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ORIGINAL PAPER

Anatomical and physiological evidence for polarisation vision in the nocturnal bee *Megalopta genalis*

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Abstract The presence of a specialised dorsal rim area with an ability to detect the *e*-vector orientation of polarised light is shown for the first time in a nocturnal hymenopteran. The dorsal rim area of the halictid bee *Megalopta genalis* features a number of characteristic anatomical specialisations including an increased rhabdom diameter and a lack of primary screening pigments. Optically, these specialisations result in wide spatial receptive fields ($\Delta\rho = 14°$), a common adaptation found in the dorsal rim areas of insects used to filter out interfering effects (i.e. clouds) from the sky. In this specialised eye region all nine photoreceptors contribute their microvilli to the entire length of the ommatidia. These orthogonally directed microvilli are anatomically arranged in an almost linear, anterior–posterior orientation. Intracellular recordings within the dorsal rim area show very high polarisation sensitivity and a sensitivity peak within the ultraviolet part of the spectrum.

Keywords Insects · Dim light vision · Dorsal rim area · Polarisation sensitivity · Nocturnal navigation

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Introduction

Although invisible to most vertebrate eyes, a pattern of linearly polarised light, centred around the sun, is present in the sky throughout the day. In linearly polarised light the electromagnetic waves oscillate in parallel planes. This polarisation plane defines the direction of the electric field component, or *e*-vector, of the electromagnetic wave. It has long been known that many diurnal insects use the sun's polarisation pattern for compass navigation, that is, they are able to use the pattern to select a course and to maintain it (von Frisch 1949, 1965; Waterman 1981; Wehner 1981; Rossel 1989; Nilsson and Warrant 1999). In order to analyse the celestial polarisation pattern, diurnal insects possess a specialised region of ommatidia in the dorsal part of the compound eye known as the dorsal rim area (DRA), the photoreceptors of which are highly sensitive to polarised light (review: Wehner and Labhart 2006).

The solar polarisation pattern persists in the evening until astronomical twilight ends (when the centre of the sun's disk is 18° below the horizon), after which the direct contribution from the sun is no longer detectable in the sky (Rozenberg 1966). Animals that use this solar polarisation pattern during twilight navigation avoid the complex geometrical changes that are present when the sun travels across the sky, because the twilight polarisation pattern is relatively simple and constant (Rozenberg

1966; Coulson 1988; Cronin et al. 2006). On the other hand, its light intensity is low, and orienting at such times requires a highly sensitive polarised light detector. The halictid bee *M. genalis* forages exclusively during twilight, where one foraging trip is typically made in the evening, and between one and four trips in the morning (Kelber et al. 2006). Interestingly, the onset of morning foraging correlates well with (and never occurs before) the beginning of astronomical twilight in the morning, while evening foraging finishes at least 25 min before the end of astronomical twilight (Kelber et al. 2006). Because of this correlation, we hypothesise that just as in their diurnal relatives, polarisation vision may play an important role in the long-distance homing flights of the nocturnal *M. genalis*.

If *M. genalis* uses celestial polarised light to orient during twilight, then they require a well-developed, polarisation-sensitive DRA in their compound eyes. In addition, a major requirement during twilight is the presence of highly light sensitive and relatively noiseless photoreceptors to detect the polarisation orientation reliably. The axes of the visual pigment molecules are constrained and aligned within the membranes of the light-sensitive rhabdomeric microvilli (Goldsmith and Wehner 1977). Thus, in order to detect the *e*-vector orientation of polarised light, these microvilli need to remain straight and aligned parallel over the entire rhabdomeric length (Wehner et al. 1975). To further enhance polarisation sensitivity, these rhabdomeres must have their microvilli aligned in one of two possible perpendicular directions. Consequently, each rhabdom possesses rhabdomeres having orthogonal *e*-vector preference, and this provides an opponent visual input to the next level of visual processing (Labhart 1980; Blum and Labhart 2000). In the present study, we used anatomical methods and intracellular electrophysiology to investigate the existence of such a polarisation-sensitive DRA in the compound eyes of the nocturnal bee *M. genalis*.

Materials and methods

Females of the halictid bee *M. genalis* were collected on Barro Colorado Island, Republic of Panama, after sunset and before sunrise, using UV light tubes mounted on white sheets. In addition, nests were collected and transported to Lund, Sweden. Individuals from these nests were used for intracellular electrophysiological recordings. These bees are most abundant during the dry-to-wet season transition (April/May) when the nests contain up to ten females (Wcislo et al. 2004). In Lund, the bees were fed with sucrose solution and kept on a reversed 12:12 h light cycle where the start of the physiological experiments was timed with the onset of "dusk".

Histology

Bees were cooled down, their head capsules opened and submerged into a fixative consisting of a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in phoshate buffer (pH 7.2–7.5). Optimal fixative penetration into the DRA was achieved by removing the ventral third of the eye. The heads were fixed for 2–3 h at 4°C, osmicated (2% OsO_4 in distilled H_2O) for one hour, dehydrated in an ethanol series and propylene oxide and subsequently imbedded in Epoxy resin (FLUKA). Five cross- and two longitudinal semi-thin section series (1 μm thickness) for light microscopy were cut from the whole DRA on a Reichert Ultracut microtome using glass and diamond knives. Moreover, we applied a re-embedding method (Ribi 1976, 1978) with semi and ultra-thin cross section series that enabled us to follow single ommatidia of the DRA throughout their entire length. In order to do this, serial frontal sections, 25–50 μm thick were cut on a Reichert sledge microtome, and embedded with Epoxy between two acetate sheets. Sections containing complete and parallel ommatidia were selected and small areas containing 6–10 ommatidia were cut out and re-embedded for serial cross sectioning. The semi-thin sections were flattened on a 60°C hot-plate, stained with toluidine blue and viewed under a Zeiss photomicroscope, while the ultra-thin sections were prepared for conventional electron microscopy. Colour pictures were taken with an Olympus DP 50 digital camera and adjusted for brightness/contrast in Adobe Photoshop.

Electrophysiology

The bees were inserted into a small tube mounted on a holder connected to a lockable ball-and-socket joint and the protruding head immobilized with melted wax. An indifferent electrode (a thin silver wire) was inserted into a small hole incised between the eyes. A small triangular hole was cut into the fronto-dorsal cornea of the eye and covered with Vaseline to prevent moisture loss. Intracellular glass (borosilicate) electrodes filled with 2 M potassium acetate were advanced through this hole to the photoreceptor cells using a Märzhäuser piezo-driven manipulator. White stimulus light (Nikon XPS-100 Xenon arc lamp) was directed to the eye via a 100 μm-wide quartz light guide, and polarised using a PUV 2 polariser (Spindler & Hoyer). This provided a point source simulation subtending 0.05°.

Recordings from the dark-adapted DRA and adjacent dorsal eye regions gave saturating responses at 30–40 and 40–60 mV, respectively. Once a cell was penetrated, the visual axis was determined using a cardan arm device, which allowed the point source to be placed at any location on an imaginary sphere with the bee's eye at its centre. To determine the cell's polarisation sensitivity, two response-intensity curves were recorded using flashes of 40 ms duration and a 10 s flash interval (V–logI curve). A V–logI curve was first recorded at the cell's preferred polarisation direction (Pol_{max}), which was determined by manually slowly turning the polarisation filter 360° whilst monitoring the cell's response, and then a second curve was recorded with light polarised in the perpendicular direction (Pol_{min}). Only cells with reliable responses and a maximum response higher than 30 mV were selected. The intensity shift S between the two curves (in log units) was subsequently used to calculate the polarisation sensitivity (PS): $PS = 10^S$. As the ascending parts of the V–logI curves were not always entirely parallel for the Pol_{max} and Pol_{min} curves, the intensity shift was measured at 1/3 of the maximum Pol_{max} response value. Labhart (1980) used a similar approach, however, his maximum response values all represented saturated maxima of V–logI curves. PS measurements within the DRA were performed at a wavelength of 350 nm, as all photoreceptors we recorded from in this eye region were UV-sensitive only, while white light was used for photoreceptors outside the DRA. Crude spectral sensitivity measurements, using a series of broad-band ($\Delta\lambda = 40$ nm) interference filters, were recorded at 50 nm intervals from 300 to 700 nm. This was achieved by stimulation with light flashes of equal quantum flux at each wavelength to reveal the spectral regions within which the cells are the most sensitive. However, as our apparatus does not have sufficient resolution to reveal the exact wavelength of maximal sensitivity, these spectral sensitivity curves are not included. After measuring the polarisation sensitivity, neutral density filters were added to the light path to set the intensity to give a voltage response about 60% of the maximum response on axis. White light was used to measure angular sensitivity and, as for the polarisation sensitivity of the photoreceptors, were dark adapted before the onset of the measurements. The point source was then moved across the cell's receptive field in angular steps of 1° and at each step a flash was delivered and the response recorded. After conversion of these responses to equivalent intensities using the axial V–logI curve, the angular-sensitivity function was calculated. Finally, the cell's impulse response—a response to a dim 2 ms flash of light giving a response amplitude of less than 3 mV—was recorded 100 times and averaged. All responses were amplified on a Biologic microelectrode amplifier and digitized using LabView software.

Results

Anatomy

Viewing *Megalopta*'s apposition eye under a dissecting microscope shows a small band of cloudy grey facets in the most dorsal eye region (indicated in Fig. 1a). As this is a common sign for the existence of a DRA in hymenopterans (Aepli et al. 1985), we further exam-

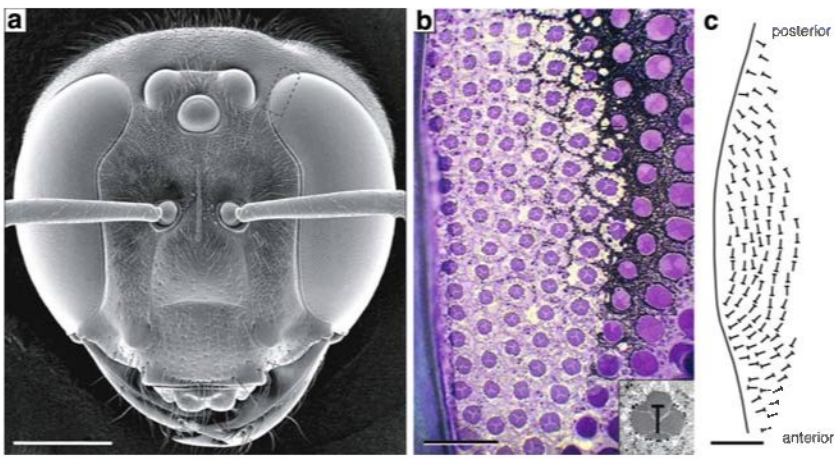

Fig. 1 **a** The head of a female *Megalopta genalis* showing the region of the left eye's dorsal rim area (DRA, *dashed rectangle*). **b** This eye region contains 5–6 rows of specialised ommatidia with large rhabdoms. The *T-bar symbol* in the insert indicates the microvillar orientation of the orthogonally aligned rhabdomeres (see Fig. 3a for detail and scale). 1 μm-thin section, toluidine blue stained. **c** The DRA of *M. genalis* showing the anterior–posterior orientation pattern of the rhabdoms . *Scale* in **a** 1 mm and in **b**, **c** 50 μm

ined this eye region in the nocturnal bee. Semi-thin section series revealed the presence of approximately 120 specialised ommatidia, aligned in 5–6 rows, containing extremely wide, bell-shaped rhabdoms with orthogonally aligned microvilli (Fig. 1b). *Megalopta's* compound eye contains approximately 4,880 ommatidia (Greiner et al. 2004), hence 2.5% of all ommatidia are located within this DRA. The rhabdom arrangement across the entire DRA was reconstructed and their microvillar directions encoded with a T-bar (inset in Fig. 1b). Its pattern shows that anatomically, an almost linear, anterior–posterior orientation dominates most of the DRA (Fig. 1c).

Semi-thin section series in tangential and longitudinal orientations revealed the structural details of these specialised ommatidia (Fig. 2), which are compared to

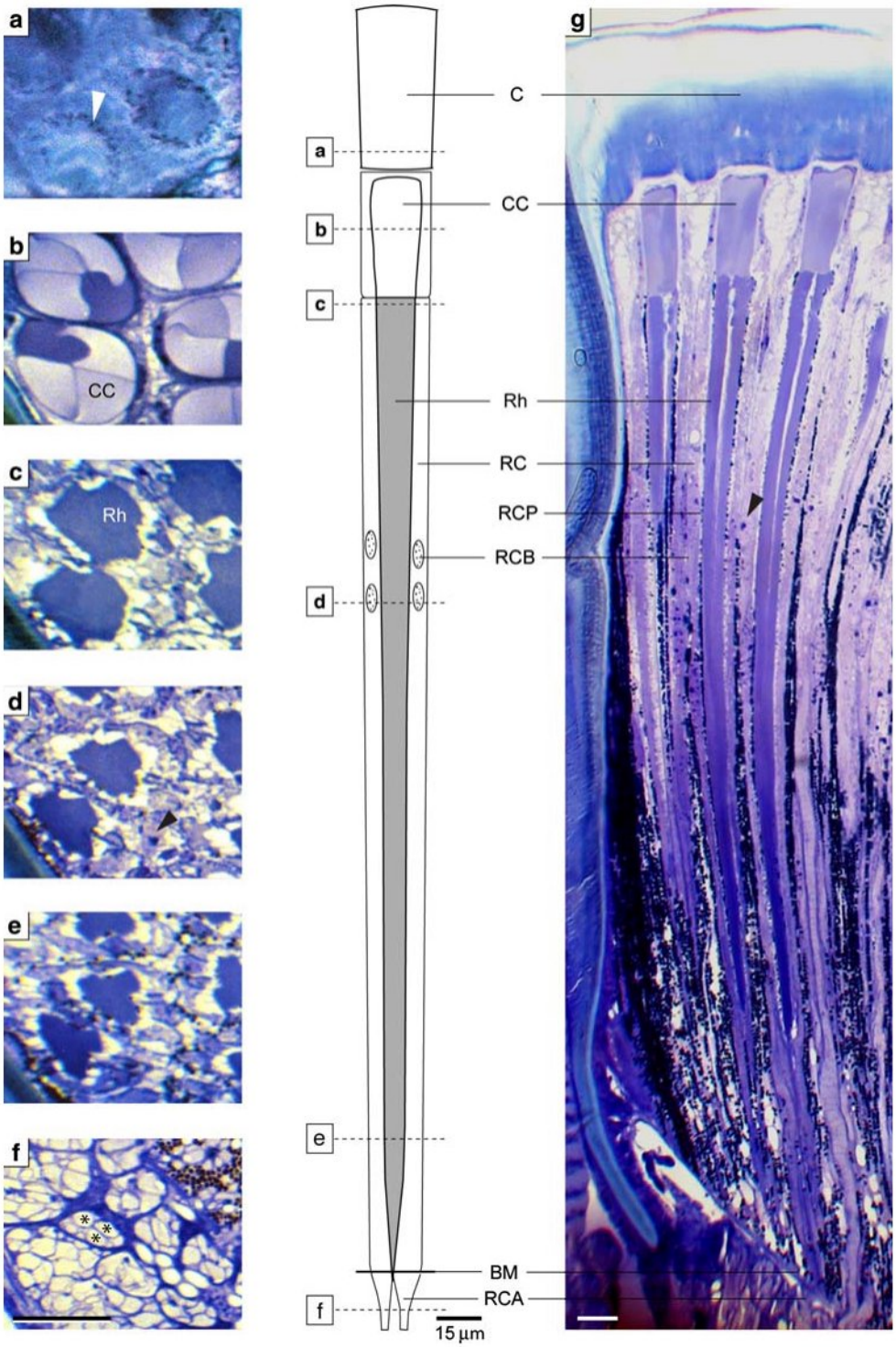

Fig. 2 The ommatidia in the DRA of *M. genalis* females: semi-schematic drawing (*centre*) with transverse (**a**–**f**) and longitudinal (**g**) histological sections. **a** The corneal facets feature corneal structures (*arrowhead*). **b** The crystalline cone (*CC*) is constructed of four Semper cells and surrounded by secondary pigment cells. Note that the optical shielding of the primary pigment cells is entirely absent (**g**). **c**–**e** Nine long retinula cells (*RC*) create the fused rhabdom (*Rh*), with the nuclei of their cell bodies (*RCB*) clustered in the distal third (*arrowheads* in **d**, **g**). **g** A ring of retinula cell pigments (*RCP*) surrounds the rhabdom over its entire length. **f** At the basement membrane (*BM*) the retinula cells form receptor axon bundles (*RCA*) with three distinctly large axons (*asterisks*). Transverse and longitudinal 1-µm sections, toluidine-blue stained. *Scale* (**a**–**f**, shown in **f**) and **g** 15 µm

Table 1 Anatomical parameters in the DRA and frontal eye region of the nocturnal bee *Megalopta genalis*

Parameter (unit)	*DRA*	Frontal[a]
Number of facets (unitless)	120	4,880 *
Maximal corneal facet diameter (μm)	28	36
Corneal thickness (μm)	55	100
Crystalline cone length (μm)	40	50
Distal rhabdom diameter (μm)	11–14	8
Rhabdom length (μm)	330	350
Ommatidial length (μm)	425	500

[a] From Greiner et al. (2004)

*Facet number represents the entire eye including the DRA

previous data of the frontal eye region in Table 1 (Greiner et al. 2004). The facets in the DRA have a diameter of 28 μm, a corneal thickness of 55 μm and contain corneal structures (Fig. 2a, arrowhead) that show similarities to the pore canals present in the DRA of the honeybee (Meyer and Labhart 1981). Beneath the cornea, the cylindrically shaped crystalline cone is composed of four Semper cells and surrounded by a sheath of secondary pigment cells. The ommatidial shielding pigments, typically pigment granules housed within the primary pigment cells, are entirely absent (Fig. 2b, g).

The rhabdoms of the DRA are each formed by nine long retinula cells (RC), with their retinula cell bodies clustered in the distal third of the retinula cells (Fig. 2g). In all other ommatidia of the eye eight long and one short basal RC are instead present. The angular orientation of the bell-shaped rhabdoms can easily be determined using light microscopy, and serial sections show that the microvillar orientation within a single rhabdomere remains constant over the entire rhabdom length (Fig. 2c–e). The rhabdom is slightly cone-shaped reducing its diameter from 11–14 μm distally to 7–8 μm proximally. Three of the nine photoreceptors contribute the largest microvillar surface over the entire rhabdom (Figs. 2c–e, 3a). There are also three markedly larger retinula cell axons present in the pseudocartridge (asterisks in Fig. 2f), which might belong to these three large RCs. Ultra-thin electron microscope sections clearly show the microvillar arrangement within *Megalopta*'s specialized DRA rhabdoms (Fig. 3b). However, since the spectral sensitivities of the individual RCs are unknown in *M. genalis*, our numbering scheme is based on that of the very similarly arranged DRA rhabdom of the honeybee (Sommer 1979). As in the honeybee, the DRA rhabdoms of *M. genalis* contain three large RCs, which are possibly homologues of the three large, UV-sensitive RCs 1, 5, and 9 of the honeybee (Fig. 3c, Sommer 1979). These three large RCs are adjacent to 6 smaller cells: RCs 2–4 being located between RCs 1 and 5, RC 6 adjacent to RCs 5 and 9, and RCs 7,8 next to RCs 9 and 1 (Fig. 3). Also in *M. genalis*, the microvilli are aligned parallel and especially in the three large RCs

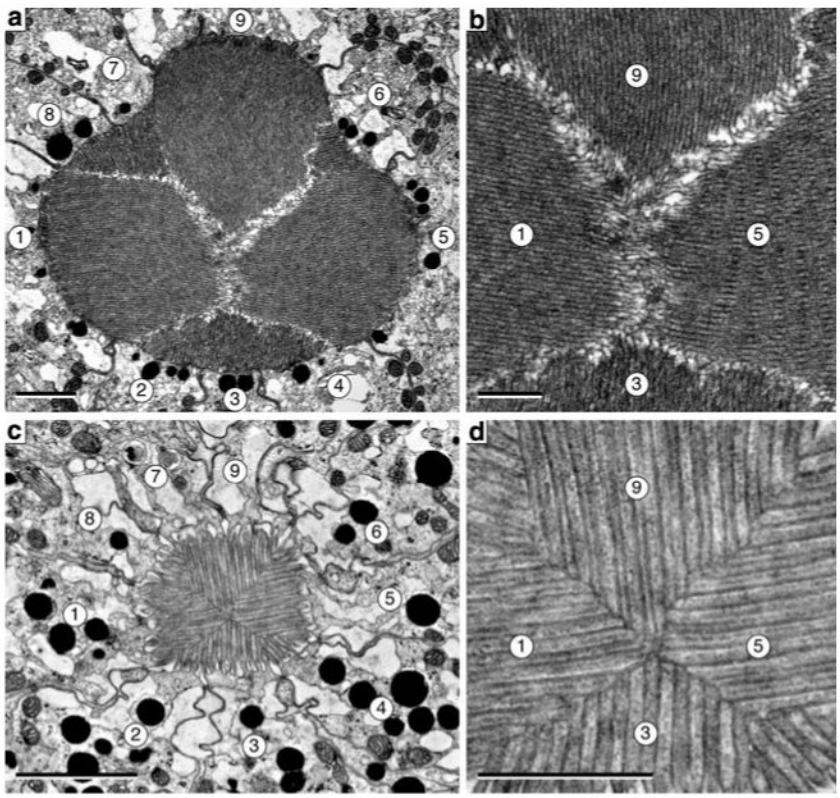

Fig. 3 The rhabdoms in the DRAs of the nocturnal bee *M. genalis* (**a**, **b**) and the worker honeybee *Apis mellifera* (**c**, **d**). The rhabdom diameter is 6–7 times wider in the nocturnal bee (**a**) compared to the worker honeybee (**c**). In both species, the microvilli of the nine retinula cells are oriented 90° towards each other (**b, d**). *Scales* (**a**, **c**) 2 μm and in (**b**, **d**) 1 μm. **c**, **d** Adapted from (Labhart and Meyer 1999)

and RC3 they are arranged in two orthogonal directions only—microvilli of RCs 1 and 5 are oriented 90.6° ± 5.1 ($n = 5$ sections) to those of RCs 9 and 3 (Fig. 3b).

Electrophysiology

Intracellular electrophysiological recordings from the DRA, as well as from dorsal ommatidia outside the DRA, showed that polarisation sensitive photoreceptors are only present within the DRA. Recordings were initially performed using white light. Subsequent spectral measurements, however, revealed hyperpolarising responses to green light in addition to strong depolarising responses within the UV. To avoid this possible electrical coupling effect from neighbouring green sensitive cells, polarisation sensitivity was recorded only within the spectrum where strong depolarisations were observed. All polarisation-sensitive cells in the DRA depolarised within the UV-range only. The V–logI curves from these recordings showed clear maxima and minima at orthogonal polarisation angles. As these two curves were generally not perfectly parallel, the intensity shifts of the most reliable cells recorded using UV light were analysed at one-third of the maximum response. Using this method we calculated a very high PS average of 21.2 ± 7.5 SD ($n = 5$ cells, Fig. 4a). However, as the non-parallel nature of the two V–logI curves represents a potential experimental uncertainty that could not be excluded, we can only estimate that the PS value lies in the range of 20 for the DRA of *M. genalis*. In contrast, all photoreceptors from the dorsal eye region outside the DRA were green sensitive and showed low PS values of 1.4 ± 0.4 ($n = 24$ cells, Fig. 4b). As no hyperpolarising effect was observed during spectral sensitivity recordings in these green-sensitive cells, recordings outside the DRA were performed using white light.

Spatial resolution of an eye is determined by the spatial receptive field (also known as angular-sensitivity function) of the photoreceptor (Warrant and McIntyre 1993). In *M. genalis*, poor lens optics, due to corneal pore canals, wide rhabdom diameters and a lack of screening pigments, result in wide spatial receptive fields, with an average acceptance angle ($\Delta\rho$, half-width of the angular-sensitivity function) of 13.8° ± 3.6° ($n = 15$ cells, Fig. 5). The noisy course and wide flanks of the curve are potentially due to the combined effects of the light scattering corneal structures and the huge diameter of the rhabdom. In the dorsal eye region outside the DRA, spatial receptive fields with $\Delta\rho = 5.9 \pm 1.7°$ ($n = 20$ cells, data not shown) were recorded, which agree well with results from measurements of the frontal eye region ($\Delta\rho = 5.6 \pm 0.8°$, Warrant et al. 2004).

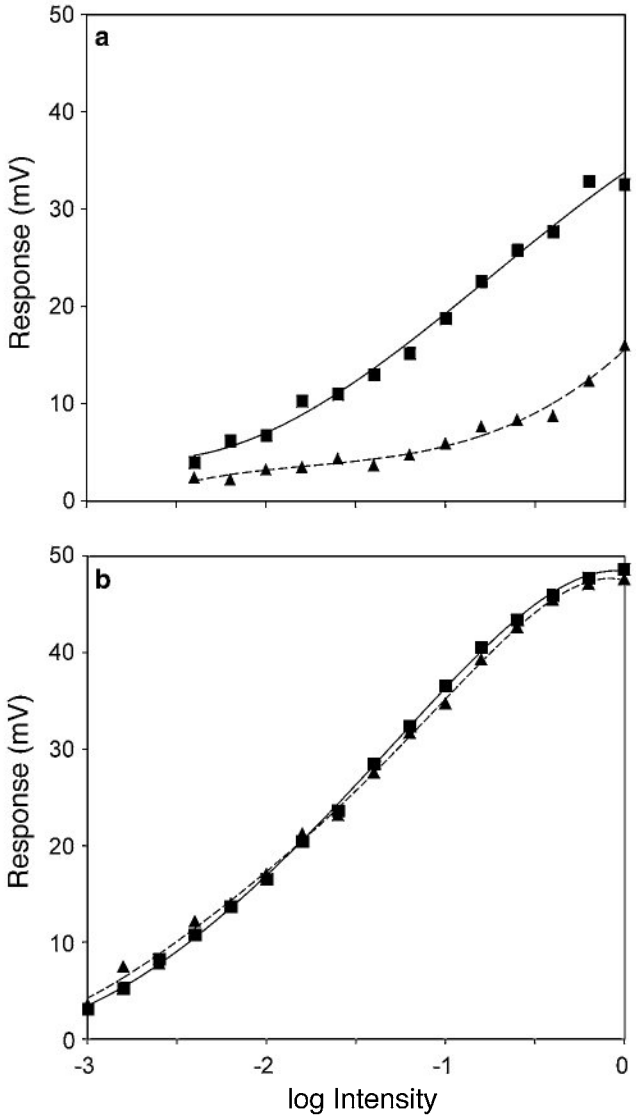

Fig. 4 Intracellular recordings from within the DRA (**a**) and the dorsal eye region bordering the DRA (**b**) of the nocturnal bee *M. genalis*. **a** The intensity shift between the maximal (max, *square*) and minimal (min, *triangle*) response to the direction of the polarisation filter leads to a polarisation sensitivity value of 18.6. **b** Outside the DRA no distinct maximal and minimal response to the *e*-vector direction of polarised light can be distinguished (PS = 1)

A good indicator of the temporal resolution of an eye is the response of the photoreceptor to a very brief and dim flash of light, known as the impulse response. The time course of the impulse response is characterised by its 'time-to-peak' (τ_p), which is the time from the onset of the stimulus to the depolarisation maximum: long τ_p indicates low temporal resolution. In the DRA of *M. genalis*, an average τ_p of 45 ± 3 ms ($n = 32$ cells) was recorded. This value is significantly different from $\tau_p = 36 \pm 4$ ms in the dorsal eye region

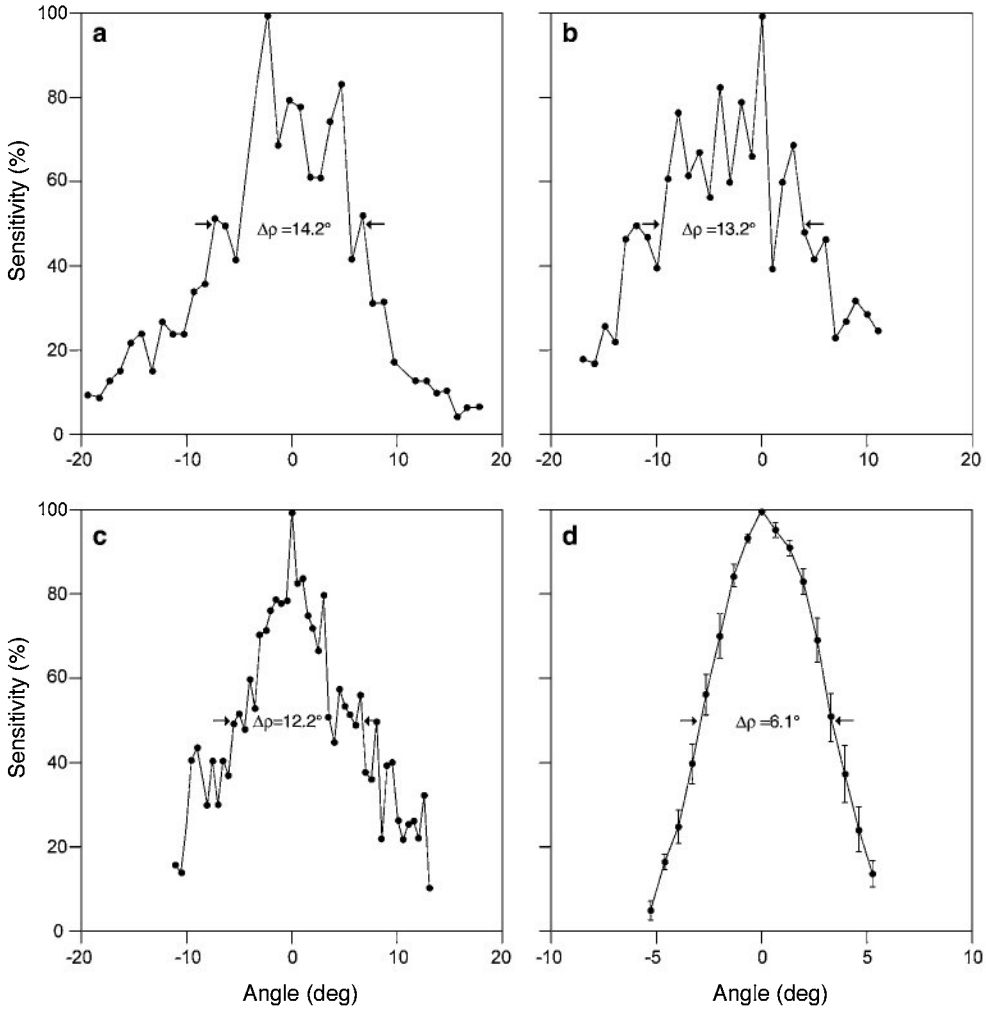

Fig. 5 **a–c** Angular-sensitivity functions from three photoreceptors in the dark-adapted DRA of *M. genalis* [the half-widths (acceptance angles $\Delta\rho$) are marked by the *arrows*]. **d** The mean angular-sensitivity curve derived from six photoreceptors in the dorsal eye region outside the DRA (*bars* show standard errors). Note the difference in angular scale compared to (**a–c**)

($n = 24$ cells, t test $P < 0.0001$). However, since the τ_p of the DRA is not different from $\tau_p = 41 \pm 8$ ms ($n = 6$ cells) measured in the frontal eye region (Fig. 4b in Warrant et al. 2004) a functional significance of the higher temporal resolution in the dorsal eye region bordering the DRA is unlikely.

Discussion

A dorsal rim area in a nocturnal bee

The halictid bee *M. genalis* is the first nocturnal hymenopteran shown to possess an anatomically and physiologically distinct DRA that is well adapted to polarisation vision at low light intensities. Anatomical specialisations include extremely wide rhabdoms, and a lack of primary screening pigments. The optical effect of these specialisations results in wide spatial receptive fields. Extensive optical integration is not a prerequisite for polarisation vision (Labhart 1986), but thought to be beneficial at low light intensities as it increases the signal-to-noise ratio and filters out interfering effects from the sky (i.e. clouds, canopy) (Labhart 1999). This signal-to-noise ratio might be further enhanced by the significantly lower temporal resolution of *Megalopta*'s photoreceptors compared to those of the worker honeybee ($\tau_p = 27 \pm 2$ ms, $n = 5$ cells; Warrant et al. 2004).

The rhabdoms of the approximately 120 specialised ommatidia contain nine long rhabdomeres each, packed with parallel microvilli throughout the length of the rhabdom. These rhabdomeres are arranged in two orthogonally oriented classes and the rhabdoms so formed are organised in an almost linear pattern across the DRA. The 11–14 μm wide rhabdoms and their long, well aligned microvilli subserve one of the highest average polarisation sensitivities (PS ≈ 20) so far recorded in an insect eye. The rather large scatter in the PS estimate may be due to our inability to determine the exact photoreceptor from which recordings were made (i.e. whether it was a small or large RC),

potential electrical interactions between photoreceptors (Shaw 1975; Labhart 1980), and/or regional differences in possible microvillar misalignments (Nilsson et al. 1987). According to theory, a perfect alignment of the rhodposin molecules within the microvilli would lead to a polarisation sensitivity maximum of 20 (Laughlin et al. 1975). However, this model is based on a single photoreceptor and does not include possible physiological interactions between photoreceptors, which could further increase PS substantially (Nilsson et al. 1987; Blum and Labhart 2000). As *Megalopta*'s average PS reaches this maximum, our physiological data show that these nocturnal bees have an extremely sensitive DRA, which should optimise signal-to-noise ratio.

Several of the optical and retinal specializations present in *Megalopta* are also found in the DRAs of diurnal bees. The honeybee's DRA is very similar to that of *M. genalis*, where the rhabdoms of about 125 specialised DRA ommatidia cover 2.6% of the entire eye surface (Sommer 1979). However, the microvilli within the DRA of the honeybee show a fan-shaped pattern compared to the almost linear anatomical orientation present in *Megalopta*'s, which might represent a specific adaptation to the unidirectional polarisation pattern present at twilight. Moreover, the much smaller rhabdom diameter in the DRA of the honeybee ($d = 2$–3 μm) (Sommer 1979) strongly indicates the importance of high light sensitivity in the DRA of the nocturnal bee. The DRAs of many bees and wasps, including the honeybee *Apis mellifera*, contain corneal structures (Meyer and Labhart 1981; Aepli et al. 1985) that result in wide visual fields (Labhart 1980). The narrow peak of the honeybee angular sensitivity function most likely reflects the approximately five-times smaller rhabdom diameter compared to *M. genalis*. The nine long photoreceptors of the honeybee DRA show a similar microvillar arrangement (Schinz 1975), with the rhabdomeres of the three large RCs (1, 5 and 9) dominating the rhabdom (Sommer 1979). Also in the honeybee, those few photoreceptors with strong UV sensitivity and hyperpolarising responses to green light showed high PS values of up to 18 (Labhart 1980). According to Labhart (1980), however, these isolated hyperpolarisations in the honeybee may derive from possible artificial electrical coupling with neighbouring green-sensitive cells.

Apart from nocturnal bees, also other animals have evolved visual specialisations for analysing celestial polarisation patterns at night. It has recently been demonstrated that there is a similar polarisation pattern formed around the moon (Gál et al. 2001), and even though this pattern is a million times dimmer than that formed around the sun, some some species of nocturnal dung beetles use this pattern to extend their foraging time into moonlight nights (Dacke et al. 2003). In the upper part of their dorsal pair of superposition eyes, specialised ommatidia contain rhabdoms possessing two classes of rhabdomeres with orthogonally oriented microvilli (Dacke et al. 2002, 2004). However, in contrast to *Megalopta*'s DRA, the dung beetle DRA reveals no difference in the optics of the cornea or the distribution of pigment compared to the rest of the eye. While the width of the photoreceptor's receptive field is unknown in dung beetles, two sets of highly polarisation sensitive cells (UV and UV/green receptors) gave high PS values of approximately 13 (Dacke et al. 2002, 2004). The highest polarisation sensitivity has been measured behaviourally in crickets, where the absolute *e*-vector sensitivity threshold lies below the light intensity of a clear moonless night sky (Herzmann and Labhart 1989). Despite a large measured scatter in PS values in the most reliable single cell recordings (ranging from 5 to 29), crickets reveal a high median PS value of 10 in their blue-sensitive DRA photoreceptors (Labhart et al. 1984; Blum and Labhart 2000). As in *Megalopta*, the cricket DRA lacks screening pigment (and thus optical screening between the ommatidia) and possesses rhabdoms of greater diameter than found in the rest of the eye (Burghause 1979). Thus, compared to visual fields of 6° in the dorsal, unspecialised part of the eye, the cricket DRA has wide acceptance angles of around 20° (Labhart et al. 1984; Blum and Labhart 2000). The well-developed DRA of the desert locust is also optically specialised with fairly high PS (Eggers and Gewecke 1993; Homberg and Paech 2002), suggesting an important navigational role during nocturnal migration (Riley and Reynolds 1986). An extreme case is the DRA of the Canarian cricket *Cycloptiloides canariensis*, where in addition to the absence of screening pigment, the DRA also lacks corneal faceting and crystalline cones, features that would endow their gigantic rhabdoms with huge, overlapping visual fields (Egelhaaf and Dambach 1983). In addition to insects, nocturnal spiders also have eyes specialised for extensive optical integration coupled with high polarisation-sensitivity. The secondary eyes of the spider *Drassodes cupreus* possess built-in polarisers that form part of a compass organ of high polarisation sensitivity (PS 9), which is used for nocturnal navigation (Dacke et al. 1999). In crustaceans, stomatopods show one of the highest polarisation sensitivities (PS 11–15: Kleinlogel et al. 2006).

Polarised light as a nocturnal navigation cue

As already mentioned, a highly light-sensitive DRA enables crepuscular beetles to analyse the dim

polarisation pattern formed around the moon (Dacke et al. 2002), a navigational cue that is most likely also used by other nocturnal insects with sufficiently high polarisation sensitivity. During twilight, however, the solar polarisation pattern persists in the evening until astronomical twilight ends (when the centre of the sun's disk is 18° below the horizon), after which the direct contribution from the sun is no longer detectable in the sky (Rozenberg 1966). During the dawn, the solar polarisation pattern is visible from the beginning of astronomical twilight. Just around sunset and sunrise the *e*-vector direction of light throughout this entire polarisation pattern has an essentially unidirectional orientation roughly parallel to the North–South axis of the sky. An intensity and spectral gradient across the sky removes the 180° ambiguity that would occur for animals using the polarisation pattern alone to find a compass direction (Brines and Gould 1982; Wehner 1997; Cronin et al. 2006). At the latitude of Panama near the equator, astronomical twilight starts about 1 h before local sunrise and ends about 1 h after local sunset. During the entire twilight period the polarisation pattern varies little in angular distribution, degree of polarisation, or spectral content, reaching an 80% degree of polarisation at wavelengths of 600 nm (Coulson 1988; Cronin et al. 2006). Thus, with the high polarisation sensitivity present in the DRA of the nocturnal bee *M. genalis*, the polarisation pattern during this twilight period represents a simple and reliable orientation cue. Indeed, the onset and offset of *Megalopta*'s activity periods correlate well with the beginning and end of astronomical twilight (Kelber et al. 2006). However, since the orientations of the ommatidia of the DRA and their visual overlaps with neighbouring ommatidia could not be measured, we do not yet know how this array of analysers exploits the simple *e*-vector pattern of twilight.

The spectrum of celestial polarised skylight at night, both from the sun and the moon, peaks at middle to long wavelengths (Coulson 1988; Gál et al. 2001; Barta and Horvath 2004; Cronin et al. 2006). Nevertheless, like other bees and ants (Duelli and Wehner 1973; von Helversen and Edrich 1974), *Megalopta*'s DRA is dominated by UV-sensitive photoreceptors. Barta and Horvath (2004) argue that UV-sensitivity is advantageous when light is scattered relatively close to the observer (e.g. beneath clouds or the forest canopy), which is strongest in the UV spectrum. In addition, measurements in the bee's environment show no dramatic decrease in the degree of polarisation at short wavelengths (Cronin et al. 2006). Thus, the UV-sensitive photoreceptors of *Megalopta*'s DRA are well suited to the skylight polarisation present during its activity periods.

Thus, in addition to landmark navigation close to the nest entrance (Warrant et al. 2004), the celestial polarisation pattern of tropical skies provides a reliable orientation cue throughout the twilight period (Cronin et al. 2006). Based on the anatomy and physiology of *Megalopta*'s specialized DRA, we conclude that these bees are able to detect the *e*-vector direction of light. As *M. genalis* invests a considerable amount of energy in the maintenance of extremely wide and highly polarisation-sensitive photoreceptors, these nocturnal bees are most likely able to use polarisation vision for course maintenance and stabilisation during long-distance foraging and homing flights.

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