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Natural phototaxis and its relationship to colour vision in honeybees

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Summary. 1. Honeybees are positively phototactic when they leave a feeding place and start to fly back to the hive. The strength of this natural phototactic response in individually marked bees was measured without interfering with their foraging behaviour.

2. Absolute sensitivity of this phototactic response to a point light source is in the range of $8.3 \cdot 10^7$ quanta s^{-1} for 537 nm. This corresponds to about 5 absorbed quanta in 28 green receptors over the integration time of 60 ms.

3. We conclude that the properties of the monopolar cells or higher order visual interneurons rather than those of the photoreceptors control the intensity dependence of the response because the slopes (n) of the response intensity functions ($R/\log I$) are steep (n: 1.0–2.65) and wavelength dependent. Blue light (439 nm) causes the steepest function.

4. The effect of residual light adaptation on the $R/\log I$-function and the spectral sensitivity ($S(\lambda)$) is negligible under the experimental conditions chosen, since the time course of dark adaptation is fast ($\tau \leqq 1$ min).

5. The blue and green receptors contribute about equally to the $S(\lambda)$ of this natural phototactic response, the UV receptors somewhat less (Fig. 5).

6. Colour mixing experiments, used to test colour vision in phototaxis, reveal no significant deviation from a simple linear summation of the quantal fluxes, irrespective of the spectral mixture used. We conclude, therefore, that under the experimental conditions colour vision is very unlikely to play a role in the phototactic behaviour of the honeybee.

7. All our results (steep $R/\log I$-functions, fast dark adaptation, $S(\lambda)$ and the absence of colour vision) support to notion that the natural phototactic response is controlled by neuronal pooling, most likely in the lamina M1 monopolar cells.

Introduction

Phototaxis is a convenient measure of visual capacities in animals, particularly in insects, because this stereotyped behaviour is easily elicited and relatively stable. The stimulus parameters influencing the response are mainly light intensity (I), wavelength (λ) or spectral composition ($I(\lambda)$). The duration and spatial properties of the stimulus are also important. The questions of relevance in our study are: how do light intensity and wavelength interact, and do bees discriminate colours in phototaxis?

The sensory and motivational state of the test animals influence phototactic response. The level of light-dark adaptation obviously controls the response, and can be the parameter of central interest, if it is the only one changed (e.g. Labhart 1974; Wolf and Zerrahn-Wolf 1935). Our concern will be to test whether the level of light adaptation effects the spectral properties of phototaxis in bees. Motivational factors are rarely studied or controlled in phototaxis experiments, because in most studies the animals were 'made strongly phototactic' by keeping them in a dark box for an extended period of time. We have chosen a different approach. Honeybees were tested during their natural cycle of food collection, when they are positively phototactic, namely when they leave a dark food source and prepare to fly back to the hive. Our test procedure does not interrupt or prevent the bee's ongoing activities. Their foraging behav-

iour is not slowed down, and the motivation for foraging is not altered. The advantage of this procedure is that naturally occurring phototactic responses are tested as opposed to experimentally induced ones.

Colour vision in honeybees is well established (rev.: Menzel 1979). The bee's compound eye contains three spectral types of receptors (UV-, blue and green receptors, Autrum and von Zwehl 1964), which are assembled in each ommatidium (Menzel and Blakers 1976). They use their colour vision at the food source and at the hive entrance (von Frisch 1914, 1967; Menzel 1985). Bees are colour blind in the optomotor response (Kaiser and Liske 1974, Kaiser 1974). Celestial orientation is organized in a colour antagonistic fashion with polarized light orientation to the sky (extended light source) being restricted to the UV region ($\leqq$410 nm), and sunlight (point light source) orientation controlled by longer wavelengths ($\geqq$410 nm) (von Helversen and Edrich 1974; Edrich et al. 1979; Brines and Gould 1979). Phototaxis in bees seems to be elicited by all three spectral receptor types, but dominated by the UV-receptors (Bertholf 1931a, b; Heintz 1959; Kaiser et al. 1977; Labhart 1974; Sander 1933; Weiss 1943). A closer examination of these papers reveals a number of important discrepancies: The response-intensity functions ($R/\log I$) are steep ($\geqq$50% response changes within one log I increment) in the studies by Heintz and Labhart, and shallow ($\leqq$30%) in Kaiser et al. The gradient of the $R/\log I$-function is wavelength dependent in all experiments but the dependency varies: it is steeper at 410 and 492 nm in Kaiser et al.'s experiments, whereas in Heintz's and Labhart's experiments it is steeper at 360 and 550 nm. Labhart and Heintz conclude that UV and green receptors contribute to the phototactic response, because they find sensitivity peaks in the UV and green, but not in the blue. Kaiser et al. suggest that all 3 colour receptors are involved, because they find an additional spectral sensitivity peak around 440 nm at low response level (but not at higher response levels). Sander finds no sensitivity peak in the UV at all, but a high peak in the blue and a secondary one in yellow. These discrepancies indicate that the contribution of the 3 colour receptor types to phototaxis may vary with the test procedure. It might well be that different response types have been summed up under the heading 'phototaxis'. We interprete this as a request for an experimental procedure which controls not only the sensory parameters but also the motivational parameters.

The question, as to whether bees perceive colour contrast in phototaxis has so far not been examined. The wavelength dependence of the $R/\log I$-function found in all studies might suggest colour effects, but there are other explanations (Menzel 1979). The dominance of one wavelength region (UV) argues against colour vision in phototaxis, since an unbalanced contribution of one receptor type distorts the perceptual colour space and eventually eliminates colour discrimination (Rushton 1972; Rodieck 1973). We show here that the contribution of the 3 spectral receptor types to the bee's natural phototactic response is balanced, and thus colour contrast perception is potentially possible. However, colour mixing experiments provide evidence only of summation of all receptor inputs, making colour vision unlikely in phototaxis.

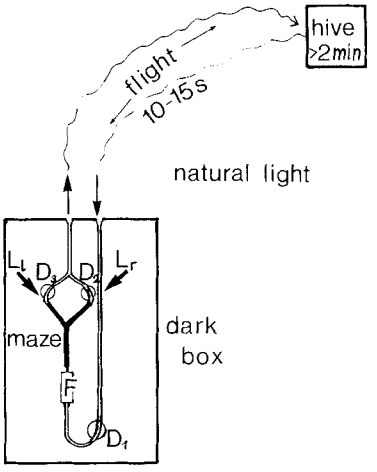

Fig. 1. Test apparatus and experimental procedure. The bees fly in the open from the hive to the test apparatus. The flight lasts 10–15 s. 2 min or longer is spent in the dark hive between the foraging flights. When a marked bee enters the tube in the dark box it is detected and individually identified by the detector D_1. The computer activates a gate, which guides the bee to the feeding place F. Access to the sucrose solution is controlled to allow feeding for 2–5 min. In this way, the period for dark adaptation can be varied. After feeding, bees are positively phototactic. They run into the Y-maze and choose the right or left arm depending on the illumination coming from the light guides L_l and L_r. After their choice they leave the Y-maze and are detected by detectors D_2 or D_3. They leave the test apparatus by an exit separated from the entrance (see Menzel and Greggers 1983 for further details)

Methods

Honeybees (*Apis mellifera carnica*) were trained to collect sucrose solution at a feeding place inside a dark box (Fig. 1). The feeding place was located 40 m south-east from the hive, and the trained bees needed in an average 10–15 s to fly from the hive to the entrance of the box. During the flight, the frontal part of the eye faced the sky and green leaves of trees. It also

faced the white background surrounding the entrance to the box with the feeding place. Bees walked inside the box in tubes (⌀16 mm), to reach the feeding place and to move on to the exit. Entrance and exit were separated, so that bees learned to move straight on after feeding. A group of bees (10–20) shuttled continuously between the feeding place and the hive throughout three summers.

One to four bees of the group were marked individually by glueing small stainless steel balls of different sizes (0.2–1.0 mm⌀) to their thoraces. The size of the steel ball on each bee was detected by an electronic device when the marked bee walked through a detection coil (Menzel and Greggers 1983). The signal was fed into a computer, programmed to select certain bees from the group for an experiment. The computer activated a gate which guided the selected bee into a tube adjacent to that used by the non-selected bees (Fig. 1). The selected bee reached a separate feeding place. After feeding the bee walked towards the exit and reached the intersection of a Y-maze, either arm of which could be illuminated with a light guide. Since the bees are positively phototactic when they have finished feeding they have a strong tendency to choose the illuminated arm of the Y-maze. The choice of the bee was recorded again by detecting coils on their way to the exit (D2, D3 in Fig. 1). We collected data from more than 20,000 runs of 115 different individually marked bees. Some bees performed many test runs (>300) others only a few.

The spectral stimuli delivered to the Y-maze were produced by two quartz halogen lamps (150 W) driven by a stabilized DC current source. The spectral filters used were DIL interference filters (Schott, Mainz) with λ_{max} at 409, 439, 489, 537 nm and an UV-IL filter with λ_{max} at 341 nm. The UV-filter was illuminated by an UV-reflecting mirror that cut off all wavelengths above 390 nm. An additional green light emitting diode (LED) λ_{max} 537, halfbandwidth 44 nm) was used in the double stimulus experiments. The LED was driven by a current stabilized power supply and could be set to different intensity levels. A calibrated radiometer (IL 700 with detector PM 270D) was used for light measurements. The photomultiplier was positioned at the intersection of the Y-maze, and measured the light flux under geometrical conditions equivalent to those at the bee's eye.

Bees are exposed to varying light intensities in their flight from the hive to the test box. In our experiments outside light intensity was measured continuously and the current value stored by the computer when a selected bee was guided into the upper tube. Flight time was found to be 10–15 s. Dark adaptation time was measured for every test run of each individually detected bee.

All experiments were run fully under the control of the computer. Regular observation of the entrance and exit was carried out in order to make sure that the computer worked properly.

Results

Basic findings: The R/logI-functions

Honeybees arriving at the feeding place are initially negatively phototactic and tend to run to darker places to search for nectar or sucrose solution. This is the biological background for the ease with which bees can be trained to enter dark tubes. After they have filled their crop they become positively phototactic and choose the illuminated arm of a Y-maze. Figure 2 gives two examples of the intensity dependence of this response when one arm is illuminated (409 nm, 439 nm) and the other one is kept dark. Each data point in Fig. 2 represents an independent series of runs of several bees (3–7 bees, 70–150 choices per bee), collected during three summers. The $R/\log I$-function is steep, with a dynamic range of 1–1.5 $\log I$. Above a certain intensity, all bees reliably choose the illuminated arm, and a further increase of the intensity does not change this 100% response.

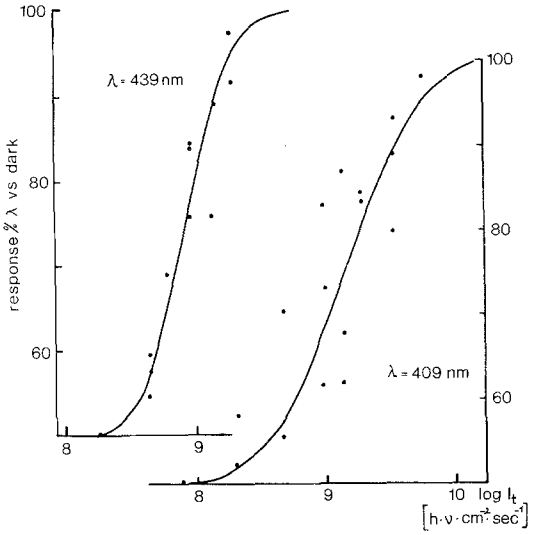

Fig. 2. $R/\log I$-functions for $\lambda = 409$ nm and $\lambda = 439$ vs a dark alternative. The response is expressed in % choice of λ relative to the dark alternative (ordinate) and is plotted against log quantal flux (abscissa). Each point is the result of 70–150 choices. The total number of choices is $n = 960$ for $\lambda = 439$ nm and $n = 1{,}710$ for $\lambda = 409$ nm. The number of different test bees is $N = 52$ for $\lambda = 439$ nm and $N = 72$ for $\lambda = 409$ nm. The smooth line is the best fit of the data to the function (1) given in the text. The slope is $n = 1.56$ for $\lambda = 409$ nm and $n = 2.65$ for $\lambda = 439$ nm

The $R/\log I$-functions of all 5 test wavelengths are given in Fig. 3. Again one arm was illuminated with varying intensities of the spectral light and the other arm was kept dark. The $R/\log I$-functions follow very well a power function of the form

$$\frac{R}{R_{max}} = \frac{(K \cdot I)^n}{(K \cdot I)^n + 1} \quad (1)$$

where I is the stimulus intensity, R the response in percent of choice of the illuminated arm, R_{max} is the saturated response (100%), K is the reciprocal value of the intensity at a response of 75%, n is the slope of the function (Baylor and Fuortes 1970; Laughlin 1981; Lipetz 1971). The value K will be used to calculate spectral sensitivity (see below). The slope n is very similar for wavelengths

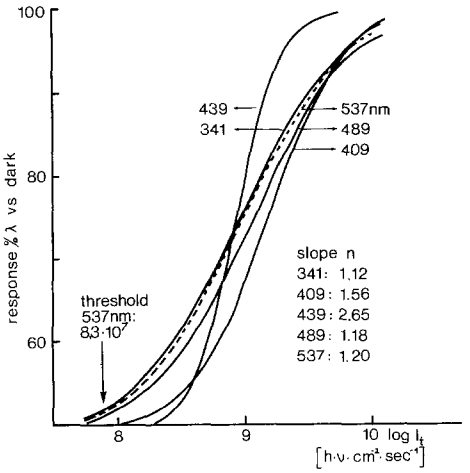

Fig. 3. $R/\log I$-function for the 5 wavelengths tested. One arm in the Y-maze was illuminated with one of the 5 wavelengths at various intensities. The other arm was kept dark (see Fig. 2). The curves are the best fit of Eq. (1) to the respective data (see text). Each function is based on 1700–2000 choices made by 50 to 80 bees

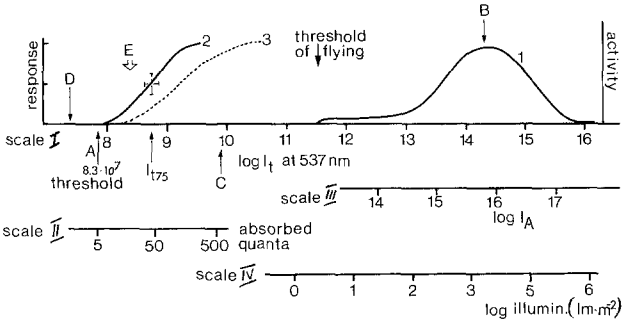

Fig. 4. Position of the $R/\log I$-function (left side, curves 2 and 3) and the frequency of foraging flights (right side, curve 1) on a common scale of quantal light flux. Scale I gives on the left side, the quantal flux of the test light at 537 nm, 8–11 log I_t in h ν/cm^2 · s and on the right side, the quantal flux at 537 nm of the light outdoors ($>10^{11}$). Scale III gives the effective light flux outdoors in log I_A, the adapting light intensity, and expressed in number of effective quanta/cm^2 · s (see text for calculation). The frequency of foraging flights (curve 1) shows, how the flight activity depends on light intensity outdoors. Threshold of foraging flights is at 10^{13} effective quanta cm^{-2} s^{-1}, optimum of foraging activity is at $7 \cdot 10^{15}$ quanta cm^{-2} s^{-1} (arrow B). Curve 2: $R/\log I$-function from Fig. 2 ($\lambda = 537$ nm), bars at the 75% response level give standard deviation for the response and the long time accuracy of the light source. Curve 3: $R/\log I$-function for 537 nm in an experiment in which the alternative arm of the Y-maze is illuminated with green light. Arrow A indicates absolute threshold for 537 nm in the experiments with a dark alternative (8.3 10^7 h · ν cm^{-2} s^{-1}, see also Fig. 3); I_{t75} is the light intensity which produces a response of 75% in a λ vs dark test (this value is used to calculate sensitivity in Fig. 5); arrow C gives the threshold as determined by Kaiser et al. (1977). Arrow *D* and *E* relate to colour training experiments, in which thresholds for achromatic vision (*D*) and for colour vision (*E*) were determined (Menzel 1981). The positions of these latter thresholds along the intensity scale are less reliable, because spatially extended light sources were used (see calculation in Menzel, 1981). Scale II gives estimated absolute number of quanta absorbed in the 7 ommatidia looking at the stimulus during the integration time of 60 ms (see text). Scale IV gives the luminance (in lm/m^2) of the light outdoors, to which the bees are exposed during their flight to the set-up. This scale allows a correlation of the easily measurable natural illumination with the quantal flux in scale I and II. Foraging frequency (curve 1) with respect to scale IV correlates well with earlier observations (Rose and Menzel 1981)

341, 409, 489 and 537nm but significantly steeper for 439 nm. (A statistical analysis of the n's of individual $R/\log I$-functions with the *t*-test reveals, for example, a significant difference at a level of $P < 0.01$ between the curves for 537 nm and 439 nm, and a significant difference at a level of $P = 0.05$ between the curves for 409 nm and 439 nm).

Absolute sensitivity

Before considering the parameters influencing the $R/\log I$-function in more detail, we need to locate this function in an absolute scale of quantal flux and relate this scale to naturally occurring illuminations. It should be kept in mind that our test bees reach the apparatus after a flight of about 10–15 s through open air at varying illumination, and that they are dark adapted for a short time (2–6 min) before performing a test. Figure 4 gives the result for I_t, the test intensity, in number of quanta (h · ν cm^{-2} · s^{-1}) for the narrow double interference filter $\lambda_{max} = 537$ nm, together with the corresponding $R/\log I$-function (Fig. 4, scale *I*, left side). Response threshold was found at $8.3 \cdot 10^7$ h · ν/cm^2 s for $\lambda = 537$ nm (arrow *A*). As a criterion for the response threshold we read from the best fitting function of formula (*I*) the log*I*-value at which response level reaches 1%.

Next we want to estimate the number of absorbed quanta in single photoreceptors at the response threshold. The light source appears to the bee at the choice point at an angle of 1.0°. This means that 1 facet lens catches 50% of the effective light, 6 facet lenses about 8% each, because the visual acceptance angle of frontal ommatidia is approximately 1.5° and the interommatidial angle is also approximately 1.5° (Baumgärtner 1928; Portillo 1936; Laughlin and Horridge 1971; Wehner 1981). This is equivalent to 2 fully illuminated facet lenses. A facet lens has a diameter of 20 µm. A threshold of 8.3 10^7 h · ν/cm^2 · s at 537 nm corresponds to 250 quanta/s in 2 fully illuminated facet lenses. Since this quantal flux is caught by 7 ommatidia, 28 green receptors point towards the light source. Therefore, the sensitivity at threshold is nearly 10 quanta/s in each of the 28 green receptors simultaneously. The time spent within the light

beam (decision time) measured with an *IR*-sensitive TV-camera is 100 ms on average. This value is close to the integration time of dark adapted photoreceptors (Raggenbass 1983, Fig. 5, 70 ms in unidentified photoreceptors in the drone bee eye). It is possible to estimate from these data an average of 20 quanta per integration time in two fully illuminated ommatidia or 28 green receptors. Assuming that half the quanta reach the photopigment in the rhabdom and that quantum efficiency is equal to 50%, we find a threshold value of about 5 quanta absorbed in 28 green receptors over the integration time (see scale III in Fig. 4).

To determine the effect of dark and light adaptation on choice behavior, we need to correlate the light measurements outdoors with respect to the intensity scale of the phototactic choices. The same interference filter (537 nm) under the same geometrical conditions (same light guide, same distance from the photomultiplier tube) as in the measurements of the light flux in the test apparatus, was used to measure the light flux at 537 nm outdoors under the same natural light conditions, experienced by bees during their approach to the entrance. Figure 4 gives that light flux as an extension of scale I to the right. This scale I is continuous with that for the light flux of the test light (I_t) because the light measurements were carried out under similar conditions, and in both cases light flux is expressed in $h \cdot v\,cm^{-2} \cdot s^{-1}$ for the same wavelength 537 nm. The effective light flux outdoors (scale III in Fig. 4) was determined in the following way. The spectral distribution of diffuse daylight (Henderson 1970) was multiplied at corresponding wavelengths with a smoothed function of the spectral sensitivity found for the phototactic response (see below, Fig. 5). The resulting function was then normalized at 537 nm to the reading from the actual spectral measurements outdoors as described above. Since the integral under the spectral transmission function for the 537 nm filter corresponds to 0.05 of the integral under the spectrally weighted daylight function, we multiplied the measurements at 537 nm with a factor of 20. This factor did not change within the accuracy of our method for varying weather conditions and daytime. Therefore, the scale for the adapting light (I_A) outdoors (Fig. 4, scale III) is shifted by a factor of 20 to the scale for I_t (537 nm). This procedure allows to compare the adapting light intensity, I_A directly with the intensity of the monochromatic test light, I_t. It should be noticed that scale I in Fig. 4 applies only to the wavelength (537 nm) for which it was measured. Since the ratio of light flux between UV (360 nm) and green (537 nm) in natural light was found to be 1:15 with very little change over daytime and weather, the scale I for UV light would have to be shifted by 1.3 log to the left.

Since flight activity of bees is determined by temperature and illumination, the sample frequency of our data varies with these parameters. Figure 4 curve 1 shows the frequency distribution of all tests with respect to illumination. It is apparent that most tests were performed at a median effective light intensity (see scale III), which corresponds to about 8 log units above threshold (for 537 nm, arrow B). On several days in the summer the temperature was high enough so that the flight activity depended only on light intensity in the evening. In this case the lowest effective illumination level for flight is 5 log units above threshold. The lowest illumination level was found to be the same (within ± 0.1 log) as that determined in the experiments by Rose and Menzel (1981) (see scale IV).

Darkadaptation

Since the animals were exposed to varying light intensities outdoors (Fig. 4 Curve 1) and then tested in the dark, we have to examine whether sensitivity differences are the result of different degrees of darkadaptation. Darkadaptation time varied between 1.5 and 4.5 min; most animals performed the test run after 2.6 min in the dark. Experiments on the time course of dark adaptation in bees by Wolf and Zerrahn-Wolf (1935), Goldsmith (1963), Autrum and Seibt (1965) and Kindermann (1983) indicate that dark adaptation after a long exposure to bright light follows an exponential function with a time course $\tau = 2.5$ to 5 min. We measured the time course of dark adaptation by comparing sensitivity to identical test situations and similar intensities of light adaptation outdoor but different dark adaptation times and found $\tau = 1$ min. This means that most of the test animals were very well or fully dark adapted.

Next we examined whether the slope of the $R/\log I$-function depends on the state of dark adaptation. This was tested by comparing the extremes of the residual light adaptation for similar test situations (animals exposed to low light intensity outdoors and long dark adaptation time vs animals exposed to very high light intensity outdoors and short adaptation time). These extremes differed by an average sensitivity difference of less than 0.5 log. The slope of the corresponding $R/\log I$-function does not depend on this small amount of residual light adaptation.

Learning

Honeybees learn quickly to associate visual, chemical or mechanical stimuli with rewarding situations. We made sure in our experiments that bees did not learn to choose one or the other arm of the Y-maze, according to chemical marks or by repeated turns to the same side, by frequently changing the tubes of the Y-maze and by randomly alternating the side of the illuminated arm. In spite of these precautions, however, bees may have learned to choose the illuminated side due to a rewarding component associated with exit out of the dark box.

Learning should improve the choice behavior as a consequence of an increasing number of correct choices. In addition, if bees see the colour of the spectral light, they may learn to turn towards the colour. We would expect, therefore, a change of the response level with the number of consecutive phototactic runs depending on the test situation prior to the new test situation. For example, if bees are first tested in a situation in which 90% of the bees choose the illuminated side and only 10% the dark side, they might have learned to run to the illuminated side and should then choose a dimmer light at a higher proportion than a group of bees which first run to the illuminated side only to 60%. We, therefore, analysed our data with respect to the following questions: Does the response level change during the 20 phototactic runs following a *change (upwards or downwards) of the intensity* of the test wavelength? Does the response level change during the 20 phototactic runs following a *change of the wavelength?* These data were compared with an equal number of runs without any prior change of the test situation. Several hundred runs were analysed.

There is no indication of learning, either for the intensity changes or for the wavelength changes. The latter result means that bees have not learned any colours under these conditions, possibly because they did not see the spectral lights as colours.

Spectral sensitivity

The sensitivity to the 5 wavelengths 341, 409, 439, 489 and 537 nm is calculated from the respective $R/\log I$-function. These calculations are complicated by the fact that the slope of the $R/\log I$-functions is wavelength dependent. This means that the probability of quantum absorption is not the only factor influencing spectral sensitivity. Various parameters have been examined to see if they affect

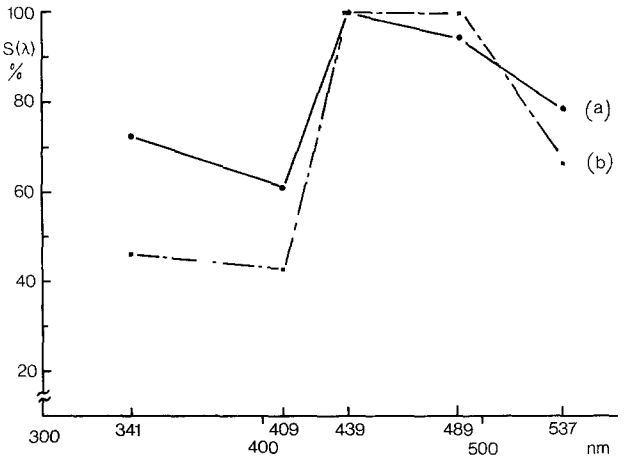

Fig. 5. Spectral sensitivity calculated from the $R/\log I$-functions of Fig. 3 for a response value of 75%. The Spectral light was tested against a dark alternative (curve a). Curve b: Spectral sensitivity calculated from $R/\log I$-functions for a response value of 50% in an experiment, in which the spectral light was tested against an alternative of green light (LED, $\lambda_{max} = 537$ nm, quantal flux 1.1 10^9 h · v cm^{-2} s^{-1})

the slope and position of the $R/\log I$-function, namely: time of the day, season, weather conditions, duration of flight between hive and set-up, chromatic distribution of natural light, and learning. We find that none of these facors affect the slope and position of the $R/\log I$-function under the experimental conditions. The only parameter of significant influence may be the state of dark adaptation (see above). We, therefore, excluded the few experiments in which the test animals were not very well dark adapted. The results are given in Fig. 5 for experiments with a dark alternative arm of the Y-maze (curve a) and those with an alternative arm illuminated by a green LED ($\lambda_{max} = 537$ nm, quantal flux $1.1 \cdot 10^9$ h$\nu \cdot cm^{-2} \cdot s^{-1}$).

Spectral sensitivity is highest in the green but not very different at other wavelengths in both series of experiments. Since the $R/\log I$-function for blue light (439 nm) tested against dark is steeper than that of the other wavelengths (see Fig. 3) sensitivity depends on the response criterion used for calculating sensitivity. At a higher response criterion than that used in Fig. 5 (>75%) $S(\lambda)$ is highest in blue. $S(\lambda)$ does not change with the response criterion in experiments with an alternative arm illuminated with green light, because $R/\log I$-functions have the same slope at all wavelengths tested.

Spectral mixing experiments

Do bees see colours in their phototactic response? We have shown that the strength of the phototactic

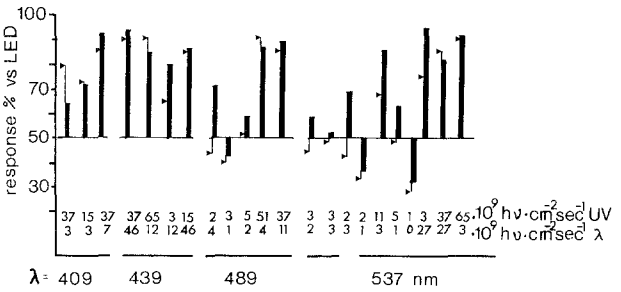

Fig. 6. Results of colour mixing experiments. In all experiments one arm of the Y-maze was illuminated with green light from a LED ($1.1 \cdot 10^9$ hν cm^{-2} s^{-1}). The other arm was illuminated with a mixture of UV (341 nm, upper row) with one of 4 spectral lights (λ, lower row). Numbers below each bar give the quantal flux for UV (upper number) and that of the respective wavelength (lower number). Response is expressed in % choice of that arm which is illuminated by the mixed light. Bars give measured response values. The triangle at each bar indicates the response value which is to be expected, if the quanta from each of the two mixed wavelengths weighted according to the $S(\lambda)$ in Fig. 5 add linearly (see text)

response depends both on intensity and wavelength of the test light. In contrast to training experiments there is no way of separating the effects of the two parameters in phototaxis, but there may be an indirect way of approaching this question. If the chromaticity of a spectral light stimulus is detected separately from intensity, then the response strength to a mixture of two wavelengths may differ from that expected for the added effects of each wavelength. Such an experiment was successfully carried out with *Drosophila* in slow phototactic responses, and the absence of an intensity dependence on a chromaticity effect was demonstrated by additional experiments (successive and simultaneous colour contrast, Fischbach 1979). The positive outcome of a mixing experiment argues against a simple additivity of weighted quantal fluxes and strongly supports the conclusion that the chromaticity of a spectral light is an independent parameter. A negative result of a mixing experiment, however, does not disprove chromatic effects, and additional results are needed to reject the existence of colour effects.

We have carried out a series of wavelength mixing experiments, in which UV light of varying intensities was mixed with one of four other spectral lights (409, 439, 489, 537 nm) also of varying intensities. One side of the Y-maze was illuminated with the mixed lights and the other with a constant intensity, broadband green light (LED, λ_{max} = 537 nm). The intensity of the LED light ($1.1 \cdot 10^9$ quanta/cm$^2 \cdot$ s^{-1}) caused a response level of 80% relative to a dark arm. The intensities of the two mixed spectral lights were chosen in such a way that saturating responses were avoided. The results and the actual intensities are given in Fig. 6. Several intensity ratios of UV light were mixed with one of the four other wavelengths. Figure 6 includes a marker for the response level that is expected if the response values for each spectral component add linearly. These calculated response values are derived from experiments in which each of the 5 wavelengths were tested against the same constant LED light at various intensities.

The procedure of calculating the expected values for additive effects include the following steps. The actual quantal fluxes of each of the 3 wavelengths (see numbers in Fig. 6 for UV, varying λ; LED light constant $1.1 \cdot 10^9$ h ν/cm^2) were multiplied by a factor derived from the $S(\lambda)$ in Fig. 5b. The sum of the spectrally weighted light fluxes of the mixed lights were used to read the corresponding response level using the $R/\log I$-function for 489 nm vs a constant LED light of $1.1 \cdot 10^9$ h ν/cm$^2 \cdot$ s.

The measured responses to the mixed lights deviate strongly in a few cases from the calculated values without an indication of systematic dependence on the kind of spectral light or the ratios of the spectral lights. Although there are a more positive deviations (measured response higher than calculated response), the effect is statistically not significant and not related to any spectral parameter. In *Drosophila,* Fischbach (1979), on the other hand, found strong effects even when very little long wavelength light was mixed with UV. If anything comparable were present in phototaxis of bees, our experiments should have shown it. We conclude that simple additivity rules apply to spectral phototaxis in bees, and that colour effects are unlikely.

Discussion

Phototaxis can be seen as a subject of visually guided behaviour. Insects switch to phototaxis in certain behavioural contexts and use the properties of the light source to steer their way towards the light. What neural strategy controlls this behaviour? Selective receptor contribution is a potent peripheral mechanism to specify neural subroutines for visually guided behaviour in bees. Since the bee's compound eye contains 3 spectral classes of photoreceptors, UV (λ_{max} = 340 nm), blue (λ_{max} = 440 nm) and green receptors (λ_{max} = 540 nm) (Autrum and von Zwehl 1964; Menzel and Blakers 1976), an analysis of the spectral properties of this behaviour may indicate the selective wiring of the spectral inputs. Behavioural experi-

ments with honeybees have been successfully used to describe such selective contributions of photoreceptors and may also be a powerful technique to uncover more central neural strategies. Our analysis of spectral phototaxis aims for such a goal. Large field motion detection, for example, receives input from the green receptors (Kaiser and Liske 1974); polarized light detection is elicited selectively by UV-receptors (von Helversen and Edrich 1974; Edrich 1977); the dorsal light reflex is controlled by the UV receptors (see Menzel 1979, Fig. 11); a point light source is interpreted as the sun through the joined action of blue and green receptors (Edrich 1977); goal-directed behaviour at the feeding place and at the hive entrance involves colour vision with an equal contribution of all three colour receptors (von Frisch 1914; Daumer 1956; von Helversen 1972; Menzel 1985).

So far, little is known about the combination of visual capacities in phototaxis, namely the sensitivity to increments of light (contrast sensitivity), the time course of dark adaptation, the wavelength selectivity or the pooling of spectral inputs, the colour discrimination and/or other features of colour vision (e.g. colour contrast, colour constancy). UV was found to be most attractive to bees, tested on escape runs towards spectral lights at relative high light intensities (Lit. see Introduction). The experiments reported here show that phototactic runs in a natural context and at low light level are controlled by the balanced input from all 3 spectral receptor types resulting in a slightly higher sensitivity to blue-green light than to UV light. Furthermore, the sensitivity to increments of light is much higher in our experiments than in those of earlier authors (see below). The perception of colour in phototaxis was not tested in earlier studies, and was considered unlikely because an unbalanced contribution of spectral receptor types would distort the colour space so much that colour vision would be of little use. The balanced contribution of all receptor types found in our experiments re-opens the question, althrough the results of spectral mixing experiments give no hint of colour vision. Mixtures of UV and one of 4 other wavelengths produce response rates, which indicate no deviation from the additive action of each monochromatic light separately. This result does not exclude the possibility of colour vision in phototaxis but makes it unlikely.

Other results support the conclusion that bees do not use colour information in phototaxis. Although bees learn to use colours in a maze as markers for correct turns on their way towards a feeding place (Menzel 1981), they did not learn the colour of the spectral stimuli in the Y-maze used here. The same Y-maze was used to train bees (Lieke 1984), and they quickly learned to associate the colour of a point light source with food reward and to discriminate colours at least as well as freely flying bees. Since the same light intensities of the point light sources were used in the colour training and the spectral phototaxis experiments, we conclude that the negative outcome in our experiments indicates that bees do not see colours in their phototactic responses.

The spectral sensitivity function (Fig. 5) makes pooling of all 3 spectral receptor types very likely, although this conclusion is based only on 5 wavelengths. We know from intracellular markings (Menzel and Blakers 1976) that each ommatidium is composed of 4 green, 2 blue and 2+1 UV receptors. The pooling of such a set of spectral types should result in a broad blue to green sensitivity maximum and an additional UV side band, and this is what we found. Most other spectral measurements of phototaxis in bees have revealed a higher sensitivity in the UV, e.g., 3–4 times higher UV sensitivity than to green light in Kaiser et al. (1977).

What might be the reason for these different experimental results? In Kaiser et al. (1977) and the experiments reported here dark adapted bees were tested. The eye region (median, frontal-lateral) and the number of ommatidia facing the stimulus (approximately 28 in Kaiser et al., 7 in our experiments) were not too different. However, response threshold is 2 log$I$ lower in our study and response increase to increments of light (slope n of the $R/\log I$-function) is much higher in our experiments. Bees performed their phototactic runs in our experiments during the natural sequence of their foraging cycle, whereas Kaiser et al. tested escape phototaxis of fixed walking bees. We have no results yet to decide whether the different intensity levels or/and the different test procedures are the main reason for the different spectral sensitivities. We suspect the latter to be of greater importance, since $S(\lambda)$ did not change very much when higher intensities were used. Evidence for strong dependencies of the spectral sensitivity on the behavioural context and the procedure of the test comes from colour training experiments. For example, Thomas and Autrum (1965) verified that the spectral sensitivity of bees depends strongly on the level of adaptation, Menzel (1967) and von Helversen (1972) found a 10 times or even higher UV than green sensitivity in a teste situation, in which the horizontally arranged spectral lights appeared on a UV-free background. Dark adapted

bees trained in a Y-maze under stimulus conditions very similar to our test situation were only 2.5 times more sensitive in the UV (Lieke 1984).

Absolute sensitivity in the phototactic response is estimated to be in the range of $8.3 \cdot 10^7$ quanta (537 nm)/cm$^2 \cdot$ s in our experiments. We arrive at this value by reading the quantal flux at the intersection of the threshold response criterion (1% above random choice) with the best fitting power function of formula (I) for the response to 537 nm at various intensities vs a dark alternative. The accuracy of this value should be better than the standard deviation of the data points at one intensity (compare Fig. 4, curve 2: $\pm 6\%$ at a response level of 75%, which corresponds to ± 0.15 log$I$), because all data points define the best fitting function and thus support its position along the log$I$-axis.

Other data on the absolute sensitivity of lens and compound eyes are in reasonable agreement with this value. For example, the human eye detects stars down to the 6th magnitude. The light flux of those stars is 1.5 quanta (507 nm)/cm$^2 \cdot$ s (Seliger and McElroy 1965, p. 295). Since the aperture of a single retinula in the bee eye is 10^5 times smaller in area than that of the human eye, bees should be 10^5 times less sensitive to point light sources (Kirschfeld 1984). It is not surprising that the difference in sensitivity of the human and the bee eye to a point light source exceeds the value of 10^5 ($5.5 \cdot 10^7$), because the bee is exposed to the test light only for 100 ms in our experiments, whereas threshold measurements on star detection in humans were not time limited.

We argued that the threshold of $8.3 \cdot 10^7$ hν/cm$^2 \cdot$ s corresponds to about 100 absorbed quanta/s in 28 green receptors of 7 ommatidia. This value is in the same range as the threshold for the fly's optomotor response. Reichardt (1969) determined threshold responses for a quantal flux of 100–250 quanta/s in 6 rhabdomeres (puls frequency 1 Hz, puls duration 100 ms). Using the same quantum efficiency of 0.5 as in our calculation one reaches a threshold value of 8–20 hν/s per receptor in the fly and about 4 hν/s per receptor in the bee.

Another issue addressed by the experiments reported here concerns the time course of dark adaptation. The test bees were exposed to a light intensity on their flight outside of up to 8 log$I$ above response threshold (Fig. 4). Dark adaptation was very fast (time course $\tau \leqq 1$ min), leaving most of the bees very well dark adapted when they performed a test run. Wolf and Zerrahn-Wolf (1935) measured sensitivity increase in bees in the darkness by recording antennal responses to moving stripes. They found a time course of $\tau = 15$ min. Kindermann (1983) found a $\tau = 3.1$ min in phototaxis experiments after extended period of strong light esposure. The reason for the faster rate of dark adaptation in our experiments is the short (about 15 s) exposure to the adapting light, and the long periods in the dark during a foraging cycle (an average of 2.5 min in the dark set-up, 3 min in the dark hive). We have shown that neither the wavelength dependence of the R/log$I$-functions nor the $S(\lambda)$ of phototaxis depend on the level of residual dark adaptation in the range as used in our experiments.

Phototaxis has been studied with mixed spectral lights in three insect species. *Drosophila* responds in slow phototaxis much stronger to a mixture of UV and long wavelength light than to the sum of the two spectral components. Successive colour effects support the interpretation that *Drosophila* analyses light in a spectrally antagonistic fashion in phototaxis, and thus may see colour in phototaxis (Fischbach 1979). The results in the white fly *Trialeurodes* are the same as in the bee, since mixed spectral lights act in the same way as the sum of the components (Coombe 1981). This is of particular interest, because the white fly reacts negatively to blue light and positively to yellow light, and thus shows wavelength selectivity in its phototaxis. The picture emerging from these 3 insect species is quite complicated. Wavelength selective behaviours exist besides true colour vision (bee, *Drosophila*), spectrally weighted additivity in one behaviour co-exists with wavelength selectivity in other behaviours (bee, white fly), and strong non-linear effects to mixed spectral lights together with colour vision is found in the same behaviour (*Drosophila*: slow phototaxis and learning). It appears that behavioural subroutines have their species specific and behaviourally specific processing of inputs from spectral photoreceptor types. Generalization both across the species and across different behaviours of one species are inadequate. For a given species (e.g. the bee), however, the specificity of input processing for a particular behaviour allows to postulate a framework for the underlying neural processes, which hopefully are ultimately accessible with neurophysiological methods.

The natural phototactic response as measured with our experimental procedure in the honeybee is characterized by (1) very high absolute sensitivity, (2) contribution of all 3 spectral receptor types with the weight of their frequency in the median frontal eye, (3) very steep R/log$I$-functions, (4) fast dark adaptation, (5) lack of colour vision. These

features reflect those of the monopolar cell M1, which collects the output of all receptors of one ommatidium (Menzel 1974; Ribi 1976). We conclude, therefore, that monopolar cells M1 or a functionally equivalent type of visual interneuron at higher level may control the phototactic response of the honeybee at low light levels.

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References

Autrum H, Seibt U (1965) Die Dunkeladaptation des Bienenauges. Naturwissenschaften 52:566

Autrum H, Zwehl V von (1964) Spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z. vergl. Physiol 48:357–384

Baumgärtner H (1928) Der Formensinn und die Sehschärfe der Bienen. Z. vergl. Physiol 7:56–143

Baylor DA, Fuortes MGF (1970) Electrical responses of single cones in the retina of the turtle. J Physiol 207:77–92

Bertholf LM (1931a) Reactions of the honey bee to light. J Agr Res 42:379–419

Bertholf LM (1931b) The distribution of stimulative efficiency in the ultraviolet spectrum for the honey bee. J Agric Res 43:703–713

Brines ML, Gould JL (1979) Bees have rules. Science 206:571–573

Coombe P (1981) Wavelength specific behaviour of the white fly *Trialeurodes vaporariorum* (Homoptera, Aleyrodidae). J Comp Physiol 144:83–90

Daumer K (1956) Reizmetrische Untersuchungen des Farbfernsehens der Bienen. Z vergl Physiol 38:413–478

Edrich W (1977) Die Rolle einzelner Farbrezeptoren bei den verschiedenen Lichtreaktionen der Biene. Verh Dtsch Zool Ges 70:236

Edrich W, Neumeyer Ch, Helversen O von (1979) 'Anti-sun orientation' of bees with regard to a field of ultraviolet light. J Comp Physiol 134:151–157

Fischbach KF (1979) Simultaneous and successive colour contrast expressed in 'slow' phototactic behaviour of walking *Drosophila melanogaster*. J Comp Physiol 130:161–171

Frisch K von (1914) Der Farbensinn und Formensinn der Biene. Zool J Physiol 37:1–238

Frisch K von (1967) The dance language and orientation of bees. Cambridge University Press, Cambridge

Goldsmith TH (1963) The course of light and dark adaptation in the compound eye of the honey bee. Comp Biochem Physiol 10:227–237

Heintz E (1959) La question de la sensibilité des abeilles a l'ultraviolet. Insect Soc 6:223–229

Helverson O von (1972) Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. J Comp Physiol 80:439–472

Helversen O von, Edrich W (1974) Der Polarisationsempfänger im Bienenauge: Ein Ultraviolettrezeptor. J Comp Physiol 94:33–47

Henderson ST (1970) Daylight and its spectrum. Adam Hilger, London

Kaiser W (1974) The spectral sensitivity of the honey bee's optomotor walking response. J Comp Physiol 90:405–408

Kaiser W, Liske E (1974) Optomotor reactions of stationary flying bees during stimulation with spectral light. J Comp Physiol 89:391–408

Kaiser W, Seidl R, Vollmar J (1977) The participation of all three colour receptors in the phototactic behaviour of fixed walking honey bees. J Comp Physiol 122:27–44

Kindermann U (1983) Verlauf der Dunkeladaptation bei der Honigbiene, gemessen mit ERG-Ableitungen und in Phototaxisexperimenten. Examensarbeit, Freie Universität Berlin

Kirschfeld K (1984) Linsen und Komplexaugen: Grenzen ihrer Leistung. Naturwiss Rundschau 37:352–362

Labhart T (1974) Behavioural analysis of light intensity discrimination and spectral sensitivity in honeybees *Apis mellifera*. J Comp Physiol 95:203–216

Laughlin SB (1981) Neural principles in the peripheral visual system of invertebrates. In: Autrum H (ed) Vision in invertebrates (Handbook of sensory physiology, vol VII/6B). Springer, Berlin Heidelberg New York, pp 133–280

Laughlin SB, Horridge GA (1971) Angular sensitivity of the retinula cells of dark adapted worker bees. Z Vergl. Physiol 74:329–335

Lieke E (1984) Farbensehen bei Bienen: Wahrnehmung der Farbsättigung. Dissertation, Freie Universität Berlin

Lipetz LE (1971) The relation of physiological and psychological aspects of sensory intensity. In: Lowenstein WR (ed), Principles of receptor physiology (Handbook of sensory physiology, vol I Springer, Berlin Heidelberg New York, pp 191–225

Menzel R (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene, *Apis mellifica*. Z Vergl Physiol 56:22–62

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

Menzel R (1979) Spectral sensitivity and colour vision in invertebrates. In: Autrum H (ed), Vision in invertebrates (Handbook of sensory physiology, vol VII/6A), Springer, Berlin Heidelberg New York, pp 504–566

Menzel R (1981) Achromatic vision in the honeybee at low light intensities. J Comp Physiol 141:389–393

Menzel R (1985) Learning in honeybees in an ecological and behavioural context. In: Lindauer M, Hölldobler B (eds) Experimental behavioural ecology, Fortschr Zool 31:

Menzel R, Blakers M (1976) Colour receptors in the bee eye – morphology and spectral sensitivity. J Comp Physiol 108:11–33

Menzel R, Greggers U (1983) Automated behavioural experiments with individually marked bees. Behav Res Meth Instr 15:569–573

Portillo J del (1936) Beziehungen zwischen den Öffnungswinkeln der Ommatidien, Krümmung und Gestalt der Insektenaugen und ihren funktionellen Aufgaben. Z Vergl Physiol 23:145

Raggenbass M (1983) Effects of extracellular calcium and of light adaptation on the response to dim light in honey bee drone photoreceptors. J Physiol 344:525–548

Reichardt W (1969) Transduction of single-quantum effects (Evidence from behavioral experiments on the fly *Musca*). In: Proceedings of the Intern. School of Physics "Enrico Fermi" Course XLIII, Processing of Optical Data by Organisms and by Machines, Academic Press, New York London, pp 176–186

Ribi WA (1976) The first optic ganglion of the bee. II. Topographical relationship of the monopolar cells within and between cartridges. Cell Tissue Res 171:359–373

Rodieck RW (1973) The vertebrate retina. Freeman Co., San Francisco

Rose R, Menzel R (1981) Luminance dependence of pigment color discrimination in bees. J Comp Physiol 141:379–388

Rushton WAH (1972) Pigments and signals in colour vision. J Physiol (Lond) 220:1–31

Sander W (1933) Phototaktische Reaktionen der Bienen auf Lichter verschiedener Wellenlängen. Z Vergl Physiol 20:267–256

Seliger HH, McElroy WD (1965) Light: Physical and biological action. Academic Press, New York

Thomas I, Autrum H (1965) Die Empfindlichkeit der dunkel- und helladaptierten Biene (*Apis mellifica*) für spektrale Farben: Zum Purkinje-Phänomen der Insekten. Z Vergl Physiol 51:204–218

Wehner R (1981) Spatial vision in arthropods. In: Autrum H (ed) Vision in invertebrates (Handbook of sensory physiology, vol VII/6C) Springer, Berlin Heidelberg New York, pp 287–616

Weiss HB (1943) Colour perception in insects. J Econ Entomol 36:1–17

Wolf E, Zerrahn-Wolf G (1935) The dark adaptation of the eye of the honey bee. J Gen Physiol 19:229–237

Wu CF, Pak WL (1978) Light-induced voltage noise in the photoreceptor of *Drosophila melanogaster*. J Gen Physiol 71:249–268