Acta Biologica Hungarica 55 (1–4), pp. 81–89 (2004)

NEUROBIOLOGY OF POLARIZATION VISION IN THE LOCUST *SCHISTOCERCA GREGARIA**

U. HOMBERG,[1,2]** SABINE HOFER,[1] MARTINA MAPPES,[1] H. VITZTHUM,[2] K. PFEIFFER,[1] S. GEBHARDT,[1] MONIKA MÜLLER[2] and AGNES PAECH[2]

[1] Department of Biology, Animal Physiology, University of Marburg, D-35032 Marburg, Germany
[2] Institute for Zoology, University of Regensburg, D-93040 Regensburg, Germany

(Received: August 31, 2003; accepted: December 1, 2003)

The polarization pattern of the blue sky serves as an important reference for spatial orientation in insects. To understand the neural mechanisms involved in sky compass orientation we have analyzed the polarization vision system in the locust *Schistocerca gregaria.* As in other insects, photoreceptors adapted for the detection of sky polarization are concentrated in a dorsal rim area (DRA) of the compound eye. Stationary flying locusts show polarotactic yaw-torque responses when illuminated through a rotating polarizer from above. This response is abolished after painting the DRAs. Central stages of the polarization vision system, revealed through tracing studies, include dorsal areas in the lamina and medulla, the anterior lobe of the lobula, the anterior optic tubercle, the lateral accessory lobe and the central complex. Physiological analysis of polarization-sensitive (POL) neurons has focussed on the optic tubercle and on the central complex. Each POL neuron was maximally excited at a certain e-vector (Φ_{max}) and was maximally inhibited at an e-vector perpendicular to Φ_{max}. The neurons had large visual fields, and many neurons received input from both eyes. The neuronal organization of the central complex suggests a role as a spatial compass within the locust brain.

Keywords: Polarization vision – insect brain – central complex – compass navigation – locust

INTRODUCTION

The polarization pattern of the blue sky serves as an important reference for spatial orientation in insects and other animals [16, 17]. Work particularly on the honeybee and the desert ant *Catglyphis* showed that small patches of blue sky suffice to guide these animals back to their nests [14, 18]. Photoreceptors adapted for the detection of the sky polarization pattern are concentrated in a specialized dorsal rim area (DRA) of the compound eye and have been detected in various insect species inluding honeybees, ants, crickets and flies [9]. Polarization-sensitive (POL) interneurons have been characterized especially in the optic lobe of the cricket [8, 10]. All neurons

* Presented at the 10th ISIN Symposium on Invertebrate Neurobiology, July 5–9, 2003, Tihany, Hungary.

** Corresponding author; e-mail: homberg@staff.uni-marburg.de

0236-5383/$ 20.00 © 2004 Akadémiai Kiadó, Budapest

show polarization-opponency, i.e. e-vectors eliciting maximal tonic inhibition were oriented perpendicularly to e-vectors eliciting maximal tonic excitation (Φ_{max}). The neurons receive monocular visual input, have eccentric visual fields and do, therefore, not provide unequivocal compass information independent of solar elevation. Anatomically, most of the recorded neurons in the cricket brain are heterolateral interneurons with arborizations in both medullae of the optic lobes, but without projections to the median protocerebrum [8].

In order to identify higher brain areas involved in sky compass orientation we have analyzed the polarization vision system in another orthopteroid insect, the desert locust *Schistocerca gregaria*. We show that the locust compound eye has a prominent dorsal rim area that is essential for polarotactic yaw-torque responses in tethered flight. Tracing studies identified several protocerebral processing stages in the polarization vision pathway, which were partly characterized electrophysiologically.

MATERIAL AND METHODS

Experiments were performed on adult locusts *(Schistocerca gregaria)* obtained from a crowded laboratory colony. For behavioral analysis of polarotaxis, animals were mounted on a rod connected to a yaw-torque meter [13]. Stationary flight was induced by laminar frontal wind of 3.0 m/s. Yaw-torque responses were recorded while a linear polarization filter above the animal, illuminated from the back, was slowly rotated (Polaroid HN 38S; visual angle 87.3°; irradiance 25.8 $\mu W/cm^2$; speed 5.3°/s).

Ultrastructural studies on the DRA of the locust compound eye were performed through conventional electron microscopic techniques as described by Homberg and Paech [5]. Horseradish peroxidase was injected into the DRA to trace the projections of DRA photoreceptor axons. Central processing stages in the polarization vision pathway were revealed through pressure-injection of dextran-biotin (lysine-fixable, 3,000 MW; Molecular Probes) into the dorsal rim of the medulla or into the anterior optic tubercle of the median protocerebrum [7].

Intracellular recordings combined with Lucifer Yellow or Neurobiotin dye injection were obtained using glass micropipette electrodes as described by [15]. Neurons were stimulated from dorsal with light directed through a polarization filter (Polaroid HN38S; visual angle 2–8°; irradiance 5–20 $\mu W/cm^2$). The orientation of the polarizer changed either stepwise (stationary stimuli) or continuously (rotating polarizer). Orientations of Φ_{max} and Φ_{min} were calculated by fitting $\sin^2$-functions to the e-vector response plots of the neurons [15].

RESULTS

Morphological specializations of the DRA

In the compound eye of the locust, a distinct dorsal rim area (DRA) can be identified even with the unaided eye. The DRA shows uniform dark pigmentation which contrasts markedly from the light brown and vertically striped appearance of the rest of the eye. Ultrastructural studies show that DRA photoreceptors are highly adapted for polarization vision [5]. In contrast to the regular hexagonal appearance of facets in lateral areas of the compound eye, facets in the DRA have an irregular shape. The corneae contain pore canals and have a cloudy appearance probably from light scattering. Screening pigment in the region of the dioptric apparatus is missing suggesting that retinula cells have strongly expanded visual fields. Ommatidia differ considerably in size, shape, and microvilli orientation from those in the rest of the eye. Rhabdoms in the DRA are only one-third of their length in the rest of the eye, but their cross-sectional area is four times as large [5]. Each rhabdom contains two sets of orthogonally oriented mircovilli, one formed by the microvilli of retinula cell 7, and the other, by retinula cells 1, 2, 5, 6 and 8 (Fig. 1). Throughout the length of the rhabdom, the microvilli of each set are highly aligned, which should result in high polarization sensitivity. In contrast, microvilli of retinula cells 3 and 4 are irregular and make only a minor contribution to the rhabdom. Their polarization sensitivity is

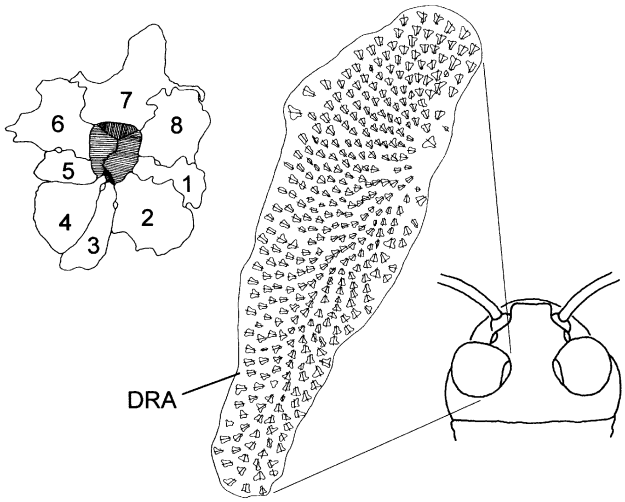

Fig. 1. Distribution and orientation of rhabdoms in the dorsal rim area (DRA) of the locust compound eye. The DRA consists of about 400 ommatidia. Rhabdoms are oriented in a fan-shaped pattern as indicated by the orientation of microvilli in retinula cell 7 (line across each rhabdom). In each ommatidium (schematic cross section, upper left) microvilli of retinula cell 7 are perpendicular to the microvilli of cells 1, 2, 5, 6 and 8. Rhabdomeres of cells 3 and 4, in contrast, are short and show irregular orientation of microvilli. Adapted from [5]

expected to be low. The DRA consists of about 400 ommatidia in total which are arranged in a fan-like manner (Fig. 1). This organization ensures that all microvilli orientations are represented in different parts of the DRA.

Polarotaxis in tethered flight

In order to demonstrate polarization vision in the locust, we have developed a behavioral assay for polarotaxis in stationary flying locusts. Animals mounted on a rod were flown in a laminar air current. A slowly rotating polarizer induced periodic changes in yaw-torque corresponding to the 180° periodicity of the stimulus (Fig. 2A). Control experiments with a rotating diffusor, a rotating intensity gradient, and a stationary polarizer showed that the response is not induced by intensity gradients in the stimulus. Polarotaxis was abolished after painting the DRAs of the compound eyes black (Fig. 2B), but remained unchanged after painting the eyes except the DRAs (Mappes and Homberg, submitted). The results demonstrate polarization vision in the locust and show that polarized light eliciting the polarotactic behavior is perceived only by the DRA of the compound eye.

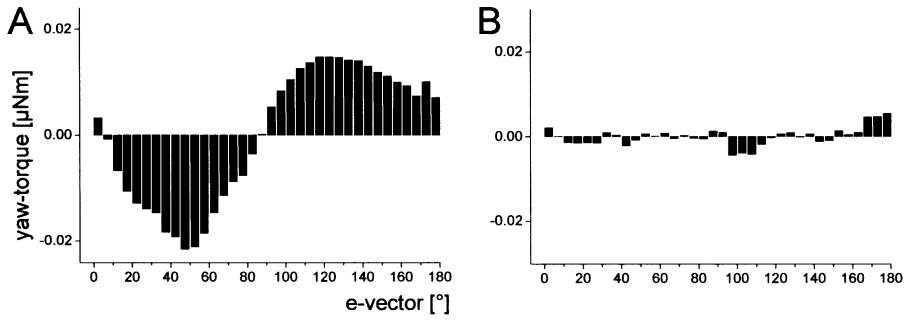

Fig. 2. Yaw-torque responses of tethered flying locusts stimulated dorsally through a slowly rotating polarizer (visual angle, 87.3°, rotatory speed 5.3°/sec). Data are means from four 180° rotations. Positive and negative yaw-torque values correspond to right- and left-hand turning. A: The yaw-torque response changes periodically corresponding to the 180° periodicity of the rotating polarizer. B: DRA's painted black. Yaw-torque reactions are small and irregular and do not correspond to the 180° periodicity of the stimulus

The polarization vision pathway in the locust brain

Tracer injections into the DRA of the compound eye, into the dorsal rim of the medulla, and into the anterior optic tubercle revealed central processing stages of the polarization vision pathway (Fig. 3). Photoreceptors from the DRA of the compound

eye project axons to distinct dorsal areas in the lamina and medulla, the first and second-order visual neuropil in the locust optic lobe [5]. Dextran injections into the dorsal rim of the medulla showed that line tangentials of the medulla with ramifications in the dorsal rim of the medulla have axonal projections through the anterior lobe of the lobula, and via the anterior optic tract into the lower unit of the anterior optic tubercle [7]. Dextran injections into the anterior optic tubercle, finally, demonstrated that neurons from the lower unit of the tubercle project to small areas in the lateral accessory lobe, termed the lateral triangle and the median olive. These areas are selectively innervated by dendritic processes of tangential neurons of the lower division of the central body [11]. Neuronal processing stages in the polarization vision pathway, therefore, include dorsal rim areas of the lamina and medulla, the anterior lobe of the lobula, the lower division of the anterior optic tubercle, the lateral triangle and median olive of the lateral accessory lobe, and the lower division of the central body [7]. Connections to descending pathways and possible parallel connections to other brain areas have not yet been elucidated.

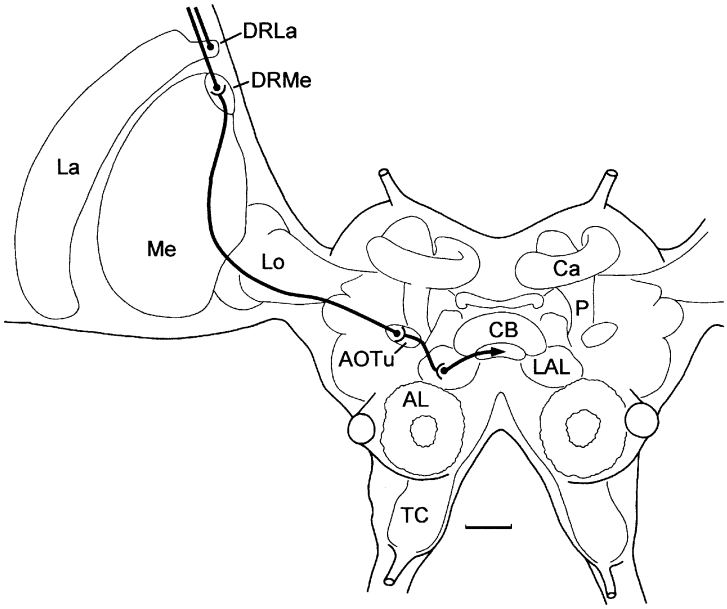

Fig. 3. Polarization vision pathway in the brain of the locust, as revealed through tracer injections into the dorsal rim area of the eye, into the dorsal rim of the medulla (DRMe), into the anterior optic tubercle (AOTu), and through dye injection into single neurons of the central body (CB). Central processing stages for polarized light include the dorsal rim of the lamina and medulla (DRLa, DRMe), the anterior lobe of the lobula (Lo), the AOTu, the lateral accessory lobe (LAL), and the lower division of the central body. AL, antennal lobe; Ca, P, calyx and pedunculus of the mushroom body; La, lamina; Me, medulla; TC, tritocerebrum. Scale bar = 200 μm

Polarization-sensitive interneurons

Intracellular recordings from polarization-sensitive (POL) interneurons in the locust brain have largely focussed on neurons of the anterior optic tubercle and central body (Fig. 4). In the anterior optic tubercle, all neurons of the lower unit were sensitive to polarized light [12]. Most recordings were obtained from heterolateral interneurons, connecting the tubercle of the right and left brain hemisphere (Fig. 4A). In all recordings, e-vectors that elicited maximum spike activity (Φ_{max}) were perpendicular to e-vectors eliciting no response or maximal inhibitory responses (Φ_{min}). Interestingly, all neurons were tonically inhibited by dorsal unpolarized light. Antagonistic responses to perpendicular e-vectors were also found in neurons of the central complex. Polarization sensitivity was found in four types of columnar and in three types of tangential neuron. All neurons, except one type of columnar neuron of the upper division of the central body had ramifications in the lower division of the central body, in the median olive, or in the lateral triangle of the lateral accessory lobe (Fig. 4B) [7]. Orientations of Φ_{max} in central-complex neurons are broadly distributed from 0–180° without obvious correlation with innervated layers or columns of the central body (Fig. 4C, D).

DISCUSSION

Through a combination of behavioral experiments, anatomical and physiological studies, we have analyzed the polarization vision system in the locust *Schistocerca gregaria*. Polarotaxis to dorsally presented polarized light depends on photoreceptors in the DRA of the locust eye, suggesting that this eye region is essentially involved in sky compass navigation as demonstrated for other insect species [9, 10]. The DRA of the compound eye shows all specializations which are characteristic of polarization analyzers in many insect species [9], and recordings from DRA photoreceptors [1] have directly demonstrated high polarization sensitivity (PS ≈ 6). These authors, furthermore, showed that the spectral sensitivity of polarization-sensitive DRA photoreceptors has a maximum in blue light, similar to the spectral sensitivity of DRA photoreceptors in crickets [1, 9].

Through tract tracing studies and intracellular dye injections, we identified several stages in the central processing of polarized light signals from the DRA, including the anterior lobe of the lobula, the lower unit of the anterior optic tubercle, the lateral accessory lobe, and parts of the central complex. Several features of the central complex suggest that this brain area serves a role as an internal sky compass and thus plays a cardinal role in spatial navigation. The broad distribution of Φ_{max} orientations in the central complex implicates that, depending on the orientation of the locust relative to the sky polarization pattern, different populations of neurons are active and might, thus, encode for head direction of the animal relative to the solar azimuth. The central complex is directly connected through the lateral accessory lobes to descend-

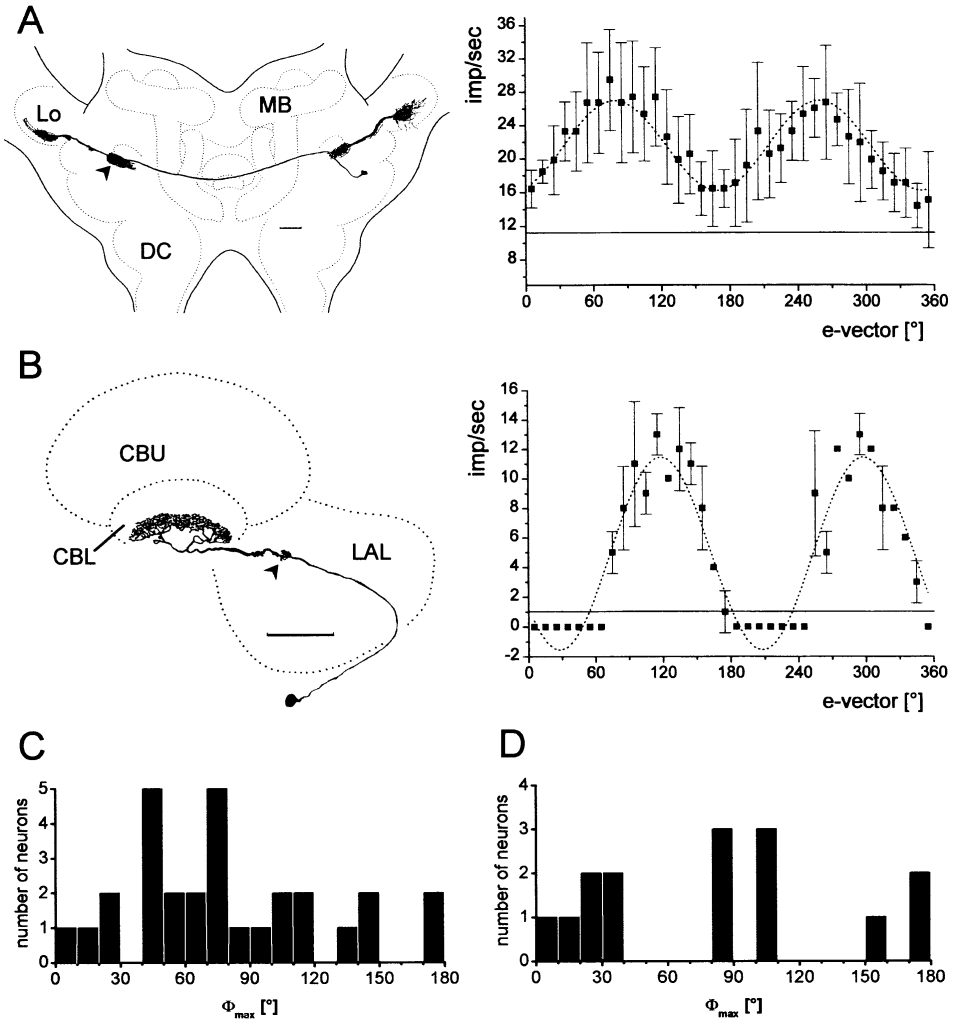

Fig. 4. A, B: Frontal reconstructions (left) and e-vector-response plots (right) of a polarization-sensitive interneuron from the anterior optic tubercle (A), and from the central body (B). A: Heterolateral interneuron with ramifications in the anterior lobe of the lobula (Lo) and in the lower unit of the anterior optic tubercle (arrowhead) of both brain hemispheres. The neuron is maximally excited at an e-vector of 79° (Φ_{max}). Solid line indicates background activity. B: Tangential neuron with ramifications in the median olive of the lateral accessory lobe (arrowhead) and tangential processes in the lower division of the central body (CBL). The neuron is maximally excited at an e-vector of 117.7° (Φ_{max}) and is maximally inhibited at an e-vector perpendicular to Φ_{max}. Solid line indicates background activity. C, D: Distribution of Φ_{max}-orientations of 29 tangential neurons (C) and 15 columnar neurons (D) of the central body. CBU, upper division of the central body; DC, deutocerebrum; LAL, lateral accessory lobe; MB, mushroom body. Scale bars = 100 µm. A and B adapted from [15]

ing interneurons to thoracic flight motor centers [3] and could affect flight direction through the balance of signals in right and left descending neurons. Finally, the accessory medulla of the locust [4, 6], the site of the internal circadian clock in the brain of cockroaches and flies [2] is closely connected to the protocerebral bridge of the central complex. The physiological properties of this connection have not been explored, but an input from the circadian clock to the internal sky compass is required to explain time compensation in sky compass navigation for maintenance of navigational direction during daily changes in solar position.

ACKNOWLEDGEMENT

This work was supported by DFG grants HO 950-4, HO 950-13 and HO 950-14 to U.H.

REFERENCES

1. Eggers, A., Gewecke, M. (1993) The dorsal rim area of the compound eye and polarization vision in the desert locust *(Schistocerca gregaria)*. In: Wiese, K., Gribakin, F. G., Popov, A. V., Renninger, G. (eds) *Sensory systems of arthropods*. Birkhäuser, Basel, pp. 101–109.
2. Helfrich-Förster, C., Stengl, M., Homberg, U. (1998) Organization of the circadian system in insects. *Chronobiol. Int. 15*, 567–594.
3. Homberg, U. (1994) Flight-correlated activity changes in neurons of the lateral accessory lobes in the brain of the locust *Schistocerca gregaria*. *J. Comp. Physiol. A 175,* 597–610.
4. Homberg, U., Würden, S. (1997) Movement-sensitive, polarization-sensitive, and light-sensitive neurons of the medulla and accessory medulla of the locust, *Schistocerca gregaria*. *J. Comp. Neurol. 386,* 329–346.
5. Homberg, U., Paech, A. (2002) Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. *Arthropod Struct. Dev. 30,* 271–280.
6. Homberg, U., Würden, S., Dircksen, H., Rao, K. R. (1991) Comparative anatomy of pigment-dispersing hormone-immunoreactive neurons in the brain of orthopteroid insects. *Cell Tissue Res. 266,* 343–357.
7. Homberg, U., Hofer, S., Pfeiffer, K., Gebhardt, S. (2003) Organization and neural connections of the anterior optic tubercle in the brain of the locust, *Schistocerca gregaria*. *J. Comp. Neurol. 462,* 415–430.
8. Labhart, T., Petzold, J. (1993) Processing of polarized light information in the visual system of crickets. In: Wiese, K., Gribakin, F. G., Popov, A. V., Renninger, G. (eds) *Sensory systems of arthropods*. Birkhäuser, Basel, pp. 158–168.
9. Labhart, T., Meyer, E. P. (1999) Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech. 47,* 368–379.
10. Labhart, T., Meyer, E. P. (2002) Neural mechanisms in insect navigation: polarization compass and odometer. *Curr. Opin. Neurobiol. 12,* 707–714.
11. Müller, M., Homberg, U., Kühn, A. (1997) Neuroarchitecture of the lower division of the central body in the brain of the locust *Schistocerca gregaria*. *Cell Tissue Res. 288,* 159–176.
12. Pfeiffer, K., Homberg, U. (2003) Neurons of the anterior optic tubercle of the locust *Schistocerca gregaria* are sensitive to the plane of polarized light. In: Elsner, N., Zimmermann, H. (eds) *The neurosciences from basic research to therapy*. Thieme, Stuttgart, pp. 567–568.
13. Preiss, R., Gewecke, M. (1991) Compensation of visually simulated wind drift in the swarming flight of the desert locust (*Schistocerca gregaria*). *J. Exp. Biol. 157,* 461–481.

14. Rossel, S. (1993) Navigation by bees using polarized skylight. *Comp. Biochem. Physiol. 104 A,* 695–708.
15. Vitzthum, H., Müller, M., Homberg, U. (2002) Neurons of the central complex of the locust *Schistocerca gregaria* are sensitive to polarized light. *J. Neurosci. 22,* 1114–1125.
16. Wehner, R. (1984) Astronavigation in insects. *Annu. Rev. Entomol. 29*, 277–298.
17. Wehner, R. (1992) Arthropods. In: Papi, F. (ed.) *Animal homing.* Chapman and Hall, London, pp. 45–144.
18. Wehner, R. (1997) The ant's celestial compass system: spectral and polarization channels. In: Lehrer, M. (ed.) *Orientation and communication in arthropods*. Birkhäuser, Basel, pp. 145–185.