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REGULAR ARTICLE

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Neural organisation in the first optic ganglion of the nocturnal bee *Megalopta genalis*

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Abstract Each neural unit (cartridge) in the first optic ganglion (lamina) of the nocturnal bee *Megalopta genalis* contains nine receptor cell axons (6 short and 3 long visual fibres), and four different types of first-order interneurons, also known as L-fibres (L1 to L4) or lamina monopolar cells. The short visual fibres terminate within the lamina as three different types (svf 1, 2, 3). The three long visual fibres pass through the lamina without forming characteristic branching patterns and terminate in the second optic ganglion, the medulla. The lateral branching pattern of svf 2 into adjacent cartridges is unique for hymenopterans. In addition, all four types of L-fibres show dorso-ventrally arranged, wide, lateral branching in this nocturnal bee. This is in contrast to the diurnal bees *Apis mellifera* and *Lasioglossum leucozonium*, where only two out of four L-fibre types (L2 and L4) reach neighbouring cartridges. In *M. genalis*, L1 forms two sub-types, viz. L1-a and L1-b; L1-b in particular has the potential to contact several neighbouring cartridges. L2 and L4 in the nocturnal bee are similar to L2 and L4 in the diurnal bees but have dorso-ventral arborisations that are twice as wide. A new type of laterally spreading L3 has been discovered in the nocturnal bee. The extensive neural branching pattern of L-fibres in *M. genalis* indicates a potential role for these neurons in the spatial summation of photons from large groups of ommatidia. This specific adaptation in the nocturnal bee could significantly improve reliability of vision in dim light.

Keywords Visual system · Compound eye · First optic ganglion · Retinula cell axons · First-order interneurons · Spatial summation · *Megalopta genalis*, nocturnal bee, female (Insecta)

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Introduction

Because of our preference for bright light, we tend to forget that most animals are active in dim light, either at night or in the dark depths of the sea. The nocturnal halictid bee *Megalopta genalis*, found in Central and South America, shows an astonishing ability to forage in dim starlight intensities and visually to learn landmarks around the nest entrance, implying that it sees well at night (Warrant et al. 2004). All this is accomplished with apposition compound eyes (Greiner et al. 2004), an eye design adapted for bright light (Nilsson 1989). We have recently discovered that the apposition eyes of *M. genalis* females show distinct retinal and optical adaptations (large facets and wide rhabdoms), which increase their optical sensitivity by a factor of 27 times compared with those of diurnal bees (Greiner et al. 2004). This is a remarkable improvement for an apposition eye; however, it is still insufficient considering the eight log-unit difference in light intensities experienced by nocturnal and diurnal bees.

Theoretical modelling suggests that nocturnal animals with small eyes can significantly improve visual reliability in dim light by employing a strategy of photon summation

in time and/or space in order to improve photon capture (Warrant 1993, 1999; Warrant et al. 1996). Temporal summation involves a summation of photons over an extended period of time, similar to a camera with an extended exposure time. This lengthened integration time is partly controlled in the retina by the photoreceptor transduction cascade but higher circuits that sum photons for even longer periods of time are also possible. A disadvantage of making vision slower, however, is that the internal spatial details of quickly moving objects cannot be perceived (Srinivasan and Bernard 1975). Temporal summation alone could therefore be detrimental for fast-moving animals such as bees and, in *M. genalis*, it is likely to contribute to photon capture to a lesser extent than spatial summation. By summing signals from large groups of neighbouring ommatidia, the apposition eyes of *M. genalis* could significantly improve photon capture in dim light. Such spatial summation, however, simultaneously widens the receptive fields of visual channels and leads to coarser spatial resolution. Nevertheless, as this is probably less costly for a fast-flying nocturnal insect, we hypothesise that *M. genalis* has developed, for spatial summation, neural mechanisms that are not present in diurnal bees. For instance, one might expect that, in nocturnal bees, laterally branching neurons are adapted for summing signals from neighbouring ommatidia.

Spatial summation most probably takes place early in visual processing and we might therefore expect to find cells mediating summation in the lamina, the first optic ganglion in the optic lobe of the insect brain. The lamina consists of a number of cell types that all have the potential spatially to sum signals from large groups of ommatidia. The retinula cell axons (RCA), reaching the lamina from the retina, and the first-order interneurons, also known as L-fibres or lamina monopolar cells, form the first synaptic connections within the optic lobe and are therefore prime candidates for spatial summation. Various types of laterally spreading L-fibres are common in insect laminas and, in the worker honeybee, two out of four L-fibres have the potential to sum information from several ommatidia (Ribi 1976). The RCAs of the worker honeybee nevertheless show no lateral branching (Ribi 1975a) and rare examples of branching RCAs can be found in the backswimmer *Notonecta glauca* (Wolburg-Buchholz 1979) and the skipper butterfly *Parnara guttata* (Shimohigashi and Tominaga 1999).

According to our hypothesis, lateral branching of the RCAs and/or the L-fibres is a necessary neural substrate for spatial summation in dim light and one would therefore expect significantly wider branching patterns in *M. genalis* than in diurnal bees. To discover whether this is indeed the case, we have investigated the architecture of the lamina by means of Golgi-stained light-microscopy sections. A complete anatomical catalogue of the nine RCAs and four L-fibre types of *M. genalis* is directly compared with equivalent catalogues for the worker honeybee *Apis mellifera* (Ribi 1987b) and the more closely related diurnal halictid bee *Lasioglossum leucozonium*. The results show that the nocturnal bee *M. genalis* possesses wide laterally branching neurons that could potentially be employed for spatial summation.

Materials and methods

Females of the facultatively social halictid bee *M. genalis* were collected at night by using UV light during the dry season on Barro Colorado Island, Republic of Panama (for details of its biology and the study site, see Warrant et al. 2004). For comparison, we studied the diurnal halictid bee *L. leucozonium*. This bee is generally abundant between the beginning of May and mid-August (Amiet et al. 2001) and was collected near the ecological field station of Uppsala University, situated on the Swedish island of Öland, and in southern Sweden near Lund.

A rapid Golgi staining method for light microscopy was employed in order to stain and identify the neural components of the lamina, viz. the first optic ganglion (Ribi 1987a). Bees were immobilised by cooling them to 4°C. Their head capsules were then opened by cutting away the mouth parts and the frontal head cuticle. Small openings in the retinas enabled optimal fixative penetration. The heads were fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in phosphate buffer (pH 7.2–7.5) for 2–3 h at 4°C, osmicated (2% OsO_4 in distilled H_2O) for 1 h and then transferred into 2.5% potassium dichromate (in distilled H_2O) for another 2–3 h. They were subsequently washed briefly in buffer and treated with a 0.75% silver nitrate solution (in distilled H_2O) for 1–2 h. After dehydration in an ethanol series and propylene oxide, the heads were imbedded in Epoxy resin (FLUKA) and sectioned at 25–50 μm on a Reichert sledge microtome. The sections were placed on microscope slides and flattened on a 60°C hot-plate before being embedded with DPX mounting medium or Epoxy resin.

Frontal section series of 200 *M. genalis* females and 110 *L. leucozonium* females were analysed by using a Zeiss Axiophot microscope. The sections containing complete and identifiable neurons were photographed with an Olympus DP 50 digital camera. From selected neurons, a stack of images was taken manually. These image stacks were then digitally reconstructed on a PC by using Adobe Photoshop 7.0 and subsequently redrawn manually on transparency paper.

Results

The optic lobe of the insect brain consists of three optic ganglia, the lamina, the medulla and the lobula, and two chiasmata between these ganglia. The outer chiasm is situated between the lamina and the medulla, and the inner chiasm between the medulla and the lobula (Fig. 1). In *M. genalis* females, each eye consists of approximately 4,880 ommatidia (Greiner et al. 2004), representing the optical units of the compound eye. Each ommatidium has a dioptric apparatus (the corneal facet and the crystalline cone) and nine retinula cells forming the fused light-

sensitive rhabdom (Greiner et al. 2004). The nine RCAs of each ommatidium pass the retinal basement membrane together as an axon bundle known as a pseudocartridge. Within each pseudocartridge, one can distinguish the two large RCAs 3 and 7, the four medium RCAs 2, 4, 6 and 8 and the three small RCAs 1, 5 and 9 (Greiner et al. 2004; see below). After penetrating the fenestrated layer, which is a region below the basement membrane containing trachea and the cell bodies of lamina cells, the pseudocartridges reach the lamina in a straight projection.

The lamina of *M. genalis* is a curved neuropile measuring about 650 μm in dorso-ventral length and 100 μm in thickness (Fig. 1). A network of tangential glia cells pervades the lamina and defines the boundaries of the RCA bundles within their cartridges, the functional units of the lamina (Fig. 2). These cartridges arise as a consequence of the appearance of the L-fibres. Each cartridge receives an RCA bundle from the overlying ommatidium, thus maintaining a retinotopic projection from the retina to the lamina. The synaptic region of the lamina (the external plexiform layer) can be divided into three layers: A, B and C. These layers are the result of the different levels of RCA terminations and the branching patterns of the L-fibres. Most distally lies the prominent 55-μm-thick layer A. The 30-μm-thick layer B is situated proximal to layer A and exhibits a characteristic striated pattern by light microscopy. The dense layer C is the most proximal and thinnest stratum with a thickness of 15 μm. The cartridge diameter measures about 14 μm in the dorsal–ventral orientation in layers A and B and about 7 μm in layer C where a prominent network of tangential glia cells occupies the 7 μm gap between cartridges (Fig. 2).

Of the nine RCAs that leave the retina, the two large RCAs 3 and 7, and the four medium RCAs 2, 4, 6 and 8 terminate as short visual fibres (svf) in the lamina (Fig. 3a–c). The three thin RCAs 1, 5 and 9 pass through the lamina as long visual fibres (lvf; Fig. 3d), terminating in the medulla. The six svf of *M. genalis* could be classified by determining their position within the pseudocartridges based on serial cross sections of Golgi-impregnated fibres for light and electron microscopy (B. Greiner et al., in preparation) and are in accordance with the classification found in the worker honeybee (Ribi 1975a). The main axonal trunk of svf type 1 (RCAs 3 and 7) divides into two bifurcated sub-branches in proximal layer A, both terminating in the proximal region of striated layer B (Fig. 3a). Short branches can occasionally be found on the main axonal trunk in layer A (Fig. 3a, arrowhead) and axonal protrusions (knots) are visible along the bifurcation in layer B (Fig. 3b). All branches of svf type 1 stay within the parental cartridge. The svf type 2 (RCAs 4 and 8) possesses a main axonal trunk with 4–5 side branches containing spines and knots (Fig. 3b). The axonal trunk, which also shows protrusions, has a fork-like end in proximal layer A. The 25-μm span of svf type 2 indicates that contact with adjacent cartridges takes place. This is supported by results from cross-section series for light and electron microscopy taken from Golgi-impregnated neurons (B. Greiner et al., in preparation). The svf 3 (RCAs 2 and 6), with its blunt termination, has only a few axonal protrusions and no side branches along the main axonal trunk that terminates in proximal layer A (Fig. 3c). Two

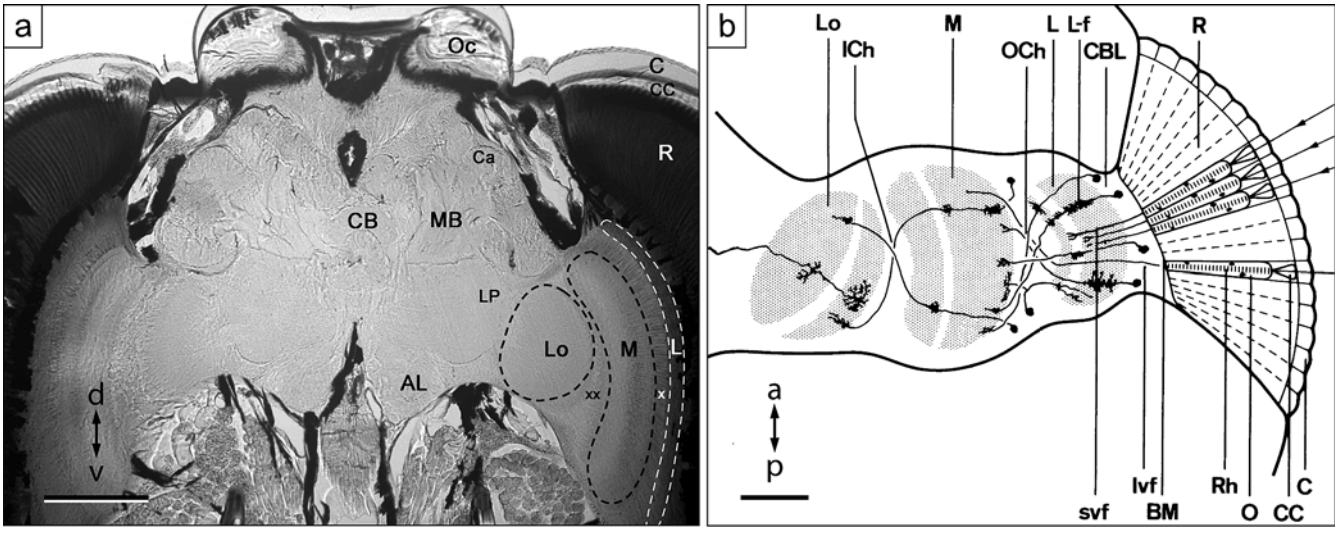

Fig. 1 **a** Frontal view of a section (25-μm-thick, Golgi stained) of the brain of *M. genalis*. The visual system features a peripheral retina (*R*) and three optic ganglia—the lamina (*L*), medulla (*M*) and lobula (*Lo*). The lamina and medulla are connected by the outer chiasm (*x*; *OCh* in **b**), whereas the inner chiasm (*xx*; *ICh* in **b**) is situated between the medulla and the lobula (*AL* antennal lobe, *Ca* calyces of the mushroom body, *CB* central body, *LP* lateral protocerebrum, *MB* mushroom body, *Oc* ocellus). The *axis* shows the dorso (*d*) to ventral (*v*) orientation. **b** Semi-schematic drawing of the optic lobe (horizontal view). The retina consists of a large number of repeated visual units, the ommatidia (*O*). Each ommatidium contains a dioptric apparatus, viz. the corneal facet (*C*) and crystalline cone (*CC*), and a light-sensitive fused rhabdom (*Rh*) made up of rhabdomeres from nine retinula cells. The axons of the retinula cells reach into the lamina (*L*) and terminate as six short visual fibres (*svf*) within the lamina and three long visual fibres (*lvf*) in the medulla. These axons synapse with first-order interneurons (*L-f*) in the lamina. The *axis* shows the antero (*a*) to posterior (*p*) orientation. Adapted from Ribi 1987b. *Bars* 200 μm

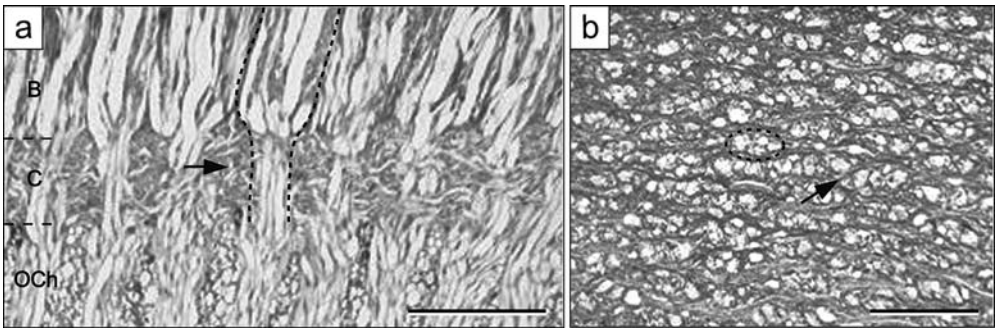

Fig. 2a, b General organisation within the lamina, viz. the first optic ganglion (1-µm-thick sections, toluidine-blue staining). The neural units (cartridges, *dashed lines*) are surrounded by a dense network of glia cells (*arrows*) as shown in longitudinal section (**a**) and cross section (**b**). These glia cells define the boundaries of the cartridges within the lamina. Note the tight packing of the cartridges in **b** (*B* layer B, *C* layer C, *OCh* outer chiasm). *Bar* 25 µm

neurons of each svf type lie within each cartridge. The three lvf types correspond to RCAs 1, 5 and 9 (Fig. 3d). We were unable to differentiate between them within the lamina because of the absence of characteristic branching patterns. Their differentiation was achieved by classifying their terminations in the medulla according to the classification developed for the worker honeybee (Ribi 1975a; see below).

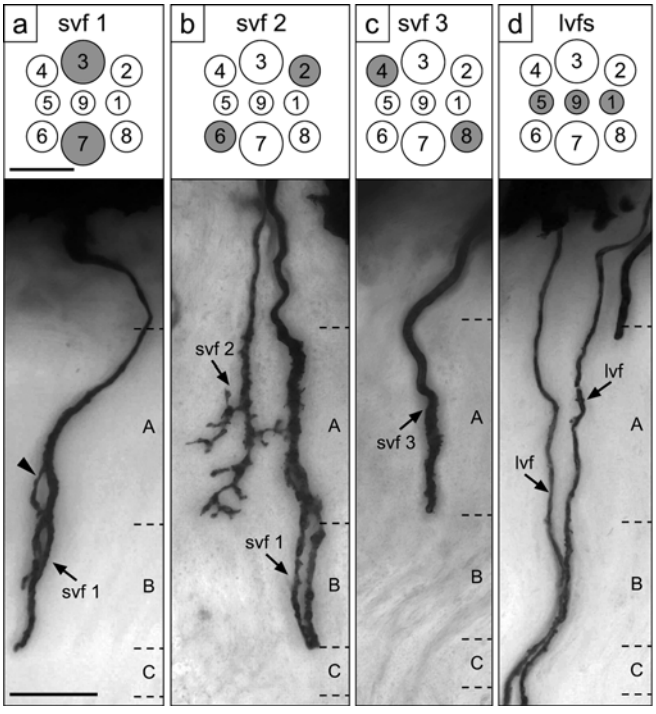

Fig. 3a–d Frontal 25-µm-thick sections, Golgi impregnation. **a** The large retinula cell axons (RCAs) 3 and 7 in the pseudocartridge (the schematic drawing shows the positions of the 9 RCAs within the pseudocartridge) terminate as svf type 1 (*svf 1*) with a bifurcated ending in layer B (*B*) of the lamina. Occasionally, short branches are visible in layer A (*A*, *arrowhead*). **b** The medium RCAs 2 and 6 end as *svf 2* in layer A (*A*), where forked lateral branches reach into neighbouring cartridges. **c** The medium RCAs 4 and 8 terminate as *svf 3* with a blunt ending in layer A (*A*). **d** The small RCAs 1, 5 and 9 form three long visual fibres (*lvf*), which pass the lamina and end in the medulla. The three types of lvf could not be differentiated within the lamina because of the absence of characteristic branching patterns (*C* layer C). *Bars* 5 µm (**a**, *top*), 25 µm (**a**, *bottom*)

At the level of the distal lamina a set of L-fibres joins each cartridge containing the nine RCAs. These can be classified by their specific arborisation patterns within the three layers of the lamina (Fig. 4). According to the description in the honeybee worker (Ribi 1976), L-fibres with branches in all three layers are categorised as being of the L1-type, L-fibres with processes in layers A and B are of the L2-type, L-fibres arborising only in layer B are of the L3-type and L-fibres branching exclusively in layer C are of the L4-type. In the lamina of *M. genalis* females, we discovered a similar L-fibre architecture, with L-fibre type L1 (in two subtypes), L2 and L4. The L-fibre type L3, as described in the worker honeybee, was not present in the lamina of *M. genalis*. Instead, we discovered a new L3-fibre type with wide lateral branches that lie in layer A and that reach into layer B, with a few short branches occasionally in layer B and branches in layer C (Fig. 4d). The cell bodies of all L-fibres are situated in the fenestrated (or cell body) layer distal to the lamina (Fig. 4).

There are two L1-subtypes present in *M. genalis*. L1-a resembles the L1-fibre of the worker honeybee and shows 14-µm-wide forked arborisations throughout layer A. These branches form numerous knots and spines. Layer B features short isolated unilateral processes, whereas in layer C, the branches spread laterally over a range of up to 23 µm and have forked terminations with knots and spines (Fig. 4a). The processes in layers A and B are most probably restricted to the parental cartridge, whereas the branches in layer C can potentially connect to adjacent cartridges. L1-b is similar to L1-a but, in addition to the short branches, also possesses branches in layer A that are up to 46 µm wide and reach into neighbouring cartridges. L1-b also has significantly wider branches (50 µm) in layer C (Fig. 4b). Our classification of L1 into two subtypes is supported by ultra-thin cross sections from proximal layer C, in which seven main axonal stems are visible. This indicates that, other than the three lvf, only four L-fibre types are present in each cartridge (B. Greiner et al., in preparation). No differences between the medullar

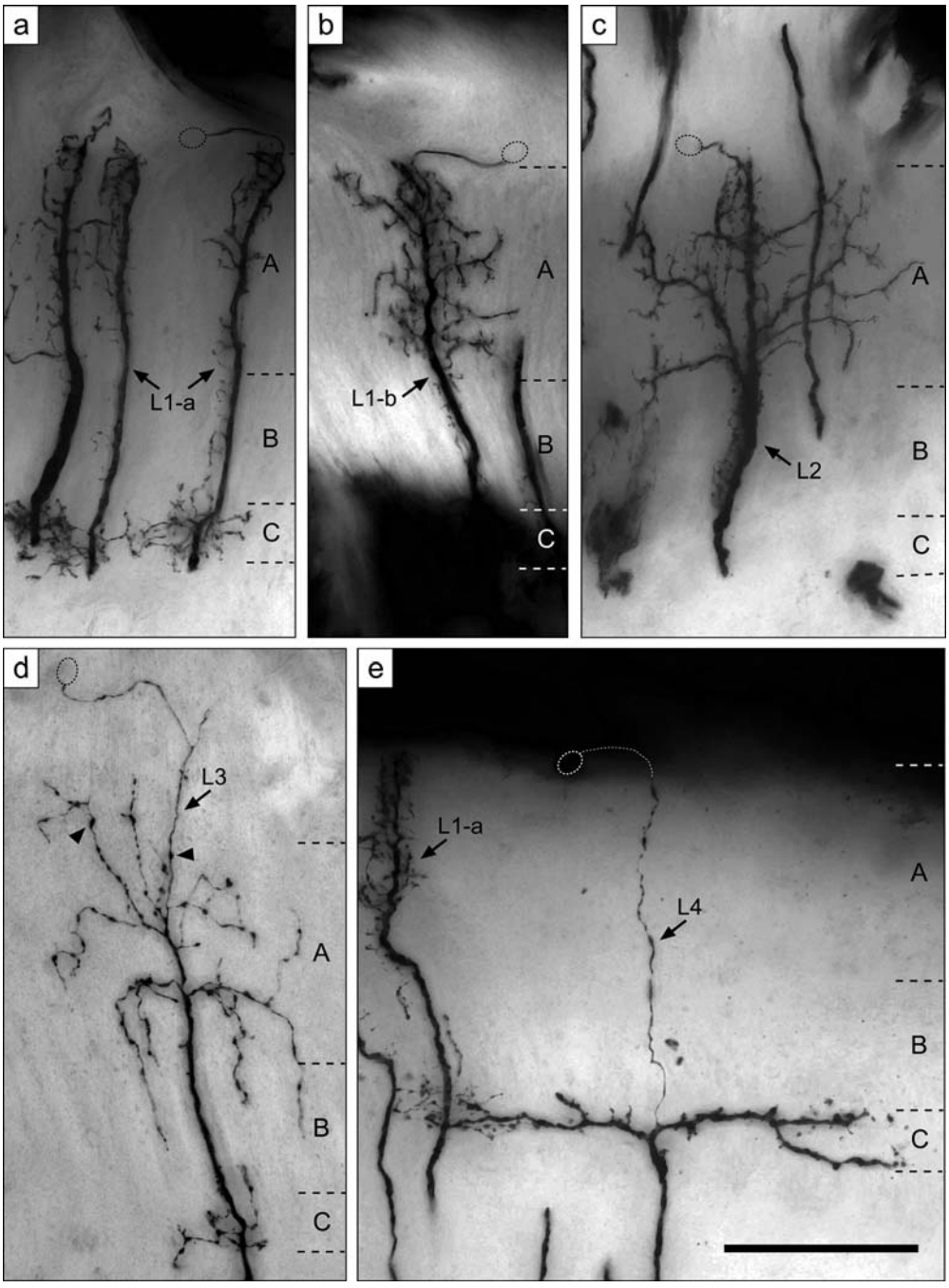

Fig. 4a–e Frontal 25-μm-thick sections, Golgi impregnation. The four types of L-fibres in the lamina of *M. genalis* (*ovals* cell bodies). **a** The L1-a subtype shows narrow branching in lamina layers A (*A*) and B (*B*) and wider branching in layer C (*C*). **b** The L1-b subtype differs mainly in layer A where it shows markedly wider branches than L1-a. **c** L2 has wide arborisation in layer A, a few short branches in layer B and no branches in layer C. **d** L3, with its wide branches in layer A that reach into layer B and few branches in layer C, is a newly discovered L-fibre type unique to the nocturnal bee (*arrowheads* blebs). **e** L4 only shows branches in layer C with an impressive dorso-ventral extent of 125 μm. *Bar* 50 μm (**e**)

terminations of these two subtypes were visible and both subtypes seem to occur across the entire lamina.

The L-fibre type L2 has a prominent axonal stem throughout the lamina. In layer A, L2-fibres show forked processes up to 81 μm wide and orientated dorso-ventrally. These have the potential to contact a wide array of neighbouring cartridges and are covered with short branches, spines and knots. In addition, short branches are also visible along the main axonal trunk. In layer B, unilateral short (5 μm) branches are visible and remain within the cartridge (Fig. 4c). L2-fibres form no branches in layer C.

The newly discovered L3-fibre type in *M. genalis* is characterised by the presence of forked processes, up to 63 μm wide, orientated dorso-ventrally in layer A; these processes reach distally towards the fenestrated layer. Furthermore, branches originating from the main axonal stem in proximal layer A project into layer B. Occasionally, a few short (4 μm) arborisations can be observed in layer B. L3 forms branches up to 20 μm wide in layer C (Fig. 4d). Compared with L2, the branches of the L3-fibre are markedly thinner and form clear blebs along their side-branches (Fig. 4d, arrowheads). These side-branches in layer A have, like those of L2, the potential to span over a wide range of neighbouring cartridges, whereas the

processes in layer C most probably only reach as far as the adjacent cartridges. Whether the wide branches in layer A are oriented only dorso-ventrally or radially is under current investigation (B. Greiner et al., in preparation).

The L4 type found in the nocturnal bee is especially interesting as it exhibits the most extensive horizontal processes in layer C with an impressive width of 125 μm and a dorso-ventral orientation (Fig. 4e). This width is equivalent to the dorso-ventral extent of nine neighbouring cartridges. No branching is present in layers A and B. The main axonal stem is extremely thin all the way from the cell body until it reaches the prominent side-branches in layer C. These branches are symmetrical and possess few spines and knots. The branches do, however, possess isolated short branches. The spines and knots described for RCAs and L-fibres indicate locations of synaptic activity and are under current investigation with the Golgi-electron microscopy technique (Ribi 1983). The dorso-ventral extent of the four L-fibre types in all three layers is shown in Table 1.

In addition to RCAs and L-fibres, various accessory cells (e.g. amacrines, tangentials, centrifugal and Y-cells) can be found within the lamina of *M. genalis*. Because of our limited understanding of their functions, these are not shown or discussed in this study.

Discussion

The lamina of the nocturnal bee *M. genalis* has the same general organisation as that described for the worker honeybee (Ribi 1987b). Each cartridge, representing the basic neural unit within the lamina, contains a particular set of neurons. These include six svf, three lvf and four L-fibre types (Fig. 5). In the nocturnal bee, svf type 2 targets adjacent cartridges and all four types of L-fibres have extensive lateral arborisations that could potentially sum photons from large groups of ommatidia. In the studied diurnal bees (*A. mellifera*, *L. leucozonium*), no branching of RCAs into neighbouring cartridges is visible and only the L2 and L4-fibre types spread laterally. Moreover, the dorso-ventral extent of L2 and L4 in the nocturnal bee is twice as great as that in the diurnal bees. In addition, a new type of L3 has been discovered with particularly wide branches that lie in layer A, project into layer B and have branches in layer C. This unique lateral branching pattern in *M. genalis* can be regarded as a specific adaptation to dim light and is a preliminary strong indication that spatial summation takes place in the lamina.

The discovery of lateral branching in svf type 2 of *M. genalis* is unique for bees and is not present in the diurnal halictid bee *L. leucozonium* or the worker honeybee (Fig. 5). So far the only other known example for lateral branching in a conventional apposition eye is in the cockroach *Periplaneta americana* (Ribi 1977), which is also active at nocturnal light intensities. Furthermore, lateral spreading of RCAs can be found in the nocturnal scorpionfly *Panorpa* sp. (Melzer et al. 1997) and in the backswimmer *N. glauca* (Wolburg-Buchholz 1979), which is also active in dim light. In these animals, laterally branching RCAs are thought to be the nocturnal evolutionary precursors for the neural superposition of higher flies (Nilsson and Ro 1994; Melzer et al. 1997). Recently, laterally branching RCAs have also been found in the diurnal superposition eyes of the skipper butterfly *P. guttata* (Shimohigashi and Tominaga 1999).

With regard to the branching patterns of the four L-fibre types, a clearly larger amount of horizontal branching can be found in the nocturnal bee compared with that of the honeybee (Fig. 5) and the diurnal halictid bee *L. leucozonium* (Fig. 6, Table 1). In *L. leucozonium*, L1 has short branches in all three layers that are restricted to the parental cartridge (Fig. 6a). L2 possesses short branches that stay within the cartridge and wide horizontal branches that lie in layer A and that have the potential to contact neighbouring cartridges (Fig. 6b). Short branches are also seen in layer B, whereas no branches are formed in layer C. L3 only forms branches in layer B (Fig. 6c); these are restricted to the parental cartridge. L4 branches exclusively in the C-layer with a dorso-ventral extent of 52 μm (Fig. 6d). L4 therefore has the potential to contact several neighbouring cartridges in layer C (for the difference in the dorso-ventral extent between the diurnal and nocturnal halictid bee, see Table 1).

As cartridge sizes differ between the different bee species, we transformed the lateral range of branching from its actual width in micrometers (Table 1) to the number of neighbouring cartridges that can potentially be covered in the dorso-ventral orientation (Table 2). This allows us to compare the differences in width between the L-fibres of the various bee species studied.

In both the diurnal bees discussed, the L-fibre type L1 is restricted to the parental cartridge in all three lamina layers. In *M. genalis*, however, subtype L1-b branches into four adjacent cartridges in layer C in addition to wider dorso-ventral arborisations in layer A. The potential for spatial summation of photons is therefore present in L1-b of the nocturnal bee. L1 fibres in the honeybee form

Table 1 Dorso-ventral extent (in μm) of the four L-fibres in the nocturnal *M. genalis* and the diurnal *L. leucozonium* (*A, B, C* three layers of the lamina, *0* no branches). Note that the data for L1 in *M. genalis* are given for L1-a (*left column*) and L1-b (*right column*)

L-fibre	Layer	*M. genalis*		*L. leucozonium*
L1 a/b	A	16	46	8
	B	6	7	5
	C	27	50	11
L2	A	81		45
	B	5		10
	C	0		0
L3	A	63		0
	B	4		10
	C	20		0
L4	A	0		0
	B	0		0
	C	125		52

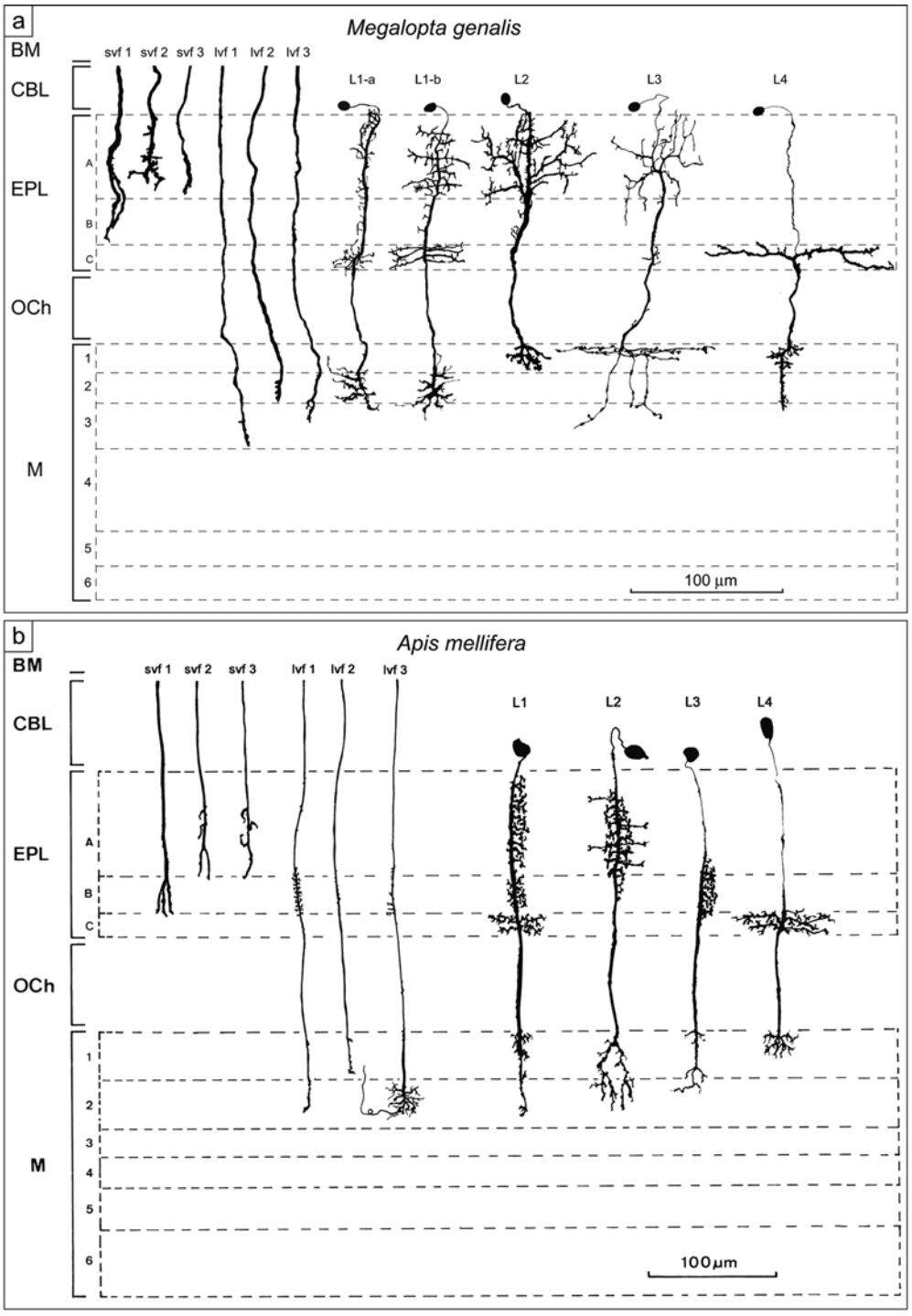

Fig. 5 Comparison of the retinula cell axon (svf 1–3, lvf 1–3) and L-fibre types (L1–L4) of *M. genalis* females (**a**) and the worker honeybee *A. mellifera* (**b**; adapted from Ribi 1975b). In *M. genalis* (**a**), sfv 2 targets adjacent cartridges and all four L-fibres have wide lateral branches up to twice as great as in *A. mellifera* (**b**). *BM* Basement membrane, *CBL* cell body layer, *EPL* external plexiform layer, *OCh* outer chiasm, *M* medulla, *A* layer A, *B* layer B, *C* layer C, *svf* short visual fibre, *lvf* long visual fibre, *L* L-fibre

synapses with L4 in layer C (Ribi 1981); this might also be the case in *M. genalis*. The two subtypes of L1 could indicate the presence of different types of functional cartridges in the lamina. L2 branches in the same layers (A and B) in all three bees studied. Nevertheless, it spreads significantly more widely in the dorso-ventral direction in *M. genalis*. Compared with just two neighbouring cartridges in the worker honeybee and three in the diurnal halictid bee, L2 has a dorso-ventral width of five neighbouring cartridges in the nocturnal bee. In the diurnal bees, L3 only branches in layer B and is restricted to the parental cartridge, whereas *Megalopta* possesses a new type of L3. These L-fibres have extensive branches in layer A (5 dorso-ventral cartridges), some of which terminate in layer B, and also have arborisations in layer C (2 dorso-ventral cartridges). In the worker honeybee, L3 connects to svf type 1 in layer B and is thought to function as a green monochromatic small-band neuron (Ribi 1981, 1987b). The new L3-type in *M. genalis* could, however, potentially sum visual signals from all three svf types and

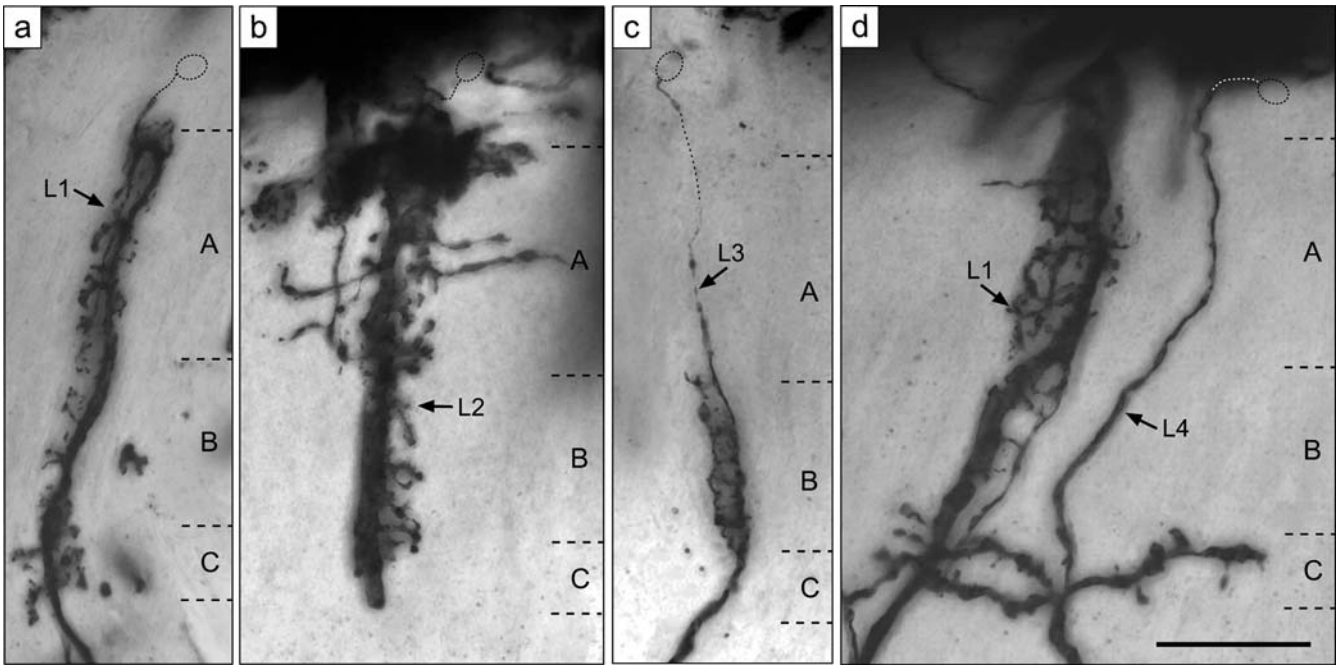

Fig. 6a–d The four types of L-fibres in the lamina of *L. leucozonium* (25-µm-thick frontal sections, Golgi impregnation; *ovals* cell bodies). **a** L1 shows narrow branching that lies in lamina layers A (*A*), B (*B*) and C (*C*) and that stays within the parental cartridge. **b** L2 has lateral arborisations in layer A reaching into neighbouring cartridges, short branches in layers A and B and no branches in layer C. **c** L3 has short branches only in layer B. **d** L4 branches only in layer C and reaches into neighbouring cartridges. *Bar* 25 µm (**d**)

Table 2 Dorso-ventral extent of the four L-fibres of the three bee species shown as the number of neighbouring cartridges that this width covers dorso-ventrally (*A, C* layers in the lamina, *0* no spreading over neighbouring cartridges). The parental cartridge of the main axonal stem is not included in the numbers. Values for *A. mellifera* are from Ribi (1976). Note that the data for L1 in *M. genalis* are given for L1-a (*left column*) and L1-b (*right column*)

L-fibre	Layer	*M. genalis*		*L. leucozonium*	*A. mellifera*
L1 a/b	A	0	3	0	0
	C	2	4	0	0
L2	A	5		3	2
L3	A	5		0	0
	C	2		0	0
L4	C	8		4	4

contact other L-fibres. In all the bee species examined, the L4-fibre type branches exclusively in layer C. Compared with the worker honeybee and the diurnal halictid bee, in which the dorso-ventral branching spreads over four neighbouring cartridges, the horizontal branches of L4 in the nocturnal bee are twice as wide and instead span over eight cartridges. In the worker honeybee, L4 connects to the L1 fibre type and is thought to modify the L1 output in response to sudden intensity fluctuations (Ribi 1981, 1987b). The same situation might be present in the nocturnal bee in which L4 might widen the visual field in dim light and, with its wide extent, sum the visual signals of a significantly larger group of ommatidia. The lateral axonal spreading in the medullar terminations of L1 and, more significantly, of L3 should also be noted. Additional spatial summation might thus occur in the second optic ganglion, viz. the medulla. There are limitations to the Golgi-light microscopy technique for quantifying the actual number of cartridges targeted. However, by using the Golgi-electron microscopy technique for serial cross sections (Ribi 1983), the three-dimensional branching pattern of the L-fibres can be revealed. Preliminary results indicate that the number of cartridges targeted by the laterally branching neurons in *M. genalis* are significantly greater than their dorso-ventral extents imply (B. Greiner et al., in preparation).

By comparing the L-fibre branching patterns of the nocturnal bee with those of other insects possessing apposition eyes with fused rhabdoms, it is noticeable that, in strictly diurnal species such as the dragonfly *Sympetrum* sp. (Meinertzhagen and Armett-Kibel 1982) and the cabbage butterfly *Pieris brassicae* (Strausfeld and Blest 1970), the branches of all L-fibre types are confined to the parental cartridge. In the lamina of the diurnal desert ant *Cataglyphis bicolor*, one of three L-fibres reaches into adjacent cartridges (Meyer 1979) and, in the locust *Schistocerca gregaria*, which is active both during the day and at night, one of six described L-fibre types extends over several cartridges (Nowel and Shelton 1981). Even the diurnal European worker honeybee has a limited ability to see in dim light (Warrant et al. 1996), possibly because two of their four L-fibres span a number of neighbouring cartridges. This may also explain why an African race of the honeybee *A. mellifera* and another honeybee, the Asian *Apis dorsata*, can continue to forage on nights when a half-full moon or larger is present in the sky (Fletcher 1978; Dyer 1985). In addition to the

spreading RCAs previously discussed, all L-fibre types in the nocturnal cockroach *Periplaneta americana* show wider lateral branching (Ribi 1977). Nocturnal insects possessing highly sensitive superposition eyes (Nilsson 1989) also have a larger portion of L-fibres with wide lateral spreading. In the strictly nocturnal firefly *Phausis splendidula*, three out of five L-fibres spread to several neighbouring cartridges (Ohly 1975) and, in the nocturnal hawkmoth *Sphinx ligustri*, all L-fibres show lateral extensions into neighbouring cartridges (Strausfeld and Blest 1970). A detailed comparison of the laminae of various insect species can be found in Kral (1987). Thus, there seems to be a strong correlation between the light intensity normally experienced by an insect and the extent to which cells in the lamina have laterally spreading branches, with insects active in dimmer light having wider branches. These observations support our current hypothesis that spatial summation is important for vision at low light intensities and that it is most likely mediated by laterally branching RCAs and/or L-fibres.

We have shown that svf type 2 and all four L-fibre types have a significantly wider branching pattern in the nocturnal *M. genalis* than in the two diurnal bees *A. mellifera* and *L. leucozonium*. L-fibres in particular are probably to receive visual input from a considerably larger number of ommatidia. Golgi-electron microscopy studies of the three-dimensional branching patterns of these cells should shed further light on the extent to which these cells are involved in spatial summation (B. Greiner et al., in preparation) and the way in which the cellular architecture of the lamina enhances nocturnal vision in the halictid bee *M. genalis*.

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