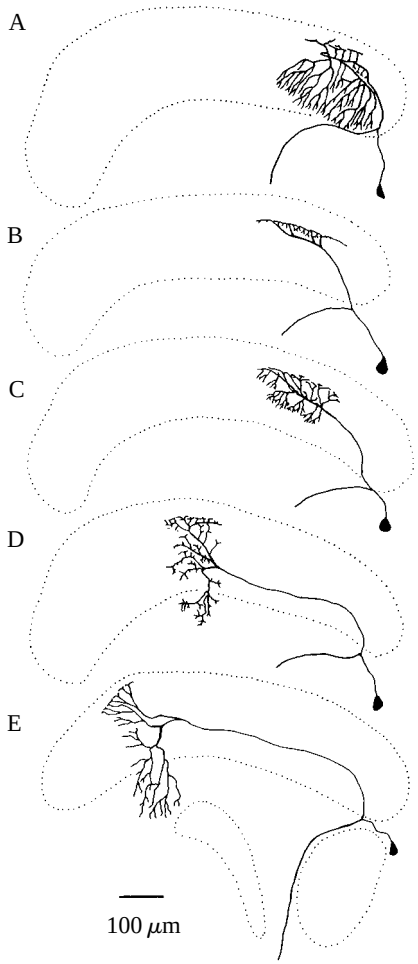

Fig. 7. *Camera lucida* drawings showing variations of dendritic fields of Mm neurones.

the axon of an Mm neurone does not connect two bilaterally symmetrical areas in the right and left medullas.

Mm neurones were also maximally sensitive to vertical downward movement of the horizontal grating and the directional motion-selectivity of the neurones was indistinguishable from that of Mp neurones (Fig. 8B), though Mm neurones were often characterized by their phasic response to a flash of light on the ipsilateral eye (Fig. 8A). Mm neurones may consist of another set of vertical downward-sensitive neurones in the medulla.

Lobula plate neurone

The lobula plate neurones spread dendritic branches into the dorsal and ventral regions of the lobula plate and project diffuse axonal branches to the lateral protocerebrum near the optic lobe (Fig. 9). Because the lobula plate neurones identified in this study were few

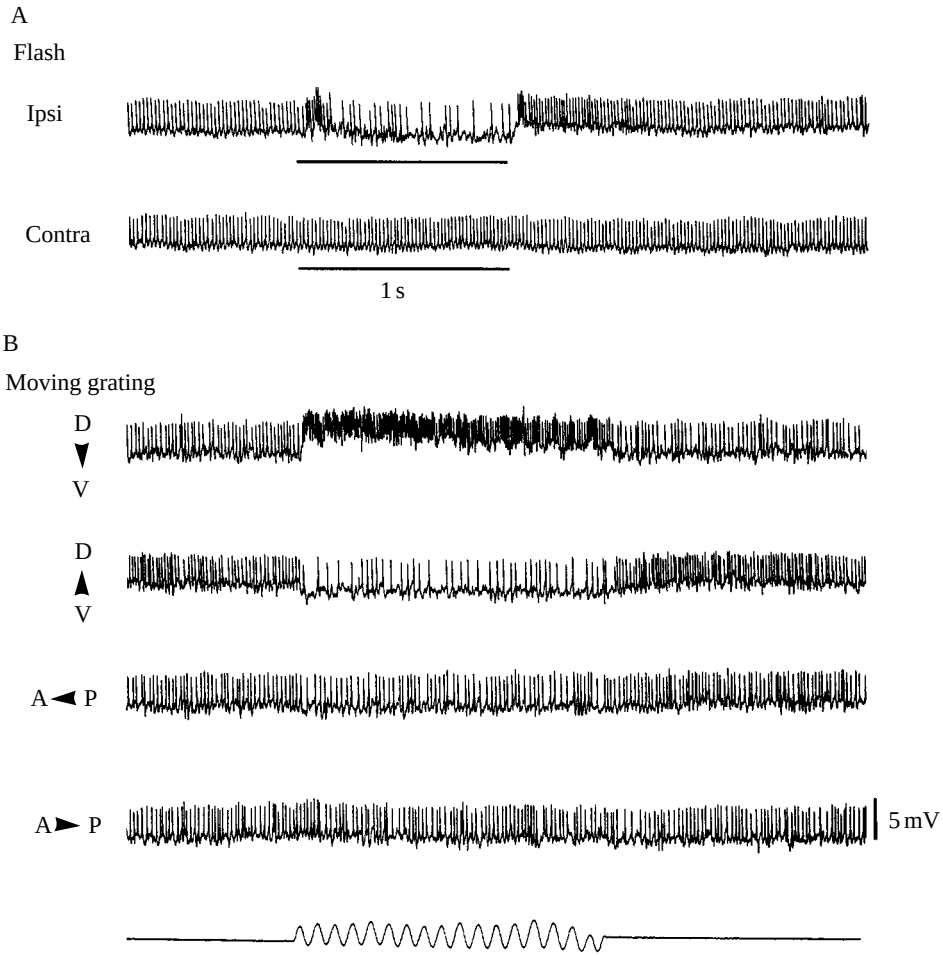

Fig. 8. (A) Responses of an Mm neurone to illumination (horizontal bar) of the ipsilateral (Ipsi) and the contralateral eye (Contra). (B) Responses of the cell to movement of grating patterns projected on a tangent screen placed beside the left eye. D, dorsal; V, ventral; A, anterior; P, posterior.

in number (*N*=4), these neurones may be a unique element or one of a very small group of similar neurones in the lobula plate.

In contrast to the two sets of medulla neurones, the lobula plate neurones were strongly excited by vertical upward motion of the grating and inhibited by motion in the opposite direction (Fig. 10B). They were almost insensitive to horizontal movement of a vertical grating in a frontal visual field. Although the simultaneous illumination of both compound eyes did not elicit a strong response, small IPSP-like deflections of the membrane potential were seen at the onset and offset of the illumination (Fig. 10A). Because the small deflection at the offset of illumination is produced by illumination of the ipsilateral eye, whereas that at the onset of illumination is produced by illumination of the contralateral eye, the receptive field of the neurone may be binocular.

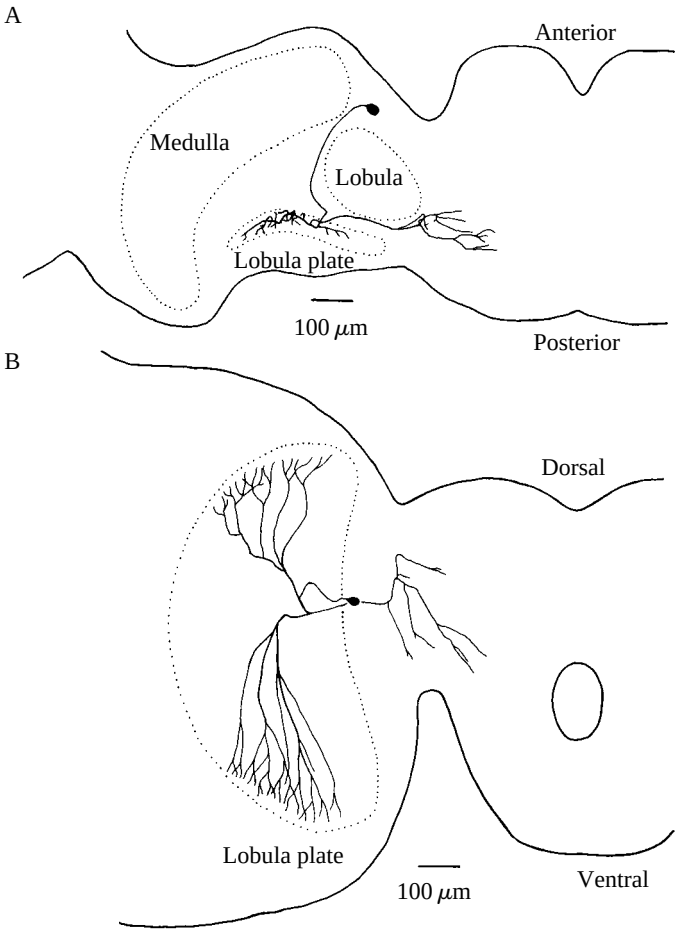

Fig. 9. *Camera lucida* drawings showing dorsal (A) and frontal (B) views of the lobula plate neurone.

Lobula neurone

The primary neurite of the lobula neurone wraps around the lobula and gives off a dense arborization in the outer superficial layer of the neuropile (Fig. 11). Several fine processes extend diffusely to the midbrain. Few fine structural specializations of branches were seen.

The lobula neurone was physiologically characterized by a lack of directional motion selectivity, i.e. it responded to motion of grating patterns in any direction (Fig. 12B). Because the neurone responds with significant excitation to stimulation of both compound eyes (Fig. 12A), its receptive field, like that of the lobula plate neurone, may be binocular. Similarly, the neurone might also be a unique element or belong to a family of only a few members, since few neurones of this type were identified from recordings (N=2).

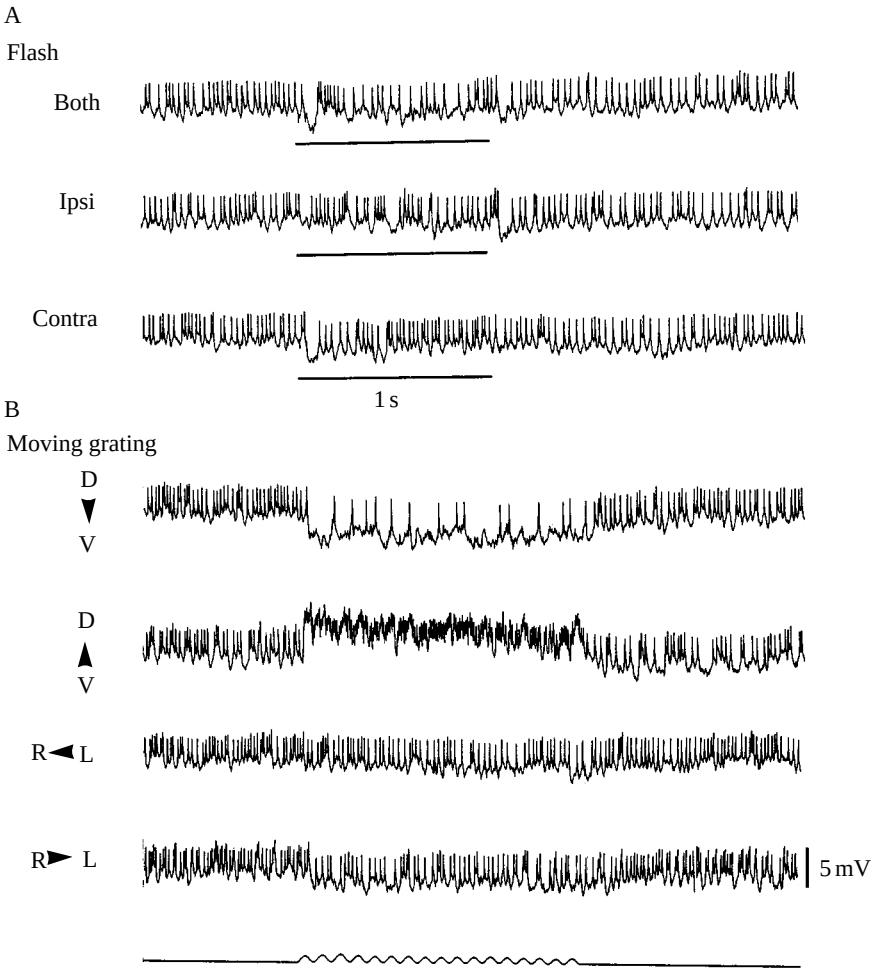

Fig. 10. (A) Responses of the lobula plate neurone shown in Fig. 9 to simultaneous illumination of both compound eyes (Both) or separate illumination of the ipsilateral (Ipsi) and the contralateral eye (Contra). (B) Directional selectivity of the cell. Stimuli were projected on a frontal screen. D, dorsal, V, ventral; R, right, L, left.

Profiles of the contrast frequency response

The contrast frequency response characteristics of the four groups of neurones were analyzed by moving the grating at various velocities in the quasi-preferred direction in a vertical or a horizontal plane. Although levels of the maximal response of the four types of neurones differ, the neurones all have similar tuning properties (Fig. 13). The excitatory responses increase with increasing contrast frequency of the stimulus, reach a maximum at 12–20 Hz and decrease rapidly with a further increase in the frequency.

Other neurones

There were at least three groups of neurones with a large soma in the anterior medulla and they innervated the lobula or the lobula complex. These neurones responded to a

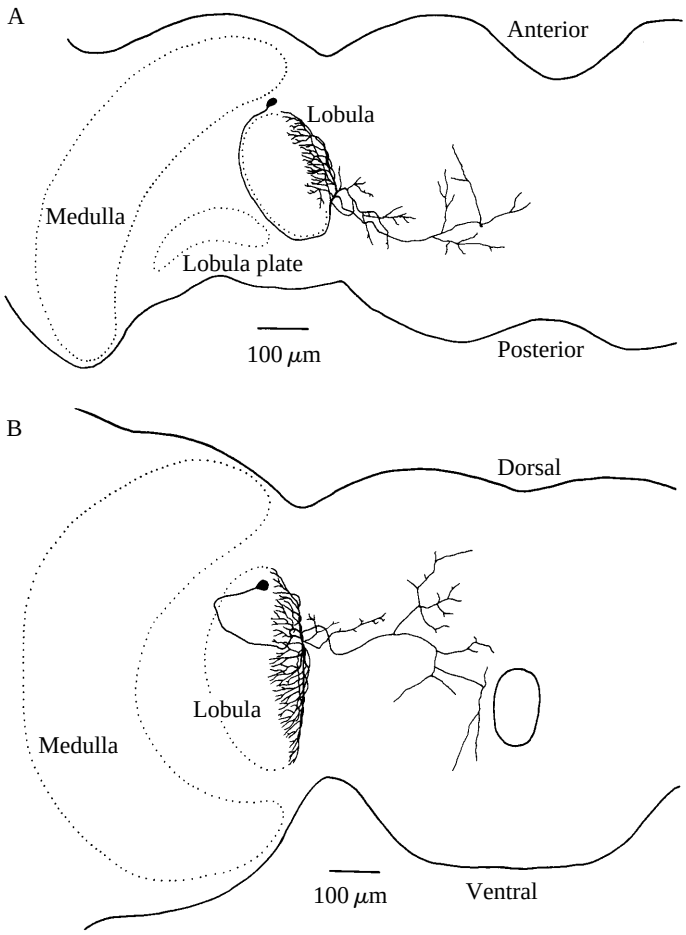

Fig. 11. *Camera lucida* drawings showing dorsal (A) and frontal (B) views of a lobula neurone.

stationary flash of light but not to motion of grating patterns (data not shown). Of these neurones, which should be characterized by other visual parameters, one was serotonin-immunoreactive and its functional properties have been described elsewhere (Ichikawa, 1994*b*). In the anterior edge of the medulla, no evidence was obtained for a neurone that was preferentially sensitive to horizontal motion or that had dendritic branches in the ventral half of the medulla.

Discussion

In the swallowtail butterfly, there are about 100 visual interneurones that have somata significantly larger than those of the surrounding imaginal cells and that innervate the proximal larval optic neuropile (medulla) (Ichikawa and Tateda, 1984; Ichikawa, 1991). It is easy to follow most of the larval medulla neurones throughout metamorphosis because their large somata are always found at a fixed position, at the anterior margin of

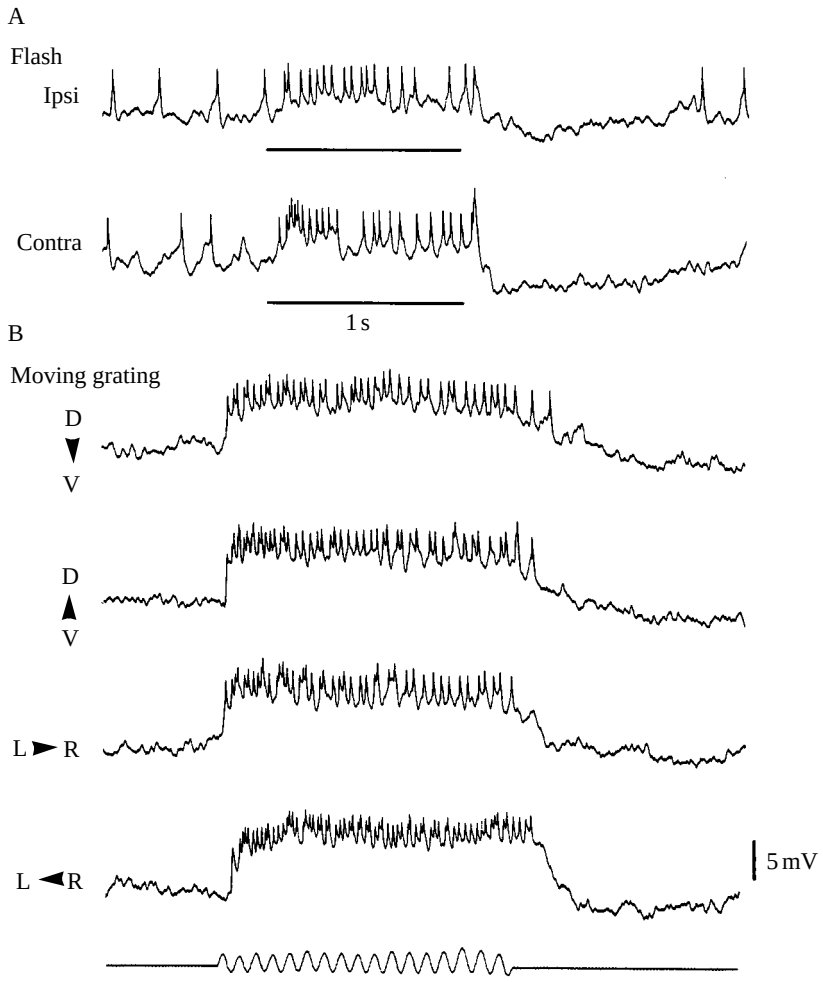

Fig. 12. Responses of the lobula neurone illustrated in Fig. 11 to stationary flashes of light (A) and moving grating patterns (B). Illumination of either the ipsilateral (Ipsi) or the contralateral eye (Contra) excites the neurone and motion of the grating in any direction produces a strong burst of action potentials superimposed on a large depolarization of the membrane. D, dorsal; V, ventral; R, right, L, left.

the imaginal medulla where the larval medulla neuropile remains as a small glomerulus, the 'accessory medulla' (Ichikawa, 1994*a*). At the adult stage, the large somata are 30–40 μm in diameter. Since no sign of degeneration of the persistent larval medulla neurones was seen during metamorphosis (T. Ichikawa, unpublished observation), it seems reasonable to assume that all the large somata were persistent larval neurones. Many medium-sized somata (20–30 μm in diameter) are visible in the same region. Although some must be of larval origin, it is difficult to determine whether they are persistent larval neurones or imaginal ones produced during postembryonic development. The motion-sensitive neurones described here had a soma that could be classified into the large size range. Thus, they are probably remodelled larval visual interneurones, although

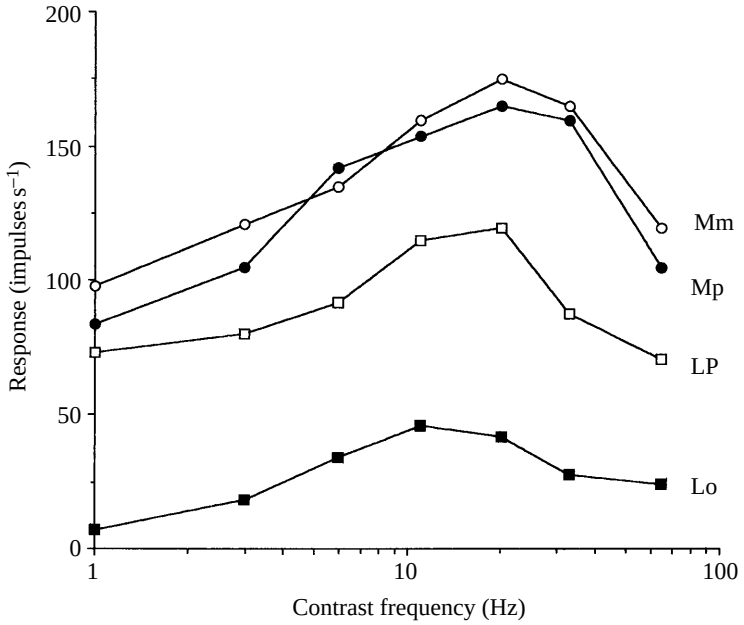

Fig. 13. Contrast frequency response functions of four groups of neurones. The spatial wavelength of the grating patterns at the closest distance to the eye was 10 °. Mp, Mp neurone; Mm, Mm neurone, LP, lobula plate neurone; Lo, lobula neurone.

some imaginal neurones may be included in other groups of neurones insensitive to motion, because the latter sometimes have a soma of medium size. Mm neurones showed the same anatomical profile as a class of persistent larval neurones that were revealed by GABA immunocytochemistry and were characterized by a tangential process invading the serpentine layer of the medulla and an axon running down the valley between the lobula and lobula plate (Ichikawa, 1994*a*). In a preliminary experiment, I used GABA immunocytochemistry to study Lucifer-Yellow-filled neurones, but found no obvious GABA immunoreactivity in the Mm neurones. However, this does not mean that the Mm neurones are not GABA-immunoreactive, because injection of a large amount of Lucifer Yellow into a cell often suppresses immunoreactions (Beltz and Kravitz, 1987; Ichikawa, 1994*b*).

The medulla neurones appear to fall into two classes, each member of a class detecting vertical motion in a particular area of the visual field of the ipsilateral eye. Since the dendritic fields of most neurones extend over 15–20 % of the width of the medulla in a horizontal plane, at least 5–7 neurones may be needed to cover the dorsal half of the medulla neuropile. Taking into account that the dendritic fields of neighbouring elements may overlap, 8–12 may be a reasonable estimate for the number of family members, as expected from the distribution of the dendritic branches of the medulla neurones (Figs 4 and 7). If all members of the Mm neurones are GABAergic, the estimate is reasonable, because about a dozen persistent larval visual interneurones are GABA-immunoreactive (Ichikawa, 1994*a*). Physiological analyses of the receptive field of individual neurones

are important for estimating the number of class members as well as for evaluating their functional properties.

The medulla neurones respond strongly to stimulation of the ipsilateral eye (Figs 5 and 8). They show a morphological polarity, i.e. branches in the medulla are smooth in appearance, whereas those in the midbrain are blebby (Fig. 2). It is generally accepted that smooth branches have mainly postsynaptic structures and blebby branches have presynaptic structures (e.g. Ibbotson *et al.* 1991; Milde, 1993). The physiological and anatomical findings suggest that the medulla neurones may be centripetal elements that relay visual information from the ipsilateral medulla to the midbrain (and to the contralateral medulla). Because the lobula and lobula plate neurones have binocular visual fields and there are few fine structural specializations of branches (Figs 9–12), it is difficult to discuss the input and output branches of the neurones. The arborization in the lobula or the lobula plate is likely to be dendritic (input) branches because (relatively large) depolarizations or hyperpolarizations during stimulation may be summed postsynaptic potentials integrated at the thick processes in the optic neuropiles. Further study is needed to determine the direction of information flow in these neurones.

Directionally selective responses have been fully analyzed for neurones present in the third optic neuropile, the lobula plate, of flies (e.g. Hausen, 1984; Hausen and Egelhaaf, 1989). The lobula plate contains a variety of motion-sensitive neurones that integrate retinal images of horizontal and vertical motion and send integrated signals to the midbrain and contralateral optic lobe (Eckert and Bishop, 1978; Eckert, 1981, 1982; Hengstenberg *et al.* 1982). Motion-sensitive responses have been recorded from the second optic neuropile, the medulla, of several insect species (butterfly, Swihart, 1968; Ibbotson *et al.* 1991; Maddess *et al.* 1991; moth, Collett, 1970; Milde, 1993; fly, Mimura, 1971; DeVoe and Ockleford, 1976; DeVoe, 1980; Gilbert *et al.* 1991; locust, Osorio, 1986). Most directionally selective medulla neurones seem to be centrifugal, and anatomically identified centripetal neurones are relatively small in number (DeVoe and Ockleford, 1976; Osorio, 1986; Gilbert *et al.* 1991; Ibbotson *et al.* 1991). No coherent set of directionally selective neurones has been described in the medulla of any species of insects, although anatomically homologous neurones were seen as tangential cells (type Mtan 2) in the butterfly *Pieris brassicae* and the moth *Sphinx ligustri* (Strausfeld and Blest, 1970; Collett, 1970). A vertical-motion-sensitive neurone (MV2) in the medulla of *Papilio aegeus* has a stripe-like dendritic field in the dorsal medulla, but this neurone may not correspond to the Mp neurone in *Papilio xuthus*, because the former is sensitive to upward motion and its axon terminates in the ipsilateral side of the medial protocerebrum (Ibbotson *et al.* 1991). The moth *Manduca sexta* has at least five types of tangential cells that connect the medulla *via* the posterior optic tract to the contralateral medulla (Milde, 1993). They appear to be homologous to the Mm neurones in *Papilio xuthus* because of their anatomical similarities: (l) a large soma localized at the anteromedial edge of the medulla, (2) an axonal pathway running down and up the valley between the lobula and the lobula plate, (3) axonal branches in the medial protocerebrum. However, the tangential cells are physiologically insensitive to motion in any direction and their dendritic branches are markedly large or oriented horizontally along the equator of the medulla, if the dendritic field is restricted to a narrow band of the medulla (Milde, 1993).

Comparative studies may reveal whether the tangential cells in the moth and the Mm neurones in the butterfly are phylogenically homologous and whether anatomical and physiological differences between the two species are the result of adaptation to diurnal and nocturnal life styles.

The presence of a variety of large-field motion-sensitive neurones in the lobula plate of the fly and a lack of such neurones in the medulla suggest that spatial integration of signals for local motion occurs intensively in the lobula plate (Hausen, 1984; Gilbert *et al.* 1991). A fly has a set of vertical (motion-sensitive) neurones that together cover the entire visual field of the eye (Eckert and Bishop, 1978; Hengstenberg, 1982; Hengstenberg *et al.* 1982). Each vertical cell usually has a vertically oriented stripe-like dendritic field, which covers the entire dorso-ventral area of the neuropile. The finding that the dendritic fields of the motion-sensitive medulla neurones in the butterfly are restricted to the dorsal half of the medulla raises a few questions. (l) Are there neurones responding to motion in the remaining half of the visual field of the eye and, if so, what is their location? (2) Where are the horizontal-motion-sensitive, afferent neurones localized? (3) Are different aspects of motion detection assigned to different optic neuropiles in the butterfly? One cannot at present answer these questions because data on the distribution of motion-sensitive neurones in the optic lobes of butterflies and moths are limited to the medulla neurones in *Papilio aegeus* (Ibbotson *et al.* 1991) and the lobula and lobula plate neurones in *Papilio xuthus* (Figs 9–12). GABA immunocytochemistry revealed that the lobula plate of a butterfly and a moth contained at least several large neurones anatomically similar to the motion-sensitive lobula plate neurones in the fly; thus, large-field motion detectors might be present (Ichikawa, 1994*a*; Homberg *et al*. 1987).

A motion stimulus often elicits small ripples or oscillations superimposed on the depolarizing or hyperpolarizing postsynaptic potentials, the frequency of which is identical to the contrast frequency of the grating pattern (Fig. 5). A similar oscillatory component superimposed on a d.c. component was obtained from a directionally selective neurone in another species of butterfly and it was enhanced when stimulation was restricted to a small area of the receptive field of the unit (Ibbotson *et al.* 1991). Because the position of the screen on which a stimulus pattern was projected could not be moved to centre the receptive field of the neurone recorded in the present study, the oscillation may be due to partial stimulation of its receptive field. An oscillatory response modulated at the stimulus contrast frequency has been predicted from a model of a local motion detector of the correlation type (Reichardt, 1961; Borst and Egelhaaf, 1989; Ibbotson *et al.* 1991). The response properties of the medulla neurones in the butterfly strongly suggest that spatial integration of output signals from a retinotopic array of such local motion detectors may be completed in the distal medulla.

Optomotor reactions are important for stabilizing the head and body of the insect during flight (Egelhaaf *et al.* 1988; Hausen and Egelhaaf, 1989). The responses of the giant motion-sensitive (V and H) cells in the fly lobula plate usually become maximal at a contrast frequency of 1–7 Hz, and the contrast frequency characteristics are often close to those of optomotor torque responses compensating yawing, rolling or pitching of the body (Eckert, 1982; Hengstenberg, 1982), suggesting that the neurones may be involved in large-field motion-processing for the optomotor reactions. When a fly lacks most of its

V and H cells, as a result of a mutation or ablation of their precursors by laser microsurgery, its optomotor torque response to wide-field motion is greatly reduced, whereas its turning response to a small moving object and an optomotor thrust and its landing response are little affected (Heisenberg *et al.* 1978; Geiger and Nässel, 1982). The results suggest that there may be another motion-sensitive pathway that is not mediated by the V and H cells. The second pathway appears to be tuned to a higher contrast frequency than the first pathway (Eckert and Hamdorf, 1981; Egelhaaf, 1987; Egelhaaf *et al.* 1988). Horridge and Marcelja (1992) reported two types of motion-sensitive descending neurones with different contrast frequency characteristics in the fly, butterfly, dragonfly and locust: 'slow' neurones tuned to 1–10 Hz and 'fast' neurones tuned to 15–20 Hz. Although the directionally selective neurones in the butterfly may be involved in the optomotor circuit for controlling rolling and pitching of the body, their higher contrast frequency (Fig. 13) favours the hypothesis that they may be involved in a second pathway that possibly mediates optomotor lift/thrust responses. The medulla neurones can provide a direct (fast) pathway from the medulla to the medial protocerebrum, the dendritic area of premotor descending neurones, for such fast optomotor responses. The swallowtail butterfly often flutters slowly (or hovers) under or within a clump of bushes while searching for a fertile female or a young shoot of a host plant for oviposition. In such a situation, the insect may have to control its height and position to avoid surrounding objects. The motion-sensitive neurones detecting relative motion of objects in a limited area of the lateral and upper visual field may play an important role in such a visual control mechanism. Physiological and behavioural studies may elucidate the functional roles of motion-sensitive neurones and synaptic connections to efferent neurones; homologous efferent neurones have been found in the *Papilio* butterfly (Ibbotson *et al.* 1991). Because the larval visual interneurones, putative precursors of the motion-sensitive neurones, are localized to a particular region between two imaginal optic lobe anlage (Ichikawa and Tateda, 1984), one may be able to investigate their function in insects missing particular neurones (Geiger and Nässel, 1982).

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