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Chromatic Properties of Interneurons in the Optic Lobes of the Bee

I. Broad Band Neurons

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Summary. The chromatic properties of single units in the optic medulla and lobula of the worker bee were examined. This paper describes the spectral sensitivity, S (λ) and the receptive fields of "broad band" units, ie. those neurons which receive qualitatively similar inputs from 2 or 3 colour receptor types.

The simplest broad band unit responds with sustained excitation or inhibition to light of all colours. Intracellular staining has identified the sustained excitatory unit as the Y8 cell of the proximal medulla.

More complex broad band units may receive a variety of colour inputs which sum with different weighting factors or the colour inputs may have different temporal patterning.

Receptive fields tend to be large (diameter greater than 60°). The simplest broad band units show homogenous receptive fields which are uniform for all colours. More complex receptive fields contain different areas where different colours evoke an optimal response. No centre-sourround spatial antagonism was found.

Introduction

Our present knowledge of the neuronal basis of colour vision is derived mainly from vertebrates. Little is known of the mechanisms of colour coding in other animals which possess highly developed colour vision. For example, several insect species can discriminate wavelengths very accurately. But while both behavioural colour discrimination and the colour receptors have been described

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(for review see Menzel, 1975) there is no information on the stages in between. The insect best characterised in this way is the honey bee which has a trichromatic colour vision with UV ($\lambda_{max}=350$ nm), blue ($\lambda_{max}=440$ nm) and green receptors ($\lambda_{max}=540$ nm) (Daumer, 1956; Autrum and v. Zwehl, 1964; Menzel and Blakers, 1976) and which discriminates wavelengths as close as 4–8 nm in the violet and blue-green wavelength region (v. Helversen, 1974). The three colour receptor types are combined in a group of receptors called an ommatidium in which all receptors share the same field of view. Thus each point of view is simultaneously analysed trichromatically. Such an input system differs basically from that of vertebrates and the first question which must be asked is whether or not this system uses neuronal mechanisms comparable to those in vertebrates.

In the vertebrates, studies in the retina and lateral geniculate nucleus in a wide variety of species (see Abramov, 1972, for review) support a two-channel hypothesis (Granit, 1947) in which one channel is thought to code luminosity more or less independently of wavelength, while the second channel codes colour more or less independently of intensity. Luminosity type ganglion cells (the "photopic dominator" of Granit, 1947) have broad spectral sensitivity functions without spectral antagonism. Their spatial organisation with its centre-surround antagonism involves similar spectral components for both centre and surround. On the other hand the "colour channel" of retinal ganglion cells and LGN-units is characterised by a combination of both spectral and spatial antagonism. This two channel hypothesis is supported not only by recordings from such units but by psychophysical experiments as well (See particular DeValois et al., 1967).

It is usual in vertebrate physiology to give the name 'colour coding' or 'chromaticity neuron' to those neurons which give a qualitatively different response to different spectral regions or which respond only to a narrow spectral range. As the response of such chromaticity neurons are also dependent on light intensity, such neurons cannot be, in the strictest sense, pure colour coders. Similarly, the luminosity neurons also show some dependence on wavelength but this is simple and broad band. In spite of the fact that this terminology is not completely justified, we will also use it for the following reports. Our luminosity neurons are those which, because of their intensity dependence and receptive field arrangements, appear more suited for intensity coding. We will call neurons colour coding or chromaticity neurons when the discrimination of different spectral regions appears to be their main property. Such a procedure has the advantage that unsolved problems are not apparently clarified by definition; i.e. that colour coding must be intensity independent neuronally because by definition, colour vision is the recognition of colour independent of its intensity.

Does colour vision in the bee require a two channel mechanism; i.e., are there luminosity type interneurons and do they receive input from one colour receptor type or all three? Is spectral antagonism a basic principle of the neuronal mechanisms as in vertebrates, and if so, is colour opponency combined with spatial opponency? We have studied these questions by recording from several hundred visual interneurons in the 2nd and 3rd optic ganglion and in the protocerebrum of the worker bee. We will first describe those neurons which receive qualitatively similar input from all three or from at least two receptor types. Such "broad band" neurons, as we shall call them, seem most suited

for light intensity and contrast coding and are, therefore possibly directly comparable to the luminosity type of retinal ganglion cells in vertebrates. All spectrally more complex neurons are discussed in the following paper (Kien and Menzel, 1977). There we describe spectrally opponent neurons, narrow band neurons and neurons which respond only to wavelengths where two receptors have overlapping spectral sensitivity functions. Recordings from even higher order neurons in the brain of the bee are reported in a third paper (Erber and Menzel, in preparation).

Methods

The animals used were worker bees, *Apis mellifera*, taken directly from the hive. The animals were firmly waxed to a stand and the frontal head capsule removed to expose the brain for recording. Both intra- and extracellular recordings were made with KCl filled glass microelectrodes of resistance 20–40 MOhms. Marking electrodes were filled with 5% Procion solution for intracellular staining and with 30% cobalt nitrate solution for extracellular marking. Procion was injected for 1–5 min by negative DC current of 4–9 nA. After injection the animal was kept at 4 °C for 2–12 h and then at room temperature for 1 h. The tissue was fixed in AAF fixative (85% ethanol, 5% acetic acid, 10% formalin, i.e. 32% formaldehyde) dehydrated and embedded in wax for sectioning. The sections were viewed and photographed through an epifluorescence microscope (Zeiss). Extracellular spots marking the electrode position were made with the cobalt electrodes using positive 1 Hz, 0.5 s pulses of c. 30 nA for 1 h. The units could not be held long enough to achieve extracellular staining of the fibre. Tissue was removed immediately after injection, the cobalt precipitated and the tissue fixed in Bouin's solution. After dehydration and clearing the preparation was viewed in whole mount and the location of the spot was drawn using a Zeiss drawing tube.

Colour stimuli consisted of a 1 s flash which was projected onto a flat diffusion screen (ground UV-transmitting plexiglass) 2 cm from the eye. The light source was a 900 watt xenon lamp from which up to 3 outputs could be taken. The light beam passed through a bank of quartz neutral filters, and interference filters (type DIL or DAL, Schott u. Gen. Mainz) reaching the diffusion screen via a quartz light guide. This light guide could be moved towards and away from the screen to change the size of the light spot as well as being moved horizontally and vertically across the screen to plot receptive fields. A second light beam from the xenon lamp, with its own neutral density and interference filters could similarly be positioned anywhere on the screen.

For quick testing of an unit's possible colour specificity three broad band filters were used (UG 14, BG 12, OG 530, Schott, Mainz). These are labelled UV, blue and yellow, and approximate the spectral regions in which each colour receptor type is active. In contrast to the narrow band filters their light flux output can not be expressed in numbers of quanta, because of their broad band or edge transmission characteristics. The light energy measured with a thermopile was: UG 14: 17 mW/cm^2, BG 12: 9,5 mW/cm^2, OG 530: 44 mW/cm^2. Quartz neutral density filters used to reduce the light intensity, are indicated in the figures in log intensity reduction (e.g., -2 means two log units of intensity less than the given intensity for each filter).

Data were stored on tape and then either spikes were counted manually or were fed through a frequency-to-voltage converter (Teledyne). Spectral sensitivities were calculated using the log-I-response curve as described in detail in Menzel (1975). In contrast to the photoreceptors, many interneurons have $R/\log I$ curves which are not invariant with intensity and have falling as well as rising segments. Therefore we were especially careful to use equivalent parts of the $R/\log I$ curves for our calculations. In some cases the slopes of the $R/\log I$ curves altered with wavelength and we will point out in the text where $S(\lambda)$ was calculated from non-parallel I curves. Receptive fields were evaluated only qualitatively from the responses heard over the audioamplifier during the experiment.

Results

1. Electrode Location

Units were sampled throughout the medulla and lobula of the bee optic lobe. Data were only collected from neurons which showed a stable and non-habituating response to a flash of white or monochromatic light. None of the units described in these papers showed motion sensitivity.

The optic lobes of the bee can be easily exposed by removal of the tracheal air sacs on the frontal part of the brain. Of the three neuropiles, lamina, medulla and lobula, only the medulla and lobula are conspicuous and the electrode can be positioned in either the medullary or the lobula region by use of external landmarks such as the junction with the brain. However because the medulla is a curved roughly hemispherical structure which overlaps the lobula in places, an accurate localisation of the electrode tip can only be achieved by some sort of marking. This was achieved here by cobalt or Procion dye injection. The cobalt sulphide precipitate showed only a spot which marked the position of the electrode tip, whereas the intracellularly injected Procion stained at least part of the neuron and showed the location of the electrode tip by additional extracellular dye flare. Figure 1 shows a sample of marked recording sites in the medulla and lobula reconstructed from serial sections and superimposed onto a schematic frontal section through the anterior-posterior midline of the left optic lobe. All recordings with KCl-filled electrodes could be judged by external landmarks to fit within the dotted line in Figure 1. No narrow band neurons could be found in the dorsal medulla and they were infrequent in

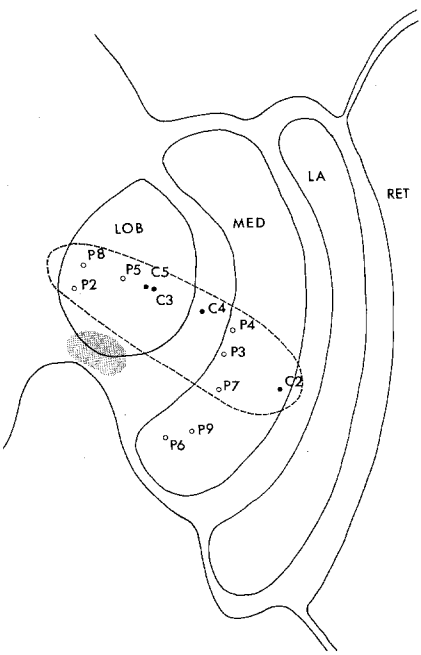


Fig. 1. Schematic drawing of the recording sites which were identified by a CoS precipitate (•C) or intracellularly injected Procion yellow (○P) superimposed on a frontal section through the optic lobe. C2, P3 and P8 are broad band units. Narrow band units which will be described in detail in the following paper (Kien and Menzel, 1977) are P6, P7, C5 (green monochromatic units), P2, P5 (green polychromatic) C3, P9 (UV monochromatic units) and P4, P9 (UV excited colour opponent cells). All recordings with KCl-filled electrodes come from the area surrounded by the dotted line. The grey area at the ventral part of the lobula indicates the large field motion detectors (Kaiser and Bishop, 1970; Kaiser, 1972; Menzel, 1973)

the extreme ventral medulla and most broad band neurons from which $S(\lambda)$ curves could be obtained were recorded in the medullary region indicated in Figure 1. The neurons appeared to be recorded at all depths from the anterior to the posterior surface.

2. *Response-Intensity Functions*

The neurons we recorded from in the medulla and lobula are high order neurons. It is therefore not surprising that their response pattern and intensity dependence is complex and latency is relatively long (sometimes up to 200 ms, e.g., Fig. 8). In all experiments the animal was kept in darkness and one eye was stimulated once every 5 s with 1 s square pulses of monochromatic or white (xenon-arc) light at different intensities over 5 log units. The response pattern to the 1 s light flash typically contains 1. a transient change in spike activity, the ON-effect; 2. a long lasting increase of spike frequency when compared with spontaneous frequency (in dark), the sustained response or if the cell is inhibited by light, a sustained decrease of spike frequency and 3. a transient change in spike activity at light off—the OFF-effect (see Figs. 3, 5–8). Some neurons may contain only one response component, the majority showed 2 and some even showed both ON and OFF effects as well as a sustained response. One aspect of the complexity of the neurons' responses is that the various components may have different intensity and wavelength dependencies. We will first discuss a few examples of variations in intensity dependence.

The basic intensity-response function (R/log I function) displays a well defined intensity threshold followed by an even rise (excited neurons) or fall (inhibited neurons) over $\frac{1}{2}$ to 3 log I units. At a certain intensity level the response saturates or may even decline slightly (Fig. 2A). The more complex the properties of the neuron the more the R/log I function tends to deviate from this simple form. Figure 2A–D show several common types of variation. For example the neuron described by the solid line in Figure 2A has ON, OFF and sustained components in its response. Both the ON and OFF components have the same R/log I function while the sustained response saturates earlier. In some neurons the slopes of the functions for the different components may vary as well. Typically such neurons show complexity in their other properties, and the neuron in Figure 2A is excited by UV and inhibited by blue and green light (unit 29, Kien and Menzel, 1977).

There is also extreme variability in the slope and extent of the rising phase of the R/log I functions. Figure 2B and C show the two extremes. Figure 2B shows an extremely steep function which rises from threshold to saturation in 0.25 log intensity unit (Fig. 2B, unit 27). Frequently such units also show a similarly steep response reduction to further increases of light intensity above saturation point (e.g. sustained response of unit 27 in Fig. 2B) so that the unit displays a preferential intensity band to which it responds. Such neurons were also found in the protocerebrum and have been called "I-band-neurons" (Erber and Menzel, in prep.). In the optic lobe such neurons have narrow spectral sensitivity functions (e.g. unit 27 is blue sensitive) whereas in the brain

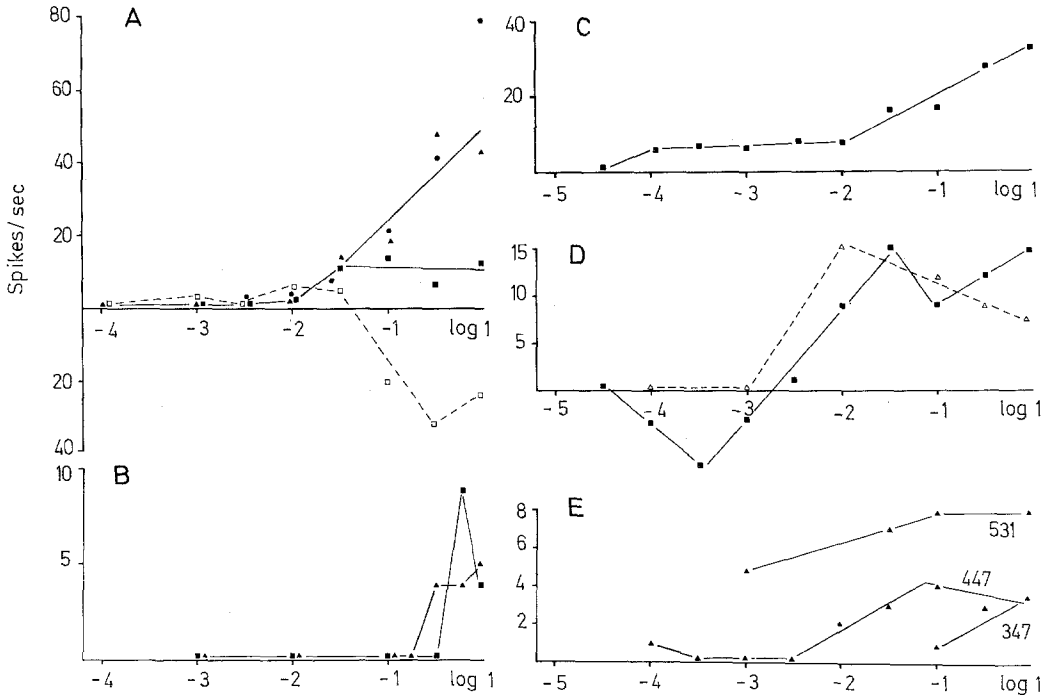


Fig. 2A–E. Variations in *R*/log *I* functions obtained with white or monochromatic light. Intensity is expressed in arbitrary log units. Response magnitudes represent the difference between spike frequency during and 500 ms before stimulation. In this and all other figures the response components are plotted as ON (▲,△) sustained (■,□) or OFF (●,○), closed symbols for excitation, open symbols for inhibition. **A** The basic form of the *R*/log *I* function for an unit excited (solid lines, unit 29) or inhibited (dashed lines, unit 32) by light. The excited unit shows different *R*/log *I* functions for the various response components. **B** An exceptionally steep *R*/log *I* function, rising from threshold to saturation in 0,25 log units for both response components. Unit 27. **C** An especially flat *R*/log *I* function showing two distinct linear regions. Unit 21. **D** Complex *R*/log *I* functions from two units showing more than one phase. Unit 22 -■-■-, unit 50 --△--△--. **E** An *r*/log *I* function which is wavelength dependent. Note the different slopes and saturation points in the short wavelength (347 nm and 447 nm) and the long wavelength region (531 nm). Unit 26

the majority of excitatory type of *I*-band neurons have broad spectral sensitivities.

The other extreme is the very gradual slope seen in Figure 2C (unit 21) and the 531 nm curve in 2 E. Here there can be more than 4 log units of intensity between threshold and saturation. Such broad curves may contain further variations as in Figure 2C where there appear to be two distinct linear regions of the curve. Generally these flat *R*/log *I* functions belong to broad band neurons.

In complex units the basic *R*/log *I* function may give way to complex curves with several rising and falling phases as in Figure 2D. The one neuron (unit 50), excited by UV light and inhibited by blue and green shows a rapid rising phase (0.75 log units) followed by a falling phase where the function declines

linearly with increasing log-light intensity. The other neuron (an UV polychromatic cell (unit 22), see Kien and Menzel, 1977) shows an initial inhibitory phase followed by an excitatory phase and then a further depression and rising phase. The fact that units with complex spatial or spectral properties show complex $R/\log I$ functions as well, is hardly surprising. The implication is merely that the complex response is derived from several inputs each with its own basic and different $R/\log I$ function. This supplies also to broadband cells which also can receive several different but positive inputs from different colour channels. An example is given in Figure 2E where a broad band neuron (unit 26), responds to different wavelengths with different slopes and saturation levels.

Variation of the $R/\log I$ function with intensity (i.e. as in Fig. 2D) or with wavelength (as in Fig. 2E) poses considerable difficulties for the measurement of spectral sensitivities. Ideally, absolute separation of intensity from spectral dependency requires complete intensity runs for every wavelength tested. Such experiments become almost impossible if the neuron can only be held for 10–20 min and information on the receptive field is also required. Various compromises become necessary. Firstly, in order to simplify the experimental procedure we restricted ourselves to neurons that responded well to stationary illumination of large parts of the ipsilateral eye. Thus the stimulus was preset and could be applied as soon as the unit was selected. Secondly, intensity runs were performed only for some but not all colours.

Spectral sensitivity $S(\lambda)$ was calculated from the relative number of quanta required to produce a 50% maximal response at each wavelength (see Menzel, 1975). This constant response criterion applies not only to response magnitude but the criterion response must always lie on the same phase of the $R/\log I$ function. If the intensity functions are parallel for all wavelengths, only being shifted along the intensity scale, this method allows a very quick determination of $S(\lambda)$ since only one intensity run need be made and all other wavelengths can be tested at only one intensity. But as we see from Figure 2, one cannot expect high order interneurons to show parallel intensity functions and so extreme caution must be used in interpreting any $S(\lambda)$ plot derived in this way. Certainly in the experiments here, if not a complete $R/\log I$ function could be made for many colours, at least a part of the curve was plotted, sufficient to clarify whether or not the $R/\log I$ function was wavelength dependent and to locate the spectral response on a particular phase of a complex curve. If the $R/\log I$ function was wavelength dependent each part of the $S(\lambda)$ plot was calculated from the intensity curve for the appropriate colour (e.g., Fig. 6 shows the $S(\lambda)$ for the neuron in Fig. 2E).

3. Broadband Neurons with Three Colour Receptor Inputs

We have recorded a variety of neurons which gave excitatory or inhibitory responses to illumination of the ipsilateral eye, did not respond to movement and were sensitive through the whole visible spectrum of the bee (300–650 nm). Two typical examples of the simplest type are shown in Figures 3 and 5. The latency of the maximal response is in the range of 30 ms. The response-intensity

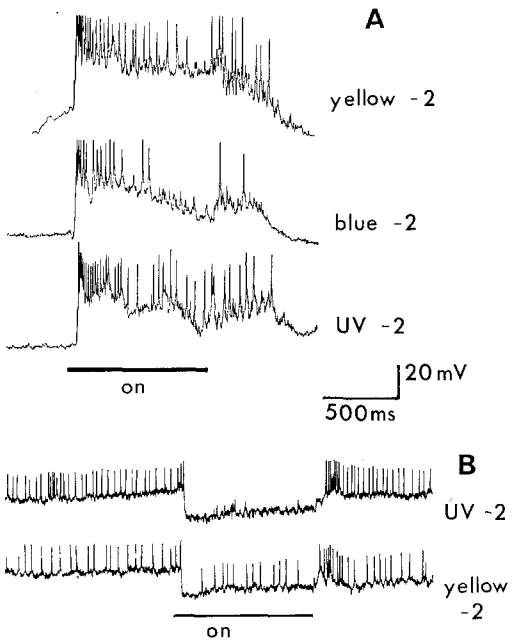
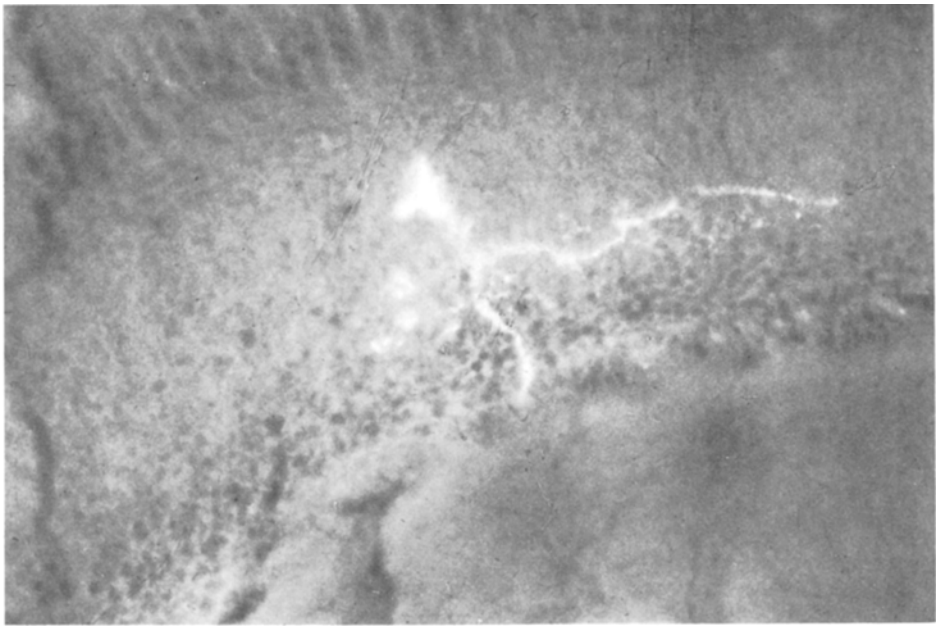


Fig. 3A and B. Intracellular recordings from broad band neurons.
A unit P3 excited by light,
B unit 90 inhibited by light. In this and all following traces, the dark bar indicates the duration of the light flash. The responses are shown to broad band stimulation, in the yellow, blue or UV wavelength region. Neutral density filters were used to decrease light intensity by 2 log units (see Methods). Note in **A** the sustained depolarisation of up to 20 mV and the excitation, which persists after the flash. In both cases the responses do not differ qualitatively for the different colours

functions of these neurons are of the simple type given in Figure 2A with rising phases covering up to 2 log units for both the sustained response of the excitatory unit and the sustained effect of the inhibitory units. The slope of the intensity functions of the sustained response is similar for all wavelengths. The ON-response of the excited neuron and the OFF-response of the inhibited neuron give similar but flatter intensity functions. The phasic OFF-effect of the light inhibited fibres is most probably a post-inhibition rebound effect, because it has similar wavelength dependence to the sustained inhibition as well as a similar intensity function. Figures 3 and 5 show that the light activated neurons have a low spontaneous (dark) frequency whereas the light inhibited neuron has a high spontaneous frequency. Intracellular recordings from units with comparable response patterns (Fig. 3) demonstrate, that an increase in spike frequency is the result of a depolarisation from summed EPSP's whereas a decrease in spike frequency is associated with a hyperpolarization of the membrane caused by summed IPSP's. Furthermore, the intracellularly recorded responses demonstrated similar patterns of EPSP's or IPSP's for different wavelengths. This result and the finding that the $R/\log I$-functions are similar for different wavelengths suggests that these neurons simply receive or produce summed inputs from the 3 colour receptor channels.

This interpretation is consistent with the measured spectral sensitivity functions. The scatter of individual neurons of these types is quite large but the main features are: 1. sensitivity through the whole visual wavelengths from 300–650 nm, 2. dominant peak in the bluish-green to green (490–530 nm) region, 3. secondary peak or shoulder in the long UV-violet (370–410 nm) region and the green (540 nm) region. The dominant peak and the increased sensitivity



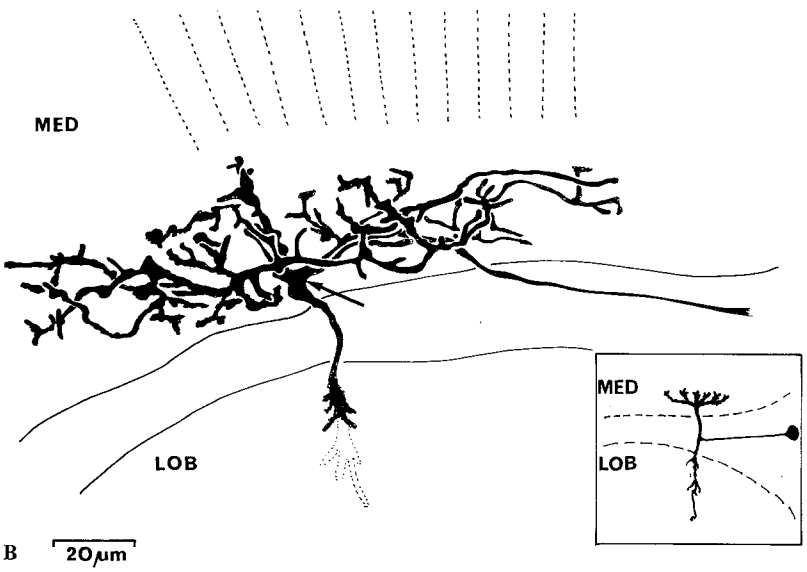


Fig. 4A and B. Reconstruction of a procion injected broad band neuron (P 3) **A** Photograph of a 15 µm section cut in the frontal plane. **B** The reconstructed neuron. There is a dense plexus of horizontal fibres spreading across the proximal medulla. Dashed lines in the medulla indicate the size and spacing of the medullary cartridges. A short thick branch enters the lobula. The dashed branches are very faintly stained arborisations. A long neurite passes laterally between the medulla and the lobula. Arrow marks location of recording site. Inset shows the cell from Cajal and Sánchez (1915) which corresponds best with the filled interneuron

between 370–410 nm strongly suggest that all 3 colour receptor channels (with λ_{max} at 350 nm, 440 nm, 540 nm) are summed inputs, the weight of the green receptor input being the largest.

We can rule out the possibility that the neuron receives input from green receptors only and that the high UV sensitivity results from electrical coupling between green and UV receptors (Menzel and Blakers, 1976). The UV sensitivity of this neuron is very much higher than that of the green receptors. In the receptors, a UV sensitivity which is 60% of green sensitivity is rarely found whereas it appears to be the rule for broad band neurons. Also recordings from the axons of green receptors have demonstrated a low UV sensitivity (<15%). This result shows that the high secondary spectral sensitivity of the receptors is a purely retinal phenomenon (Menzel and Blakers, 1976).

All the markings of this type of neuron located the recording site in the proximal layer of the medulla. Figure 4 gives a reconstruction of an intracellularly marked neuron of the excitatory type. This neuron has a large arborisation in the most proximal layer of the medulla (about 20 medullary cartridges wide) and projects into the 1st, possibly also into the 2nd layer of the lobula. Cajal and Sánchez (1915) describe such neurons in the bee, fly, dragonfly and beetle (Figs. 54, 57, 58, 59 in Cajal and Sánchez) and characterise them as "pluristratified T-shaped fibres connecting the proximal medulla and the lobula". Strausfeld (1975) classifies this neuron as the Y8 neuron. We have not yet filled any light inhibited broad band neurons sufficiently to determine if they belong to the same morphological type.

There are also broad band visual neurons recorded in the medulla and lobula which show more complex response characteristics. Figure 6 for example shows a neuron which responds with transient spike frequency increase in light ON (large field illumination of ipsilateral eye), sometimes one or two spikes at light OFF and has a very low (1 Hz) spontaneous activity. This neuron is sensitive to all wavelengths between 300 and 650 nm. In contrast to the units described above it displayed different slopes of the $R/\log I$ function for different wavelengths and different saturation levels for different wavelengths (see Fig. 2E): longwave stimulation (>480 nm) causes flat $R/\log I$ functions with high saturation level, short wave stimulation (<460 nm) steep $R/\log I$-function with low saturation level. Calculation of $S(\lambda)$ from the same response criteria for all wavelengths is therefore misleading. In Figure 6 we show the $S(\lambda)$ curve in two parts to take into account the different response characteristics in the long and short wavelength regions. The $S(\lambda)$ curve implies that this unit receives a separate green input in addition to a blue and UV input.

Another example of a broad band neuron with more complex response characteristics is given in Figure 7. The unit responds to wide field illumination of the ipsilateral eye with an increase of spike frequency. The ON-response and the sustained response have similar $R/\log I$-functions which show no appreciable differences for different wavelengths. The $S(\lambda)$-functions however, demonstrate drastic differences (Fig. 7). The sustained response indicates excitatory input from UV and green receptor channels, possibly also from the blue receptors. In contrast, the ON-response shows no contribution from the UV, a shift of the green maximum to 531 nm and a secondary maximum in the bluish

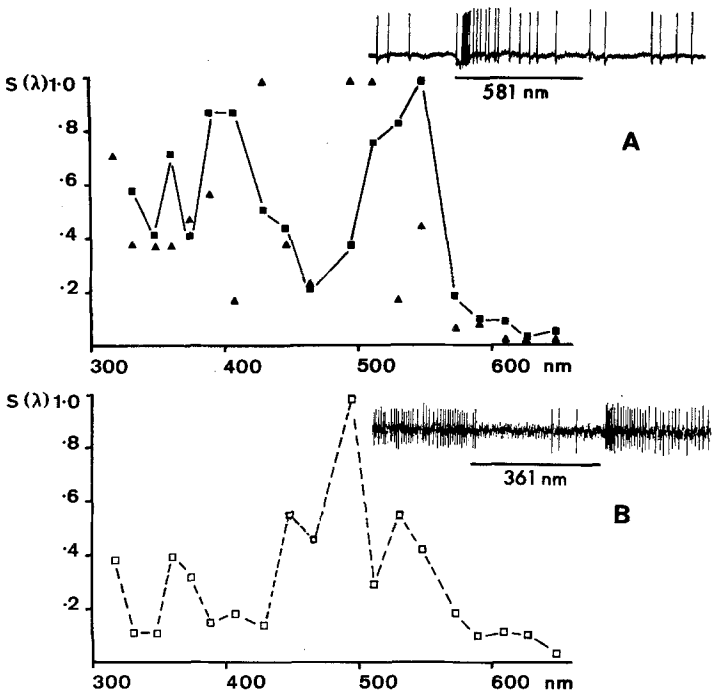


Fig. 5. Spectral sensitivities, $S(\lambda)$ of two broad band units excited by light (**A** unit 14) and inhibited by light (**B** unit 16). Sample traces of each neuron, with the wavelength at which that particular recording was made, are shown. Flash duration is 1 s. In this and all following cases the sensitivity is normalised to a value of 1 for the wavelength where the lowest relative number of quanta were needed to elicit a half maximal response. In **A** the triangles show the $S(\lambda)$ of the ON response, -■-■ excitatory sustained response, -□---□- inhibitory sustained response. Spikes are retouched

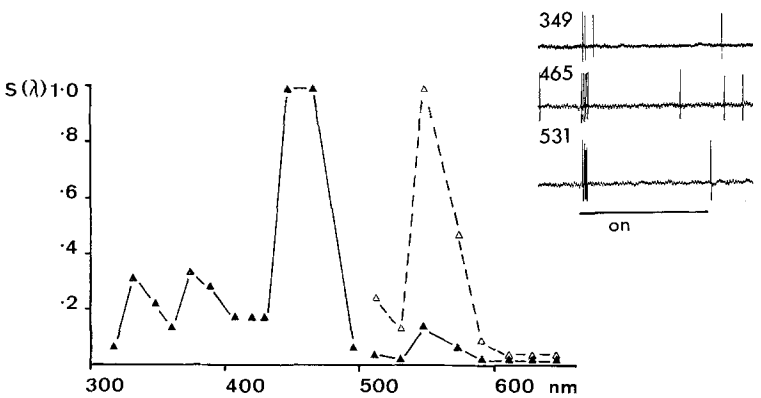


Fig. 6. Spectral sensitivity function of a unit (unit 26) responding only with a short burst at onset of illumination. The $R/\log I$ function of this unit is shown in Figure 2E. Representative responses to a 1 s flash are shown in the inset. As this unit showed different $R/\log I$ functions for long and short wavelengths the $S(\lambda)$ is calculated in 2 parts with a break between 490 nm and 512 nm. The heavy line is the long wave length function normalized to the most sensitive blue response. The dashed curve is that where the long wavelength function is normalized to its own maximum value

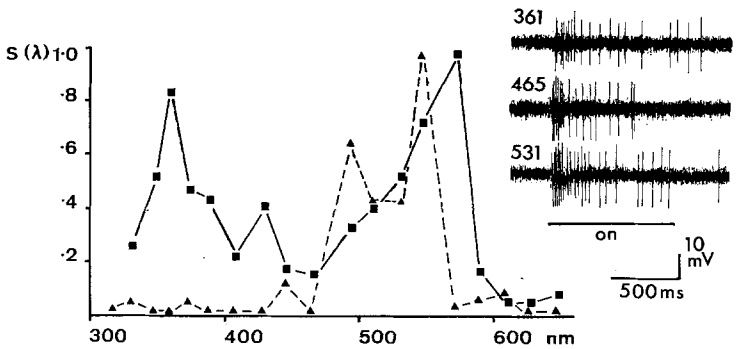


Fig. 7. Spectral sensitivity of the sustained responses (■) and the ON-responses (▲) of the complex broad band unit shown in the inset. Note the different functions of the two response components. Unit 21

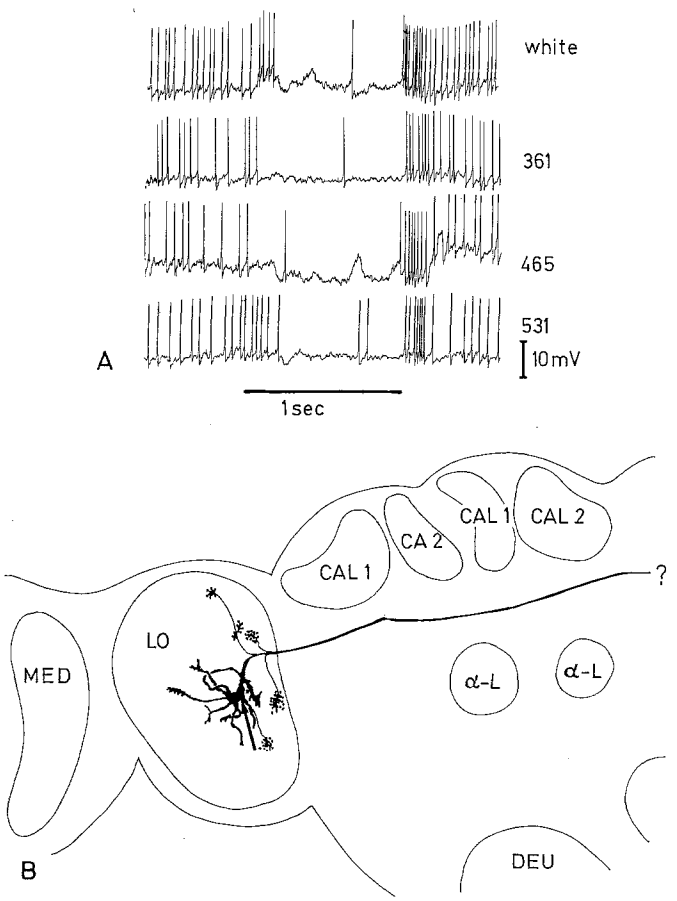


Fig. 8. Responses and Procion reconstruction of a broad band neuron (*P* 8) recorded in the lobula. Arrow in the reconstruction indicates recording site. See text for explanation

green. We conclude that this neuron receives phasic input from blue and green receptor channels and tonic input mainly from UV and green receptor channels. Thus not only may a neuron receive colour inputs with different weighting but the temporal patterning of these inputs may also vary.

Only one complex broad band was filled with Procion yellow and no complete spectral run was obtained. However, the traces in Figure 8 show that for all wavelengths there is a sustained inhibition which is the result of complex integrative processes. Note the fluctuation of membrane potential during stimulation, especially with white and blue light, and also the discrete bursts of EPSP's and IPSP's during the inhibition. Also spikes or a packet of EPSP's occur 500–800 ms after onset of illumination and there is an enormous variation in latency. The greater potential variation during blue light, plus the appearance of a negative DC response component allow the speculation that the electrode lies closer to the blue inputs than to green and UV inputs.

The procion marking demonstrates (Fig. 8B) that this neuron samples information from a large area of the lobula. Relatively thick branches point more centrifugally, whereas thin, faintly stained branches with brushlike endings run lateraly within the proximal layers of the lobula. A thin axon crosses the brain, but without any arborisations. The dye filling was not complete so we could not trace the axon further than shown. From the relative position of the axon in the brain we can conclude that it runs along the tract which may connect the two lobules or pass to contralateral ventral protocerebrum (Jawlowski, 1958; Vowles, 1955; Strausfeld, pers. comm.).

4. Broad Band Neurons with 2 Colour Receptor Inputs

Figure 9 shows a typical spectral sensitivity of a neuron which receives inputs from 2 non-overlapping receptors. The sustained response is generated by an input from UV and from green receptors, with the UV being the most heavily weighted. The UV light is most effective at the wavelength where the UV receptors absorb maximally (350 nm) and the secondary peak in the green coincides with the peak of green receptor absorption (540 nm). A neuron which receives inputs with the same sign from 2 non-overlapping receptors can in no way discriminate colour and therefore is still classed as a broad band neuron. On the other hand such a neuron could specifically code 'bee purple', and in this case the neuron could be of the colour coding type. Behavioural experiments (Daumer, 1956) have shown that the mixture of the two extremes of the bee's colour spectrum (UV and yellow) produces a unique colour which the bee can distinguish from every other spectral colour. In these experiments UV was shown to be more effective than yellow, a finding which would fit well with the neuron described here.

5. Receptive Fields of Broad Band Neurons

Receptive fields were characterised by successively moving a small light spot (diameter ca. 5°) horizontally and vertically across the diffusion screen. Simulta-

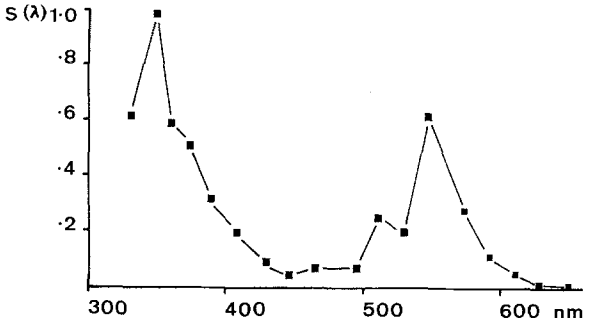


Fig. 9. Spectral sensitivity of an unit (32), which receives input from two non-overlapping colour receptor channels. Note the two clear peaks which coincide with the maximum absorption of the UV and green receptors

neous stimulation with neighbouring light spots was used to test for the presence of inhibitory areas. The screen was divided into a grid of X–Y coordinates and Figure 10A shows the geometry for their conversion to angular distances from the head axes. The response at each point was classed as "strong", "weak" or "absent" (judged from the response heard over the audioamplifier) and was plotted onto a map of the whole visual field.

The simplest field organisation (Fig. 10B) is found in broad band sustained excited or inhibited neurons whose spectral sensitivity is best understood if one assumes about equal weight of excitatory or inhibitory input from all 3 colour receptors (e.g., as in Fig. 5). The receptive field of the example given in Figure 10B for a sustained inhibited neuron is an approximately circular field with diameter of 60–80°. The sensitivity is even over the whole field and there are sharp boundaries with no sensitivity outside. The receptive field has the same size and organisation for all wavelengths. All fields of broad band units were positioned around the eye centre (asterisk in Fig. 10B–E, arrow in Fig. 10A) thus facing the frontal-lateral, medio-dorsal part of the whole visual field. As the intracellular marking (Fig. 4) has shown in an excitatory unit the medullary branching at the input side of the neuron is about 20–25 medullary cartridges wide. As each medullary cartridge can be associated with a single point of the visual environment and these visual points are determined by the retinal mosaic (Braitenberg and Strausfeld, 1973) one can estimate a visual field of 50–70° for 20–25 medullary cartridges. The measured receptive field size and width of the dendritic tree are therefore in good agreement. Furthermore this finding, coupled with the similar $S(\lambda)$ functions of these neurons suggests that excitatory and inhibitory response of this neuron type reflects subclasses of the generally the same neuron group.

The other 3 examples of receptive fields have a more complex organisation. The unit in Figure 10C displays relatively small concentric areas of maximal response to UV and green stimulation but these are of different size (UV 10–20°, green 40–50°), the response strength fading gradually from the field centre to the edges which are not sharp. It is possible that the different size of the maximal response field for UV and green reflects an intensity effect. Detectable

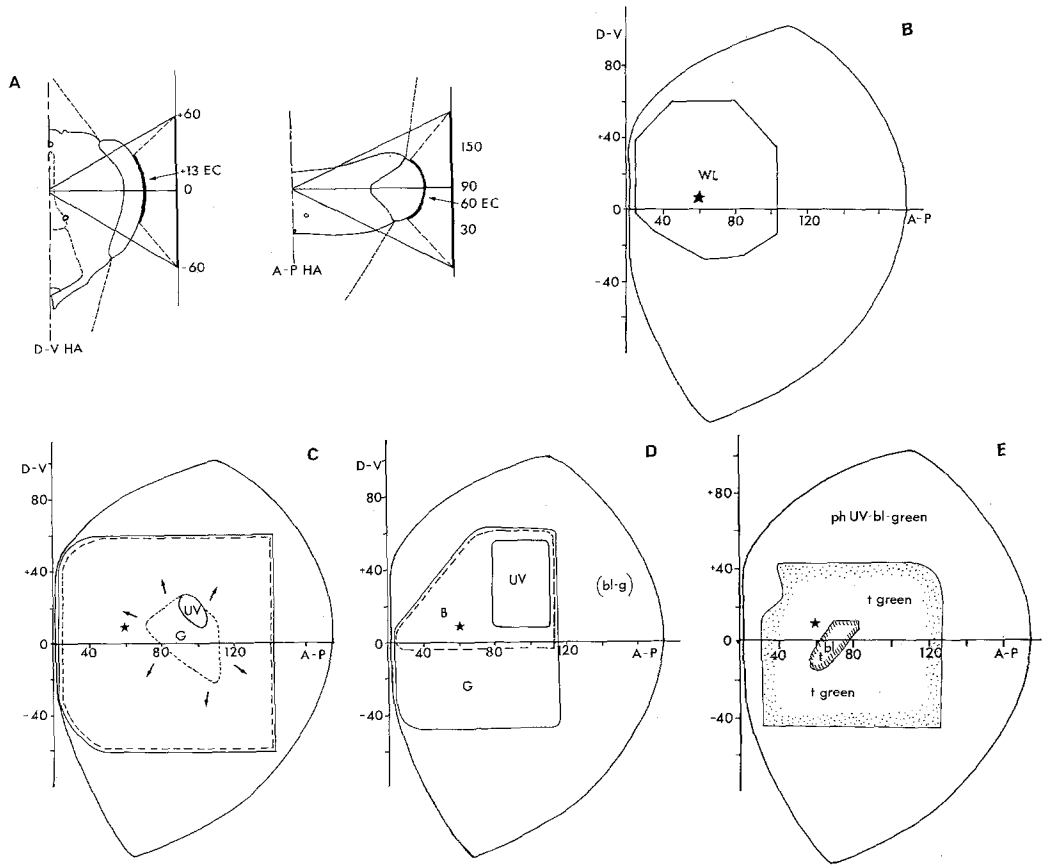


Fig. 10A–E. Receptive fields of broad band neurons. **A** schematic illustration showing how positions on the diffusing screen are related to the head axes in both the vertical (dorso-ventral) and horizontal (anterior-posterior) axes. Arrow marks position of eye centre (*EC*) on these coordinates. *D-VHA*: dorso-ventral head axis; *A-P HA*: anterior-posterior head axis. Co-ordinates on the screen were converted to angular distances from the head axes by standard trigonometry. Because of the proximity of the screen to the head these angular distances will bear no relationship to the angle subtended at the eye. This is shown by marking the projection of the diffusing screen boundaries onto the eye (thickened line shows screen projection) and relative to the head. The screen subtends, relative to the head, vertically from $-60°$ to $+60°$ and horizontally from $+30°$ to $+150°$. **B–E** Receptive fields of various units enclosed in an outline of the visual field of one eye referenced to the head axes. The asterisk marks the position of the eye centre. *WL*, white light, *B* or *bl*, blue; *G*, green. Letters in upper case indicated a "strong" response, in lower case a "weak" response. ph, phasic; t, tonic. B, unit 47; C, unit 22a; D, unit 20; E, Unit 21

response can be monitored even at about 100° from the centre. The spatial sensitivity in blue is not qualitatively different from that of UV and green but no clear maximal response centre could be found. This unit responds with a sustained excitation and its $R/\log I$ function is very steep (from threshold to saturation within one log I step) and its $S(\lambda)$ displays a peak in UV and green and weak or no input from blue receptors.

Units 20 (Fig. 10D) and 21 (Fig. 10E) have clearly different field sizes for different colours. Unit 20 responds to light flashes with ON and OFF excitation throughout its whole visual field. The receptive field is smallest in UV, larger in blue and even larger in green. Outside these strong response areas the unit is weakly sensitive over the whole eye to blue and green light. The receptive fields for the 3 different colours overlap but are not exactly concentric. Unit 21 (Fig. 10E) responds to light flashes with a phasic-tonic excitation (Fig. 7) where the phasic response is more sensitive (about 1,5 log I) than the tonic response and differs in its $S(\lambda)$ function (Fig. 7). A phasic response can be found for UV, blue and green over the whole eye (note a phasic response in the UV is still predicted even though the $S(\lambda)$ curve shows no UV input: green receptors still have 10% sensitivity throughout the UV). The tonic response area is larger to green than to UV and blue. If different receptive field sizes plotted with each colour related from an intensity rather than a colour dependence, one would expect that the receptive field size for UV would be only a little smaller than that in green because sensitivity calculated for the sustained response was only a bit lower at 349 nm than at 548 nm. The receptive field to UV light however, is even smaller than that to blue. This indicates an inhomogeneous wavelength dependent organisation of the receptive field.

Thus, in summary, our receptive field measurements of broad band neurons have revealed two general classes: 1. a simply organised sharply bordered field which is typical for the simplest broad band sustained inhibited or excited medullary neuron, and 2. a complex field with more or less concentric parts of different field sizes in UV, blue and green with relatively small diameter for strong responses and large (in most cases the whole eye) diameter for the weakest responses. This type of field is typical for neurons which have multicomponent excitatory responses and spectral sensitivity functions which differ for these different response components.

Discussion

We have examined broad band units to see if they could serve as mean light intensity references for intensity-independent colour coding neurons. The bee does not have specialised low intensity type photoreceptors comparable to the rods in the vertebrate eye, and has colour vision even at the lowest light intensity to allowing any visually oriented behaviour (Menzel, unpubl. observations). A highly sensitive luminosity-coding system should receive input from all colour receptor types or at least from the long-wavelength sensitive green-receptors and the short wavelength sensitive UV receptors. Neurons in this system would be equipped with high absolute sensitivity because of the multiple input for each point of view and the broadening of their spectral sensitivity. One would also expect that the response pattern of such neurons and the spatial organisation of their inputs would be independent of wavelength if the input is simply a summation of 2–3 different colour receptors within each commatidium and the spatial organisation is a summation of several ommatidia with positive or negative sign but without wavelength antagonism.

We have found only one type of neuron which fullfills the conditions for a high sensitivity luminosity coding system. This neuron type receives summed

input from all 3 colour receptors, has a response pattern which is qualitatively independent of wavelength (univariant only to intensity), displays a $R/\log I$ function (response rise or fall over 2 log intensity units) which codes naturally occurring intensity contrast with high efficiency and has the simplest receptive field structure possible (excitatory or inhibitory throughout the field with even sensitivity over the whole field, sharp edges, no differences for different wavelengths). Anatomically the neuron appears to be a 3rd order interneuron which receives inputs from the long UV receptors and the lamina monopolars via the medullary intrinsic fibres (Strausfeld, 1975). This anatomical finding is confirmed by a comparison of the $S(\lambda)$ function with that of the lamina monopolar cells (Menzel, 1974). In both cases the dominant maximum is located near 500 nm in the region of overlap of blue and green receptors. The differences between the short wavelength sensitivity of the two neuron types is be explained by the presence of an additional UV input parallel to the monopolar cells.

A luminosity system which receives input from all 3 receptor types is less complex than a luminosity reference system for each colour channel but it would be perturbed by differential sensitivity changes in the different colour receptors due to chromatic changes of illumination. Colour constancy under varying illumination conditions could then be seriously injured. The drawback of such a system is probably counteracted by the following retinal mechanisms which guarantee similar sensitivity changes in the different receptor types: 1. The pupil mechanism (radial-pigment movement) in each photoreceptor is not completely independent of excitation of the other photoreceptors in an ommatidium (Menzel and Knauth, 1973). This partial coupling causes an almost equal light attenuation in all receptors of one commatidium. 2. As all 3 photoreceptors posses a similar blue light absorbing thermostable metarhodopsin the relationships of sensitivity between the three receptor types will remain constant (Hamdorf et al., 1972; Hamdorf and Schwemer, 1975; Hamdorf, 1975).

All other broad band neurons showed more complex sensitivities which do not allow us simply to assign these all to the luminosity group. Their $R/\log I$-function may differ for different wavelengths, their $S(\lambda)$ functions may be different for the ON-response, the sustained and OFF-response, their receptive field was often found to be not identical for different colours. Even so we cannot conclude that these neurons exhibit differential response to different wavelengths because a given response can be produced in most cases by any wavelength in nearly any part of its receptive field merely by adjusting the intensity. It is possible that these 3rd, 4th or even higher order neurons have very specific optimal stimuli, e.g., specific colour patterns or arrangements. However, we know that they were not sensitive to movement of large or small objects, stripes or light points, and they did not prefer the flash to illuminate any particular proportion of their receptive field area. Although our experiments in no way indicate a function for these neurons, the stationary light flash can be used as a tool to analyse their colour inputs. Different $S(\lambda)$ functions for ON-, sustained, and OFF-responses prove the existence of multiple and separate colour inputs even to the broad band neurons, as do the differences in $R/\log I$-functions at different wavelengths. The variety of receptive field organisations also reflect the spatial separation of these inputs, although no evidence for a centre-surround organisation, as in vertebrates was found.

In summary, the broad band neurons in the medulla and lobula of the bee appear to be a heterogeneous group of interneurons involving typical luminosity type neurons as well as neurons receiving different colour inputs with

different spatial and temporal properties. It remains to be determined whether these neurons are non-colour specific as their flash-evoked response suggest, or are complex feature detectors as is perhaps suggested by the complicated receptive fields.

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References

Abramov, J.: Retinal Mechanisms of colour vision. In: Handbook of sensory physiology, Vol. VII/2, Physiology of photoreceptor organs (ed. M.G. Fuortes), pp. 567–608. Berlin-Heidelberg-New York: Springer 1972

Autrum, H., Zwehl, V. v.: Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z. vergl. Physiol. **48**, 357–384 (1964)

Braitenberg, V., Strausfeld, N.: Principles of the mosaic organisation in the visual system's neuropil of *Musca domestica*. In: Handbook of sensory physiology, Vol. VII/3A, Central processing of visual information (ed. R. Jung.). Berlin-Heidelberg-New York: Springer 1973

Cajal, S.R., Sánchez, D.: Contribucion al conocimiento de los centros nerviosos de los insectos. Trab. Lab. Invest. Biol. Univ. Madrid **13**, 1–164 (1915)

Daumer, K.: Reizmetrische Untersuchung des Farbensehens der Biene. Z. vergl. Physiol. **38**, 413–478 (1956)

DeValois, R.L., Abramov, J., Meads, W.R.: Single cell analysis of wavelength discrimination at the lateral geniculate nucleus in the macaque. J. Neurophysiol. **30**, 415–433 (1967)

Granit, R.: Sensory mechanisms of the retina. London: Oxford University Press 1947

Hamdorf, K.: Primärprozesse beim Sehen der Wirbellosen. Rhein. Westf. Akad. Wiss. N 253, 1–42 (1975)

Hamdorf, K., Höglund, G., Langer, H.: Mikrophotometrische Untersuchungen an der Retinula des Nachtschmetterlings *Deilephila elpenor*. Verh. dtsch. zool. Ges. **65**, 276–280 (1972)

Hamdorf, K., Schwemer, J.: Photoregeneration and the adaptation in insect photoreceptors. In: Photoreceptor optics (eds. A.W. Snyder, R. Menzel), pp. 263–289. Berlin-Heidelberg-New York: Springer 1975

Helversen, O. v.: Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. J. comp. Physiol. **80**, 439–472 (1973)

Jawlowski, H.: Nerve tracts in bee (*Apis mellifica*) running from the sight and antennal organs to the brain. Ann. Univ. M. Curie-Sklodowska (C) **12**, 307–323 (1958)

Kaiser, W.: A preliminary report on the analysis of the optomotor system of the honey bee: single unit recording during stimulation with spectral light. In: Processing of information in the visual system of arthropods (ed. R. Wehner), pp. 167–170. Berlin-Heidelberg-New York: Springer 1972

Kaiser, W., Bishop, L.G.: Directionally selective motion detecting units in the optic lobe of the honey bee. Z. vergl. Physiol. **67**, 403–413 (1970)

Kien, J., Menzel, R.: Chromatic properties of interneurons in the optic lobes of the Bee. II. Narrow band and colour opponent neurons. J. comp. Physiol. **113**, 35–53 (1977)

Menzel, R.: Spectral response of motion detecting and "sustaining" fibres in the optic lobe of the bee. J. comp. Physiol. **82**, 135–150 (1973)

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

Menzel, R.: Colour sensitivity in insects. In: The compound eye and vision in insects (ed. G.A. Horridge), pp. 121–153. Oxford: Clarendon Press 1975

Menzel, R., Blakers, M.: Functional organisation of an insect ommatidium with fused rhabdom. Cytobiol. **11**, 279–298 (1976)

Menzel, R., Knauth, R.: Pigment movement during light and chromatic adaptation in the retinula cells of *Formica polyctena* (Hymenoptera, Formicidae). J. comp. Physiol. **86**, 125–138 (1973)

Strausfeld, N.I.: Atlas of an insect brain. Berlin-Heidelberg-New York: Springer 1976

Vowles, D.M.: The structure and connexions of the corpora pedunculata in bees and ants. Quart. J. micr. Sci. **96**, 239–253 (1955)