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Colour vision in butterflies

I. Single colour experiments

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Summary. 1. The retinula cells of *Vanessa* are similar in physiological properties, including spectral sensitivity $S(\lambda)$ and negative electrical coupling, to those already described in *Papilio*, except that red-sensitive cells have not been found in either retina or lamina.

2. In the dark-adapted eye, with a stimulus of one colour, lamina ganglion cells yield only hyperpolarizations.

3. Some lamina ganglion cells (LMC's) have a broad flat $S(\lambda)$ with angular sensitivity showing that they receive summed input from only one ommatidium. Others have narrow $S(\lambda)$ and narrow field suggesting that primary receptors from single ommatidia interact on or before reaching them. Narrow peaks are near 500 or 550 nm, but not spread through the spectrum, suggesting colour specific behaviour rather than colour vision.

4. *Vanessa itea, V. kershawi, Precis villida* and *Heteronympha merope* all have optomotor $S(\lambda)$ similar to the curves for green-sensitive photoreceptors, with broad peak between 500 and 550 nm.

5. Optomotor responses of butterflies fall off rapidly around 0.1 $cd \cdot m^{-2}$, whereas insects with superposition eyes are 100 times more sensitive. Calibrations suggest that the butterfly optomotor threshold is well above the photon flux that yields abundant bumps in the retinula cells.

6. There is difficulty in reconciling the $S(\lambda)$ of the optomotor response with the $S(\lambda)$ of any of the individual LMC's.

7. The physiological properties of receptors, LMC's and deep optic lobe units are brought together in a discussion of insect colour vision.

Abbreviations: *LMC* lamina monopolar cell; *PS* polarization sensitivity; $S(\lambda)$ spectral sensitivity; $\Delta\rho$ angular width of the field at 50% contour

Introduction

The relatively large effects of extracellular currents in the butterfly retina (Horridge et al. 1983) could represent some kind of far-forward processing of colour if the inverted potentials that can be recorded with a microelectrode in the retinula cells make themselves felt in any way at the first synapses on the visual pathway. Therefore, following that study of the retina, we turned our attention to the responses of the second-order visual neurons, knowing that the retinal interactions are significant only if they are passed on to the neurons which are the next ones down the line. Whether or not that is so, we are able to show for the first time the colour coding in the second-order neurons in the insect visual pathway. Alternatively, the negative coupling in the retina would be significant if it could be revealed in a behavioural response. Therefore we also examined the optomotor response in colour to see how the spectral properties of the photoreceptors and of the lamina neurons are related to the spectral sensitivity of motion perception.

The ommatidia of the compound eye each contain a group of primary photoreceptors that lie on one optical axis in fused-rhabdomere apposition eyes such as dragonflies, bees and butterflies. Together these axons run to a single neuropil unit called a cartridge in the first optic neuropil layer, the lamina. Each cartridge contains a number of

second-order cells, of which the largest and most commonly penetrated with an electrode are the lamina monopolar cells (LMC's). This retina-lamina projection preserves angular information exactly and one criterion of an LMC is its narrow angular sensitivity.

The general properties of LMC's are known from microelectrode studies on locust (Shaw 1968), fly (Laughlin and Hardie 1978), dragonfly (Laughlin 1974, 1975) and bee (Menzel 1974). The outstanding feature is that the LMC postsynaptic response is a hyperpolarization with a fast rise time and rapid fall to a plateau. Negative-going bumps are recorded at low light levels which cause positive bumps in response to single photons in the retinula cells. The peak response of the hyperpolarization is graded over a range of only about a tenfold change in light intensity, but is moved along the intensity axis by background light so that the LMC's are able to follow a modulation over the whole ambient range up to bright sunlight (Laughlin and Hardie 1978).

Accounts of the neuron anatomy of the butterfly lamina (reviewed in Strausfeld and Blest 1970, and seen in our own preparations) show that most of the retinula axons end in the lamina upon the cartridges of lamina ganglion cells, and per ommatidium there are probably three long visual fibres which pass through the lamina to the medulla (Meinertzhagen 1976:p.562). In the bee (Ribi 1981) and the dragonfly (Meinertzhagen et al. 1983) the different lamina monopolar cells each receive a selective anatomical input from particular retinula cells, but colour coding of LMC's has been found by electrophysiology so far only in a few cells in dragonflies (Meinertzhagen et al. 1983). Bees have one or two LMC's per cartridge with broad spectral sensitivity $S(\lambda)$, as if these cells receive additive inputs from several receptor types (Menzel 1974). In the dragonfly, despite the careful mapping of morphological synaptic contacts, the interpretation of $S(\lambda)$ of the lamina neurons is still tentative (Meinertzhagen et al. 1983). Without knowing the pattern of synaptic contacts and without marking cells, we are unlikely to do better in the butterfly.

The correlation between $S(\lambda)$ of motion perception and of the LMC responses is as yet unresolved. In the bee, the broad $S(\lambda)$ for LMC's (Menzel 1974) does not agree with the $S(\lambda)$ for motion perception (Kaiser and Liske 1974), which must therefore be done via another set of neurons. Relevant to this is the conclusion (Meinertzhagen et al. 1983) that the two largest LMC's in the dragonfly also have broad $S(\lambda)$, as if they receive input from several retinula cell types. Presumably the corresponding fibres with broad $S(\lambda)$ in the bee were the easiest to penetrate, and other LMC's in the bee were not recorded. The optomotor $S(\lambda)$ of the bee could also be derived from as yet unknown LMC's with inputs only from the photoreceptors with a green peak. In the dragonfly, Laughlin (1974) had also recorded only broad $S(\lambda)$ for the lamina hyperpolarizing responses. All previous work agrees with the conclusion that the electrode is selective when penetrating lamina neurons.

Material and methods

Butterflies were captured in Canberra and either used the same day or kept overnight in a cage in natural daylight. To make long-lived preparations they are fed with dilute honey. Methods, including electrodes, calibration of the stimulating light and recording, were as described in previous publications from this laboratory in this journal (Horridge et al. 1983; Matic 1983). The flash duration was 40 ms, and interval between flashes 6 s to give a reasonable compromise between light-adapting the cell and losing it. In recording the lamina ganglion cells, the main difficulty encountered is to locate the correct place in the optic lobe.

For the optomotor responses, we copied the equipment used by Kaiser and Liske (1974). The essential part is a white reflecting cone with cut-out stripes which face one beam of the lamp. This cone oscillates within an outer cone which faces another beam from the same 1,000 W Xenon arc lamp. Interference filters are placed in the paths of the two beams which illuminate the two cones from opposite directions. By this means, stripes of any colour can be made to oscillate against a background of any colour or black, but in the experiments reported here we used only monochromatically illuminated stripes against a black background. The intensities are controlled by neutral density filters. All filters are calibrated separately and the whole light pathway is calibrated again with a IL700 spectroradiometer (International Light, Massachusetts) with SEE 400D vacuum photodiode detector. For white light, the brightness of the stripes which the butterfly sees was also calibrated with a luxmeter with spot of angular diameter 1°.

Results

Retinula cells of Vanessa itea (The Admiral)

The spectral sensitivity of four different types of retinula cells has already been described in *Papilio* (Matic 1983). In *Vanessa* we find the same four types, except that the red-sensitive cells known to be present (Bernard 1979) have so far not been encountered. In *Vanessa* the electrical interaction between receptors is similar to that already described in *Papilio* (Horridge et al. 1983).

UV receptors. The spectral sensitivity of a typical UV receptor has a peak near 350 nm and quite a long tail through the visible region (Fig. 1), possibly due to positive coupling with other cells. The polarization sensitivity (PS) was 5 measured at 350 nm on-axis, and it was greater when measured

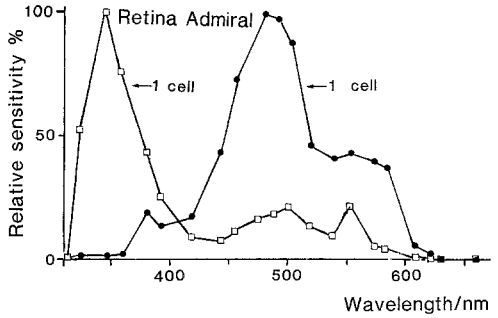

Fig. 1. Retinula cell $S(\lambda)$ of the Admiral. A UV cell with peak near 350 nm and a blue-sensitive cell with peak near 490 nm

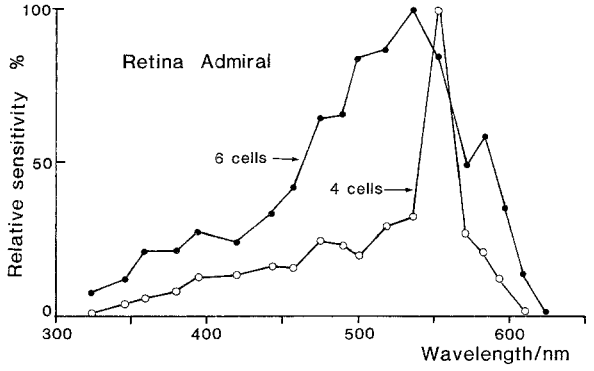

Fig. 2. Average $S(\lambda)$ for two groups of green-sensitive retinula cells of the Admiral, one with broad peak near 530 nm, and shape similar to the rhodopsin absorption curve, the other with narrow peak near 550 nm, explained only by interaction within the retina

off-axis. The example shown is a cell from the side of the eye, with visual axis almost at right angles to the body axis. We mention this point to emphasize that the side of the eye contains receptors of at least three colour types, UV, blue and green. The $V/\log I$ curve from 10% to 90% of the response is over a range of 1000-fold intensity.

Green receptors. These are the commonest units in the retina, with a peak at 500 to 550 nm. The shape of the $S(\lambda)$ curve (Fig. 2), is similar to that of the rhodopsin absorption curve, except that the β peak in the UV is sometimes missing, as if there is a negative interaction from UV cells on the same optical axis. A notable feature of the green receptors is that the $V/\log I$ curve from 10% to 90% of the response has a slope over a 10,000 fold range of intensity, as if there is strong influence of the ERG because the $V/\log I$ curve is measured at peak λ which is also the peak of the ERG. The indifferent electrode is in the proboscis, so that the effective recording is across the basement membrane of the eye or across the photoreceptor terminals (Horridge et al. 1983). The best angular sensitivity was $\Delta\rho = 1.8°$.

Blue receptors. Cells with peak in the range 450–500 nm were seldom encountered: we have only one record in *Vanessa itea* (Fig. 1), and even that has a shoulder at 550 nm as if positively coupled to green receptors.

Cells with broad spectral sensitivity. We find a large proportion of units (as distinct from cells) with several spectral sensitivity peaks in the UV, blue and green. These multiple peaks of retinula cells are probably artefacts caused by the electrode penetrating several retinula cells, a conclusion which is supported by the wide range of the $V/\log I$ curves, over a 10,000 fold range from 10% to 90% of the maximum response in some of the examples.

Cells with narrow peak near 550 nm. As in the lamina, we find a class of cells with a very narrow spectral peak near 550 nm (Fig. 2). It is difficult to find a mechanism for such a narrow spectral sensitivity unless we assume an antagonistic interaction between a penetrated cell with peak in the green and other cells with peaks towards the blue. The $V/\log I$ curve at 550 nm is over a range of 1,000 fold from 10% to 90% and angular sensitivities are narrow: the best $\Delta\rho = 1.3°$ to $1.6°$ without subsidiary lateral peaks or shoulders.

$V/\log I$ curves. For the comparison with lamina cells, it is important to note the similarity of the ranges and slopes of the plots of V/V_{max} for the different types of retinula cells (Fig. 3). Antagonistic electrical interaction between cells in the retina can only reduce the slope of the V/V_{max} curve, and extend its range. We find that the intensity range in which the lamina cell responses and the optomotor responses are graded lies over only a part of the range of the retinula cells, measured with the same equipment and the same calibrations (Figs. 3 and 8).

Lamina recordings in Vanessa itea (The Admiral)

Electrolaminagram. With the electrode moved out of a cell into extracellular space in the lamina, the response to a flash can be large (up to 40 mV) and positive-going. The waveform is slower and the angular sensitivity broader than that of a lamina cell. When the maximum amplitude is plotted against the intensity of the flash, the resulting curve extends over an intensity range of 10^4 or more, and shows no sign of saturating at the highest intensity

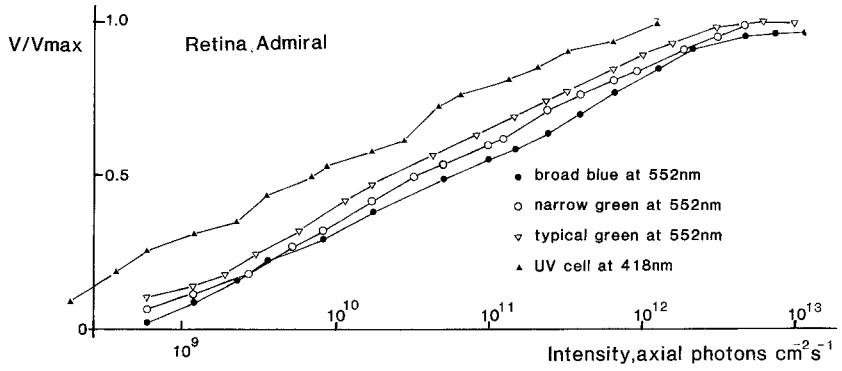

Fig. 3. Representative V/V_{max} curves for four retinula cell types of the Admiral as a function of peak axial photon flux. The intensity calibrations are the same as those for diurnal moth, locust and blowfly cells in Horridge et al. (1983, Fig. 18)

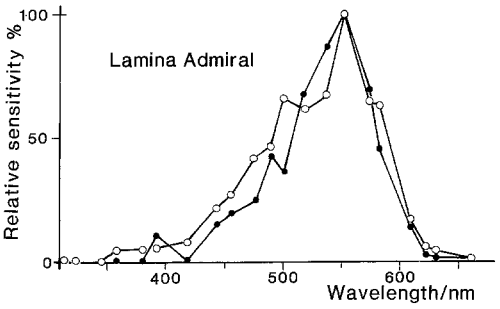

Fig. 4. $S(\lambda)$ of the electrolaminagram of the Admiral, two runs

available because increasing intensity recruits further receptors. The $S(\lambda)$ of this response has a peak at 550 nm (Fig. 4), resembling the $S(\lambda)$ of the green receptors, but it lacks sensitivity in the UV. There must be some interaction in the generation of the extracellular currents in the lamina such that the contribution of the green receptor cells (which are in the majority) is reduced in the ultraviolet.

Intracellular records. Records from butterfly lamina cells are hyperpolarizations and very similar to those recorded from locust and dragonfly. We saw no suggestion that the polarity of the response could be influenced by the angle, wavelength or polarization plane of the stimulus. This implies that the negative lateral interaction between retinula cells does not pass into the lamina cells by synapses or by current flow, and that lamina cells do not have obvious negative interaction among themselves. The responses are hyperpolarizing and noisy, with a rapid rise from the baseline and a much slower decay (Fig. 5). For measuring $S(\lambda)$ we always used a brief flash of 40 ms duration, which does not allow time for the response to come to a plateau.

Types of spectral sensitivity of lamina cells

Broad blue peak below 500 nm. Four lamina cells had a broad peak in the blue between 450 and 500 nm (Fig. 6) and a not particularly sharp angular sensitivity ($\Delta\rho = 2.0° - 2.2°$). Curiously, in all four cells, there was a low sensitivity at 550 nm, as if green cells peaking sharply at 550 nm cause some inhibition. Apart from that, these cells appear to receive inputs from blue receptors with little interaction. The $V/\log I$ curve from 10% to 90% response is over only a fifteen fold range of intensity (1.2 log units).

Broad spectral sensitivity. Seven lamina cells had a broad spectral sensitivity right across the range from 350 to 600 nm (Fig. 6) falling rapidly to zero below 323 nm and above 620 nm with no input from red-sensitive retinula cells. The only cell in which we measured $S(\lambda)$ by counting bumps at low light levels had a curve of this type. The angular sensitivity is not remarkable: $\Delta\rho = 1.7°$ in two examples measured, showing that input is from a single ommatidium.

This is the type described in dragonfly (Laughlin 1974) and bee (Menzel 1974) and it is the type most easily penetrated in the butterfly lamina. There is no marked polarization sensitivity (PS) at most wavelengths, but sometimes a wavelength can be found where PS reaches 2 or 3. In the retina a recording with these properties would be considered to arise from several receptors penetrated by the one electrode; in the lamina it is very difficult to be sure that the recording is in fact from a single neuron without marking it.

A feature of these cells is that the $S(\lambda)$ curve has a spiky profile as if large changes in inter-receptor interaction are caused by small changes in wavelength. This fine structure of the $S(\lambda)$ curve deserves further analysis by adjustment of incident angle and polarization plane.

Narrow peak near 500 nm. Four cells had a narrow spectral peak at 500 nm (Fig. 7) and very narrow angular sensitivity curves, two with $\Delta\rho = 1.2°$ and two with $\Delta\rho = 1.4°$. These properties would make

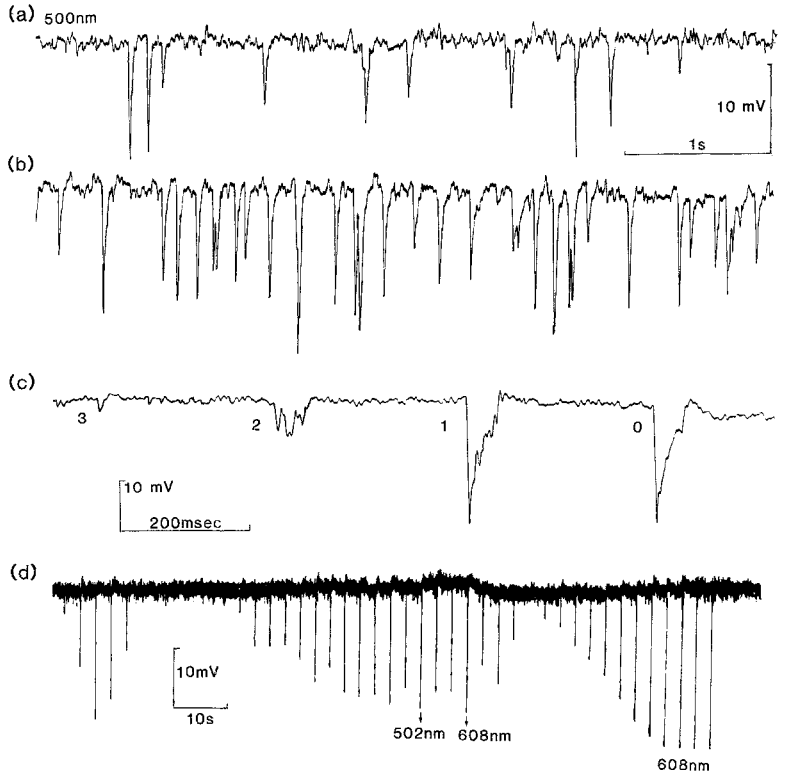

Fig. 5a–d Sample recordings from a lamina cell of the Admiral, (**a, b**) bumps in continuous weak illumination at the intensities shown. **c** responses to flashes at four intensities, each ten times brighter than the last. **d** typical recording of angular and spectral sensitivity, and calibration to different intensities

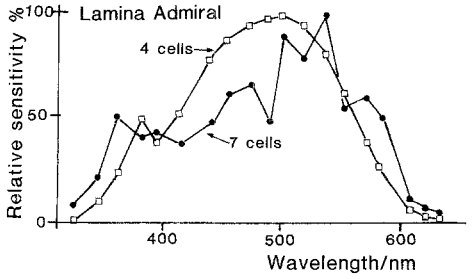

Fig. 6. $S(\lambda)$ of two lamina cell types of the Admiral, with blue peak (average of four cells) and with broad spectral sensitivity (7 cells)

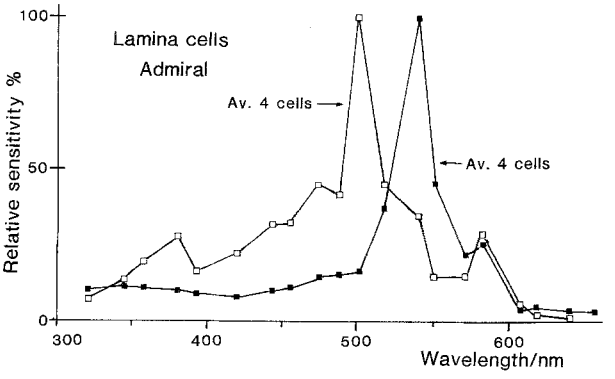

Fig. 7. $S(\lambda)$ of Admiral lamina cells with narrow peak near 500 and 550 nm

sense if these cells had their main inputs from a blue-peak retinula cell of one ommatidium which is antagonised in either retina or lamina by green-sensitive cells of its own and surrounding ommatidia. These cells would be especially sensitive to small objects of a different colour against a background of greenish blue.

Narrow peak near 550 nm. Four cells had a narrow peak in the green between 520 and 550 nm with relatively low sensitivity through the blue and ultraviolet (Fig. 7). Angular sensitivity was not remarkable, $\Delta\rho = 1.5°$ and $1.8°$ in two examples measured.

It is clear that this type of cell has its principal input from the retinula cells with peak near 550 nm, but like the previous type it is impossible to distinguish as yet whether the very narrow spectral peak originates in retina or lamina interactions.

These cells would be especially sensitive to small coloured objects against a green background. It is an attractive idea that the above types with narrow peaks at 500 and 550 nm, together with others with broad $S(\lambda)$, are especially significant in visual behaviour concerned with the discrimination of foliage by colour for egg-laying.

Missing groups. In the lamina we found no UV-sensitive or red-sensitive cells, although retinula

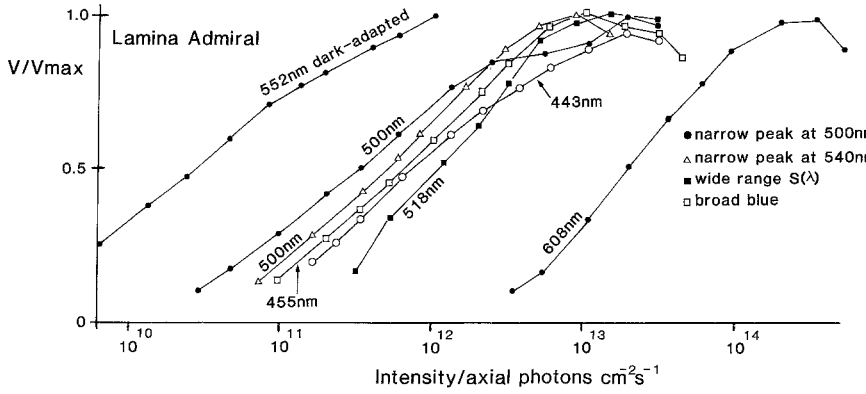

Fig. 8. Representative curves for V/V_{max} as a function of peak axial photon flux in lamina cell types of the Admiral, showing the intensity range over which they are graded, and that the curves are parallel irrespective of cell type or wavelength

cells occur with peaks near 350 nm and near 610 nm. This negative evidence suggests that we are discussing long visual fibres, or if there are UV and red-sensitive LMC's, they are difficult to penetrate. We also failed to find lamina cells with $S(\lambda)$ curves similar to the green receptors which are the most abundant type in the retina. At present we are unable to indicate whether this is a fundamental feature of lamina organisation or merely an incidental result of fibre size so that such green-sensitive cells are never penetrated.

Polarisation sensitivity (PS). All the lamina neurons recorded had no polarisation sensitivity at most wavelengths when tested on axis, but occasionally there was a wavelength or an angle of the stimulus at which the PS rose to 2 or at most 3. This is exactly what would be expected if several photoreceptors, but not all of an ommatidium, excite each lamina neuron; and if only some of the interactions described in the retina come through into the lamina. We saw nothing of the electrical effects that can be demonstrated in the retina by selection of a critical stimulus wavelength with polarised light.

Bumps in lamina cells. Relatively large bumps of more than 10 mV can be recorded from dark-adapted lamina cells (Fig. 5a). They are clearly derived from retinula cell bumps but the problem in working with them is to find the axis of the cell and identify its main properties without causing light-adaptation which rapidly and persistently reduces the amplitude of the bumps. The units could not be held sufficiently long for the long periods of recording in low continuous illumination that is required for measurement of physiological properties by counting bumps. A superficial inspection shows that the lamina bumps are apparently not each caused by several packets of transmitter, but this is an important point that could be further tested by analysis of the waveforms.

$V/Log\,I$ curves. When the response (as a fraction of the maximum response) is plotted against the log of the intensity of the flash for lamina cells, there is about a 30-fold range of intensity (1.5 log units) between the 10% and 90% values of the response (Fig. 8). The curve is less steep, therefore, than that recorded for the fly and dragonfly (Laughlin and Hardie 1978).

All experiments were upon preparations that had been dark-adapted for an hour but had then been stimulated by the process of making measurements of angular and spectral sensitivity. The stimulus was always a flash of 25 ms at intervals of 6 s, with long waits between each series of flashes. The preparation was therefore partially dark-adapted. Most of the $V/\log I$ curves (Fig. 8) of the lamina cells are bunched together so that 50% saturation at peak wavelength is in the region of 10^{12} photons $cm^{-2}s^{-1}$, which compares well with light-adapted lamina cells in the fly and dragonfly.

No attempts were made to change the state of adaptation of the lamina cells, or to apply a background light in the present series of experiments, for several reasons. The cells are difficult to find and to hold for more than 30 min, and adaptation experiments were not attempted until absolute, angular and spectral sensitivity had been examined.

Lack of inverted responses in the lamina. The strong field effects in butterfly retina (Horridge et al. 1983) can be explained by a circuit diagram (Shaw 1975) in which current generated by a group of (say) green-sensitive receptors that are stimulated by green light completes its circuit back to the retina via the terminals of less strongly stimulated UV and blue receptors, so hyperpolarizing them. Butterfly retinula cells yield inverted electrical responses

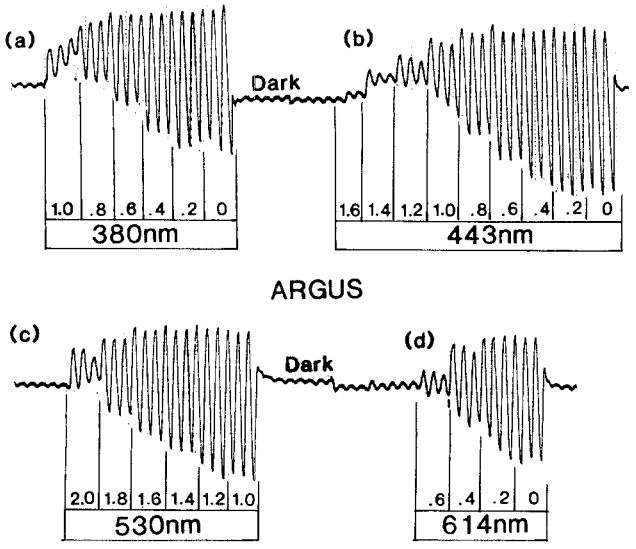

Fig. 9a–c. Optomotor response of the Argus butterfly to the oscillating drum in the intensity ranges where amplitude depends on intensity, for four different wavelengths at 380, 443, 530 and 614 nm. The drum oscillates at 0.25 Hz with amplitude 20°

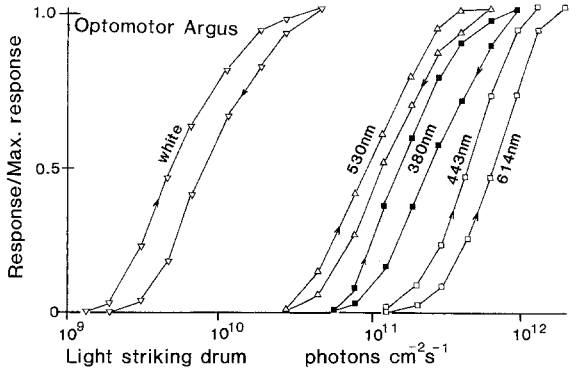

Fig. 10. Optomotor response amplitude of the Argus as a function of intensity, showing that the curves are parallel irrespective of the wavelength of the stimulating light. From 10% to 90% they extend over less than a 10 fold intensity range. The light is calibrated as the photon flux arriving at the white drum surface. Upward arrows show experimental runs that were started at the dim side (dark-adapted), downward arrows show runs started at the bright side (light-adapted). The direction of the run must be consistent for measurement of $S(\lambda)$

which are explicable in this way (Matic 1983) and can even yield inverted bumps when suitably stimulated. Possibly several reasons contributed to our failure to observe inverted responses in lamina cells. It is a reasonable generalization at the present time that retinula cell synapses upon lamina cells in all dark-adapted insects generate transynaptic hyperpolarizations. An electrode firmly sealed in a lamina cell sees no extracellular field if the indifferent electrode is effectively in the extracellular fluid immediately outside the cell. Even so, in the light-adapted eye, with the lamina cells hyperpolarized, the retinal field effects should turn off the transmitter and produce depolarizations in response to suitable stimuli. In practice, we never saw depolarizations. In defence, we can say only that in this investigation any possible antagonistic effects may have been masked in one-colour experiments in the dark-adapted state. Also, we searched first for cells which gave hyperpolarizations, as being credible lamina units.

Spectral sensitivity of the optomotor response

When butterflies are placed in a drum oscillating at 0.25 Hz with the body axis along the axis of oscillation, they continue for hours with responses of the head, which rotates on the neck (Fig. 9). This optomotor response is the head stabilization reflex for roll in flight.

The amplitude of the response is a function of light intensity over a rather narrow intensity range of about 10 fold (Figs. 9, 10, 13, 14). The response amplitude plotted against the log of the intensity gives an S-shaped curve which is very similar to the $V/\log I$ curves of the lamina ganglion cells. In normal flight in daylight the response is presumably always saturated. When the responses over a range of intensities are measured at different wavelengths, the resulting family of curves are parallel (Fig. 10). Therefore the amplitude of the response can be used to calculate the optomotor $S(\lambda)$ curve as a linear plot, with values proportional to photon effectiveness, and it can be related to visual pigment absorption curves.

Butterflies live in a world of colour. Numerous behaviour patterns concerned with finding flowers, mates, foliage for egg laying, avoiding predators, finding resting places and recognizing their own eggs, have perhaps all played a part in the evolution of the peak wavelengths of the photoreceptors. Each of these behaviour patterns makes use of a different combination of colours, but because the optomotor response is fundamentally the same irrespective of other behaviour, we expect it will show a constant spectral sensitivity for a number of butterflies of different habits. We therefore tested a number of species.

Vanessa itea. The Australian Admiral. The average optomotor $S(\lambda)$ for 12 animals has a main peak near 520 nm and a subsidiary peak near 390 nm (Fig. 11) like the $S(\lambda)$ of a green receptor with the same peak. Almost all the individual animals had the peak at 520 nm and the subsidiary peak also. The optomotor $S(\lambda)$ curve is quite smooth, unlike

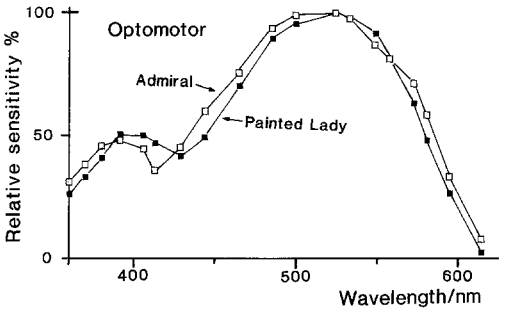

Fig. 11. Optomotor $S(\lambda)$ curves for the Admiral and the Painted Lady

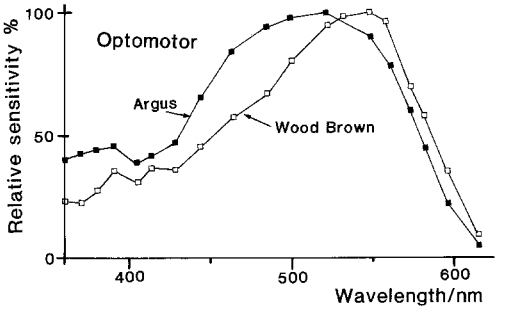

Fig. 12. Optomotor $S(\lambda)$ curves for the Meadow Argus and Wood Brown

the $S(\lambda)$ curve of the lamina cells with broad $S(\lambda)$ (Fig. 6).

The plots of response (plotted as response/maximum response) rise steeply from 10% to 90% in a tenfold intensity range (Fig. 13). At higher intensities the optomotor response is usually less than maximum.

Vanessa kershawi. Australian Painted Lady. The average optomotor $S(\lambda)$ is indistinguishable from that of *V. itea* (Fig. 11). When we compare the responses at different intensities (Fig. 13), *V. kershawi* is less sensitive than *V. itea* i.e. its response range is in brighter light, in agreement with its habit of always flying in open sunlight.

Precis villida. The Meadow Argus. The Argus is a close relative of *Vanessa,* with a similar habit to *V. kershawi,* but with a later season in Canberra. Actual responses are shown in Fig. 9, optomotor $S(\lambda)$ in Fig. 12, response range in Fig. 10, and adaptation in Fig. 14. The optomotor $S(\lambda)$ is indistinguishable from that of the Admiral, but the eye is slightly less sensitive in our test apparatus. The response curve can be moved by dark-adaptation to make it 10 times more sensitive (Fig. 14).

Heteronympha merope. Common Brown. The peak optomotor $S(\lambda)$ in this species is near 550 nm, with a broad shoulder falling steadily to a small subsidiary peak at 380 nm (Fig. 12). Some of the individual animals do not show the subsidiary peak at all.

The intensity range of the response (Fig. 13) shows that *Heteronympha* is more sensitive than *Vanessa kershawi,* which is to be expected as *Heteronympha* flies in shady places whereas *V. kershawi* flies in open sunlight.

Adaptation. The effect of adaptation was tested at the end of the butterfly season, when only the Common Brown (*Heteronympha merope*) and the Meadow Argus butterfly (*Precis villida*) were still available.

By starting in the dark, and working from the threshold towards the stronger response with progressively more light, we plotted a dark-adapted curve. Alternatively, by starting in bright light, which was reduced step by step until the response started to fall, we plotted a light-adapted curve (Fig. 14). Now we see a difference between *Heteronympha* (which flies in shady places) and *Precis* (which flies in open sunlight). The *Heteronympha* dark-adapted night eye and the light-adapted day eye are both more sensitive than

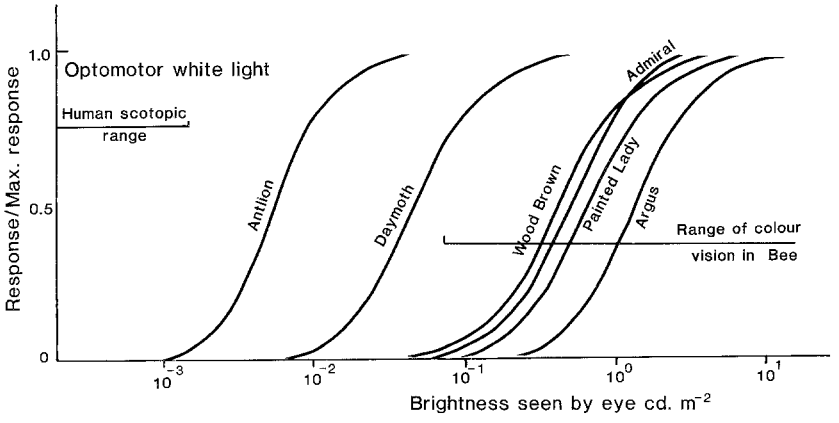

Fig. 13. Amplitude of optomotor response as a function of white light intensity for various insects tested in the same conditions. The range of intensity over which the response is graded is quite different for different insects, and is below the ambient intensity at which they normally fly. The white light, including UV, was from a standard xenon arc with quartz optics

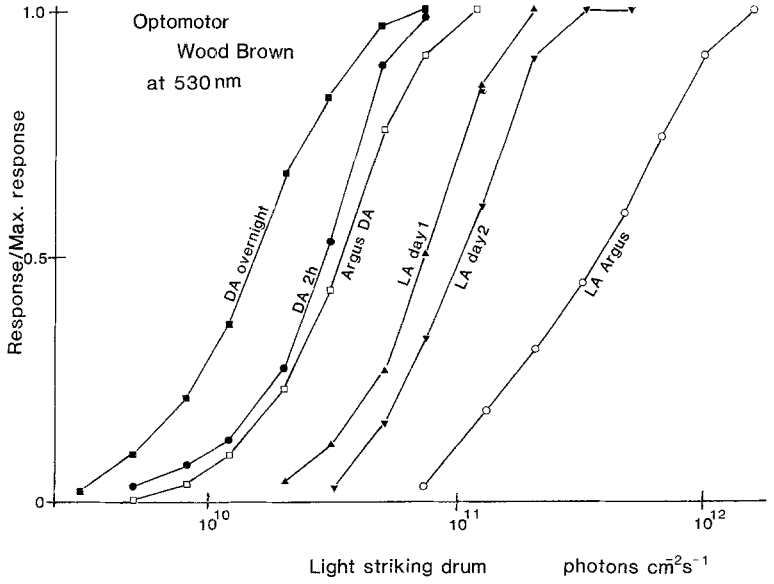

Fig. 14. Efforts to extend the range of intensity over which the optomotor response is graded. The butterfly (Wood Brown) was light-adapted on the first day, dark-adapted for 2 h, dark-adapted overnight and finally light-adapted on the 2nd day. The extremes for light-adapted and dark-adapted Argus are also shown. The stimulus drum was illuminated by green (530 nm) light on a black background. Calibrations as in Fig. 10

the corresponding states of the eye of *Precis*, although the ranges overlap.

The curves of response plotted against intensity have similar slope and shape but the dark-adapted night eye is only about ten times as sensitive as the light-adapted day eye. Even by extreme dark-adaptation, we could not make the optomotor response function over a wider intensity range (Fig. 14).

These results show that the butterfly optomotor response does not function in dim light and that it is saturated in normal bright daylight. But the butterfly retina is functional at low intensities, as bumps can be recorded from retina and lamina cells in dark-adapted preparations. In contrast, in the fly (Fermi and Reichardt 1963) and insects that fly in dim light, the optomotor response is effective at very low light levels as well as in bright light. Different again, some insects with superposition eyes show optomotor sensitivity at spectacular low intensities (Fig. 13).

The range of intensity in which the optomotor response is graded is only about 10 or 20 times, and is similar to that of the LMC's. The shapes of the curves of the lamina and optomotor responses as a function of intensity are also similar, and one could suggest that the intensity/response curves of the LMC's could act as the bottleneck which limits the optomotor response. We can easily be led into two errors here, however. First, the LMC responses are in answer to fixed flashes at different intensities, but the optomotor response is generated by the edges of the striped pattern sliding over hundreds of visual axes. Secondly, the LMC response is an amplified inverted transform of the retinula cell response, which is limited at the lower end by photon noise: it is most unlikely that the shape of the curve for the optomotor response can be limited at the lower end by photon noise because it lies in quite a different intensity level. For the fly, of course, Fermi and Reichardt (1963) did make this assumption.

Phalaenoides tristifica (Agaristidae) Daymoth. For comparison we measured the optomotor $S(\lambda)$ of this diurnal moth with a superposition eye (Fig. 15) and the intensity range within which the response depends on intensity. This moth gives a 50% response at about a tenth of the brightness required for *Vanessa* (Fig. 13). The moth and the butterfly have eyes of similar size but the moth has a superposition eye which admits light through a patch 12–14 facets wide, subtending an angle of more than 20° in the central region of the eye. When we compare the data for diurnal butterflies in Fig. 3 with previous data for *Phalaenoides* retinula cells (Horridge et al. 1983) we see that they have the same sensitivity when dark-adapted and tested in the same equipment. It is not certain, therefore, that all of the additional aperture contributes to lowering the optomotor intensity threshold. The optomotor $S(\lambda)$ has a peak near 520 nm and a subsidiary peak near 380 nm (Fig. 15), corresponding exactly to the peaks of the two types of retinula cells (Horridge et al. 1983). In this moth, therefore, the simplest conclusion would be that the optomotor response receives inputs from all the receptor cells, not just those peaking in the green. There are as yet no lamina recordings from the Daymoth.

Nymphes myrmeleonides (Neuroptera) Ant lion. We will add a result from a nocturnal superposition eye

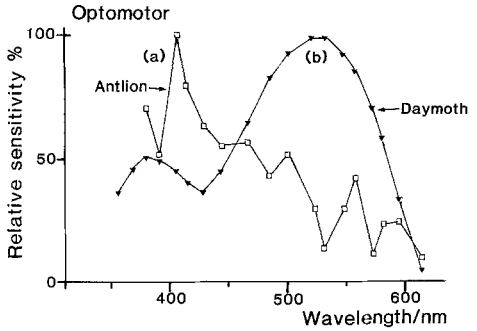

Fig. 15. Optomotor $S(\lambda)$ curves of nocturnal Ant lion and diurnal Daymoth, which have superposition eyes of similar size

for comparison. With the same calibrations and the same experimental set-up, the intensity range where the optomotor response is dependent upon intensity is about 500 times higher for the Admiral butterfly than it is for the ant lion, each measured at peak spectral sensitivity (Fig. 13). The ant lion was attracted to light in the evening, and clearly it is graded over a limited range at low intensities in the same way as the butterfly optomotor response is graded over a short range at higher intensity.

Unexpectedly the ant lion optomotor $S(\lambda)$ peaks near 400 nm, with a progressive fall towards 600 nm (Fig. 15). The $S(\lambda)$ is not tuned to the diurnal background which is predominantly green, but perhaps to the general background of the sky, which becomes a deep blue soon after sunset when these insects are active.

Discussion

Relations between retina and lamina

Depolarization of retinula cell terminals always causes a hyperpolarization of the lamina cell: no other post-synaptic effect from retinula cells has been observed. We can speculate that the lack of excitatory synapses at this junction means that the integration of retinula endings upon LMC's to give spectral opponency in the second-order neurons is not possible in insects. As the butterfly visual system took on the task of processing more visual behaviour involving colour, perhaps antagonistic electrical interaction in the retina became essential. Unequal depolarization of retinula cells in butterflies is accompanied by extracellular currents that flow through the terminals of the less depolarized cells, perhaps switching off their synapses (Horridge et al. 1983). We have seen no sign of such effects in the lamina, perhaps because the antagonistic effects are masked in one-colour experiments with a flashing point source in the dark-adapted state.

The lamina units with flat $S(\lambda)$

These abundant units, also reported as common in the worker bee (Menzel 1974) and dragonfly (Laughlin 1974) are at present identified with lamina ganglion cells M1 or M2 which both have synaptic inputs from all retinula cells of their own ommatidium in those two groups of insects. The correlation between anatomy and physiology is not so easily tested, however. The only four cells that have been marked in the dragonfly lamina proved to be other types, and their physiological properties were not obviously explained by any known pattern of synaptic input (Meinertzhagen et al. 1983). Perhaps synaptic connectivity patterns really are obscured by extracellular currents.

The function of lamina units with flat $S(\lambda)$ remains a puzzle. They appear to receive inputs from all receptors, but if so they should mediate the optomotor response if in fact absolute sensitivity is at a premium for insects that fly. Relevant to that issue, however, Srinivasan (1983) has argued that motion detection is optimized by using only receptors with a peak in the green, and in line with his idea we have abundant evidence that the optomotor response is green sensitive. Also, butterflies do not have a particular low optomotor threshold. That leaves M1 and M2 with no known behavioural output unless we postulate that they are visual pathways which combine resolution, angular direction, contrast detection and rapid adjustment of intensity range (i.e. ordinary form vision) but with flat $S(\lambda)$ and therefore colour blind. Perhaps form vision is colour blind in that sense, but we lack data from behavioural tests.

The neural pathway to the optomotor response

We measured spectral sensitivity at three different levels not examined by previous authors and now consider how the retinula cell properties reveal themselves in the lamina and in the optomotor response. Let us bring together the curves of the response amplitude as a function of intensity for retinula cells, lamina cells and the optomotor response.

1. The curves for lamina cells and optomotor response are similar in shape, slope and range whereas those for retinula cells are over a wider range.

2. The curves for the lamina cells can be shifted along the intensity axis by adaptation, as discussed by Laughlin and Hardie (1978).

3. In different insects the range over which the optomotor response depends on intensity is near the lowest light level at which the insect normally flies, and the response curves are not shifted far along the intensity axis by adaptation.

Therefore, if some or all of the lamina cells are on the pathway of the optomotor response, there must be some other bottleneck which limits the intensity range over which the optomotor response is graded.

Now let us consider spectral sensitivity, $S(\lambda)$.

1. There is a variety of colour types of lamina cells, some derived from a single retinula colour type, some by summation of retinula colour types and some by more complex interactions that could be negative electrical interaction in the retina.

2. The optomotor $S(\lambda)$ fits approximately the $S(\lambda)$ of the retinula cells with green peak, but axons of these cells terminate upon LMC's in the lamina.

3. We do not find LMC's with $S(\lambda)$ similar to the retinula cells with green peak. Perhaps we simply missed them because of the selective properties of the electrode. One LMC with green peak was recorded in the dragonfly by Meinertzhagen et al. (1983) who discuss the point. Further search is required for the motion-perception pathway at lamina level.

We have already noted that in the bee the $S(\lambda)$ of the motion detection system corresponds to that of the green receptors and less closely to the LMC cells with flat $S(\lambda)$. It is unlikely that the discrepancy can be explained by proposing that only the side of the eye functions in the optomotor response and that the side of the eye contains only green receptors. Both these propositions have been tested in the butterfly and found to be untrue. In the bee, Milde (1978) finds receptors of all colour types in all parts of the eye. We could assume that in the bee the $S(\lambda)$ of the green receptors is mimicked only in the final optomotor output, but that would be a rather complex way to arrive at a highly adaptive spectral sensitivity in the behavioural response when the eye already contains a predominance of green receptors.

The axon terminals of the green receptors end in the lamina in all groups of insects where this point has been investigated, notably bee (Ribi 1981) and dragonfly (Meinertzhagen et al. 1983). Most likely, green receptors also terminate upon dendrites of neurons that are on the motion-perception pathway but which we have not encountered. A possible explanation is that the optic medulla of the bee contains motion-detecting neurons with lamina inputs similar to those recorded in the fly by Arnett (1972) and that these in turn have inputs from retinula cells with a green peak (see discussion in Laughlin 1981, p.249). For the dragonfly there is no data on the optomotor $S(\lambda)$. For the butterfly *Vanessa* it is possible that lamina cells with broad $S(\lambda)$ (Fig. 6) contribute with others to the optomotor response, as will be seen by examination of the $S(\lambda)$ curves, bearing in mind that hundred of LMC's are summed in this response.

The necessity for behavioural analysis

The coding of colour at all levels in the butterfly optic system is by the simultaneous activity of neurons, whether they are primary receptors, wide field or narrow field neurons. At every level there are colour types (4 in the retina) and colour is coded by the relative excitation of these cell types with differing $S(\lambda)$. On the theory presented below, narrow $S(\lambda)$ units and narrow fields units are excitatory, while wide $S(\lambda)$ and wide field units are inhibitory to the next neurons down the line. With parallel processing of this type, the understanding of the neuronal mechanism is entirely dependent upon arousing the appropriate behaviour, for that is the only way to pick out the appropriate combination of active neurons. The visual behaviour pattern depends on a particular combination of coloured cues, such as chasing a mate in flight. The group of neurons that select the particular combination of coloured cues could be almost at the motoneuron level and excited only when the behaviour pattern is initiated, so that their wavelength specific responsiveness would be hard to catch in the absence of the behavioural act.

Colour-specific neurons and behaviour

The discovery of units with narrow $S(\lambda)$ in the lamina, when put together with abundant data on wide-field spiking colour-coded interneurons in butterfly optic lobe and brain (Swihart 1970, 1972a, b) leads us to new ideas about mechanisms of colour vision in insects that are also relevant to vertebrates and even man.

All theories of colour vision are based upon the three types (4 in butterfly) of primary photoreceptors with $S(\lambda)$ peaks that are necessarily broad on account of the absorption properties of rhodopsins. Among insects the worker bee is the classical animal for behavioural work on colour vision, and recently a new colour triangle was calculated for the worker honey bee (Menzel and Lieke 1983) following the method set out by Rodieck (1973 p.710 based on Maxwell 1855) for tricone space in human colour vision. They calculate the relative contributions of the different receptor types at each

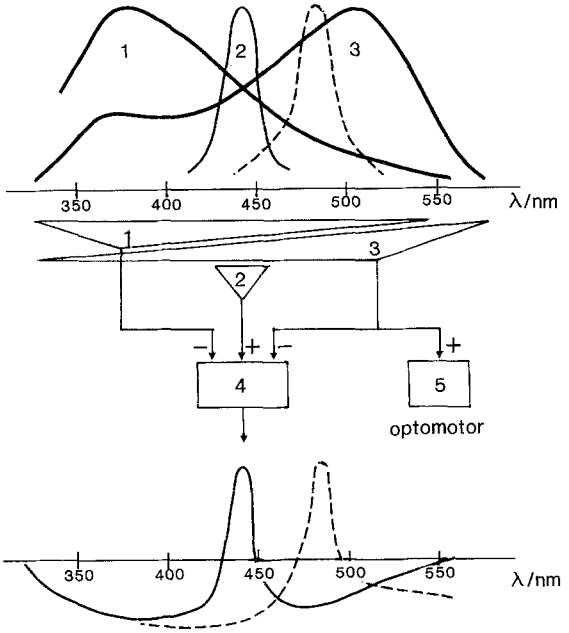

Fig. 16. A possible function of interneurons with (1,3) wide and (2) narrow $S(\lambda)$. Wide $S(\lambda)$ units are inhibitory to the next neuron (4) and negate responses to inappropriate stimuli. Narrow $S(\lambda)$ units are excitatory to the next neuron, and preserve specificity. Dashed line shows the effect of moving the optimum of neuron 2. The optomotor response is driven by neuron 3 alone and is 'colour blind'

λ, and the resulting colour triangle fits the ability of bees to discriminate two similar wavelengths anywhere in the spectrum (the $\Delta\lambda$ function of von Helversen 1972). This is not surprising, because there must be agreement if the neuronal colour processing is making full use of the receptor input, but nothing new is uncovered about the actual mechanisms.

Let us now turn to the intermediate processing which leads to an entirely different conclusion.

As *components* in the mechanism for the discrimination of wavelength differences in butterfly optic lobes and brain, we have available numerous interneurons with broad fields and broad $S(\lambda)$. Any model has to account for all of these (see Fig. 16). A puzzling feature in the earlier work of Swihart on *Papilio* was that only about a dozen spiking neurons out of 2,000 tested had narrow $S(\lambda)$, and they were in the protocerebrum. A few cells showed various kinds of colour opponency, occasionally with 3 colours involved e.g. V^+, G^-, Y^+ as described by Swihart (1970) in *Papilio*. Usually they have long and short wavelengths in opposition, either way round. We can rule out the broad $S(\lambda)$ neurons as unsuitable for colour discrimination, and we can see that neurons with colour opponency are similar to those with narrow $S(\lambda)$, in that a small change in λ causes a large modulation of the response over a narrow range of λ. Such colour-specific neurons are characteristic of optic lobes of insects with colour specific behaviour (Swihart 1970, 1972a, b; Hertel 1980). In fact, in insects the three (or 4) receptor types do not feed into a very large number of interneurons that divide the spectrum between them, one or more 'capable of vibrating in perfect unison with every possible undulation' (Young 1802). There are sufficient central neurons to do this in mammals with colour vision, but such versatility does not correspond to the facts in insect optic lobes. They appear to have a limited number of colour-specific neurons.

When we turn to the visual behaviour of insects towards coloured objects, we find that they have many sharply specific responses to objects of particular colours (along with other attributes of the objects). For example, male butterflies chase other butterflies, especially the females of their own species. They use specific wing colours besides other visual features such as wing beat frequency. Bees and butterflies learn to visit flowers or papers of any colour where there is a sugar reward. Individual neurons, however, appear to be colour specific. The varied properties of colour-coded interneurons so far recorded in insects are clearly not what would be expected if the butterflies or bee had an appreciation of colour space, or a generalized ability to discriminate throughout the spectrum.

We believe that the difficulty in matching the behaviour of the animals with that of the neurons originates in the lumped treatment of the discrimination experiments. Individual bees and butterflies that have learned a flower colour, or the particular green of the foliage of the food plant, do not discriminate all colours. Insects with a colour-specific behaviour discriminate one or a few colours, and they could do that by relying on a small number of narrow $S(\lambda)$ interneurons. In fact, one neuron with narrow $S(\lambda)$ and two wide band neurons would be sufficient for each colour, the narrow one at the critical λ and the other two on each side of it (Fig. 16).

The difference between learnt and innate colour discrimination then becomes the general problem of how neurons have their properties temporarily modified in any learning process. If a colour triangle can be plotted from the behaviour of any one individual it is because colour-specific neurons are reduplicated close together through the spectrum in the optic lobes or brain of that animals. In the case of the bee, the specific neurons for the whole spectrum are not necessarily all in one bee. The ability to discriminate through the spectrum is a statistical

property of all the insects in the experiment, not in any way demonstrating an ability of one insect to generalize colour in the way that a human can be asked to demonstrate.

The differences between wavelength-specific behaviour and colour vision in insects has been surveyed by Menzel (1979, p.551). To demonstrate colour vision requires the discrimination of any colour from all similar colours irrespective of their intensity and from all shades of grey. In wavelength-specific behaviour the insect responds specifically to one colour simply because it is more sensitive at that wavelength. We doubt whether these definitions are useful when the neuron properties are considered. A system as in Fig. 16 will select it's favourite colour from all others but it only works with one colour. We think it more likely that insects have several units, as in Fig. 16, and that some of the narrow $S(\lambda)$ units can have their peak shifted by learning. Such a system undermines the distinction between colour vision and wavelength-specific behaviour. The whole animal may have several colour-specific behaviour patterns under different circumstances. For each behaviour only one colour is discriminated, and it acts as a colour cue which is processed in pathways parallel to those of the spatial vision.

The same model (Fig. 16) may apply in spatial vision of objects by insects, in that a group of narrow field 'on'-units could interact with widefield inhibitory units to define shapes in visual space, and trigger off a number of appropriate feature-specific responses. Exactly as for colours, it is doubtful whether a single bee can demonstrate an ability to generalize a class of shapes from other classes of shapes (Wehner 1981; pp.469 and 538). It is a semantic question whether such a system has 'form-vision'.

This model accounts for the large proportion of broad field or broad $S(\lambda)$ units at all levels. With our specific model (Fig. 16), the narrow spectral band unit, with a narrow or broad visual field, is excitatory for the next higher-order interneurons. The broad $S(\lambda)$ units flanking it are inhibitory, and broad $S(\lambda)$ units commonly have wide visual fields. The flanking units can be considered as few in variety, whereas the central 'on'-unit is at a point in the spectrum corresponding to an innate colour specific behaviour, or at a flexible wavelength that is temporarily fixed by learning. Because the narrow band 'on'-unit has a small field, it can combine with similar units to make a spatial template for a particular combination of colour, shape and size. If there are several such narrow $S(\lambda)$ neurons as in Fig. 16, each triggering different responses, it is a semantic question whether such a system as a whole has 'colour vision'.

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