Polarised Light Detection in the Bee, *Apis mellifera*

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Summary. The ninth cell in each ommatidium is the only retinula cell that acts as a polarised light detector. The ninth cell is a short retinula cell which is found in the proximal third of the ommatidium. This conclusion is based on the following observations:

1. The most frequently found green sensitive cells have either no or very low sensitivity to polarised light. This is caused by electric coupling of cells with different microvilli orientations. The electric coupling was found to be independent of adaptation state.

2. There are two different types of UV receptors: the more frequently found UV cells also have a distinct spectral sensitivity at longer wavelengths (>450 nm) and a small polarisation sensitivity. A very rarely recorded UV receptor type with no sensitivity to longer wavelengths (>450 nm) has a high polarisation sensitivity (average PS = 5, maximal PS = 9). All recordings of these UV cells were made close to the basement membrane in the region of the 9th cell.

3. The short length of the 9th cell, its position in the proximal third of the ommatidium, and the orientation of the microvilli is theoretically consistent with it having a high polarisation sensitivity.

To find evidence for the mechanisms underlying the integration of polarisation information coming from the 9th cells, we examined the microvilli orientation of these cells in neighbouring ommatidia in different eye regions. While the medio-ventral eye region contains only one population, with microvilli oriented at 45° to the vertical, the dorsal and medio-dorsal eye regions contain two populations lying at 120° or 60° to each other. The pattern of microvilli orientation is a mirror image in both eyes. This pattern enables an unambiguous determination of the polarisation plane in the dorsal and medio-dorsal eye part if there is a comparison of excitation in the 9th cells of neighbouring ommatidia and/or ommatidia in the two eyes sharing the same field of view.

Our conclusions are consistent with v. Frisch’s findings on the orientation to polarised light by the worker bee.

Introduction

The orientation of the honey bee to the pattern of the linear polarised light of the sky is well known (for summary see v. Frisch, 1967). The widely accepted explanation of this polarised light detection in the bee is based on a model of the bee ommatidium devised by v. Frisch. In this
model the star-like arrangement of the 8 retinula cells combined with the polarisation sensitivity of each retinula cell (depending on its radial position) produces 8 polarisation sensitive inputs. Later v. Frisch adapted his model to Goldsmith's (1962) finding of only two directions of microvilli proposing a "four-branched polariser" in each ommatidium.

Polarisation sensitivity of single retinula cells depends on the alignment of the photopigment dipole molecules in the microvilli membrane (dichroic sensitivity) and the highly ordered arrangement of microvilli in the rhabdomeres (Kirschfeld, 1969; Snyder, 1973). The fused rhabdom of the bee ommatidium has two directions of microvilli perpendicular to each other. Goldsmith (1962) was the first to point out that this microvilli arrangement would produce two polarisation sensitive channels in each ommatidium. However, the bee is polarisation sensitive only in the UV (v. Frisch, 1967; Kirschfeld, 1973; v. Helversen, unpubl.) and the microvilli of the two UV-receptors in each ommatidium have the same orientation (Gribakin, 1969). Therefore one ommatidium would not be sufficient to code the e-vector of linear polarised light. Even input from all 8 retinula cells to a polarisation analyser can not explain the polaromotactic orientation of the bee (Kirschfeld, 1972).

All the above speculations on polarised light detection presume that the bee's retinula cells are sensitive to polarised light. Although the polarisation sensitivity of single retinula cells (PS) has been examined in several other insect species (locust; bee drone; Shaw, 1968, 1969a, b; fly; Burkhartt and Wendler, 1960; Antram and v. Zwehl, 1962; Shaw, 1966; Scholes, 1969; dragon flies: Horridge, 1966; cockroaches: Butler and Horridge, 1973; water bug: Walscot, 1971), the PS of worker bee receptors has not yet been studied. Therefore, the first step in analysis of polarised light detection is the measurement of PS in single retinula cells.

The experiments presented here demonstrate that the ninth retinula cell in the bee ommatidium plays the dominant role in polarised light detection. Snyder (1973) has already predicted a high PS for the 9th cell. On the basis of its structure and its position in the proximal third of the fused rhabdom (Philips, 1965; Schlote cited in v. Frisch, 1967; Gribakin, 1972; Gründler, 1972), we find that the 9th cell's microvilli are aligned with 4 other retinula cells, rather than at 45° to them as described by Gribakin (unpubl.). Thus an interaction between the 9th cell and the 2 groups of long retinula cells still does not solve the problem of insufficient polarisation coding with two channels. Our results show that polarised light detection depends upon the comparison of 9th cell outputs in neighbouring ommatidia and/or in ommatidia in the two eyes sharing the same field of view. Our analysis is a combination of single retinula cell recordings, spatial reconstruction of fine structural elements in different parts of the eye and a theoretical determination of the PS of the 9th cell.

Methods

Electrophysiology

KCl filled glass microelectrodes with a resistance of 150–300 MΩ were used for intracellular recording. The capacity compensation of the high impedance preamplifier (Grass P16) was used to bring the baseline to less than 300 μvec. The receptors of the left eye were exposed for electrode impalement by slicing off a small dorsal, ventral or frontal sliver of cornea. For intracellular marking, electrodes were filled with a 6% solution of Procion yellow MR. Negative current pulses (20–30 μA; pulse width 0.5 sec; frequency 1 Hz), were applied through the electrode for 5–10 min (Kaneko, 1970). The dye filled electrodes had a lower resistance and yielded better recordings if they were placed overnight in the Procion yellow solution at 50°C. After the experiment the eyes were placed in Bowman's fixative and were dissected after 1 hour. They were then fixed overnight. After embedding in Spurr's (1969) epoxy resin 12.5 μ thick sections were made with a glass knife.

The left eye was centred in a perimeter device which carried a UV transmitting flexible light guide (1 mm long, dia. 3 mm). This light guide produced a light spot which subtended less than 45° at the eye. Monochromatic light (every 16,6, 316 and 666 nm) was produced using a 90° W Xenon arc lamp and a Schott color glass monochromator (CM 250). Broad band filters (UG 5: 316–382 nm, BG 12: 399–466 nm; VG 9: 482–549 nm; OG 550: 566–666 nm) were used to attenuate stray light. A set of quartz neutral density filters reduced the light intensity over 3.5 log units in 1/3 log steps. Variable planes of polarised light were provided by a UV-transmitting polarisation filter which could be rotated in 10° steps and which was mounted close to the end of the light guide. The absolute light intensity was calibrated in quanta terms repeatedly with a Radiant flux meter (Hewlett Packard 8330 A). The thermopile was put at the position of the eye.

After mounting the animal under dim light it was left in the dark for at least 30 min before experiments were started. The stimulation program was usually 250 μsec long flashes separated by 8 sec. For light adaptation a second UV transmitting light guide was used, placed 1° beside the light guide transmitting the test flashes. White or spectral light for adaptation came from the same Xenon lamp through a second quartz condenser.

Electron microscopy

Dorsal or median parts of the left eye were fixed in Karnovsky's (1967) Paraformaldehyde-hydantoin mixture (2.5% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M cacodylate buffer, plus 0.1% CaCl₂, pH 7.3) for 2 hours at room temperature. Postfixation was in 1% OsO₄ in cacodylate buffer for 1 hour at room temperature. After rapid dehydration in acetone (with 0.5% NaCl) the specimen was embedded in Spurr's (1969) epoxy resin, cut with a Reichert ultramicrotome and examined in a Jeol electron microscope (JEM 100 B).

Intracellular Response and Definition of Polarisation Sensitivity

As in other insect photoreceptors, the penetration of a retinula cell is indicated by a sudden change in potential from 0 mV to −25 to −45 mV and the
presence of a positive going potential to illumination. Stable intracellular potentials from the worker bee retina cells are somewhat more difficult to obtain than in many other insect species, and we regarded as satisfactory only recordings where the resting potential did not change during the first 5 min. Such a change was usually shown by damaged cells in the first few minutes. With a stable penetration one could record from the same cell for several hours (up to 6.5 hours). The size of the depolarisation due to illumination was not used as a criterion for selection between recordings, because one cannot know in advance if retinula cells in one animal or retinula cells in different animals produce the same potential to a standard flash. Most cells give a response of up to 15 to 25 mV for the plateau potential and up to 20-30 mV for the peak potential. The maximal values recorded were 37 mV for the plateau and 45 mV for the peak.

The stimulating light was accurately (± 0.5°) centred on the optical axis of the cell recorded, for the acceptance angle of the bee is very narrow (2.6°; Laughlin and Horridge, 1971). Recordings were made from the medio-frontal, dorso-frontal and dorsal part of the eye. We did not find any differences in the spectral or polarisation sensitivity in different eye parts and therefore we do not distinguish here between recordings from different eye parts but pool them according to their physiological characteristics.

In calculating the polarisation sensitivity of the retinula cells it was assumed that, at any given e-vector orientation, the relative reduction in quantum capture was the same over the entire response range of the cell. This means that response/log intensity curves are displaced parallel to the intensity axis, as a result of sensitivity changes. The PS can be derived from the responses to constant test flashes of different e-vector orientation (10° steps) by use of the empirically derived response/intensity function for each cell. This derivation is achieved for each e-vector orientation by displacing the response/log intensity function parallel to the intensity axis, so that it passes through the point defined by the intensity and amplitude of the response to the test flash of polarised light. This new curve is used to derive the intensity required to elicit a 50% max response at that e-vector orientation. The sensitivity $S(\beta)$ of a cell at any e-vector angle $\beta$ is defined as:

$$S(\beta) = \left( \frac{I_{\text{max}}}{I_{\beta}} \right) \cdot 100$$

where $I_{\beta}$ is the 50% intensity and $I_{\text{max}}$ is the value of $I_{\beta}$ obtained at the maximally effective value of $\beta$. We define the PS of a retinula cell as the ratio of the maximum sensitivity $S(\beta)_{\text{max}}$ to the minimum sensitivity $S(\beta)_{\text{min}}$ by rotation of the polarised filter ($\beta$):

$$\text{PS}(\beta) = \frac{S(\beta)_{\text{max}}}{S(\beta)_{\text{min}}}$$

Sensitivity is directly proportional to the light power absorbed. The absorption is highest if the e-vector of linear polarised light is parallel to the long axis of the microvilli and smallest if the e-vector is perpendicular to the microvilli (Langer, 1965; Langer and Thorell, 1966; Eguchi and Waterman, 1998; exceptions are the 7th and 8th cell in the fly; Kirschfeld, 1980). Thus PS is given by light power absorbed (P) with the e-vector parallel ($\parallel$) over light power absorbed with e-vector perpendicular ($\perp$) to the microvilli axis (Snyder, 1973):

$$\text{PS} = \frac{P_{\parallel}}{P_{\perp}} = \frac{S(\beta)_{\text{max}}}{S(\beta)_{\text{min}}}$$

### Results

**A. Electrophysiology**

1. PS of the Green Sensitive Cells

We measured the spectral sensitivity of each cell for which PS was determined. The most commonly recorded cells in the bee eye are maximally sensitive to green light with a peak between 509-549 nm and a varying sensitivity in the UV (Artmut and v. Zweak, 1964; Menzel, unpubl.). PS was measured in a total of 92 of these green cells using 549 or 533 nm as test light and sometimes as well 366, 449 and 583 nm. Most cells show no or very small PS ($\text{PS} < 1.2$, $n = 70$ group 1 in Fig. 1). Another group of cells (group 2 in Fig. 1, Fig. 3b) has an average PS of 1.43 ranging from 1.2-2.4. No green cell was found with PS higher than 2.4.

Green sensitive cells differ in their sensitivity in the UV ranging from 5% to 80% of sensitivity to green light. It will be shown elsewhere (Menzel, unpubl.) that one can distinguish 3 groups of green cells depending on their relative sensitivity at 366 nm: group 1 has a UV sensitivity of 30%–80%; group II 15%–30%; and group III has 0%–15% sensitivity in UV. The correlation between spectral and polarisation sensitivity is shown in Fig. 1.
cells with larger potentials (maximal potential > 25 mV); c) two cells with very low PS were marked with Procion yellow; in each case the stain was found only in one cell. There was no spreading to neighboring cells; d) if one recorded simultaneously from 2 cells one should expect to find a high PS in 60% of recordings because one of the two neighbouring cells always has the same microvilli direction as the tested cell; e) some cells changed their spectral sensitivity presumably as a result of change in recording locus. This always happened suddenly, there were no gradual shifts (Menzel, unpublished).

We conclude therefore, that the poor PS is not an artefact but proves that retinula cells with different microvilli directions are electrically coupled exactly as Shaw (1969a) found in drone retinula cells.

Shaw (1969a) found a higher PS with stronger stimulation in the drone bee. This could mean that (1) the influence of the electric coupling is less pronounced if the membrane resistance is very low due to strong illumination, (2) that electric coupling is a dynamic process decreasing with the light adaptation due to repeated strong illumination.

The first alternative seems plausible because we know that the membrane resistance during strong illumination is very low, probably much lower than the coupling resistance (Shaw, 1969a). The low resistance would short circuit the cell to the extracellular space reducing the influence of the potential coming from the neighbouring cell via the coupling resistance. To test this hypothesis we compared the PS with different intensity stimulation in several cells but found only a small increase with stronger stimulation. Therefore the change in membrane resistance during strong illumination can not play an important role in changing the effect of electrical coupling.

The second alternative (dynamic electrical coupling) was tested in 11 cells. PS was first measured in the dark-adapted state, then the cell was light adapted with a continuous white background (xenon lamp) light which depolarised the cell to about half of its dynamic range (see methods). After at least 5 min adaptation, intensity and polarisation runs were made, sometimes using several wavelengths. The influence of the adaptation light on the potential form, the intensity dependence and spectral response are described elsewhere (Menzel, unpubl.). The two examples in Fig. 3 show that PS does not change with light adaptation. Stronger adaptation was not possible because the cell would not show any potential change to the test flashes. We therefore conclude that interceptor coupling is not a dynamic process depending on the adaptation state of the photoreceptors.

2. PS of Blue Sensitive Cells

Retinula cells with a maximum spectral sensitivity in the blue region (420–460 nm) was found frequently in the drone eye but rarely in the worker bee eye (Atrum and v. Zwehl, 1964; Menzel, unpubl.). In

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Table 1. Cross correlation between spectral and polarisation sensitivity in green sensitive retinula cells. The number of cells for each spectral group with no PS (1.0–1.2) or some PS (1.2–2.4) are given in percent of cells for each PS group.

<table>
<thead>
<tr>
<th>Spectral groups of green cells</th>
<th>group I</th>
<th>group II</th>
<th>group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 1.0–1.2</td>
<td>60%</td>
<td>35%</td>
<td>5%</td>
</tr>
<tr>
<td>PS 1.2–2.4</td>
<td>16%</td>
<td>16%</td>
<td>68%</td>
</tr>
</tbody>
</table>

Sensitivity (Table 1) shows clearly that PS is usually very low, in those cells which have a high secondary peak in the UV (group I) and is generally higher in green cells with a low UV sensitivity.

The insensitivity of the most common receptor in the bee eye to polarised light is astonishing for PS should theoretically be high in the fused rhabdom with optical coupling (Snyder, Menzel and Laughlin, 1973). Loss of PS as a result of simultaneous recordings from 2 cells (Atrum and v. Zwehl, 1964) or from damaged cells can be excluded for the following reasons:

a) recordings were taken only from cells with baselines stable for much longer than 5 mins; a damaged cell would not maintain resting potential this long; b) cells with small potentials (maximal potential < 10 mV) did not show a lower PS than...
Fig. 3. Two examples of the influence of a continuous background light on the polarisation sensitivity of green receptors. The cells were first tested in the dark adapted state (longer than 50 min in the dark, •) then light adapted to a white (Xenon lamp) background light. The response to the adaptation light was 0.6 (cell No. 76) and 0.45 (cell No. 62) of the maximal response. In all runs the test light was 533 nm. Polarisation sensitivity was calculated using an intensity function measured in the light adapted state immediately before the polarisation run (○). Both examples demonstrate that PS does not change with light adaptation.

agreement with Shaw (1969a) we found a low PS in the drone’s blue cells (Fig. 1, 4 cells tested, average PS = 1.43). In the worker bee we tested only one blue sensitive cell for its PS and found a value of 1.27 (Fig. 1).

3. PS of UV Sensitive Cells

Since the bee orientates to the polarisation pattern of the sky, using only wavelengths shorter than 450 nm (v. Frisch, 1967) it is of interest to test UV receptors for their PS. UV receptors are not easily recorded. Out of a total of 250 cells only 13 showed a maximal sensitivity at 349 nm. These UV receptors fall in two distinct groups (Menzel, unpubl.); one group (10 cells) has a high sensitivity at wavelengths longer than 450 nm, the other group (3 cells) has no sensitivity (less than $10^{-2}$ relative sensitivity) at wavelengths longer than 450 nm. Cells of the second group were found only when the electrode was inserted just above the basement membrane. The PS values for the two groups of UV receptors are given in Fig. 1, and Fig. 4 shows the average PS function for UV cells without long wavelengths sensitivity. The results
given in Fig. 1 come from only those receptors for which a complete spectral sensitivity function was established. As it was difficult to record from UV-receptors for longer than 15 min, PS was often measured first and the cell was lost before the spectral runs were finished. In all cases where PS was poor relative sensitivity in green (532 nm) was clearly higher than 5%. Two of the UV cells with poor PS shown in Fig. 1 were recorded in the dorsal part of the eye, two in the dorso-median part. The 3 UV cells with high PS were all recorded in the dorso median part (2 frontal, 1 lateral).

Our interpretation of these results is that the high PS recordings come from the ninth cell which is located at the base of the ommatidium. We have only indirect evidence as we have not yet succeeded in marking one of the high PS cells. However, these high PS cells were very rarely found and only near the proximal end of the ommatidium in the region of the 9th cell. In summary we have measured high PS in only 3 out
Fig. 5a—d. Cross-sections through ommatidia in the worker bee eye at different levels in the proximal third of the ommatidium. The numbering of the retinula cells used here follows that of Perrelet (1970). The cell numbers correspond to Gribakin's (1969, 1972) grouping as follows: Nos. 1 and 4 groups 1 (UV cells), Nos. 2, 3, 5, 6 group II (Green cells), Nos. 7, and 8 group III (blue cells). The 9th cell replaces cell No. 7; therefore the microvilli of the 9th cell are perpendicular to that of the two UV cells. The 4 green cells are symmetrically arranged and the two blue cells (Nos. 7 and 8) are the cells which form their axons first. A orients 9 retinula cells surrounding the rhabdom, b is a cross-section in the area of the 9th cell nucleus, the 7th cell has formed its axon (not shown on this picture); c some microns deeper, the axon of the 7th cell lies close to the ninth cell; d only few microns more proximal, the 8th cell now withdraws from the rhabdom and forms an axon; in this area the fused rhabdom is asymmetric.

Fig. 5b

of more than 280 cells. These high PS recordings were made close to the basement membrane, where the 9th cell is located. Thus we interpret the infrequent high PS to be that of the 9th cell.

B. Electronmicroscopy

1. The Ninth Cell

The existence of a ninth basal cell in the worker bee ommatidium was recently discovered simultaneously by Gribakin (1972), Grundler (1972) and Menzel (unpublished), but in fact it was first described by Philips
Our single cell recordings show that this cell plays an important role in the coding of the plane of polarised light. The theoretical analyses of the spectral and polarisation properties of insect photoreceptors by Snyder (1973), Snyder and Pask (1973) and Snyder, Menzel and Laughlin (1973) have emphasized the significance of the arrangement and fine structure of the receptive elements of the photoreceptors. We therefore give a description of the fine structure, and orientation of the 9th cell in different parts of the worker bee eye.

The 9th cell appears in the basal quarter of the ommatidium and has a total length of about 50–80 μm. It replaces the 7th cell which withdraws from the rhabdom as an axon (Fig. 5a–d). In some cross sections one finds 9 retinula cells all with a rhabdomere (Fig. 5a, 6). This observation clearly indicates that Varela’s and Porter’s (1969) interpretation of a branching 8th cell in the worker bee ommatidium is incorrect. In addition the location of the nucleus 30–50 μm above the basement membrane (Fig. 5b) makes it possible to distinguish the 9th cell from all other 8 retinula cells which have their nuclei in 3 more distal layers (Gribakin, 1969). The cell opposite to No. 9, cell No. 8 forms its axon some μm more proximal to the 9th cell (Fig. 5c, d). Below this region the rhabdom is asymmetric having only 7 rhabdomeres (Fig. 5d).
2. Microvilli Orientation of the 9th Cell

When the 9th cell takes up the position of the 7th cell in the rhabdom, the 2 directions of microvilli in the rhabdom are not changed. (Fig. 5, 6). Gribakin's (in press) statement that the microvilli of the 9th cell lie at 45° to the other rhabdomeres cannot be verified. We know from Gribakin's (1969) spectral adaptation experiments that cells No. 1 and 4 (his group 1) are UV cells. It is of great importance for the PS of the 9th cell that the microvilli of these two UV cells are orientated perpendicular to that of the 9th cell.

Looking at neighbouring ommatidia (Fig. 6, 7) it is immediately obvious that the 9th cell has different positions and the microvilli lie...
at different angles relative to the axis of the eye. This is the result of a rotation of the whole ommatidium and not of a displacement of the 9th cell relative to the 8 long retina cells. We measured the microvilli directions of the 9th cell in a large number of cross-sections normal to the long axis of the ommatidium and compared different eye regions. Fig. 6 describes our procedure and Fig. 7 gives the results in histograms. The long axis of the microvilli in the 9th cell is given in respect to the z-row of the ommatidial pattern (angle \( \alpha \) in Fig. 6, 7). The results given in Fig. 7 come from the left eye. For comparison Fig. 6 shows a cross-section through the frontal-dorsal part of the right eye. The comparison of Fig. 6 with Fig. 7b makes it clear that the left eye is a mirror image of the right eye both in their ommatidial pattern and in the microvilli orientation of the 9th cell.

In the frontal-ventral eyepart (Fig. 7) the microvilli of the 9th cell are arranged mainly at \( z = 120^\circ \) to the z-row. The scatter is quite large but the histogram Fig. 8a shows that only one group of directions exists in this eye region. As the z-row lies at about 75° to the vertical (dorsal-ventral) head axis, the median value of \( \alpha \) here is 135° in the right eye and 45° in the left eye with respect to the horizontal head axis. In the frontal-dorsal eyepart (Fig. 8b) the main group of directions shifts to \( z = 150-160^\circ \) (relative to z-row) and a second group with \( z \approx 40^\circ \) appears. Here the z-row lies at 65° to the vertical head axis, the main group therefore lies at 175°, the smaller group at 65° relative to the horizontal head axis. In the dorsal eye region there are two distinct groups of microvilli direction of about equal size (Fig. 8c), one with \( \alpha = 70^\circ \), the other with \( \alpha = 130^\circ \) to the z-row. The average angle between the two groups is about 60°. The z-row in this eye region lies at 55° to the vertical head axis; therefore, relative to the horizontal head axis the two groups have mean angles of 105° and 165°.

These results show clearly that the ommatidial pattern especially the directions of the 9th cell microvilli, differ in the various eye regions. The two eyes are mirror images of each other with respect to the orientation of the ommatidia and the 9th cell. We know from v. Frisch’s experiments that the dorsal eye part is most important for polaro-menotactic orientation. Only in this eye region does the ninth cell show clear groupings of microvilli orientations and the 2 groups lie at 120° or 60° to each other. Such an asymmetric arrangement requires only 2 receptors at 60° to each other for an unambiguous determination of the polarisation plane. Note that this is not the case with 2 receptors whose microvilli lie perpendicular to each other (Kirschfeld, 1972). Thus the simplest determination of the polarisation plane can be done by simultaneous comparison of the ninth cell outputs from two neighbouring ommatidia with broadly overlapping fields of view.

To test this hypothesis we calculated the angle (\( \beta_0 \)) between the microvilli directions of neighbouring 9th cells \( \beta_0 = |x - x_0| \) and between 9th cells in ommatidia lying 2 ommatidia apart \( \beta_2 = |x_1 - x_2| \). We studied neighbouring cells along the z-row as the arrangement of ommatidia is most accurate along each z-row. The same procedure was applied to the x- and y-row but gave less clear results, because of the greater irregularity of the ommatidial array of the x- and y-row (see Fig. 6). The histograms in Fig. 8 give \( \beta_0 \) and \( \beta_2 \) calculated from the measurements in Fig. 7c (dorsal eye region). In both cases there is a dominant group of 9th cell pairs which have the same microvilli direction (\( \pm 15^\circ \)). In contrast to \( \beta_0 \), a clear secondary group is found for \( \beta_2 \) at 120°. This indicates that the probability for two neighbouring 9th cells having their microvilli at 120° to each other is much higher than for pairs with 2 ommatidia between them. From these results it seems likely that the orientation of microvilli in neighbouring 9th cells plays an important role in the analysis of the polarisation plane of light.

C. Morphological Basis for High PS in Ninth Cell

The structure of the 9th cell and its position below the other retinula cells, led Snyder (1973) to predict that this cell has a large PS. A brief review and amplification of his arguments are given here:

1. Effects of Cell Lengths

PS is largest in the shortest cells, i.e., in cells that absorb the least amount of light. The ninth cell is shorter than 80 μm i.e. less than one third
the length of the two other UV cells. From Snyder’s (1973) Fig. 2, we can see how a cell’s length affects its polarization sensitivity.

2. Polarisation Filter Effect

The effect of one rhabdomere above another is to enhance the PS of the lower cell. If the direction of microvilli of the two cells are perpendicular to each other, the amplification effect is maximal. The longer the upper rhabdomere the greater the effect. These results can be stated mathematically as:

\[ \text{PS (of 9th cell)} = \gamma \times \left(1 - \frac{1}{\Delta} \right) \times \text{PS (of 9th cell if it were alone)} \]

where \( \gamma \) = absorption coefficient of the upper rhabdomere; \( l \) = length of upper rhabdomere; \( \Delta \) = dichroic sensitivity of microvilli medium of upper rhabdomere.

If for \( \gamma \) we take 1% absorption/\( \mu \)m, \( l = 200 \mu \)m and \( \Delta = 4 \) we find that the PS sensitivity of the ninth cell is 4.5 times larger than if it were not under the other cells. If we assume that the PS of the 9th cell alone is approximately 3 then the PS of 9th cell in the bee could be as large as 13.5.

Furthermore the rhabdomeres of both long UV cells follow the 9th cell along its full length and act like parallel or lateral polarisation filter further enhancing the PS of the 9th cell (Snyder, Menzel and Laughlin 1973). The theoretical PS value is greater than our largest PS measurement which was PS = 9. Our calculation was conservative in that the value of \( \Delta \) chosen may be too small. A larger \( \Delta \) leads to a greater PS. In other words we have theoretically predicted an enormous PS in the 9th cell. Electrical coupling may down grade the PS in the actual fused rhabdom. If electrical coupling depends on the area of membrane contact between two cells and is an unavoidable consequence of the dense packing of retinula cells then the PS of the 9th cell is only reduced whereas the PS of the 8 long cells is almost abolished.

Our results explain why the distal UV rhabdomeres have microvilli that are parallel rather than perpendicular to each other. By having parallel microvilli the distal rhabdomeres act as polarisation enhancers for cell 9. This effect would be significantly reduced if the microvilli of the distal retinula cells were perpendicular.

As a consequence of 1) and 2) above, the absolute sensitivity of the 9th cell is necessarily less than that of the longer UV cells. Our data so far collected are not sufficient to confirm this aspect, because the comparison of different recordings on the basis of absolute sensitivity is very difficult.

Snyder, Menzel and Laughlin (1973) showed theoretically, that to have PS = 9, a distal UV rhabdomere would have to be formed by a microvillar medium with a dichroic sensitivity \( \Delta \) considerably greater than 10 in addition to the complete absence of electrical coupling. Since this is highly improbable, the high PS recorded in the UV must be that of the 9th cell.

The 9th cell of the bee bears the same relationship to the other UV cells as the 8th cell in the fly to the 7th cell. Snyder (1973) has shown that the morphology of cell 8 in the fly is consistent with its having a larger PS than cells 1-6. We suggest that the general mechanism in compound eyes for amplifying polarisation sensitivity is the tiering of receptor elements.

The suggestion of PS sharpening mechanism due to detection of waveguide modes by Snyder and Pask (1972) would appear to be a small effect compared to that discussed here. Further more there is no evidence that the integrative mechanism could make use of such information.

Discussion

We owe our knowledge of the orientation of the bee to natural polarisation patterns to the famous experiments of v. Frisch (1967). Following Autrum (Autrum and Stumpf, 1950) he related this ability to the star like arrangement of the 8 retinula cells and made an eight or four branched polariser model to describe the first step in the integration of the polarisation detection.

All previous studies have assumed that each receptor in the bee’s eye is polarisation sensitive, even though no experimental evidence was available (c.f. Kirschfeld, 1973). Shaw (1966) however had already found a very small PS in drone bee retinula cells. Our recordings have shown that nearly all green, blue and UV sensitive receptors in the worker bee have very little or no PS. This finding can be explained by electrical coupling between retinula cells of one ommatidium, directly demonstrated by Shaw (1969a, b) in drone bee and locust. The effects of electrical coupling within one ommatidium are described in detail elsewhere (Snyder, Menzel and Laughlin, 1971). But how then does the bee eye detect the polarisation plane?

We have found that a UV-cell without any sensitivity to wavelengths longer than 450 nm has a high PS. This in itself accounts for polarised light detection in the UV. The recording site near the basement membrane and the rarity of the recordings (3 out of 260) indicate this receptor is the 9th basal retinula cell. Theoretical considerations (e.g. fine structure and arrangement of receptor types), also predict that the 9th cell should

18 J. comp. Physiol., Vol. 88
show the highest PS. Thus in all probability each ommatidium has only one polarisation channel, the 9th cell.

With one PS cell an unambiguous determination of the polarisation plane in one ommatidium is not possible. We shall discuss 2 general types of possible mechanisms for coding the $e$-vector of light. These are based on (1) the interaction between the ninth cells from neighbouring ommatidia with broadly overlapping fields of view; (2) the interaction between ninth cells located in the two eyes but looking in the same direction. Mechanism (1) is generally possible because the interommatidial angle in the dorsal and frontal eye region is only $0.8-1.6^\circ$ (Baumgärtner, 1928; Portillo, 1936) whereas the acceptance angle of retinula cells is in the range of $2.5^\circ$ (Laughlin and Horridge, 1971). Furthermore, the spatial arrangement of the 9th cells microvilli support this mechanism. The microvilli of neighbouring 9th cells are more likely to be orientated at $120^\circ$ to each other than are microvilli of not neighbouring cells. This means PS-detectors with nearly the same view are more likely to have different microvilli directions than other pairs of cells. Von Frisch found that a polarisation pattern must subtend at least $10^\circ$ at the eye if the bee is to orientate to it, and in a recent report (Zolotov and Frantsveitch, 1973) it was found that at least 25-50 ommatidia are necessary to determine the $e$-vector of polarised light. In the dorsal and frontal eye region between 20 and 50 ommatidia look at a $10^\circ$ field. This means that several pairs of neighbouring 9th cells look at this field even when some neighbours have the same microvilli directions.

The second alternative (2) is based on the finding that each eye is the mirror image of the other eye, microvilli in one eye lying at $135^\circ$ to the horizontal head axis appear to be at $45^\circ$ to this axis in the contralateral eye. As the dorsal and frontal eye parts have a binocular field of view there are always ommatidia in both eyes looking at the same dorsal and frontal part of the visual field. A binocular cross comparison between the 4 (2 from each eye) main groups of microvilli directions would give precise information on the polarisation plane.

Behavioral experiments testing the polaro-menotactic orientation should decide for or against one of the two models discussed above. Von Frisch (1960, 1967) studied the bee’s orientation to a polarised light pattern using their ability to direct their dances on horizontal combs with reference to polarised light. In these experiments an arteficial or natural polarised light spot was placed in a relatively low and lateral position. Assuming bees make use of the polarised light pattern only during the tail wagging pattern of their dance, these experiments prove that bees are capable of determining the polarisation plane with one eye only. It can not be excluded, however, that bees analyze the polarisation plane during other phases of the dance possibly with both eyes, and use that information to direct their tail wagging runs. In another experiment v. Frisch found that bees are disoriented, if that eye is blinded which faces the polarised light during the tail wagging run. Also this experiment is not conclusive for our question because the possibility of the bee needing both eyes to analyze the polarisation plane can be excluded only by a control experiment, in which the left eye is blinded and the polarised light is seen by the right eye during the tale wagging run.

Recently Kirschfeld (1973) reported experiments in which the bee responded to a moving pattern of polaroid filters. The optomotor response was found only if the polarisation planes of the polaroid stripes were at $45^\circ$ and $135^\circ$ to the vertical and if the polaroids were illuminated with UV light. There was no response to $0^\circ$ and $90^\circ$ polarization planes and to the $45^\circ$/135$^\circ$ pattern in green and white light. We give an alternative interpretation to Kirschfeld’s discussion, which is consistent with our findings.

Most of the 9th cells microvilli, in the frontal eye region, which was stimulated in Kirschfeld’s experiments lie at $45^\circ$ and $135^\circ$ to the horizontal head axis in the left and right eye respectively. If polaroid stripes with $45^\circ$/135$^\circ$ plane are moved around the animal this would induce an excitation modulation only in the 9th cell but not in the 8 long retinula cells. From the orientation of the 9th cell microvilli and its spectral sensitivity it is clear why the response is found only in UV light to a $45^\circ$/135$^\circ$ pattern.

To understand the lack of response in white light we assume as a first integration step the summation of all 9 retinula cells on one second order neuron per ommatidium. The summation stage would give high continuous excitation in green and white light. It would show only a very small modulation to the moving polaroid pattern. The polarisation insensitive 8 long retinula cells would mask the excitation change coming from the 9th cell. In UV, however, the excitation modulation coming from the 9th cell would be relatively large in comparison to the continuous excitation through the 8 long retinula cells, and the modulation after summation would exceed threshold. In contrast to this model Menzel (1973) assumed a connection of the summation stages only with the green receptors. This question is discussed in detail in another paper on the spectral sensitivity of monopolar cells in the bee (Menzel, unpubl.). It can be shown that as a result of the different absolute sensitivity of the colour receptors and the high gain in monopolar cells (c.f. Laughlin, 1973) the monopolar cells are dominated by the green receptors near threshold.

Basically our interpretation of Kirschfeld’s experiments is that the optomotor response is triggered through the only polarisation sensitive cell (the 9th cell). The underlying mechanism, however, is completely different from that which controls the polaro-menotactic behaviour. In this light the optomotor response to polarised light in UV is a by-product of the neuronal wiring.

Our recordings have shown that the basal 9th retinula cell in the bee ommatidium is most likely the only polarisation sensitive channel. This finding is consistent with the high polarisation sensitivity theoretically
predicted for the 9th cell the arrangement of the 9th cell in neighbouring omnidetia, the behavioural experiments of von Frisch on the polaromemotactic orientation and the optomotor response to a polarisation pattern described recently by Kirschfeld. The development of a specialised "polarisation receptor" and the elimination of the polarisation sensitivity in all other receptors in the earliest possible stage (in the retina) reduces the complexity of the neuronal wiring in the optic lobe. We suggest that this is of great importance for the efficiency of the neuronal integrative processes in the optic ganglia of the bee.

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Polarised Light Detection in the Bee, Apis mellifera

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