Spectral Response of Moving Detecting
and “Sustaining” Fibres in the Optic Lobe of the Bee

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Summary. The spectral sensitivity of motion detecting fibres was measured. Single units were recorded extracellularly in the contralateral optic lobe between the lobula and the protocerebrum. The \( \lambda_{\text{max}} \) occurred at 513 nm, a low secondary maximum at 346 nm. The zero detection method with alternating stripes of different colours and balanced intensities showed that this type of motion detecting fibre responds only to one colour. It is concluded from the spectral sensitivity that only the green receptors are connected to the motion detecting unit. A “sustaining” fibre found in the same region of the optic lobe had a spectral sensitivity with \( \lambda_{\text{max}} \) 534 nm, a shoulder at 462 nm, and a secondary maximum in the UV region (346-372 nm). It is supposed that all 3 types of colour receptors are connected to this sustaining fibre. The relevance of these findings to colour coding in the bee’s brain is discussed.


Im gleichen Gebiet des optischen Lobus wurde ein Fasertyp gefunden, der bei stationärer Beleuchtung mit anhaltender Aktivität antwortet ("sustaining unit"). Das Maximum der spektralen Empfindlichkeit liegt bei 534 nm, eine Schulter bei 462 nm und ein zweites niedrigeres Maximum im UV (346-372 nm). Es wird vermutet, daß alle 3 Farbrezeptoren im Bienenauge mit diesem Fasertyp verbunden sind. — Die Bedeutung dieser Ergebnisse für die Aufklärung der Verschaltung der Farbrezeptoren wird diskutiert.

Introduction

Colour vision in the worker bee has been studied extensively in behavioural experiments (v. Frisch, 1914; Daumer, 1950; Menzel, 1967; *).

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v. Helversen, in press) and the spectral sensitivity of the receptors has been measured using intracellular recording (Antrum and v. Zwehl, 1964). However, the intermediate mechanisms of colour coding in the optic lobe and brain of the bee are still unknown. In the bluishgreen and violet regions of the spectrum the bee is able to distinguish colours with wavelengths separated by only 4–6 nanometres (v. Helversen, in press). This means that the three types of colour receptors with \( \lambda_{\text{max}} \) at 340, 430–460 and 530 nm, must be approximately connected to higher order neurons so that very narrow band widths of the spectrum can be distinguished.

The only responses described in the bee brain are the motion detecting units of Kaiser and Bishop (1970). Bishop (1970) measured the spectral sensitivity of these units, but stimulated with stationary flashes of spectral light and used the latency to the on-response. As these units are very insensitive to stationary light flashes I measured the spectral sensitivity using a stripe pattern of spectral colours. Above that the involvement of these neurons in colour coding is studied by stimulating them with alternating stripes of different colours with variable intensities. In addition several "sustaining" fibres were found during these experiments and the spectral sensitivity of one of these units was measured.

Methods

Foraging worker bees (Apis mellifera carnica), collected at the entrance of the hive, were prepared as described by Kaiser and Bishop (1970). Extracellular recordings of single contralateral fibres were made with etched stainless steel electrodes of tip smaller than 1 \( \mu \)m, coated with Inal-X. Accurate isolation of the spikes of one unit was achieved in the following way: spikes of the highest amplitude were selected using the internal trigger level of the oscilloscope. Their waveforms were displayed at high sweep speed (Fig. 2A). If only one waveform was found, it was concluded that the signal had been isolated from a single unit. For each sweep triggered in this way a single pulse was fed to two different integrators; one (Fig. 1, SI; Fig. 2, D2), triggered by the stimulus, summed all spikes during the stimulus and during the interstimulus intervals; the other (Fig. 1, BI; Fig. 2, DI) had an internal time base and summed spikes in every 0.5 sec.

Light was provided by a xenon arc (XBO 900) with 2 quartz condensers. The standard beam (see Fig. 1) passed through neutral density filters, an interference filter (Schott, DIL 534 nm), a ground glass disc and a large glass lens, which focussed the beam on the eye through the stripes of a large striped wheel (Fig. 1A). The test beam passed through one of 18 interference filters (Schott UV-IL and DIL), quartz neutral density filters, two quartz lenses and an electronic shutter. The stimulus was a pattern of moving stripes with 10.3° period of viewed through a window (white circle in Fig. 1A) of 20°. The spectral wavelength and intensity of the stripes and the background could be varied independently to each other by using the following system: The standard beam was viewed by the animal through the gaps in a large aluminium wheel. The test beam illuminated the MgO coated surface of the sector. The geometry of these beams was such that no motion could be seen by the human eye if both beams had the same colour and intensity (zero detection method). The position of the window in front of the rotating wheel was fixed in relation to the bee's eye and ensured that only the median lateral part of the contralateral eye was stimulated. The stripes moved horizontally forwards or backwards with a contrast frequency of 2.9 Hz.

The calibration of the spectral light in relative numbers of quanta was made with a UV-sensitive selenium cell (550 UV, Lunge, Berlin) coupled to a galvanometer. The spectral efficiency and linearity of the selenium cell was estimated frequently with a Thermopile (Kipp and Zonen) and a Microwatt-ammeter (Keithley Instruments, model 149).

Results

a) Motion Detector Units

The units described below were recorded in the tract between lobula and protocerebrum. They are motion detectors with monocular contralateral visual fields and an excitatory response to horizontal movement of the stripe pattern from posterior to anterior (forward). The characteristics of these units have been specified in detail by Kaiser and Bishop (1970) as follows: spontaneous activity in the dark with a spike frequency of 5–10 per second (Figs. 2b and 3), directional sensitivity (i.e. excitation in the preferred direction, inhibition in the null direction).
Fig. 2. A) Superimposed spikes of a motion detection unit. This picture was used to decide if one unit only was recorded. Sweep time 0.2 msec/cm. At each internally triggered sweep a signal from gate-out was fed to the integrators. B) A motion detection unit with contralateral visual field and excitation by forward movement. Light (534 nm) was turned on at arrow with pattern already in motion (preferred direction). Note post-excitatory depression after light-off (arrow). Stripe period 10.3°, contrast frequency 2.9 Hz, maximal contrast (illumination from the front, black background). C) Recording of a contralateral "sustaining" fibre with small on- and off effect. The unit did not respond to movement in the visual field. Light (534 nm) was turned on (arrow) for 10 sec. The off response is not shown. D) Output of both integrators (see: method). 1. The output of the rapid integrator (spikes per 0.5 sec). 2. The slow integrator; amplitude shows total number of spikes during stimulus and inter-stimulus interval. The event marker shows light and dark periods. This record is part of a spectral run in the UV-region.

The response to movement in the preferred direction is shown in Fig. 2B. The contrast frequency in all experiments was 2.9 Hz, this is in the range of the maximal response (Kaiser and Bishop, 1970, Fig. 5). In most experiments the striped pattern always moved in the excitatory direction and was illuminated for 5 sec every 20 sec. Data are obtained as shown in Fig. 2. The rapid integrator (D1) shows the phasic-tonic response characteristic and post-excitatory depression. The slow integrator indicates the sum of all spikes during stimulation and during the interstimulus interval. There are no differences in spike pattern or dynamic response characteristics of different intensities and different spectral colours; therefore only these summed responses were used for calculating the average spike frequency.

Fig. 3. Response of one motion detection unit as a function of the intensity of the stimulus. Each point is the average of 4 measurements (- - - - 136 nm, -- 512 nm). Closed symbols (△ 356 nm, △ 512 nm) show the response to 5 sec light flashes without movement of the stripes; large open circles give spontaneous activity (Sp) in the dark. The animal was dark-adapted for longer than 30 min. Contralateral forward movement, maximal contrast with black background and stripes illuminated from the front; contrast frequency 2.9 c/sec. The light was turned on when the pattern was already in motion. The stimulus program was: 5 sec light on, 12 sec light off. Measurements were made in random order of different light intensities. Bars show standard deviation.
The response increases with increasing intensity over a range of 1.5 log units of light intensity and declines slightly after reaching a maximum. The response is plotted against the stimulus intensity for two wavelengths 356 nm and 513 nm for one unit under the standard conditions (Fig. 3). The slopes in the rising phase of these curves are not significantly different for different wavelengths (t-test).

Seven motion detection units all with the same preferred direction and nearly the same absolute sensitivity were recorded from 5 different preparations. A response-intensity curve with at least 3, mostly 5 different intensities was plotted for each of the 18 spectral colours. Each of the 3–5 points on the response-intensity curve is the average of up to 4 values obtained from each unit at different time during the experiment. Out of many recordings only these 7 units had a sufficient stable response (some hours). The spectral sensitivity of each unit was calculated from these curves by taking the reciprocal of the intensity needed to obtain a constant response of 30 spikes/sec. This method gives the relative sensitivity of each unit. The average of the 7 units is given in Fig. 4. Maximal sensitivity is at 513 nm, range from 462 to 534 nm in single units. A low secondary maximum is found in the UV-region near 356 nm; its difference from the minimum at 402 nm is not highly significant ($P = 0.055$ by t-test).

A comparison with the spectral sensitivity of the green receptor of the bee (Autrum and v. Zwehl, 1964) shows that the motion detector receives input mainly from this class of receptors. However, the maxima are not exactly at the same wavelength. Considering the deviation of the green maximum of different motion detector units and the observations that the maximum of the green receptor often shifts (Autrum and Kolb, 1968; Eguchi, 1971) the two curves fit well in the visible region. The agreement is very good, especially between 402 and 478 nm and the standard deviation small. Therefore it is supposed that the blue receptor (430–460 nm) has no connection to this class of units. As Autrum and v. Zwehl (1964) did not find a secondary maximum of the green receptor it is necessary to determine whether the UV-receptors send inputs to this motion detection system.

We can distinguish by experiment with the zero detection method (v. Buddenbrock and Friedrich, 1933; Kaiser, 1968) between two classes of motion detection systems both of which have two or more types of colour receptors at their inputs. When presented with alternating stripes of differing wavelengths the first class gives no motion response for a particular balance of intensities for each pair of wavelengths. This class is defined as showing no colour vision in this experiment. This finding can be explained by supposing that all the receptors are summed in the input to the motion sensitive system, or only one type of colour receptor acts as the input, or some combination is summed in a manner which allows a null point in the experiment. There are many possible models which allow this, but considering the response characteristics to different wavelengths and the spectral sensitivity it can be differentiated between these possible models. The other class of motion detection system is connected to colour receptors in such a way that motion of alternating stripes of differing wavelengths is always seen as motion no matter how the intensities of the wavelengths are balanced. Such a system, defined as having colour vision, is again possible in many ways. Since the motion detecting units in the bee brain are very sensitive to changes in contrast of stripe pattern, the zero detection method readily distinguishes between these two classes of mechanisms and the results on single units can be compared with the optomotor response of the whole animal.
Fig. 5. Average response of one motion detection unit as a function of the varying contrast of the striped pattern. Contrast C is defined after Fermi and Reichardt (1963) as: \( C = (a - b)/(a + b) \), where \( a \) is the relative quantal content of the standard beam and \( b \) that of the test beam. Standard beam: 534 nm constant intensity; test beam: ● 372 nm, △ 418 nm, ○ 534 nm, variable intensity. The minimum of each curve was normalised to a relative contrast of zero. 5–12 measurements were averaged for the minimum value, otherwise each point is the average of 2–3 measurements. The standard deviations for the minimum values are shown (± for 372 nm, ±± for 478 nm, ± for 534 nm). Stimulation program: standard beam and test beam are continuously on; the motion of the stripe pattern is switched on for 5 sec. Spontaneous response (SP) is the average spike frequency between movement stimuli.

Three different stimulation programs, with a test beam of 372 nm, 418 nm or 534 nm and a standard beam of 534 nm, were used. In the first (Fig. 5) both test beam and standard beam were on continuously, the motion of the striped pattern was switched on for 5 sec every 15 sec and increase of spike frequency was measured. For each of the three pairs of spectral colours a minimum response can be found which is not significantly different from spontaneous activity, which is measured without the motion, but with the test and the standard beams switched on. These experiments show that no colour vision as defined can be found in the motion detection unit. It was shown in two other experiments that this also holds true when the unit is at high level of activity on account of a continual motion of the striped pattern. When the standard beam and the motion are continuously switched on (maximal response to maximal contrast) and then the test beam is switched on regularly for 5 sec we obtain the result shown in Fig. 6. This rapidly reduces the contrast and the activity decreases to a very low spike frequency. Because of the postexcitatory depression, the minimum occurs at a low spike.
frequency and the curves are broad. The third stimulus program (Fig. 7) also reveals no colour-specific responses. Here the striped wheel was always in motion and both beams were on; the intensity of the test beam was changed nonsystematically every 10 sec. The average spike frequency in the 3 sec following the short burst evoked by the sudden change in contrast was measured.

In Figs. 5–7 the relative contrast of each pair of colours (372/534 nm, 418/534 nm, 534/534 nm) is given on the abscissa. In this way each minimum lies at zero and one sees clearly that the curves have the same slope. Different intensities in terms of relative numbers of quanta of test beam are needed to match the standard beam to produce the minimum response (for 372 nm 3.3 times and for 418 nm 2.8 times more than for 534 nm). A very exact spectral sensitivity curve can be estimated with this method (Kaiser, 1972).

These three test programs show that the motion detector unit responds only to brightness contrast, not to colour contrast, regardless of excitation level in the receptors and interneurons.

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**Fig. 7.** Average response of the same motion detection unit as in Fig. 5 and 6 as a function of varying contrast of the striped pattern. See Fig. 5 for explanation. Stimulation program: standard beam, test beam and motion of striped pattern are continuously on. The intensity of the test beam is varied every 10 sec (see text). The dashed line gives the average spontaneous response level at a medium light intensity and without motion.

**Fig. 8.** Relative spectral sensitivity of a "sustaining" unit, calculated from response—log intensity diagrams for each colour (solid line). Relative sensitivity (ordinate) is the reciprocal of the intensity of each colour which produces a constant response (25 spikes/sec). The dashed line gives the average relative spectral sensitivity of the green receptors of worker bee determined from intra-cellular recordings (Aurum and v. Zwehl, 1964)

b) "Sustaining" Fibre

Besides these motion detection units one can find fibres which respond to light with a sustaining activity. These units are not activated by any movement in their receptive field. There is a response peak at light-on and another peak at light-off of different relative magnitude of response in different fibres (Fig. 2C). Sustaining fibres were found rarely and could usually be held only for a short time. However, in 6 units it was possible to demonstrate a maximal sensitivity in the green region (513–534 nm), low sensitivity in the UV-region and no inhibition in any part of the spectrum. Compared with the motion detection units the sustaining fibres had a lower absolute sensitivity. A stimulus intensity greater by 1.5–2 log units is needed to evoke the same spike frequency as given by the motion detectors. It is possible, however, that the stimulus was outside the most sensitive region of the receptive field because its position could not be changed.
One unit was held for several hours and response-intensity curves for each of the 18 spectral colours were obtained. Each response-intensity curve was plotted from 7–12 measurements at each wavelength. The spectral sensitivity was calculated for a constant response of 25 spikes/sec (Fig. 8). The maximum at 543 nm and the steep slope of the sensitivity at longer wavelength are in good agreement with the curve for the green receptor. In contrast to the spectral sensitivity of the motion detector, a small maximum appears at 463 nm and below 386 nm the sensitivity decreases. The poor agreement with the spectral sensitivity of the green receptor in the region between 300 and 500 nm suggests that the "sustaining" fibre receives some additional inputs from the UV or blue receptors, or both.

**Discussion**

For all motion detection units a positive correlation was found between response and increasing intensities of all wavelengths. Therefore, inhibitory inputs from any colour receptor type can be excluded. This limits the possible connections between receptors and motion detection unit to only the 3 different types given in Fig. 9a–c. As each ommatidium contains more than one cell of each colour receptor type (Gribakin, 1969, 1972), it is supposed that the response of some retinula cells of one ommatidium are summed in a first stage of integration. Anatomical and electrophysiological evidences for convergence of retinula axons onto one single monopolar cell are found in flies (Boschek, 1970; Strausfeld, 1971; Zettler and Järviilehto, 1972). In bees it is known that 6 of the 9 retinular axons of one ommatidium run to the same cartridge and it is assumed that more than one retinular axon have synapses with one monopolar cell (Varela, 1970; Meinertz-Hagen, personal communication). Motion detection is thought to occur by correlation between parallel inputs (Hassenstein and Reichardt, 1956; Thorson, 1966). For simplicity only two ommatidia are shown in Fig. 9, although it is known that many ommatidia are involved.

The motion detectors have no colour specific responses as they show the same type of intensity dependency in all wavelengths tested. However, colour coding is possible as in Fig. 9c. In this case the three colour receptor types are summed separately and correlators exist for each receptor type. The unit following the correlators would react to colour contrast in the zero detection experiment even when all the colour specific correlators have excitatory input to this unit. As such responses were not found this model can be excluded.

The lack of colour vision as defined above (page 6) may be caused by two different types of connections between summing stage and correlator: either all receptors of the ommatidium are fed to the summing stage (Fig. 9a) or only one receptor type (Fig. 9b, a part of 9c). The small shift of the green maximum and a secondary maximum in the UV seem to agree with model 9a. However, the small UV-maximum is no proof of connection with the UV-receptor, because a green receptor with secondary maximum in the UV has been found in bees (Anstrum, 1968, Fig. 11) and in other insects (Aeschna: Anstrum and Kolb, 1968; Eguchi, 1971; Notonecta: Bruckmoser, 1968; Paravepsula: Menzel, 1971). The discrepancy between the maxima of the motion detectors and the
green receptors is smaller than the variation of single-motion detectors and the position of the green receptors peak is also variable (Langer and Thorrell, 1966; Autrum and Kolb, 1968; Eguchi, 1971). Further evidence against this model comes from the zero detection experiments. In some experiments, the eye was illuminated for longer than 30 minutes with the green light (532 nm) of the standard beam without any significant change in the zero point at different wavelengths when compared with measurements immediately after a long dark adaptation period. If more than one photopigment system would be involved, a selective adaptation effect with changes of the zero points should be found.

Bishop (1970) inferred a connection to all three types of photoreceptors (as in model Fig. 9a) from an action spectrum of the same motion detection unit. He stimulated with short light flashes and measured the latency between stimulus and onset of the response. The fibres are very insensitive to stationary light flashes (Fig. 3). In the present experiments, the fibres were stimulated with their normal input and the results are more likely to show their pattern of connections.

It is supposed that the motion detection unit receives input only from stage which sums the responses of the green receptors (Fig. 9b). It is not known if there are more motion detectors which are connected to the other colour receptors. Model Fig. 9b would be a part of model Fig. 9c, if there are colour-specific motion detectors for both other receptor types; but these were not found during recording. In both parallel studies with the optomotor response of the bee (Liske and Kaiser, 1972), no such evidence was found. With regard to the "sustaining" fibre, it is uncertain whether inputs other than the green receptors are involved. The spectral sensitivity for one "sustaining" unit showed a shoulder at 462 nm, a broad UV-maximum and a decrease of the sensitivity below 337 nm. Therefore it cannot be excluded that all three receptors are involved in unknown proportions. Adaptation experiments are required before further conclusions can be made.

The only other information on colour coding in the insect brain comes from recordings of interneurons in butterflies. Swihart (1969, 1970) found in *Epargyreus clarus* a dichromatic colour vision system and opponent colour coding neurons in the brain. In *Papilio troilus* there is a trichromatic system and in the brain narrow- and wide-band fibres. The motion detection unit, discussed here, are sensitive throughout the spectrum. Neurons of the system used in colour vision in the bee should have the properties of the neurons described by Swihart. As the motion detectors appear to receive inputs from only one colour receptor type it is unlikely that they are involved in more sophisticated colour coding. The motion detecting system, apparently used in the visual stabilization of flight, is therefore quite separate from the bee's system of colour vision for objects which it visits, such as flowers.

References


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