J. exp. Biol. 155, 127–145 (1991)
Printed in Great Britain © *The Company of Biologists Limited 1991*

INTEGRATION OF COLOUR SIGNALS IN THE MEDULLA OF THE SWALLOWTAIL BUTTERFLY LARVA

BY TOSHIO ICHIKAWA

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan

Accepted 11 July 1990

Summary

Spatial and chromatic properties of 25 types of medulla neurones which integrate input from different optical units (stemmata) of the larval eye in the swallowtail butterfly were examined by illuminating individual stemmata with chromatic stimuli. Eleven neurones received different types of colour (opponent) input from a few stemmata; thus, the receptive fields are spectrally heterogeneous. The stemmata dominating these complex neurones were usually located in the frontal (central) region of the eye. Seven neurones showed a relatively homogeneous spectral profile over the receptive field by receiving similar spectral input from two or three stemmata which were usually located in the dorsolateral (peripheral) region of the eye. Three of these simple units showed tonic or phasic responses. The remaining seven were also spectrally simple neurones but with larger receptive fields covering four to all six stemmata. Some units showed a spatial summation of responses or a spatial antagonism between central and peripheral or dorsal and ventral regions of the eye.

Introduction

Insects usually have compound eyes consisting of an array of optical units, ommatidia, each of which contains several photoreceptor cells. The photoreceptor cells for many insects differentiate into colour receptors with different spectral sensitivities (Menzel, 1979). The photoreceptors project axons into the first (lamina) and second neuropiles (medulla) in the optic lobe. There are relatively few species of lamina neurones and, with the lack of distinct wavelength dependency of the neuronal responses (Laughlin, 1976; Meinertzhagen *et al.* 1983; Menzel, 1974; Zettler and Autrum, 1975), it is unlikely that colour information from the photoreceptors is intensively processed in the lamina. In contrast, it is very likely that this task is performed in the next neuropile, the medulla, which has the most elaborate organization of a variety of neural elements (Strausfeld, 1976). For technical reasons, however, medulla neurones examined physiologically are too few to elucidate colour-coding mechanisms in the neuropile. Several neurones found in the honeybee and locust medulla showed colour-opponent responses

Key words: insect, medulla, visual interneurone, colour vision, *Papilio xuthus*, stemmata.

(Hertel, 1980; Hertel and Moronde, 1987; Hertel *et al.* 1987; Kien and Menzel, 1977; Osorio, 1986, 1987).

Lepidopteran larvae have a simple eye consisting of six optical units (stemmata) arranged semicircularly on each side of the head. The receptive field of a stemma overlaps partially with that of a neighbour at a particular region in the visual space (Ichikawa and Tateda, 1982). Each stemma has seven photoreceptor cells beneath a small corneal lens and a crystalline cone (Toh and Sagara, 1982). The photoreceptor cells in each stemma are spectrally differentiated into two or three types (Ichikawa and Tateda, 1980). They project axons into the lamina and medulla (Ichikawa and Tateda, 1984; Toh and Iwasaki, 1982). The close similarity in basic structure of the visual systems between the larvae with stemmata and the adults with compound eyes makes the larval visual system a simple model for insect colour vision. Investigation of the larval visual system may reveal basic or general aspects of neural integration mechanisms of photoreceptor signals in the insect optic lobe.

Response profiles of a variety of visual interneurones in the medulla of the swallowtail butterfly larva were examined by stimulating individual stemmata, separately, with monochromatic light of varying wavelengths. The results indicated that the medulla contained two major groups of functionally different neurones for integrating spectral signals from these photoreceptors. One group of neurones was mono-stemma-dominated, combining the outputs of the different classes of photoreceptors present in a single stemma in specific colour opponent mechanisms, as described in a previous paper (Ichikawa, 1986). The other group of neurones is multi-stemmata-dominated, integrating photoreceptor signals from two or more stemmata into a great variety of neural signals with specific spatial and chromatic properties. This paper describes the functional properties of 25 units, about 50 % of the multi-stemmata-dominated neurons thought to be present in the medulla.

Materials and methods

Preparation, recording and stimulation were the same as in a previous paper (T. Ichikawa, in preparation). Animals used were fifth-instar larvae of the swallowtail butterfly *Papilio xuthus* L. reared in our laboratory. After plugging the mouthparts of a larva with a quick-drying glue, the head of the larva was fixed with beeswax to an experimental chamber. The chamber was then filled with a physiological saline (NaCl 4 mmol l^{-1}; KCl 40 mmol l^{-1}; $MgCl_2$ 18 mmol l^{-1}; $CaCl_2$ 3 mmol l^{-1}; glucose 150 mmol l^{-1}; pH 6.5 with 2.5 mmol l^{-1} KH_2PO_4–$KHCO_3$), and all of the head except for the right lateral part bearing the stemmata was placed to soak in this bath of saline. The brain was exposed by removing a frontal part of the cuticle of the head capsule. Circumoesophageal connectives were cut to reduce the transmission of movement of the oesophagus to the brain. A pair of stainless-steel insect pins, which served as a platform for the brain, was placed under the brain. The tip of a glass pipette containing 1 %

Pronase was put on the brain for 20–30 s to facilitate the penetration of a glass pipette microelectrode.

A microelectrode filled with 1 mol l^{-1} potassium acetate was inserted into the somata region of the medulla neurone between two imaginal disks (Ichikawa and Tateda, 1984). An indifferent electrode was placed in the saline bath. Intracellular responses were amplified in the conventional manner and recorded simultaneously on magnetic tape and a chart recorder.

Each stemma was illuminated independently *via* a quartz optical fibre (150 μm in diameter), the tip of which was placed within 50 μm of the surface of the corneal lens of the stemma. White light from a 500 W xenon arc lamp and monochromatic test light from a grating monochromator (Bausch & Lomb) equipped with a 150 W xenon arc lamp were introduced into each optical fibre with the aid of a small quartz half-mirror. Monochromatic light for background illumination was obtained by interposing an interference filter with a transmission peak at 370, 450 or 580 nm into the pathway from the light source. A rotary metal disk with various patterns of holes was placed in each light path to select the stemmata to be illuminated. The duration of illumination was controlled by a mechanical shutter. The intensity of the light was regulated with quartz neutral density filters and a quartz neutral density wedge. The light intensities were measured by a radiometer (Sanso U-3580) or a thermopile (Kipp & Zonen). The reference intensity of white light ($\log I_w=0.0$) corresponded to 3.2 W m^{-2}, and that of monochromatic light ($\log I_m=0.0$) to 2.4×10^{13} quanta cm^{-2} s^{-1}. Contamination of stray light from a light path scattered into adjacent stemmata, determined in preliminary experiments by measuring photoreceptor responses, was less than −3 log units.

Analysis of response profiles

After successful penetration of a medulla cell in the dark, six stemmata were illuminated first simultaneously and then separately with a white test light of a constant intensity ($\log I_w=-4.0$) to determine the receptive field profile. If the cell was identified as a unit of the multi-stemma-dominated neurone, six stemmata were simultaneously exposed to monochromatic test light of increasing intensities for 1 s at 0.2 Hz. This was done to produce intensity–response functions at 370, 450 and 550 nm; these wavelengths are almost identical to the spectral location of maximum absorbance (λ_{max}) for the three classes of photoreceptors, and are most important for the determination of the influence of those photoreceptors. The stemmata were then individually exposed to the three monochromatic test stimuli at a constant intensity ($-3.0<\log I_m<-2.0$). The intensities of the test stimuli were chosen so as to elicit about 90 % of the maximum response which the unit could produce when all the stemmata were illuminated. If the unit was still responsive after this basic characterization of the chromatic properties throughout the field of view of the stemmata, an additional experiment was made to obtain either intensity–response functions measured at the three wavelengths for individual stemmata or spectral response functions, determined by illuminating all the stemmata or a particular stemma with monochromatic stimuli of various wave-

lengths at a constant intensity. Sometimes similar basic and/or additional experiments were made with white or chromatic light in the background.

Results

Intracellular potentials were recorded from 350 multi-stemmata-dominated neurones. The neurones continued to discharge in the dark and responded with an increase or a decrease in the discharge rate, associated with a slow depolarization or a hyperpolarization of the membrane. Although there was a great variety in this group of medulla neurones, identification of neurones from different preparations was often facilitated because most showed characteristic electrical activities. These included fluctuations of the resting potentials, patterns of impulses and relative amplitude of the slow postsynaptic potentials compared with the impulse height. These electrical activities were closely related to the spatial and chromatic response profiles of the neurones. Examples of intracellular responses of physiologically identified neurones are shown in Fig. 1 (also see Figs 2, 4, 6 and 11). Neurones A–C responded with an increase in the discharge rate of the impulses superimposed on the depolarizing postsynaptic potentials. Neurone A was easily distinguishable from neurone B by its relatively small depolarization and unitary EPSP- and IPSP-like fluctuations of the resting membrane potential in the dark. Neurone C was characterized by a quite regular impulse pattern and a tonic response. The other neurones were characterized by phasic excitatory responses evoked at both the onset and the offset (D) or only the offset (E) of the stimulus. Since the somata of the medulla neurones are distant from their dendrites and axons, which are located in the neuropile (a synaptic region) (Ichikawa and Tateda, 1984), these different features of the electrical activity recorded from somata may reflect different mechanisms of signal generation and different spatial properties for signal propagation from the generating sites, based on different morphology. Similar characteristic electrical activities found in another group of medulla neurones have been described previously (Ichikawa, 1986). The 25 types of neurones identified were roughly divided into two classes according to the chromatic complexity or heterogeneity of the receptive fields: simple and complex. Some were characterized by specific temporal or spatial properties.

Spectral characteristics

Simple cells

Neurones of this class received a similar type of spectral input from different stemmata; hence, the receptive fields were relatively homogeneous.

Fig. 2A illustrates the responses of a neurone that received the same type of colour-opponent input from three stemmata. In response to monochromatic stimuli applied to all six stemmata (All), the unit was excited at a short wavelength (370 nm) and inhibited at intermediate (450 nm) and long wavelengths (550 nm). Applying the same stimuli to individual stemmata (I–VI) revealed that the colour-opponent signals originated from stemmata IV–VI, the three stemmata located in

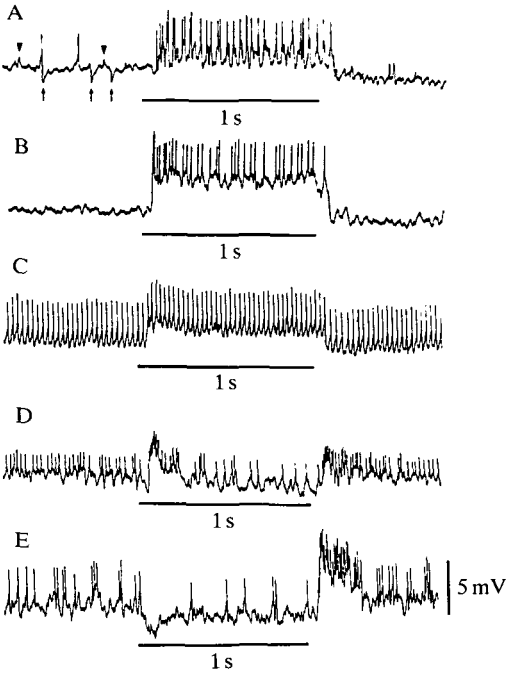

Fig. 1. Intracellular responses of five medulla neurones showing characteristic electrical activity in response to monochromatic (A) or white light stimuli (B–E) under a dark-adapted state. (A) A simple colour-opponent cell characterized by a relatively small depolarization during stimulation (550 nm). Unitary EPSP-like (arrowheads) and IPSP-like (arrows) fluctuations were distinguishable in the dark. (B) A complex colour-opponent cell responding with a relatively large depolarization. (C) A tonic cell discharging action potentials quite regularly. (D) A phasic (on–off) cell showing transient depolarizations when the light was turned on and off. (E) A phasic (off-) unit with a transient depolarization when illumination was terminated. Horizontal bars beneath each record indicate the stimulus duration of 1 s. log I_w=−4.0. log I_m=−2.5. Spatial and spectral response profiles of units A–E are represented in Figs 3A, 8A, 10A, 10B and 10C, respectively.

the dorsal and lateral regions of the eye. Fig. 2B shows a response profile of the unit obtained by illuminating individual stemmata separately (I–VI) and simultaneously (All) with monochromatic light of a constant intensity at 370, 450 and 550 nm. Illumination of stemma V produced the strongest responses, which could reach the maximum obtainable by simultaneous illumination of all stemmata. Stimulation of the adjacent stemmata (IV and VI) evoked weaker responses, saturating at 60–80 % of the maximum response. Illumination of the three other stemmata (I–III) gave a very weak excitation, even with the strongest stimuli. Fig. 2C shows the action spectrum of the unit in which impulse numbers during 1 s stimulation of all stemmata are plotted. The action spectrum and response profile indicate that ultraviolet receptors present in stemmata IV–VI mediated the excitation and that blue and green receptors in the same stemmata mediated the inhibition. Although the excitatory input from stemmata I–III was often too weak

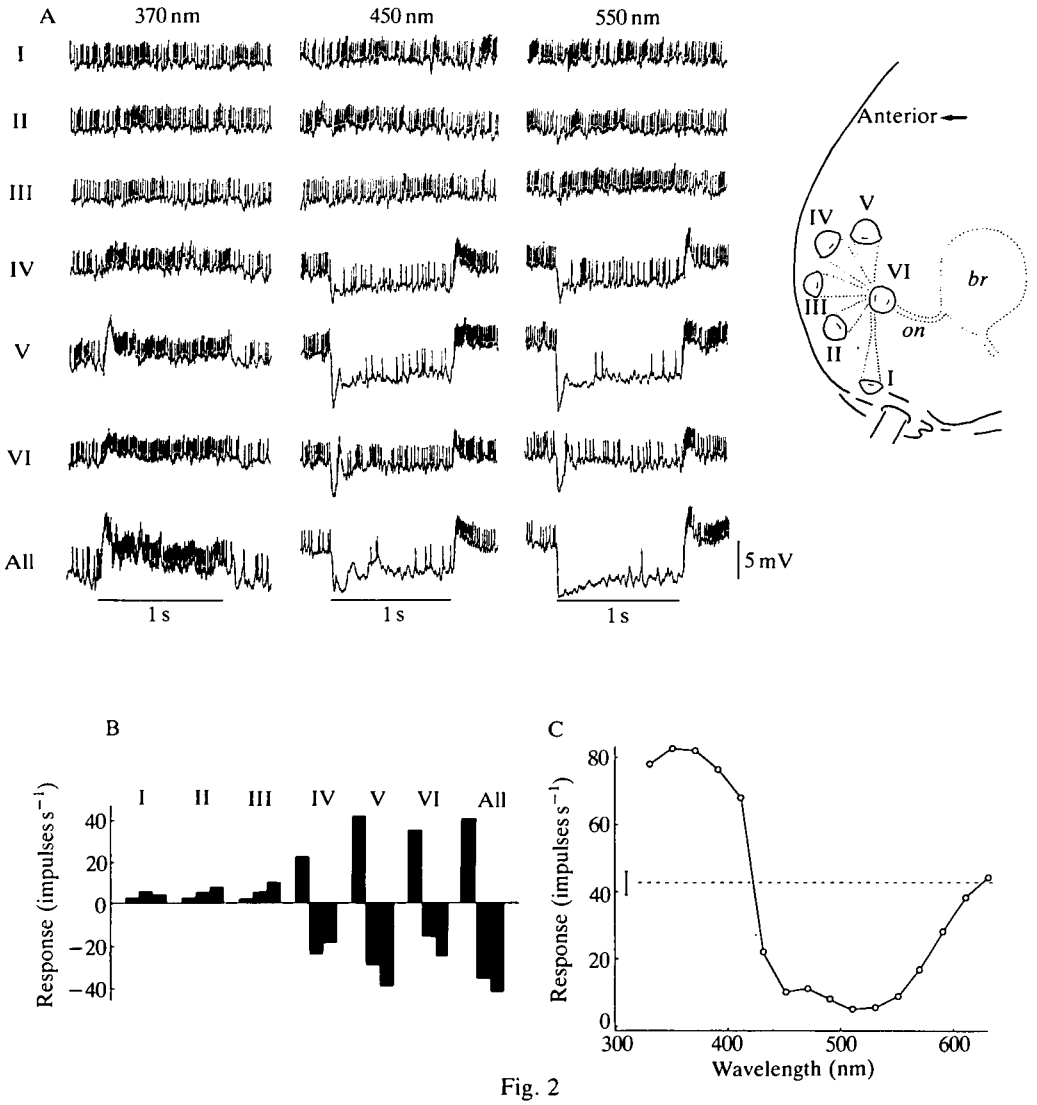

Fig. 2

to be analyzed, an analysis of such weak input made on a different preparation indicated that the excitatory signals were mainly from blue and green receptors.

Fig. 3 illustrates response profiles for four types of neurones with similar colour-opponent profiles but different receptive fields. They were excited by longer wavelengths and inhibited by shorter wavelengths: excitation was mediated by green receptors and inhibition was mediated by ultraviolet receptors in trichromatic stemmata (II, IV–VI) or blue receptors in dichromatic stemmata (I, III). Three neurones (A–C) combined the colour-opponent signals from two or three stemmata located at the frontal part of the eye (II–IV) or at the dorsal and lateral parts of the eye (IV–VI). The fourth neurone (D) received signals from all six

Fig. 2. (A) Intracellular responses of a medulla neurone with a spectrally homogeneous, colour-opponent receptive field obtained by applying monochromatic light of three wavelengths to individual stemmata separately (I–VI) or simultaneously (All). The wavelength-dependent excitatory and inhibitory responses were evoked by illuminating three stemmata (IV–VI) located in the dorsal and lateral parts of the eye. log I_m was −2.5 (about 2 log units above threshold at 550 nm). Stimulus duration (horizontal bars) was 1 s. (B) The spatial and spectral profiles of the response of the unit. In this and the following similar response profiles, left, middle and right bars indicate, respectively, the amplitudes of the response to light at 370, 450 and 550 nm applied to single stemmata separately (I–VI) or all stemmata simultaneously (All). A positive (negative) value of the response represents an increment (a decrement) of the impulse discharge rate by stimulation. (C) An action spectrum of the neurone in the dark-adapted state. Impulse numbers during 1 s of stimulation were plotted. log I_m = −2.5. The vertical bar and the interrupted line indicate the range and the mean of impulse numbers during the 1 s before stimulation, respectively. The inset shows an arrangement of six left stemmata (I–VI). Internal structures are indicated by dotted lines. Axons from photoreceptors in the stemmata join together to form an optic nerve (*on*) and enter the brain (*br*).

stemmata, but stemma IV was the most influential. As apart from stemma IV, single stemmata lost their ability to produce a strong response.

Several neurones had a weak or concealed colour-opponency which required the presence of an appropriate chromatic background to reveal colour-opponent responses. Fig. 4 shows the responses of such a cell. On a neutral background (dark or white) the unit showed inhibitory responses to all wavelengths, whereas on a yellow background it revealed an excitatory response to the intermediate wavelength (450 nm) (Fig. 4A). The action spectra obtained on the white and yellow backgrounds indicate that the inhibitory signals derived from ultraviolet and green receptors, whereas the excitatory signals were from blue receptors (Fig. 4C). The spatial response profile of the unit shows that major sources of

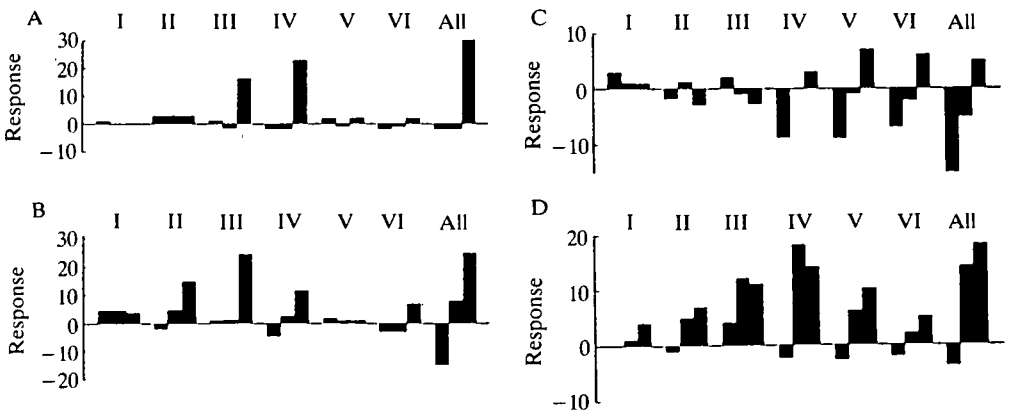

Fig. 3. Spatial and spectral profiles of four simple neurones with colour opponency. They received similar colour-opponent input from two (A), three (B, C) or all stemmata (D).

these signals were from stemmata II–V (Fig. 4B). Fig. 5 shows response profiles for three other neurones showing concealed colour-opponency. For two neurones (Fig. 5A, B), the inhibitory input from the blue receptors was concealed by a strong excitatory input from the ultraviolet and green receptors. The two were

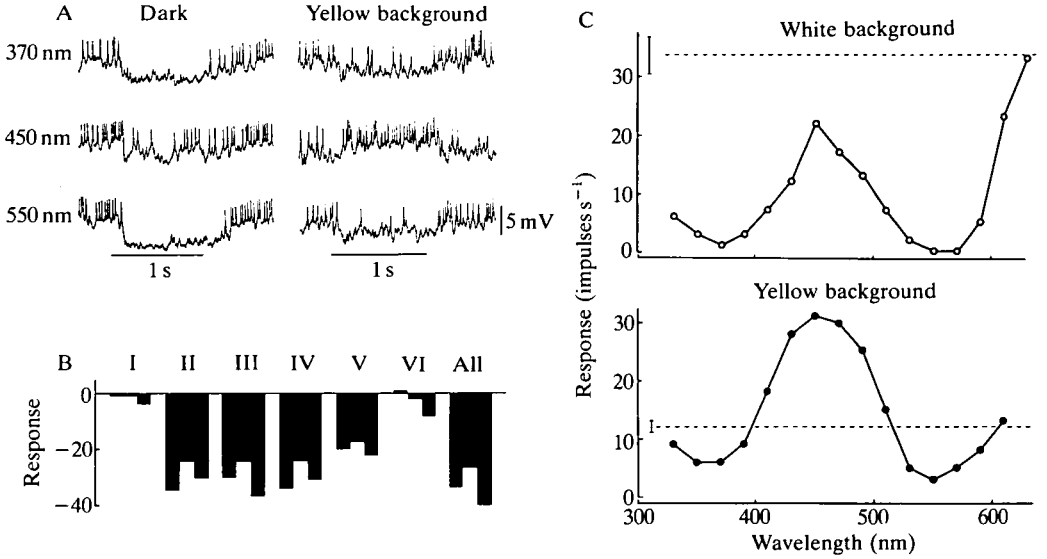

Fig. 4. (A) Intracellular responses of a neurone with concealed colour-opponency to monochromatic stimuli, recorded under two different background conditions. In the dark-adapted state, the cell was inhibited at all wavelengths, whereas it was excited at 450 nm on a yellow (580 nm) background (2.4×10^{10} quanta $cm^{-2}s^{-1}$). log $I_m=-2.5$ (dark) or -1.0 (yellow background). (B) Spatial and spectral profiles of the response of the unit in the dark-adapted state. The unit received dominant input from stemmata II–V. (C) Action spectra of the neurone measured on white (0.32 mW m^{-2}) and yellow (580 nm) backgrounds (2.4×10^{10} quanta $cm^{-2}s^{-1}$). log $I_m=-0.5$ (white background) or -2.0 (yellow background). The vertical bars and interrupted lines indicate, respectively, the ranges and means of impulse numbers during the 1 s before stimulation.

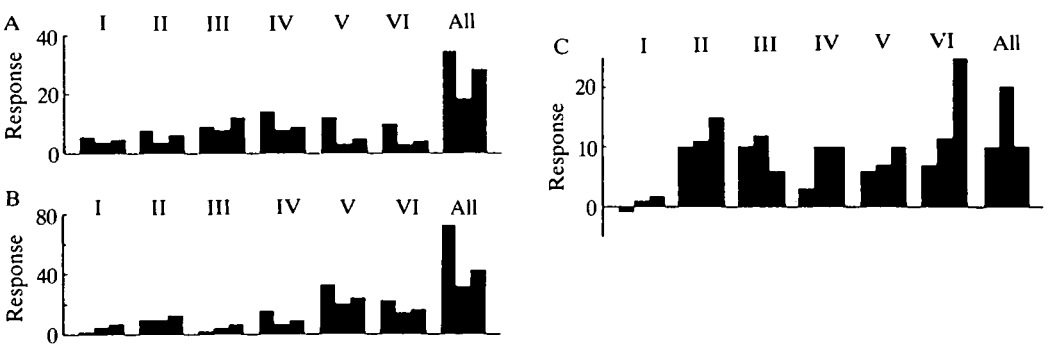

Fig. 5. Response profiles of three neurones with concealed colour-opponency. They receive similar spectral input from five or six stemmata.

easily distinguishable because the most influential stemmata and the magnitude of their maximum response differed. The third cell (Fig. 5C) appeared to receive input from green and blue receptors in stemmata II–VI. However, the response produced by simultaneous stimulation of all stemmata with a green stimulus (550 nm) was smaller than that produced by stimulation of stemma VI with the same stimulus. The depressed nature of the intensity–response function obtained by illuminating stemma III with the green light indicated that this was due to a significant inhibitory effect of green receptors in stemma III.

Complex cells

Neurones of this class receive different chromatic inputs from different stemmata, so that their receptive fields are spectrally heterogeneous.

For the unit illustrated in Fig. 6A, a green (550 nm) stimulus given to stemma II elicited a strong excitation, while the same stimulus presented to the neighbouring stemma III caused an inhibition. Ultraviolet (370 nm) or blue (450 nm) stimuli produced similar responses in both stemmata. The action spectra of the unit indicate that the type of input from stemma II is UV−B+G+ and input from III is B+G− (Fig. 6C). The changes in impulse frequencies caused by illuminating other stemmata were often too small to be distinguished from a spontaneous fluctuation of the maintained discharge (Fig. 6B). However, a small depolariz-

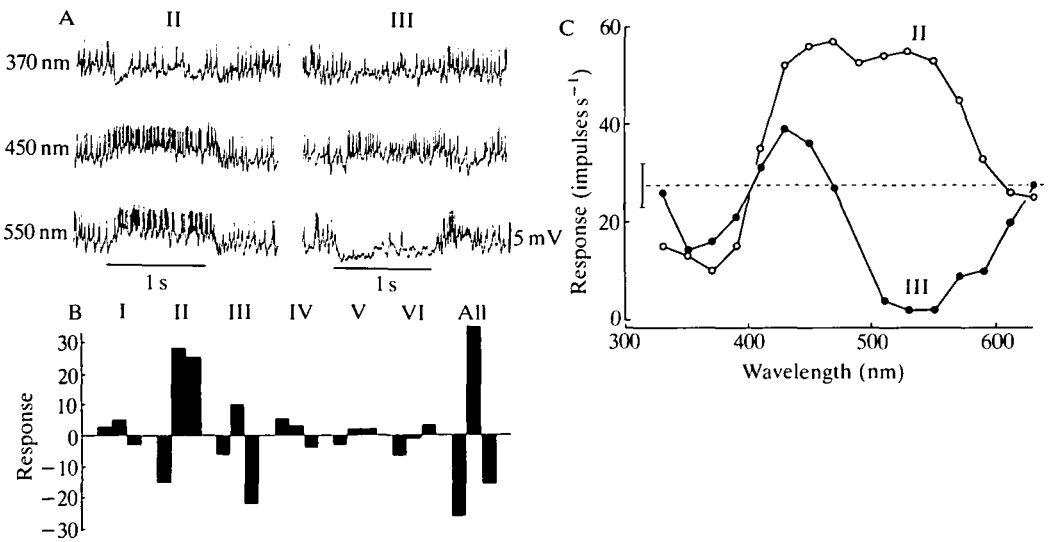

Fig. 6. (A) Responses of a complex neurone receiving different types of colour-opponent input from two adjacent stemmata. The polarities of responses evoked by illuminating stemmata II and III are the same at 370 nm (inhibitory) and 450 nm (excitatory), but different at 550 nm. (B) Response profile of the unit. The colour-opponent input provided by the two stemmata are UV−B+G+ (II) and B+G− (III). $\log I_m = -3.0$. (C) Action spectra of the neurone measured by illuminating stemmata II and III separately. The vertical bar and interrupted line indicate, respectively, range and mean of impulse numbers during the 1 s before stimulation.

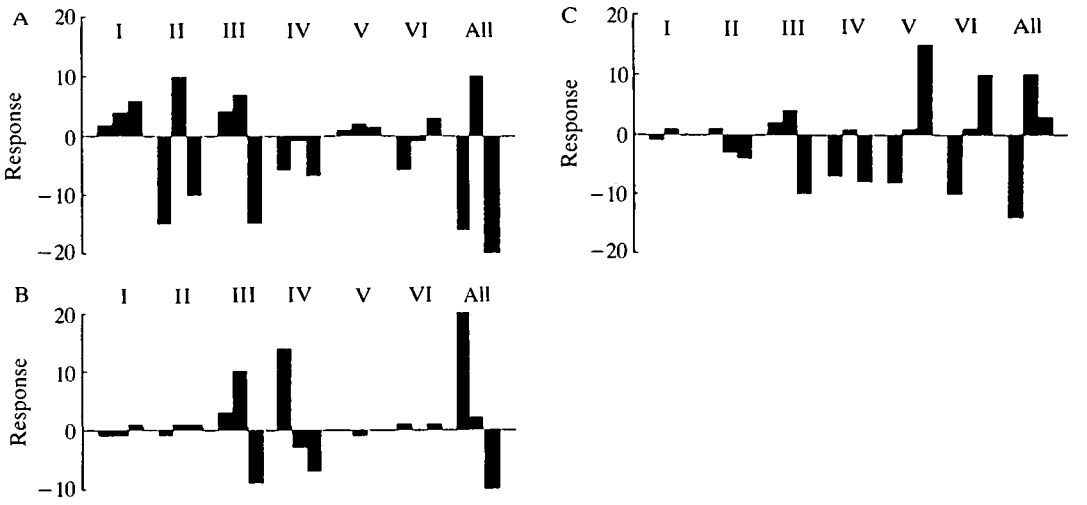

Fig. 7. Response profiles of three complex neurones receiving different types of colour-opponent input from neighbouring stemmata. Types of dominant colour-opponent input determined are as follows: (A) UV−G−B+ (II) and G−B+ (III); (B) G−B+ (III) and UV+G− (IV); (C) G−B+ (III), UV−G−b+ (IV) and UV−G+ (V, VI).

ation caused by stimulating stemmata I and V or a hyperpolarization caused by illuminating stemmata IV and VI was evident. Simultaneous stimulation of all stemmata revealed a colour opponency as if the unit was UV−B+G− (Fig. 6B).

Neurones with other combinations of opponent colours are represented in Fig. 7. The two neurones in Fig. 7A,B have the same colour-opponent input (B+G−) from stemma III as the unit shown in Fig. 6, but they have different inputs from stemmata II (UV−G−B+) (Fig. 7A) and IV (UV+G−) (Fig. 7B). The response profile for the third cell is more complicated (Fig. 7C). Excitation was elicited by shorter wavelengths given to stemma III and by longer wavelengths to stemmata V and VI. In contrast, inhibitory responses were produced by long wavelengths applied to stemma III and by short wavelengths applied to stemmata V and VI. For stemma IV, which is located between stemmata III and V, the unit was inhibited by both short and long wavelengths.

There were several neurones that received a strong colour-opponent input from one or two stemmata and weak (concealed) colour-opponent and/or non-colour-opponent input from neighbouring stemmata. The three cells shown in Fig. 8A–C received a colour-opponent signal from a single stemma (II or IV) and a non-colour-opponent input from a neighbouring stemma (I or III); some weak signals from other stemmata were evident. This response profile appears to be the same as that of some colour-opponent neurones classified as mono-stemma-dominated neurones (Ichikawa, 1986). However, they were easily distinguishable from the latter because these neurones, unlike the mono-stemma-dominated neurones, did

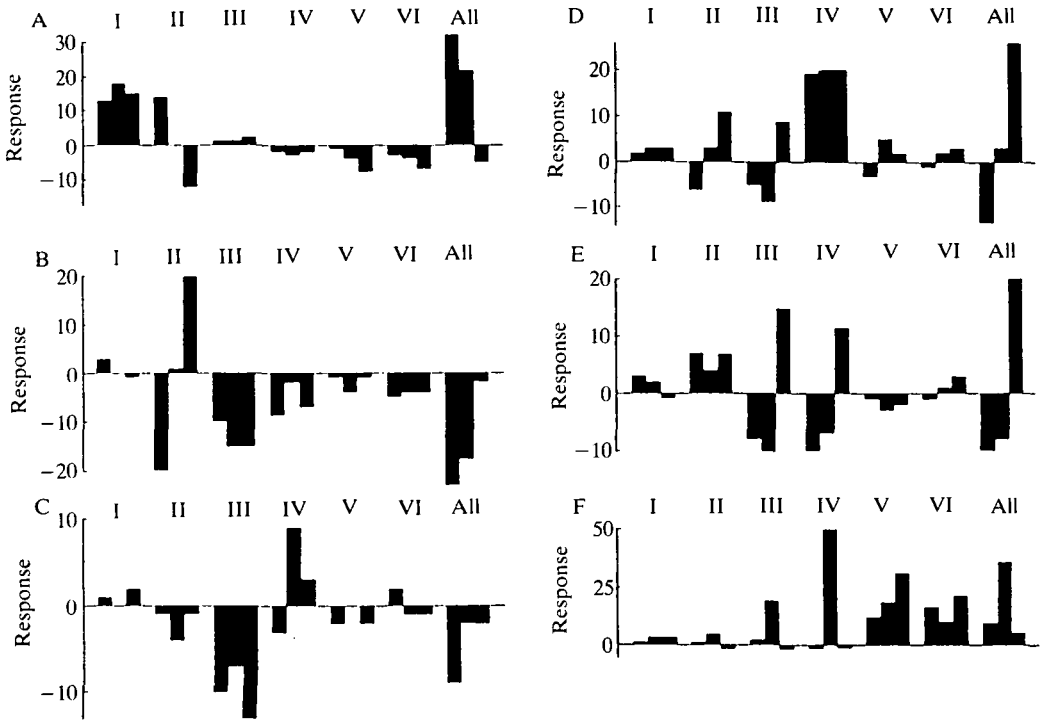

Fig. 8. Response profiles of six complex neurones receiving colour-opponent input and concealed or non-colour-opponent input from neighbouring stemmata. Types of dominant input determined were as follows: (A) B+G+ (I) and UV+G− (II); (B) UV−G+ (II), G−B− (III) and UV−b+g− (IV); (C) G−b+ (III) and B+UV− (IV); (D) UV−B+G+ (II), B−G+ (III) and B+G+UV+ (IV); (E) UV+G+ (II), B−G+ (III) and UV−G+ (IV); (F) B+g− (III), B+uv−g− (IV), G+uv− (V) and UV+G+ (VI).

not show any centre-surround-like spatial antagonism when tested with white light (Fig. 8A,B), and they lost their distinct colour opponency when all the stemmata were illuminated (Fig. 8B,C) (this means that the colour-opponent signals were no longer dominant). In the other three units shown in Fig. 8D–F, two stemmata (II and III or III and IV) provided similar colour-opponent signals which appeared to dominate the overall spectral profile of the neurones, though the neighbouring stemmata did send significant input.

One neurone showing spatially segregated colour opponency, where the opponent input derived from different stemmata, is illustrated in Fig. 9. The unit received blue-dominant, excitatory input from stemma II and green-dominant, inhibitory input from stemma III, so that simultaneous stimulation of the two stemmata or all six stemmata revealed a colour-opponent interaction.

Temporal characteristics

There were a few types of neurones characterized by temporal rather than

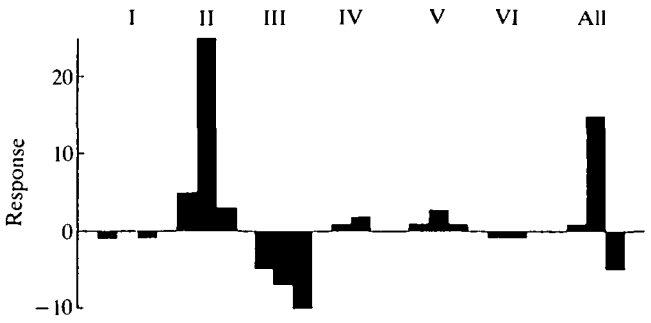

Fig. 9. Response profile of a neurone showing colour antagonism between two adjacent stemmata. The antagonistic responses were mediated by blue receptors in stemma II and green receptors in stemma III.

spectral properties, as shown in Fig. 1C–E. A great majority of the neurones usually showed considerable fluctuation in the impulse discharge rate and responded in a phasic–tonic manner (cf. Figs 1A,B, 2, 4, 6 and 11). The tonic cell discharged fairly regularly in the dark and responded with an increase in the discharge rate, to all wavelengths, superimposed on a small depolarization (Fig. 1C). Fig. 10A shows the response profile of the tonic cell. Two or three classes of colour receptors present in stemmata I, V and VI appeared to be dominant for the unit. The response profile shown in Fig. 10B was obtained from the phasic cell, which showed a transient depolarization at the onset and offset of the stimulation (cf. Fig. 1D). For such an 'on–off' unit, impulse numbers were counted for 300 ms after the start and termination of the stimulation. The spectral profile over the receptive field of the unit appeared to be relatively homogeneous

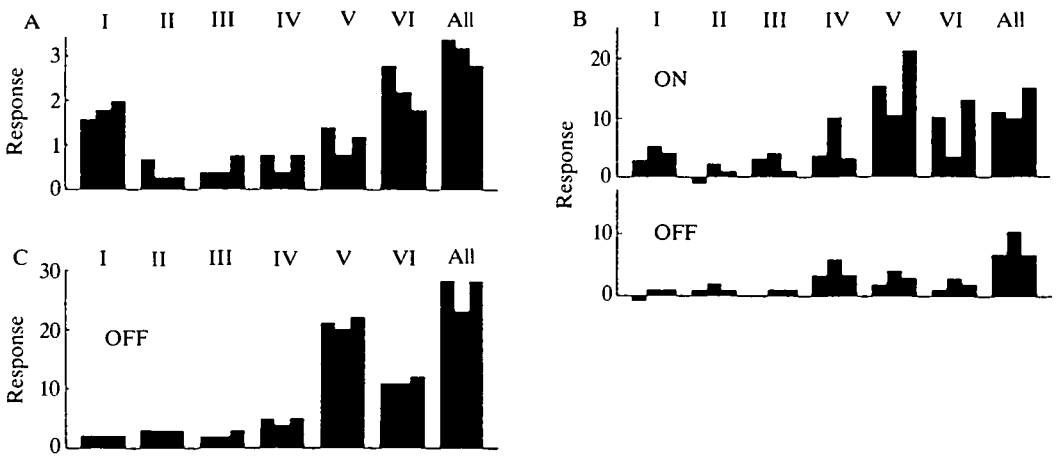

Fig. 10. Profiles of three cells showing tonic or phasic responses. (A) The tonic cell illustrated in Fig. 1C; (B) the on–off cell illustrated in Fig. 1D; (C) the off cell illustrated in Fig. 1E.

(simple), but there was a difference in the class of photoreceptors mediating different components of the response. For stemmata V and VI the on-responses were dominated by the ultraviolet and green receptors and the off-responses were blue-dominant, whereas for stemma IV both on- and off-responses were blue-dominant. Fig. 10C shows the response profile of another phasic cell, an 'off' unit, the response of which was characterized by a transient depolarization at the cessation of stimulation (cf. Fig. 1E). The ultraviolet and green receptors in stemmata V and VI dominate the cell.

Spatial characteristics

For the majority of neurones described in this paper, the responses evoked by illuminating one particular stemma serving as the centre of the receptive field could reach almost the same level as the maximum response produced when all the stemmata were illuminated. However, the activity of the neurones shown in Fig. 5A,B revealed a low saturation level when any single stemma was stimulated. When different numbers of stemmata were illuminated, the neurones showed a distinct additive property, as illustrated in Fig. 11. Illumination of stemma VI elicited a small number of impulses, superimposed on the small depolarization of

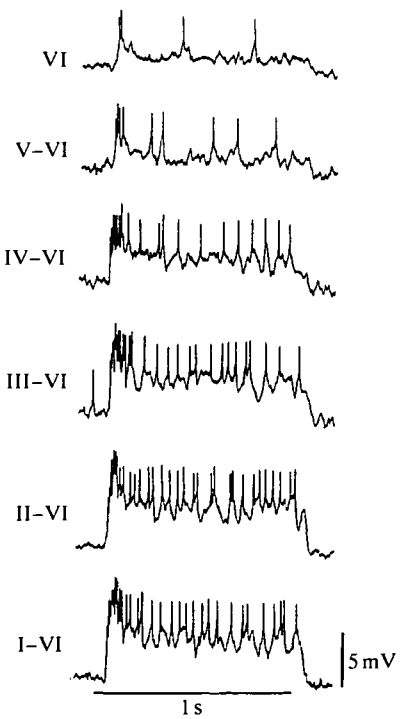

Fig. 11. Spatial summation of the response of the neurone shown in Fig. 5B. As the number of stemmata illuminated increases from one (VI) to six (I–VI), the amplitude of the slow depolarization and the impulse discharge rate increase. log $I_w = -4.5$.

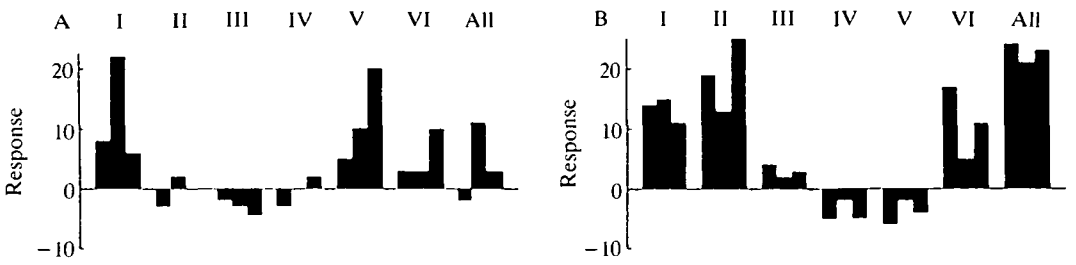

Fig. 12. Response profiles of two neurones with large antagonistic receptive fields. The units show an antagonism between (A) the centre (II–IV) and the periphery (I, V, VI) or (B) the dorsal (IV, V) and the ventral (I, II, VI) parts of the eye.

the membrane. As the number of stimulated stemmata increased, the number of impulses, as well as the amplitude of the depolarization, increased.

There were two types of neurones showing a characteristic spatial antagonism between different areas of the eye. The example shown in Fig. 12A reveals a weak, but distinct, inhibitory area (II–IV) surrounded by a strong excitatory area (I, V, VI). Such an organization of the receptive field appears to represent antagonism between the centre and the periphery of the larval eye, because structurally stemma III seems to correspond to the centre or 'fovea' of the eye (see Discussion). The unit illustrated in Fig. 12B shows a dorsoventral gradient or antagonism: stemmata IV and V, which occupy the dorsal part of the eye, sent inhibitory signals, whereas the stemmata I, II and VI, which occupy the ventral and lateral parts of the eye, sent excitatory signals. Spectral profiles of the antagonistic areas of the two neurones appeared to be similar and simple: green (and blue) dominant for the former (Fig. 12A) and ultraviolet and green dominant for the latter (Fig. 12B).

Classification overview

Fig. 13 shows a summary of the receptive field profiles of the 25 neurones identified in this study. The neurones are arranged according to the most influential stemmata (a receptive field centre) and the number of influential stemmata (width of the field). The spatial profile of each neurone was constructed by connecting the levels of relative amplitude of the response to illumination of individual stemmata (I–VI) with a smooth curve. To evaluate a colour-opponent response, the excitatory and inhibitory components of the response were summed. Neurones A–R have dominant input from a few stemmata and the remaining neurones S–Y have input from 4–6 stemmata. Within the former group, there appear to be three subgroups of neurones with partially overlapping different visual fields: neurones A–E are dominated by stemmata II and III, neurones F–K are dominated by stemmata III and IV, and neurones M–R are dominated by stemmata V and VI (and I). The abbreviation on the right of each receptive field

profile indicates the spectral complexity of the field of the neurone. Symbols in parentheses represent types of overall spectral input (and the temporal and spatial properties). There is a close relationship between spatial and spectral profiles. The neurones provided with dominant input by stemmata II–IV, including neurone L, are characterized by a spectrally complex receptive field (C) or a simple receptive field (S) with distinct colour-opponent characteristics (neurones F and I, cf. Fig. 3A,B). Neurones dominated by stemmata V and VI have a spectrally simple receptive field and some show a characteristic tonic or phasic response. The wide-field neurones S–Y also have a spectrally simple receptive field; some are

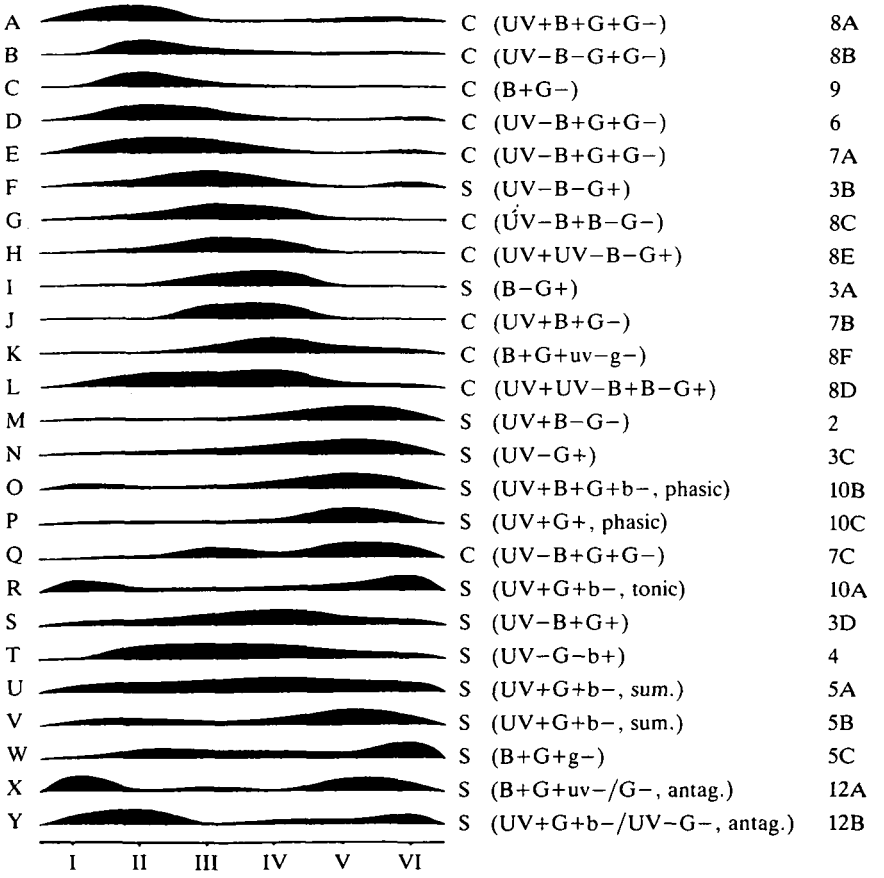

Fig. 13. A summary of the receptive field profiles of 25 types of neurones. Relative responses of a unit to stimulation of individual stemmata (I–VI) were calculated from the corresponding response profile illustrated in Figs 2–12 and the levels were connected with smooth curves. Neurones are arranged according to the most influential stemmata and the number of dominant stemmata. Abbreviations S and C denote cells with spectrally simple and complex receptive fields, respectively. The types of overall spectral input and the temporal properties of responses (tonic, phasic), spatial summation (sum.), or spatial antagonism (antag.) are in parentheses. Numbers (with a letter) in the extreme right-hand column indicate corresponding figures.

characterized by spatial summation of responses (sum., cf. Fig. 11) or by spatial antagonism between particular areas of the eye (antag., cf. Fig. 12).

Discussion

Many insects have on their compound eyes one or more specialized regions, foveae, where angles between neighbouring optical units (inter-ommatidial angles) are smaller than elsewhere. The width of the receptive field of a photoreceptor cell is smallest in the central region of the eye and it increases with the distance from the centre (Hardie, 1979; Rossel, 1979). Although in the lepidopteran larval eye there are too few units to define the fovea, the receptive fields of photoreceptors increase with the distance from one particular stemma (III) (Ichikawa and Tateda, 1982). Khattar (1977) observed in *Papilio demoleus* that even a larva with all but one stemmata blinded could orientate to coloured papers, and a larva with stemma III unblinded showed greater orientational responses than did a larva with other single stemmata unblinded. These physiological and behavioural events suggest a centre–periphery gradient in the organization of the larval eye. In the present study, there was a particular medulla neurone that showed a distinct spatial antagonism between stemmata II–IV and the other stemmata (Fig. 12A). Furthermore, the functional properties of neurones dominated by stemmata II–IV apparently differed from those of neurones dominated by stemmata IV–VI (and I), as described above. These results suggest a centre–periphery organization of the larval visual system.

The results of all the studies showed that the larval medulla consists of at least three classes of neural elements with particular degrees of convergence of photoreceptor signals. The first class consists of many neurones which make a colour-coded pathway for every single stemma (Ichikawa, 1986). These mono-stemma-dominated neurones are repetitive elements of several distinct functional (and possibly morphological) types (T. Ichikawa, in preparation). The second class of neurone integrates colour signals from a few adjacent stemmata and the third class integrates signals from almost all the stemmata. Unlike the first class of neurone, most of the second and third classes of neurones appear to be unique elements with specific functional properties, though there were a few types of cell that received similar spectral input from different sets of stemmata (Fig. 3). Functional differentiation of the second (and third) class of neurones, however, may not always indicate morphological differentiation. Although there is no available morphological evidence, it is likely that some neurones, especially in the second class, belong to a morphological type with a defined dendritic field and periodicity of appearance in the medulla neuropile. Repetitive organizations of similar elements, but in a more elaborate manner, have been observed in the medulla and lobula of adult optic lobes as well as in the lamina (e.g. Strausfeld, 1976).

The mono-stemma-dominated neurones were classified into three families, according to the combination of opponent colours: blue *vs* green, ultraviolet *vs* green and blue *vs* ultraviolet and green (Ichikawa, 1986). Each family consists of a

few neurones, or several types of neurones, which showed characteristic spectral sensitivity functions depending on background conditions (T. Ichikawa, in preparation). Most of the colour-opponent responses found in the present study also belonged to these three combinations. Since the larval optic lobe lacks the lobula, the larval medulla seems to be equivalent to a complex of the medulla and lobula in adult insects. Thus, it is possible that some multi-stemma-dominated neurones may postsynaptically integrate the output of the same or different spectral types of mono-stemma-dominated neurones with different receptive fields. No neurone examined in detail in the present study showed a spectral sensitivity function identical to that of a particular type of mono-stemma-dominated cell. Furthermore, a few neurones showed new colour combinations of opponent interactions: ultraviolet *vs* blue+green (Figs 2 and 6) and ultraviolet *vs* blue (Fig. 8C). These findings suggest (1) that a multi-stemmata-dominated neurone does not simply integrate output of some mono-stemma-dominated neurones, and (2) that the multi-stemmata-dominated neurone is the same order of interneurone as the mono-stemma-dominated neurone, rather than a postsynaptic element of the latter.

How many neurones make up the larval visual system? An eye has 42 photoreceptor cells (seven cells in six stemmata) (Ichikawa and Tateda, 1980). It is usually difficult to distinguish the somata of larval visual interneurones from those of developing cells of the imaginal disks (optic lobe anlagen) which enclose the larval optic neuropile. However, we found at most 24 somata of lamina neurones and about 100 somata of medulla neurones present in particular regions of the brain, as determined by selective incorporation of cobalt ions by larval (visual) interneurones (though all neurones in the optic lobe did not always take up the cobalt) (Ichikawa and Tateda, 1984). If each type of neurone is a physiologically unique cell, about half the medulla neurones may be mono-stemma-dominated because each stemma had at least eight neurones to be solely dominated (Ichikawa, 1986; T. Ichikawa, in preparation). Hence, I describe here about half the remaining (multi-stemmata-dominated) neurones. Among the others, which I could not test, there are probably many (complex) neurones with specific spectral profiles that differ from those of the neurones reported here. Some neurones may respond to illumination of a contralateral eye, though the neurones examined did not show any measurable response to diffuse white light presented to the contralateral eye soaked in saline.

Since stemmata V and VI have very large receptive fields in the dorsal and lateral (peripheral) areas of the visual space (Ichikawa and Tateda, 1982), medulla neurones receiving input from these stemmata, including those dominated by almost all the stemmata, seem to carry information about the luminous or chromatic conditions of a large visual field serving as a background. Their functional properties (Figs 2, 3, 10, 11 and 12) suggest the type of information the visual system needs to 'know' regarding background conditions and changes, and it is easy to speculate on their functional roles in visual processing. In contrast, it is difficult to deduce functional roles for neurones dominated by the central

stemmata (II–IV) from their complicated spectral profiles, though they should respond strongly to coloured patterns of specific configurations. When a coloured object is presented, a lepidopteran larva makes a visual scanning movement which seems to compensate for the inherently poor resolution of the eye resulting from the small number of optical units (Götz, 1936; Dethier, 1943). Hence, the temporal configuration of the stimulus may be important when considering the functional roles of the neurones. Different components of the responses of many complex neurones, including the unit shown in Fig. 6, had different time courses. Temporal properties will be described elsewhere.

The work was supported in part by grants from the Ministry of Education, Science and Culture of Japan. I thank M. Ohara for reading the manuscript.

References

DETHIER, V. G. (1943). The dioptric apparatus of lateral ocelli. II. Visual capacities of the ocellus. *J. cell. comp. Physiol.* **22**, 115–126.

GÖTZ, B. (1936). Beiträge zur Analyse des Verhaltens von Schmetterlingsraupen beim Aufsuchen des Futters und des Verpuppungsplatzes. *Z. vergl. Physiol.* **23**, 429–503.

HARDIE, R. C. (1979). Electrophysiological analysis of the fly retina. I. Comparative properties of R1–6 and R7 and R8. *J. comp. Physiol.* **129**, 19–33.

HERTEL, H. (1980). Chromatic properties of identified interneurons in the optic lobes of the bee. *J. comp. Physiol.* **137**, 215–231.

HERTEL, H. AND MORONDE, U. (1987). The physiology and morphology of centrally projecting visual interneurons in the honeybee brain. *J. exp. Biol.* **133**, 301–315.

HERTEL, H., SCHÄFER, S. AND MORONDE, U. (1987). The physiology and morphology of visual commissures in the honeybee brain. *J. exp. Biol.* **133**, 283–300.

ICHIKAWA, T. (1986). Color and spatial antagonism in the visual system of the larval swallowtail butterfly. *Brain Res.* **397**, 381–385.

ICHIKAWA, T. AND TATEDA, H. (1980). Cellular patterns and spectral sensitivity of larval ocelli in the swallowtail butterfly *Papilio*. *J. comp. Physiol.* **139**, 41–47.

ICHIKAWA, T. AND TATEDA, H. (1982). Receptive field of the stemmata of the swallowtail butterfly *Papilio*. *J. comp. Physiol.* **146**, 191–199.

ICHIKAWA, T. AND TATEDA, H. (1984). Termination profiles of photoreceptor cells in the larval eye of the swallowtail butterfly. *J. Neurocytol.* **13**, 227–238.

KIEN, J. AND MENZEL, R. (1977). Chromatic properties of interneurons in the optic lobes of the bee. II. Narrow band and colour opponent neurons. *J. comp. Physiol.* **113**, 35–53.

KHATTAR, P. (1977). Certain factors determining visual and olfactory orientation of the larvae of *Papilio demoleus* L. PhD thesis, University of Delhi.

LAUGHLIN, S. B. (1976). Neural integration in the first optic neuropile of dragonflies. IV. Interneuron spectral sensitivity and contrast coding. *J. comp. Physiol.* **112**, 199–211.

MEINERTZHAGEN, I. A., MENZEL, R. AND KAHLE, G. (1983). The identification of spectral receptor types in the retina and lamina of the dragonfly *Sympetrum rubicundulum*. *J. comp. Physiol.* **151**, 295–310.

MENZEL, R. (1974). Spectral sensitivity of monopolar cells in the bee lamina. *J. comp. Physiol.* **93**, 337–346.

MENZEL, R. (1979). Spectral sensitivity and color vision in invertebrates. In *Handbook of Sensory Physiology*, vol. VII/6A (ed. H. Autrum), pp. 503–580. Berlin: Springer-Verlag

OSORIO, D. (1986). Ultraviolet sensitivity and spectral opponency in the locust. *J. exp. Biol.* **122**, 193–208.

OSORIO, D. (1987). Temporal and spectral properties of sustaining cells in the medulla of the locust. *J. comp. Physiol.* **161**, 441–448.

ROSSEL, S. (1979). Regional differences in photoreceptor performance in the eye of the praying mantis. *J. comp. Physiol.* **131**, 95–112.

STRAUSFELD, N. J. (1976). *Atlas of an Insect Brain.* Berlin: Springer-Verlag.

TOH, Y. AND IWASAKI, M. (1982). Ocellar system of the swallowtail butterfly larva. II. Projection of retinular axons in the brain. *J. Ultrastruct. Res.* **78**, 120–135.

TOH, Y. AND SAGARA, H. (1982). Ocellar system of the swallowtail butterfly larva. I. Structure of the lateral ocelli. *J. Ultrastruct. Res.* **78**, 107–119.

ZETTLER, F. AND AUTRUM, H. (1975). Chromatic properties of lateral inhibition in the eye of a fly. *J. comp. Physiol.* **97**, 181–188.