

**Polarization vision: Behavioral studies in
tethered flying desert locusts,
*Schistocerca gregaria***

Polarisationssehen: Verhaltensversuche mit
fixiert fliegenden Wüstenheuschrecken,
Schistocerca gregaria



Dissertation

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ERKLÄRUNG:**Eigene Beiträge und veröffentlichte Teile der Arbeit**

Laut Promotionsordnung der Philipps-Universität Marburg vom 29.11.1989 müssen bei den Teilen der Dissertation, die aus gemeinsamer Forschungsarbeit entstanden, die individuellen Leistungen des Doktoranden deutlich abgrenzbar sein. Dies wird für die Kapitel I-III im Folgenden näher erläutert.

Kapitel I: Behavioral evidence of polarization vision in tethered flying locusts

- Ausarbeitung, Durchführung und Auswertung aller Experimente durch die Autorin. Die Entwicklung der Auswertungsmethode wurde in Zusammenarbeit mit Dr. Jan Dolzer vorgenommen.
- Verfassen der Veröffentlichung in Zusammenarbeit mit Prof. Dr. Uwe Homberg. Das Kapitel entspricht der Veröffentlichung.
- Veröffentlichung: Mappes M and Homberg U (2004) *J Comp Physiol A* 190: 61-68

Kapitel II: Surgical lesion of the anterior optic tract abolishes polarotaxis in tethered flying locusts, *Schistocerca gregaria*

- Ausarbeitung, Durchführung und Auswertung der meisten Experimente durch die Autorin. Einige Kontrollexperimente wurden von Iris Friedrich und Bianca Backasch im Rahmen von Laborpraktika durchgeführt.
- Verfassen der Veröffentlichung in Zusammenarbeit mit Prof. Dr. Uwe Homberg. Das Kapitel entspricht dem akzeptierten Manuskript.
- Veröffentlichung: Mappes M and Homberg U (2006) *J Comp Physiol A*, akzeptiert am 18. August 2006

Kapitel III: Polarotactic flight behavior in desert locusts: Field studies underneath natural sky light

- Auswertung, Durchführung und Auswertung aller Experimente durch die Autorin. Für die Auswertung wurde ein *Spike2*-Skript von Keram Pfeiffer erstellt und von der Autorin verwendet.

Weiterhin ist die Verfasserin Co-Autorin der Veröffentlichung:

- Homberg U, Hofer S, Mappes M, Vitzthum H, Pfeiffer K, Gebhardt S, Müller M, Paech A (2004) Neurobiology of polarization vision in the locust *Schistocerca gregaria*. *Acta Biol Hung* 55: 81-89

Die Erstellung der Dissertation in englischer Sprache wurde am 24. August 2006 vom Dekan des Fachbereiches Biologie der Philipps-Universität Marburg genehmigt.

ZUSAMMENFASSUNG

Viele Insekten sind in der Lage, sich am Polarisationsmuster des blauen Himmels zu orientieren und mit seiner Hilfe zu navigieren. Dies ist von besonderer Bedeutung, wenn die Sonne z.B. durch Wolken verdeckt wird, aber dennoch Flecken blauen Himmels sichtbar bleiben. Das Muster entsteht durch die Streuung des Sonnenlichts an Molekülen in der oberen Erdatmosphäre und ist durch konzentrische Anordnung der *E*-Vektoren um die Sonne gekennzeichnet. Da das Polarisationsmuster vom Sonnenstand abhängt, wandert es mit dieser im Tagesverlauf über den Himmel. Die Nutzung des Musters als Orientierungshilfe wurde u.a. für Bienen, Ameisen, Fliegen und Grillen nachgewiesen. Detektiert wird das Himmelspolmuster über einen speziellen Augenteil, die dorsale Randregion (dorsal rim area, DRA). Diese Augenregion ist allen zur Navigation nach polarisiertem Licht fähigen Insekten eigen und zeigt einige morphologische Spezialisierungen.

In den letzten Jahren wurden große Fortschritte in der Erforschung der Polarisationssehbahn im Gehirn der Wüstenheuschrecke *Schistocerca gregaria* gemacht. So konnte man zeigen, dass eine visuelle Bahn von der DRA ausgehend über den dorsalen Rand der Lamina und der Medulla zum anterioren optischen Tuberkel zieht. Von dort projizieren Interneurone weiter über das laterale Dreieck zum Zentralkomplex, der eine Funktion als Lokomotionszentrum innehat. Ungeklärt blieb jedoch bisher, ob *S. gregaria* auch im Verhalten polarisationsabhängige Reaktionen (Polarotaxis) zeigt. Im ersten und zweiten Abschnitt der vorliegenden Arbeit wurde eine Methode entwickelt und etabliert, mit der Polarotaxis bei stationär fliegenden Heuschrecken untersucht werden kann. Hierbei können Drehrichtungsänderungen (yaw-torque) der Tiere über einen Drehmomentmesser aufgezeichnet und somit vom polarisierten Licht abhängige Verhaltensantworten analysiert werden. Im dritten Abschnitt der Arbeit wurden Verhaltensexperimente im Freiland durchgeführt. Hierfür wurde eine bereits in Versuchen mit Monarchfaltern etablierte Methode benutzt, um das Verhalten von Heuschrecken unter natürlichen Lichtbedingungen zu studieren.

Ziel der Dissertation waren der Nachweis polarotaktischer Orientierung im Flug der Wüstenheuschrecke, die Lokalisierung des hierfür verantwortlichen Augenteils, Untersuchungen zur Beteiligung des anterioren optischen Traktes an der

Polarisationssehbahn sowie die Untersuchung des Flugverhaltens im Freiland unter natürlichen Lichtbedingungen. Die vorliegende Arbeit ist in drei Kapitel gegliedert, deren Inhalte im Folgenden zusammengefasst werden:

Kapitel I:

Verhaltensanalyse des Polarisationssehens fixiert fliegender Heuschrecken

Behavioral analysis of polarization vision in tethered flying locusts

Honigbienen und Wüstenameisen sind in der Lage, auf Futtersuchekursionen mit Hilfe des Himmelpolarisationsmusters zu navigieren und trotz eventuell sehr verschlungener Wege einen direkten Weg zurück zum Stock bzw. Nest einzuschlagen. Beide Spezies besitzen die oben beschriebene DRA, welche auch in der Heuschrecke vorhanden ist. Dies führte zu der Vermutung, dass auch Heuschrecken in der Lage sind, polarisiertes Licht zu detektieren und vielleicht auch zur Orientierung im Flug zu verwenden. Für Larven des 5. und 6. Larvenstadiums konnten bereits polarisationsabhängige Richtungsänderungen im Lauf nachgewiesen werden, doch Verhaltensversuche im Flug wurden bisher noch nicht durchgeführt. Die ventral an einem Drehmomentmesser fixierten Tiere zeigten periodische Drehrichtungsänderungen in Abhängigkeit von der *E*-Vektor-Orientierung eines rotierenden Polarisationsfilters. Periodische Drehmomentänderungen verschwanden, wenn den Tieren ein rotierender Diffusor, eine Folie mit Intensitätsgradient oder der stationäre Polarisationsfilter präsentiert wurde. Somit kann davon ausgegangen werden, dass das Verhalten der Heuschrecken tatsächlich durch das polarisierte Licht und nicht durch Nebeneffekte induziert wurde. In weiteren Versuchen wurden den Heuschrecken beide DRAs mit schwarzer Farbe übermalt, woraufhin die Polarotaxis praktisch verschwunden war. Die Experimente zeigen, dass Wüstenheuschrecken sich an linear polarisiertem Licht orientieren und dass die DRA der verantwortliche Augenteil für die Detektion polarisierten Lichts ist.

Kapitel II:

Chirurgische Läsionen im anterioren optischen Trakt unterbinden Polarotaxis in fixiert fliegenden Heuschrecken, *Schistocerca gregaria*

Surgical lesion of the anterior optic tract abolishes polarotaxis in tethered flying locusts, *Schistocerca gregaria*

Mittels Färbungen und elektrophysiologischer Ableitungen im Gehirn der Heuschrecke konnte in den letzten Jahren ein großer Fortschritt in der Analyse der Polarisationssehbahn gewonnen werden. Jedoch konnte man bisher eine Beteiligung des anterioren optischen Traktes (AOT) an dieser Sehbahn nur aufgrund anatomischer Daten vermuten. Da er die Verbindung zwischen dem optischen Lobus und dem als polarisationssensitiv nachgewiesenen anterioren optischen Tuberkel darstellt, lag die Schlussfolgerung nahe, dass über ihn Polsehinformationen ins Zentralhirn geleitet werden. Aus diesem Grund führte ich Läsionsexperimente durch und durchtrennte den AOT der linken Hemisphäre. Daraufhin wurden Verhaltensversuche, wie sie oben beschreiben wurden, durchgeführt. Zunächst wurde Polarotaxis der fixiert fliegenden Tiere wie in Kapitel I registriert. Anschließend wurde das Komplexauge der intakten, nicht operierten Hemisphäre schwarz übermalt. Dies führte zu einem vollständigen Ausfall polarotaktischer Flugrichtungsänderungen. Tiere, bei denen entweder nur die Operation ohne Läsion oder die Operation mit Schnitt im Antennallobus durchgeführt wurde, zeigten keine Beeinträchtigung im polarotaktischen Flug. Somit zeigten diese Versuche, dass der AOT ein essentieller Bestandteil der Polarisationssehbahn ist und dass diese vollständig im anterioren Teil des Gehirns verläuft.

Kapitel III:

Polarotaktisches Flugverhalten bei Wüstenheuschrecken: Feldstudien unter natürlichen Lichtbedingungen

Polarotactic flight behavior in desert locusts: field studies underneath natural sky light

Das Lauf- und Flugverhalten von Ameisen, Bienen und Monarchfaltern in Abhängigkeit von polarisiertem Himmelslicht wurde im Freiland bereits untersucht,

doch bei Heuschrecken wurde die Polarotaxis bisher nur unter Laborbedingungen nachgewiesen. So stellte sich die Frage, ob Heuschrecken sich im Freiland tatsächlich nach dem Himmelpolmuster orientieren. Neben dem Himmelpolmuster steht die Sonne im Freiland als weiterer Orientierungspunkt zur Verfügung und bisher war nicht bekannt, ob Heuschrecken primär die Sonne oder das Polarisationsmuster zur Navigation verwenden, wenn keine Landmarken sichtbar sind und ob überhaupt Himmelslicht zur Orientierung während der Wanderflüge verwendet wird. In einem Flugsimulator wurden im Freiland Flugversuche durchgeführt, wobei die Heuschrecken freien Blick auf den Himmel hatten und sich um 360° drehen und somit selbst die Relation ihrer Körperlängsachse zur Sonne bestimmen konnten. Die Tiere zeigten gerichteten Flug, sowohl wenn die Sonne für sie sichtbar war, als auch, wenn sie nicht mehr sichtbar war. Nach Übermalung beider DRAs mit schwarzer Farbe flogen sie gerichtet, wenn die Sonne sichtbar war, verloren aber an Gerichtetheit, sobald dies nicht mehr der Fall war oder wenn der Himmel komplett von Wolken bedeckt war und somit keine Polinformationen mehr vorhanden war. Zwei von drei Flügen, bei denen die Versuchsanordnung komplett im Schatten lag, zeigten ebenfalls stark reduzierte Gerichtetheit. Diese Ergebnisse lassen vermuten, dass Heuschrecken hauptsächlich die Sonne zur Orientierung verwenden aber ob sie das Polarisationsmuster dann als Navigationshilfe benutzen, wenn die Sonne nicht sichtbar ist, konnte anhand der Versuche nicht vollständig geklärt werden und muss durch weitere Experimente gezeigt werden.

INTRODUCTION

Polarized light and polarization pattern of the blue sky

The sun is the most prominent celestial orientation cue and has been used as a compass in traveling and migration for millennia by men and animals alike (e.g. Schmidt-Koenig 2001). Other orientation cues in the sky are the moon, the stars, and planets like the Venus, also called the morning or evening star. A further well-known orientation guide for long-range migrations is the earth magnetic field which provides spatial information for many migrating birds (Schmidt-Koenig 1979). In addition to the sun, the blue sky bears another valuable orientation cue: a pattern of polarization. Light waves sent from the sun are originally unpolarized and oscillate in many planes. When they impinge on air molecules of the earth's upper atmosphere, parts of the light waves are scattered (Rayleigh scattering) and oscillate in only one plane (*E*-vectors, Waterman 1981). *E*-vectors form concentric circles around the sun (Fig. 1).

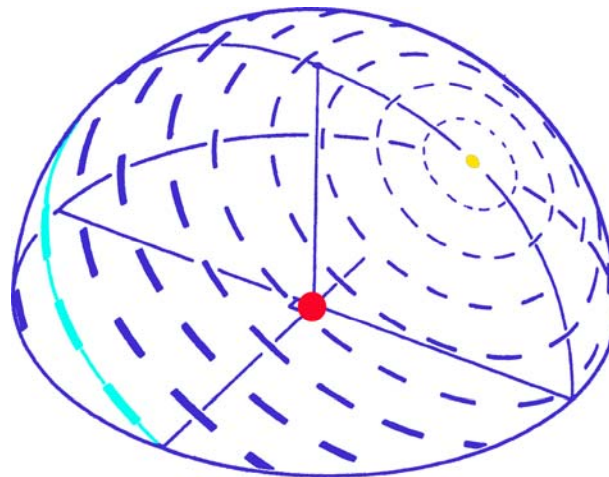


Fig. 1 Three-dimensional schematic drawing of the polarization pattern of the blue sky. Light blue: highest degree of polarization at an angle of 90° relative to the sun; yellow dot represents the sun at an elevation of 53° ; red dot represents position of observer. *E*-vectors (dark blue) form concentric circles around the sun. Modified after Petzold 2001.

The polarization pattern is characterized by defined *E*-vector orientations, degree of polarization, and radiation intensity (Wehner 1996). The degree of polarization could reach a maximum of about 75% at clear blue skies and when the sun is near the horizon (Coulson 1988). The pattern is fixed in relation to solar position and moves along the sky in the course of the day. The sun is always perpendicular to each *E*-

vector orientation and, therefore, the polarization pattern gives clear information about the sun's position. The degree of polarization is highest at an angle of 90° to the sun (Fig. 1) so that maximum polarization information is in the zenith when sun is low, i.e. at dawn and dusk.

Orientation to polarized light in insects

Karl von Frisch (1949) was the first to show that the honey bee *Apis mellifera* detects polarized sky-light and uses it for spatial navigation. Meanwhile the orientation to polarized light has been demonstrated for many insect species including another ant (*Cataglyphis bicolor*, e.g. Wehner 2003), crickets (*Gryllus campestris*, Brunner and Labhart 1987), flies (*Musca domestica*, v. Philipsborn and Labhart 1990), monarch butterflies (*Danaus plexippus*, Sauman et al. 2005), and dung beetles (*Scarabaeus zambesianus*, Dacke et al. 2002). The roles of polarization vision are manifold: the ventral part of the eyes of the backswimmers *Notonecta glauca* possesses many photoreceptors that are sensitive to polarized light (Schwind 1983) and in flight they detect water surfaces by horizontal polarization of UV light; honey bees can use polarization vision to give directional instructions for foraging to their conspecifics when they are back in the hive (waggle dances; v. Frisch 1949); dung beetles use it as an orientation guide to leave their food source in a straight line to avoid aggressive fights (Dacke et al. 2002). Desert ants were shown to make long and tortuous foraging walks, but use the sky polarization pattern to return to their nest on a straight line (e.g. Wehner et al. 2002).

Polarization sensitivity in further animal species

Besides insects, other species including crustaceans, cephalopods, marine snails, spiders, and scorpions are also sensitive to polarized light (Horváth and Varjú 2004). Among the orientation to polarized light of the celestial hemisphere, polarization sensitivity of animals in water and at the water/air interface was demonstrated (Wehner 2001). Polarization sensitivity has, likewise, been demonstrated in crustaceans, e.g. in the shore-living water flea *Daphnia pulex*. These animals were

shown to swim toward polarized light (Schwind 1999), which in nature would lead them away from the shore towards deeper water.

Detection of polarized light by insect compound eyes

In 1979 Burghause found ommatidia accumulated in a dorsalmost part of the cricket compound eye (so-called dorsal rim area, DRA) with specializations that were highly suited for the detection of polarized light. Meanwhile, DRAs have been found in many other insect species like bees, ants, flies, beetles, dragonflies (Labhart and Meyer 1999), monarch butterflies (Stalleicken et al. 2005), and desert locusts (Homberg and Paech 2002). Morphological specializations of DRA photoreceptors include a 90°-arrangement of microvilli, reduced length and enlarged cross-sectional area of rhabdoms, homochromacy of DRA photoreceptors (UV receptors in bees, ants, and flies, Labhart and Meyer 1999, v. Philipsborn and Labhart 1990; blue receptors in locusts and crickets, Homberg and Paech 2002, Labhart and Meyer 1999), and well-aligned, non-twisted microvilli in each photoreceptor (Labhart and Meyer 1999). Behavioral experiments with occluded DRAs in crickets and flies (Brunner and Labhart 1987; v. Philipsborn and Labhart 1990) resulted in loss of polarotaxis and showed that the DRA is the responsible eye part for mediating polarization vision.

Polarization vision pathways

The way of polarized light signals from the DRA via the optic lobes to the central brain has not been studied in great depth. Recently, however, more and more insights were obtained in signal processing and forwarding. Most information on neuronal connections originate from physiological studies and tracer injections in crickets and locusts (Blum and Labhart 2000; Homberg et al. 2003). In crickets a small group of polarization-sensitive neurons (termed POL1) has been studied in the optic lobe. These neurons connect the right and left medulla through a posterior commissure (Labhart and Meyer 2002). In addition, Sakura and Labhart (2005) showed polarization-sensitive tangential neurons of the cricket central complex. Photoreceptors of the locust DRA project to the dorsal rim of the lamina and medulla

and to the anterior lobe of the lobula of the optic lobe (Homberg and Würden 1997; Homberg and Paech 2002). The anterior optic tract (AOT) connects the optic lobe to a small neuropil in the anterior central brain, the anterior optic tubercle (AOTu), which consists of a lower and an upper subunit (Fig. 2). Electrophysiological studies recently showed that the AOTu is a part of the locust polarization vision pathway, because the lower as well as the upper unit contains polarization-sensitive neurons (Pfeiffer et al. 2005). Output fibers from the AOTu project to the median olive and the lateral triangle of the accessory lobe and make contact with tangential neurons that innervate the lower division of the central body (Fig. 2; Homberg et al. 2003). The central complex is an unpaired neuropil in the center of the brain and consists of the upper and lower division of the central body (CB), the protocerebral bridge and paired spheroidal noduli. Electrophysiological studies demonstrated polarization-sensitive neurons in the central complex, most of them in the lower division of the CB (Vitzthum et al. 2002). In *Drosophila* the ellipsoid body of the CB was shown to be responsible for flight control (Ilius et al. 1994). A function of the *Drosophila* CB in visual pattern memory, spatial orientation, and navigational control of locomotion was suggested by Strauss (2002) and Liu et al. (2006). These findings and the fact that neurons of the CB receive input from both eyes strongly suggests that the CB plays an indispensable role as a locomotion and navigation center in the insect brain.

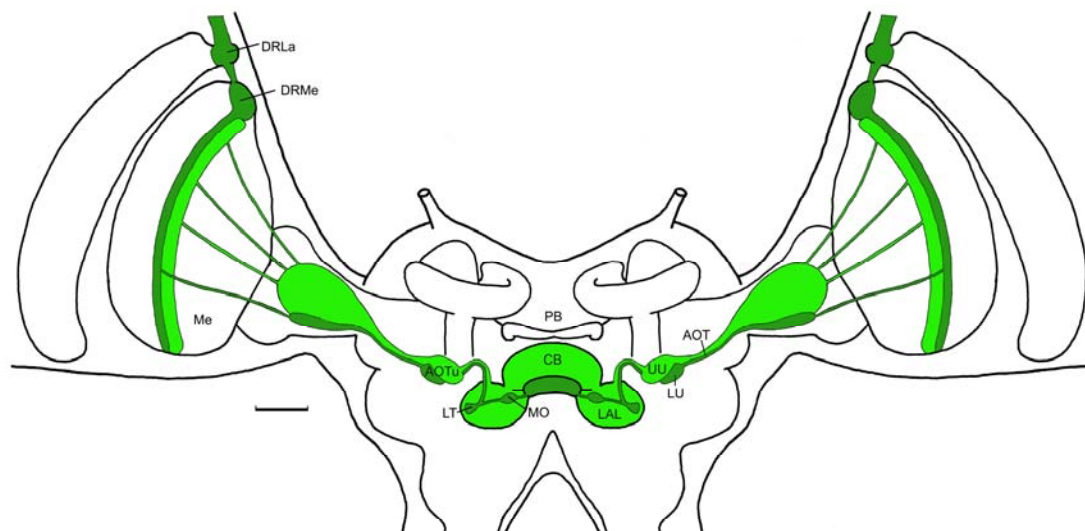


Fig. 2 Polarization vision pathways in the locust brain. The polarization vision pathway to the lower division of the central body (CB) is shown in dark grey; a parallel visual pathway to the upper division of the central body is shown in light grey. AOT anterior optic tract; AOTu anterior optic tubercle; DRLa, DRMe dorsal rim of the lobula and the medulla; LAL lateral accessory lobe; LT lateral triangle; LU, UU lower and upper unit of the AOTu; Me medulla; MO median olive; PB protocerebral bridge. Scale bar: 200 μ m. Modified after Homberg et al. (2003).

Time compensation

Using the sun as an orientation cue is a wide-spread navigation method in animals. However, the sun's position is not fixed during the day and, therefore, represents no constant guidepost for flights over several hours. For maintaining course during long-range migrations, animals, therefore, need to constantly adjust their flight direction relative to the changing solar azimuth (Homberg 2004). Recently, monarch butterflies were shown to possess a time-compensated sun compass (Stalleicken et al. 2005) and half a century earlier Lindauer (1954) and von Frisch and Lindauer (1954) demonstrated that honey bees find their way back to the hive, even if they are on their way for several hours. This competence requires the existence of an internal circadian clock and indeed, in many insect species a circadian clock has been localized and characterized. In the cockroach *Leucophaea maderae* the accessory medulla of the optic lobe houses the circadian pacemaker (Reischig and Stengl 2003). In desert locusts an accessory medulla is also present and contains polarization-sensitive neurons (Homberg and Würden 1997). Polarization-vision pathways have so far been demonstrated especially in the anterior brain, but the accessory medulla is mainly connected to the central brain by posterior fibers, e.g. through the posterior optic tract (POT). The POT projects to the posterior optic tubercle (POTu) which is further connected to the protocerebral bridge of the central complex by polarization-sensitive neurons (Heinze and Homberg 2005). Because of the proved clock function of the accessory medulla in the cockroach and its connections with the POTu and the protocerebral bridge by polarization-sensitive fibers one can presume that this neuropil is also the timekeeping center in the desert locust. Therefore, the central complex may be ideally positioned at the interface between anterior polarization pathways and a posterior time compensation pathway.

Goals and major results of this study

Santschi provided homing harvester ants from the sun and landmarks by a black cylinder (1923). These experiments suggested for the first time that not only directed sunlight but also light of the blue sky bears orientation information in some way. In 1954 von Frisch and Lindauer showed that honeybees are able to use a sky-polarization compass for spatial orientation. Three years before, first evidence for

celestial compass orientation in desert locusts was provided by Kennedy in attempts to develop effective pest control strategies for locust plagues (Kennedy 1951). He diverted flight directions by deflecting the solar azimuth with a mirror. Eggers and Weber (1993) performed preliminary walking experiments on a Kramer treadmill with larvae and adults of *Schistocerca gregaria* and *Locusta migratoria*. They showed that locusts are able to detect and use polarized light for menotactic orientation.

Whether and how desert locusts rely on the polarization pattern of the sky for maintaining navigational directions during long-range migrations, remained unclear until today. Therefore, the aim of the present PhD thesis was a behavioral analysis of of polarotaxis in tethered flying locusts. Here I present locust flight experiments with polarized light as an orientation cue for the first time. First I wanted to know, whether an inherent motivation is present to follow defined *E*-vector orientations. Hence, we tested yaw-torque responses of tethered flying locust under a slowly rotating linear polarization filter. I found periodic *E*-vector dependent changes in turning tendency whereas control experiments with a rotating diffuser, an intensity pattern and a stationary polarizer demonstrated that periodic changes in turning tendency behavior is indeed induced by polarized light. By painting both DRAs black I demonstrated that this eye region is indispensable for the detection of polarized light.

In a second set of experiments I performed microsurgical lesions to analyze the polarization vision pathway of the locust brain involved in polarotaxis. The AOT of the left brain hemisphere was transected and flight behavior under the rotating polarizer was analyzed. I showed that the anterior optic tract is an indispensable part of visual pathways mediating polarotaxis in the desert locust, because polarotaxis was abolished after painting the eye of the intact brain hemisphere black.

A third series of experiments investigated whether desert locusts indeed use sky signals as guiding cues for orientation. Tethered flying locusts were analyzed in an outdoor flight simulation under natural blue or cloudy skies. I found that locusts fly strongly uni-directional under blue sky when the sun is visible. The data strongly suggest that orientation to direct sunlight is a part of the desert locust navigational system. Orientation exclusively to polarized light could not be tested in a sufficient number of flights, but we found first evidence for that when locust flight was still directed when the sun was not visible.

References

- Blum M, Labhart T (2000) Photoreceptor visual fields, ommatidial array, and receptor axon projections in the polarizations-sensitive dorsal rim area of the cricket compound eye. *J Comp Physiol A* 186:119-128
- Brunner D, Labhart T (1987) Behavioural evidence for polarization vision in crickets. *Physiol Entomol* 12:1-10
- Burghause FMHR (1979) Die strukturelle Spezialisierung des dorsalen Augenteils der Grillen (Orthoptera, Grylloidea). *Zool Jb Physiol* 83:502-525
- Coulson KL (1988) Polarization and intensity of light in the atmosphere. Deepak, Hampton, VA
- Dacke M, Nordström P, Scholtz C, Warrant E (2002) A specialized dorsal rim area for polarized light detection in the compound eye of the scarab beetle *Pachysoma striatum*. *J Comp Physiol A* 188:211-216
- Eggers A, Weber T (1993) Behavioural evidence for polarization vision in locusts. In: Elsner N, Heisenberg M (eds) *Gene-Brain-Behaviour*. Thieme, Stuttgart, p 336
- Frisch K von (1949) Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzen der Bienen. *Experientia* 5:142-148
- Frisch K von, Lindauer M (1954) Himmel und Erde in Konkurrenz bei der Orientierung der Bienen. *Naturwissenschaften* 41:245-253
- Heinze S, Homberg U (2005) A new set of tangential neurons of the protocerebral bridge in the desert locust *Schistocerca gregaria* is sensitive to polarized light. 98. Annual meeting of the DZG, Bayreuth, <http://www.uni-bayreuth.de/dzg-gebin2005>, p. 73
- Homberg U (2004) In search of the sky compass in the insect brain. *Naturwissenschaften* 91:199-208
- Homberg U, Paech A (2002) Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. *Arthropod Struct Devel* 30:271-280
- 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
- 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

- Horváth G, Varjú D (2004) Polarized light in animal vision. Springer, Berlin, Heidelberg, New York
- Ilius M, Wolf R, Heisenberg M (1994) The central complex of *Drosophila melanogaster* is involved in flight control: studies on mutants and mosaics of the gene ellipsoid body open. J Neurogenet 9:189-206
- Kennedy JS (1951) The migration of the desert locust (*Schistocerca gregaria* FORSK.). I. The behavior of swarms. II. A theory of long-range migrations. Phil Trans Roy Soc Lond B 235:163-290
- Labhart T, Meyer EP (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
- Labhart T, Meyer EP (2002) Neural mechanisms in insect navigation: polarization compass and odometer. Curr Opin Neurobiol 12:707-714
- Lindauer M (1954) Dauertänze im Bienenstock und ihre Beziehung zur Sonnenbahn. Naturwissenschaften 41:506-507
- Liu G, Seiler H, Wen A, Zars T, Ito K, Wolf R, Heisenberg M, Liu L (2006) Distinct memory traces for two visual features in the *Drosophila* brain. Nature 439:551-556
- Petzold J (2001) Polarisationsempfindliche Neuronen im Sehsystem der Feldgrille, *Gryllus campestris*: Elektrophysiologie, Anatomie und Modellrechnungen. PhD thesis, University of Zurich, Switzerland
- Pfeiffer K, Kinoshita M, Homberg U (2005) Polarization-sensitive and light-sensitive neurons in two parallel pathways passing through the anterior optic tubercle in the locust brain. J Neurophysiol 94:3903-3915
- Philipsborn A von, Labhart T (1990) A behavioural study of polarization vision in the fly, *Musca domestica*. J Comp Physiol A 167:737-743
- Reischig T, Stengl M (2003) Ectopic transplantation of the accessory medulla restores circadian locomotor rhythms in arrhythmic cockroaches (*Leucophaea maderae*). J Exp Biol 206:1877-1886
- Sakura M, Labhart T (2005) Polarization-sensitive neurons in the central complex of the cricket, *Gryllus bimaculatus*. Neuroforum 2005 Suppl:154B
- Santschi F (1923) L'orientationsidéale des fourmis, et quelques considérations sur leurs différentes possibilités d'orientation. I. Classification des diverses

- possibilities d'orientation chez les fourmis. *Mém Soc Vaudoise Sci Nat* 4:137-175
- Sauman I, Briscoe AD, Zhu H, Shi D, Froy O, Stalleicken J, Yuan Q, Casselman A, Reppert SM (2005) Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* 46:457-467
- Schmidt-Koenig, K (1979) Avian orientation and navigation. Academic Press, London
- Schmidt-Koenig, K (2001) Zur Geschichte der Orientierungsforschung. *J Ornithol* 142, Suppl 2:112-123
- Schwind R (1983) A polarization-sensitive response of the flying water bug *Notonecta glauca* to UV light. *J Comp Physiol* 150:87-91
- Schwind R (1999) *Daphnia pulex* swims towards the most strongly polarized light: a response that leads to 'shore flight'. *J Exp Biol* 202:3631-3635
- Stalleicken J, Mukhida M, Labhart T, Wehner R, Frost B, Mouritsen H (2005) Do monarch butterflies use polarized skylight for migratory orientation? *J Exp Biol* 208:2399-2408
- Strauss R (2002) The central complex and the genetic dissection of locomotor behaviour. *Curr Opin Neurobiol* 12:633-638
- 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
- Waterman TH (1981) Polarization sensitivity. In: Autrum H (ed) *Handbook of sensory physiology*, vol VII, part 6B. Springer, Berlin Heidelberg New York, pp 281-461
- Wehner R (1996) Polarisationsmusteranalyse bei Insekten. *Nova Acta Leopoldina* 294:159-183
- Wehner R (2001) Polarization vision—a uniform sensory capacity? *J Exp Biol* 204:2589-2596
- Wehner R (2003) Desert ant navigation: how miniature brains solve complex tasks. *J Comp Physiol A* 189:579-588
- Wehner R, Gallizzi K, Frei C, Vesely M (2002) Calibration processes in desert ant navigation: vector courses and systematic search. *J Comp Physiol A* 188:683-693

CHAPTER I:

Behavioral analysis of polarization vision in tethered flying locusts

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Behavioral analysis of polarization vision in tethered flying locusts

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Abstract For spatial navigation many insects rely on compass information derived from the polarization pattern of the sky. We demonstrate that tethered flying desert locusts (*Schistocerca gregaria*) show e-vector-dependent yaw-torque responses to polarized light presented from above. A slowly rotating polarizer (5.3° s^{-1}) induced periodic changes in yaw torque corresponding to the 180° periodicity of the stimulus. Control experiments with a rotating diffuser, a weak intensity pattern, and a stationary polarizer showed that the response is not induced by intensity gradients in the stimulus. Polarotaxis was abolished after painting the dorsal rim areas of the compound eyes black, but remained unchanged after painting the eyes except the dorsal rim areas. During rotation of the polarizer, two e-vectors (preferred and avoided e-vector) induced no turning responses: they were broadly distributed from 0 to 180° but, for a given animal, were perpendicular to each other. The data demonstrate polarization vision in the desert locust, as shown previously for bees, flies, crickets, and ants. Polarized light is perceived through the dorsal rim area of the compound eye, suggesting that polarization vision plays a role in compass navigation of the locust.

Keywords Compass navigation · Compound eye · Locust flight · Polarization vision · *Schistocerca gregaria*

Introduction

Scattering of sunlight in the earth's upper atmosphere leads to partial polarization of light from the blue sky

(Waterman 1981). As a result, the blue sky shows a polarization pattern consisting of e-vectors oriented in concentric circles around the sun. The degree of polarization varies from 0 (direct sunlight) to a maximum (under optimal conditions) of about 0.75 or 75% along the great circle of the sky at an angular distance of 90° from the sun (Coulson 1988). Since the e-vector pattern of the sky is linked to the solar position, directional information in the sky is independent of the visibility of the sun, and can also be obtained from a small patch of blue sky. Many insects are able to detect the polarization pattern of the blue sky and use this capability for spatial orientation (Wehner 1984; Wehner et al. 1996). The use of skylight polarization for spatial navigation was shown in field studies on honeybees and ants (von Frisch 1949; Wehner 1982; Rossel and Wehner 1986) and, using a spontaneous orientation response, in laboratory studies in crickets and flies (Brunner and Labhart 1987; von Philipsborn and Labhart 1990).

Burghause (1979) reported ommatidia specialized to detect skylight polarization in a small dorsal rim area (DRA) of the cricket's compound eye, and similar DRAs have meanwhile been demonstrated in many other insect species (reviewed by Labhart and Meyer 1999). Specializations of DRAs favoring a role in polarized light detection are similar in all species studied and include (1) orthogonal arrangement and high alignment of microvilli, (2) reduced length and enlarged cross-sectional area of rhabdoms, (3) high polarization sensitivity of DRA photoreceptors, and finally (4) degraded optics by light scattering structures in the cornea or missing screening pigment (Labhart and Meyer 1999, 2002).

The compound eye of the desert locust, *Schistocerca gregaria*, has a particularly prominent DRA (Homberg and Paech 2002). It largely consists of blue-sensitive photoreceptors with high polarization sensitivity (Eggers and Gewecke 1993). Tracing studies have mapped central processing stages which are supplied by input from the DRA and showed that these include dorsal areas in the lamina and medulla, the anterior lobe of the lobula,

the anterior optic tract and tubercle, the lateral accessory lobe, and the central complex (Homberg et al. 2003). Single-cell recordings demonstrated polarization sensitivity in interneurons at the level of the medulla (Homberg and Würden 1997), the anterior optic tubercle, the lateral accessory lobe, and the central complex (Vitzthum et al. 2002). In order to gain further insight into the mechanisms of polarized-light-dependent spatial orientation in the locust, we have developed a behavioral assay for polarization vision in this insect. This study demonstrates polarotaxis in stationary flying locusts and shows that photoreceptors in the DRA of the eye are essential for this behavior. Parts of this study have been published in abstract form (Mappes and Homberg 2003).

Materials and methods

Animals

Animals were kept in a crowded laboratory colony under LD 12:12 h at 28°C and 50% relative humidity (RH). Adult male and female desert locusts (*S. gregaria*) at least 10 days after imaginal molt were used. A small metal pin was attached ventrally to the pterothoracic sternum by rosin wax. The pin was used to attach the locusts to a friction free thrust/yaw-torque meter (see below). In all experiments, the heads of the animals were fixed to the thorax by rosin wax.

Experimental setup

Locusts were flown in a laminar air current within the flight chamber (34 cm×25.5 cm×26 cm) of a horizontal wind tunnel (Gewecke 1975), so that the body axis was nearly horizontal (Fig. 1). The sides and bottom of the chamber were constructed from sheets of matt-black plastic material. For optimal flight performance, wind speed was adjusted at 3.0 m s⁻¹. The yaw-torque meter was provided by Dr. R. Preiss and has been described in detail by Preiss and Gewecke (1991). It consists of a pair of transducers and allows to measure thrust and yaw torque simultaneously but was used here only for measurement of instantaneous yaw torque by electronic subtraction of the signals from the two transducers. The subtracted signal was filtered (low-pass, 1 Hz) and fed into an interface (CED 1401 plus; Cambridge Electronic Design) and a laboratory computer (Pentium III processor) equipped with Spike2 software for graphic depiction of turning tendency and for later analysis. Stimulation, testing procedure and data analysis largely follow the procedure developed by Brunner and Labhart (1987) and von Philipsborn and Labhart (1990) for analysis of polarization vision in crickets and flies.

Light was produced by a fluorescent lamp (Osram Dulux EL 16 W; Fig. 1). It passed through a horizontal diffuser and evenly illuminated a circular window in the ceiling of the flight chamber (Fig. 1). The window was fitted with different rotating filters and provided a wide field stimulus of 87.3° visual angle centered around the zenith. The following rotating stimuli were used: (1) polarized light was produced by inserting a linear polarization filter (Polaroid HN 38S, irradiance: 25.8 μW cm⁻²); (2) unpolarized light was produced by inserting a diffuser (two sheets of translucent drawing paper, irradiance: 30.2 μW cm⁻²); (3) to control for the possible effects of intensity gradients produced by the polarizer, filter (2) was overlaid with the polarization filter (irradiance: 9.8 μW cm⁻²). All light intensities were measured with a radiometer (Photodyne 18XTA, silicium detector) at the position of the locust head. Filter rotation was monitored through a photodiode covered by a

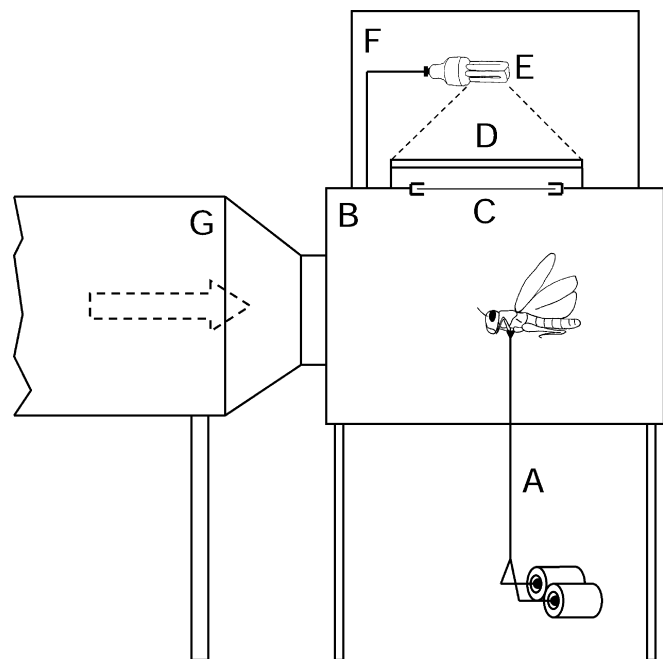


Fig. 1 Experimental setup for testing polarotaxis of tethered flying locusts (animal not drawn to scale). *A* mounting rod connected to the yaw-torque meter (for more details see Gewecke 1975; Preiss and Gewecke 1991); *B* flight chamber; *C* rotating polarization filter, diffuser, or combined polarizer-diffuser; *D* diffuser; *E* fluorescent lamp; *F* cover to ensure optimal lighting; *G* wind tunnel producing laminar air current

polarization filter (invisible to the locust). Its output was fed into the second channel of the data recording equipment (see above).

Testing procedure

Experiments were carried out at 30–32°C and 40–50% RH. In order to obtain regular flight behavior and an even flight position (Preiss and Gewecke 1991), animals were flown in complete darkness for a few minutes. After locusts became accustomed to the situation, light was turned on and the polarizer/diffuser started rotating. A single experiment consisted of three full revolutions of the polarizer (speed: 5.3° s⁻¹) either clockwise or counter-clockwise. Therefore, each e-vector orientation of the pattern occurred six times.

In control experiments (1) the polarization filter was replaced by the diffuser (see above), (2) the polarization filter was placed directly behind the diffuser (intensity gradient), and (3) animals were flown under a stationary e-vector (parallel to their longitudinal body axis). Each animal was first flown under the polarizer and subsequently under one of the control conditions. In order to identify the eye region involved in the response, locusts were flown under the polarization pattern with their DRAs painted black (Marabu Decorlack matt, water-based). In a second group, the compound eyes were painted black except for the DRAs.

Data analysis

Recordings from each flight were transferred from Spike2 to Microcal Origin 8.0 and Clampfit 8.0 (Axon Instruments, Foster City, Calif., USA) for further analysis. For each experiment, the yaw-torque responses of the six periods were averaged within 5° bins; an inherent turning tendency, defined as yaw-torque average of all bins, was subtracted from the data. For each histogram,

a periodicity score P as a measure of the animal's response to the rotating e-vector was determined empirically (for details see Appendix) following a procedure developed by von Philipsborn and Labhart (1990). It describes the degree of periodicity in the histograms by detecting periodic behavior both in cases with strong and weak modulation of yaw torque. Periodicity scores obtained under different stimulus conditions were statistically compared using the non-parametric U -test after Wilcoxon, Mann and Whitney (Clampfit 8.0).

At two or more positions of the histograms the locusts showed no turning tendency. The *preferred* e-vector orientation was defined as being followed by a turning response in the direction of e-vector rotation. Turning in the opposite direction indicates the *avoided* e-vector orientation. For statistical evaluation of the distributions of the preferred and avoided e-vectors, the χ^2 -test (SPSS for Windows 11.0; SPSS, Chicago, Ill., USA) was applied.

Results

General

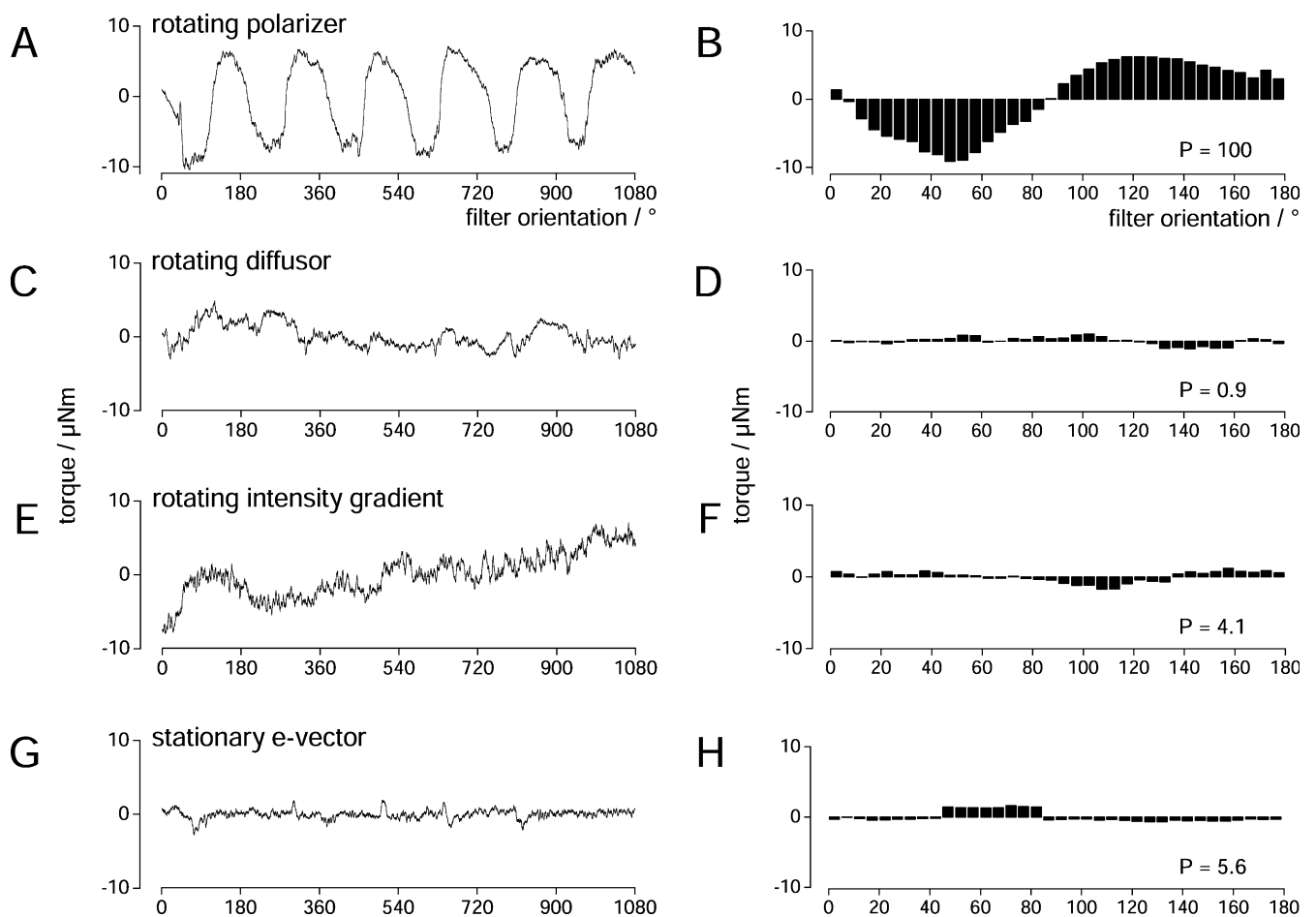
In this study we tested yaw-torque responses of desert locusts to polarized light in stationary flight. The animal's readiness to fly was highly variable and did not depend on sex. Also the motivation to respond consistently to the rotating polarizer varied and was sometimes low, even in good flyers. Only flights without any interruption were, therefore, evaluated and included in the data. There was no significant difference between

flights under the polarizer rotating clockwise or counter-clockwise (non-parametric U -test after Wilcoxon, Mann and Whitney).

Rotating polarizer

For analysis of yaw torque under the rotating polarizer, 142 flights of 92 animals were evaluated. Rotation of the polarization filter induced periodically changing yaw-torque responses in the locusts which corresponded to the 180° periodicity of the rotating polarizer (Fig. 2A).

Fig. 2 Flight traces and yaw-torque histograms under rotating polarizer (A, B), rotating diffuser (C, D), rotating intensity gradient (E, F), and stationary e-vector (G, H). Positive and negative yaw-torque values correspond to right- and left-hand turning, respectively. Inherent turning tendency was subtracted in the flight traces and in the yaw-torque histograms. **A** Test situation with rotating polarizer. The animal shows a 180° periodicity. **B** Average yaw-torque responses from the six 180° rotations in A, divided in 5° bins. The periodicity score calculated from the histogram (see Appendix) is $P=100$. **C** Rotating diffuser. **D** Histogram from flight in C. The periodicity score is 0.9. **E** Intensity gradient; the animal shows a shift in yaw torque during the experiment, but no periodic changes. **F** Histogram from flight in E; $P=4.1$. **G** Stationary e-vector. **H** Histogram from flight in G. The periodicity score of this flight is 5.6. The units on the *abscissae* in G and H are virtual numbers, corresponding in time to the angular filter positions in A–F



During 180° rotation a single period of yaw torque in the direction of the rotating polarizer was usually followed by a period of yaw torque in the opposite direction or vice versa (Fig. 2A, B). The transitions between right and left turning were moments when no yaw torque occurred. Periodicity scores of these flights ranged from 0.08 to 259.2 with a mean score of $\bar{P} = 48.9$ (Fig. 4A).

Control experiments

Controls were executed between test flights under the rotating polarizer in order to demonstrate that e-vector responses were present at that time. Yaw-torque responses during rotation of the diffuser were analyzed in 55 animals. During these flights, yaw torque was irregular and, in many flights, altered frequently between left and right turns (Fig. 2C, D). Similar results were obtained when placing the polarization filter behind the diffuser, tested in 20 experiments (Fig. 2E, F). In these controls, periodicity scores ranged from 0.03 to 129.6 (diffuser only, $\bar{P} = 14.2$) and from 0.06 to 86.4 (polarizer behind diffuser, $\bar{P} = 11.1$) (Fig. 4B, E). Statistical comparison of the periodicity histograms shows significantly increased P scores in the test situation compared to the two controls ($P < 0.001$, diffuser; $P < 0.001$, diffuser plus polarizer). The histograms of the two control situations are not significantly different from each other ($P < 0.5$).

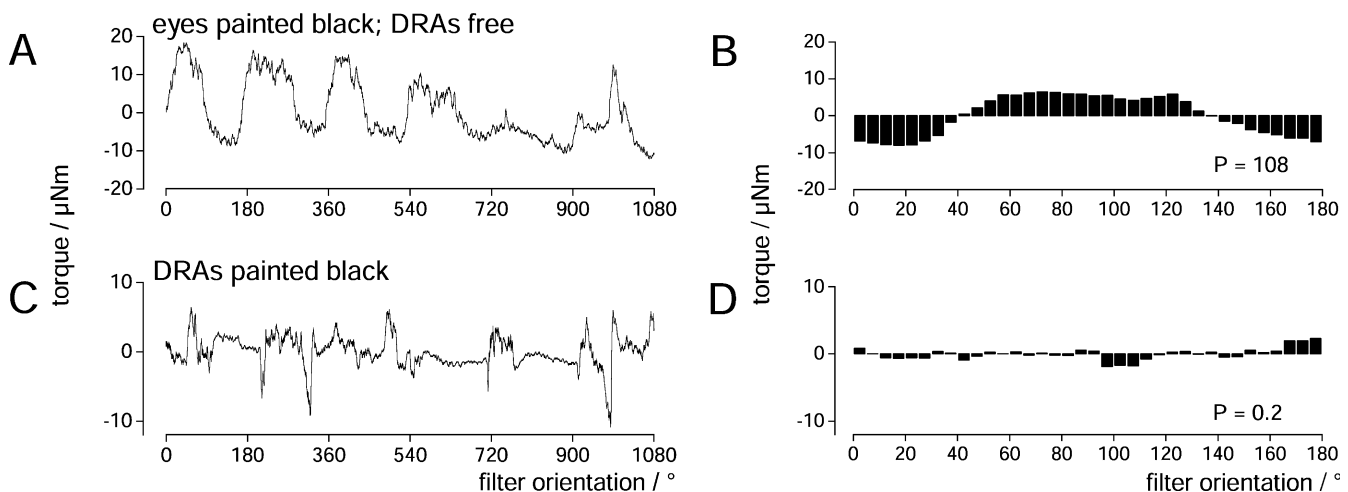
In addition to the two controls described above, 32 experiments were performed under a stationary e-vector, parallel to the animals' longitudinal axis. Since this stimulation provides no periodic information, P scores were low and ranged from 0.02 to 28.8 with $\bar{P} = 7.6$

(Figs. 2G, H and 4D). Periodicity scores of flights under stationary e-vector are significantly different from the test flights ($P < 0.001$). No significant difference occurs between the P scores of flights under the stationary e-vector and the two other controls (in all cases, $P < 0.1$).

Determination of eye region involved in polarotaxis

For many insect species, a specialized DRA of the compound eye was shown to be responsible for the detection of polarized light (see Introduction). The presence of a DRA in *S. gregaria* likewise suggests that this region plays an important role in polarization vision. To determine the eye region involved in the polarization response animals with different parts of their eyes occluded were tested under the rotating polarizer. Experiments with DRAs painted black or with compound eyes except the DRAs painted black were executed between regular test flights to show that e-vector responses existed in the untreated animal. In 31 flights the compound eyes except the DRAs were overlaid with black paint. These animals showed no difference in flight behavior from untreated animals under the rotating polarizer ($P < 0.1$) (Fig. 3A, B). Periodicity scores ranged from 2.1–324, with $\bar{P} = 35.3$ (Fig. 4C). In 32 flights the DRAs were occluded and the rest of the eyes was left open. In these experiments, the animals' turning tendency changed irregularly and was sometimes minimal (Fig. 3C, D), and periodicity scores ranged from 0.007–81, $\bar{P} = 15.7$ (Fig. 4F). Statistical comparison shows that P scores of experiments with free DRAs are significantly larger than those of experiments with DRAs painted black ($P < 0.05$). No significant difference occurred between experiments under the polarizer in untreated animals and in animals with compound eyes except DRAs painted black ($P < 0.1$). Likewise, P scores of control experiments with diffuser and with diffuser plus polarizer are not significantly different from the experiments with painted DRAs ($P < 0.1$, diffuser; $P < 0.1$, diffuser plus polarizer).

Fig. 3 Flight traces and yaw-torque histograms under rotating polarizer with compound eyes painted black and dorsal rim areas (DRAs) free (A, B), and with DRAs painted black (C, D). A, B Painting the compound eyes except the DRAs results in yaw-torque responses corresponding to the 180° periodicity of the rotating polarizer; $P = 108$. C, D With DRAs painted black, the flight trace is irregular and P is low (0.2)



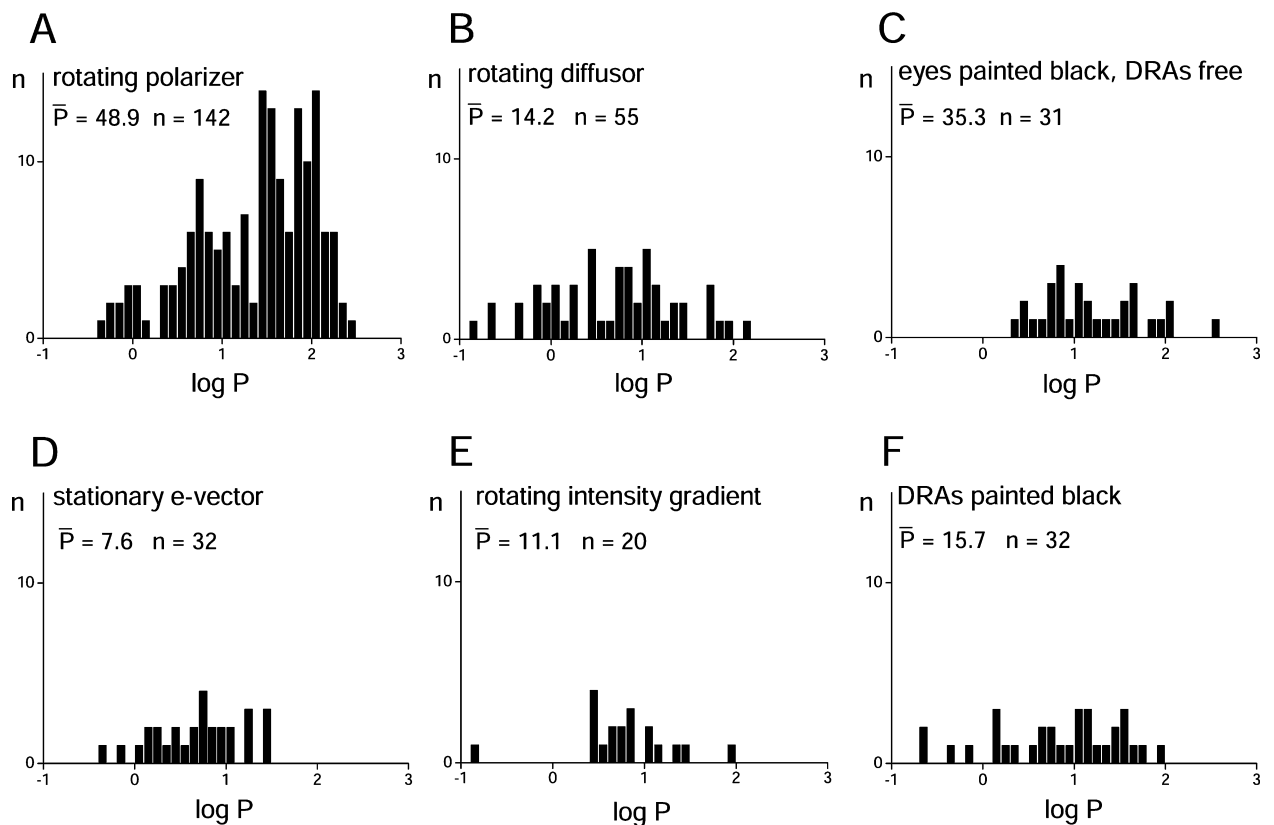


Fig. 4A–F Comparison of P scores of all test flights and controls. Number of flights and means of P scores are given for each histogram. **A** Rotating e-vector; **B** rotating diffuser; **C** rotating e-vector, compound eyes painted black, but DRAs open; **D** stationary e-vector; **E** rotating intensity gradient; **F** rotating e-vector, DRAs painted black, rest of the eyes open. Flights under rotating polarizer (**A**) have significantly higher P scores than the controls in **B** ($P < 0.001$), **D** ($P < 0.001$), and **E** ($P < 0.001$). Likewise, flights with DRAs open (**C**) have significantly higher P scores than flights with DRAs painted black (**F**, $P < 0.05$). The distributions in **A** and **C** (rotating polarizer, DRAs open) are not significantly different from each other ($P < 0.1$), and no significant difference exists between the four control situations (**B** versus **D**, $P < 0.5$; **B** versus **E**, $P < 0.5$; **B** versus **F**, $P < 0.1$; **E** versus **D**, $P < 0.1$; **E** versus **F**, $P < 0.1$; **F** versus **D**, $P < 0.1$)

Discussion

In the present study we developed a behavioral assay to analyze polarization vision in the desert locust *S. gregaria*. We examined the flight behavior of tethered flying locusts and showed that a slowly rotating polarizer above the animals induced periodic changes in turning responses with and against the direction of rotation. Locusts flying under a rotating diffuser or under a rotating intensity gradient showed yaw-torque responses which were indistinguishable from those to a stationary e-vector. Thus, intensity gradients inherently connected to polarized light stimulation do not contribute to the observed behavior. In an earlier study, Eggers and Weber (1993) demonstrated polarotaxis in locust larvae (*S. gregaria* and *Locusta migratoria*) walking on a Kramer treadmill. Polarization vision in the locust is, therefore, present in larvae and in adults and is not restricted to a specific behavioral context such as flight.

For a better comparison with previous studies, our experimental design and procedure for data evaluation closely followed experiments performed in crickets, *Gryllus campestris*, and flies, *Musca domestica* (Brunner and Labhart 1987; von Philipsborn and Labhart 1990). In contrast to polarotaxis in flight, as demonstrated here, Labhart and coworkers used spontaneous changes in walking direction correlated with changing e-vector directions. In the present study (Fig. 4) and that by von Philipsborn and Labhart (1990) on walking flies, there is

Preferred and avoided e-vectors

Under the rotating polarizer, the animals' turning tendency changed periodically every 180° . However, at two positions of the histogram the stimulus induced no turning tendency. After the appearance of these e-vector positions, the locusts either tried to follow the rotating polarizer (*preferred* e-vector) or to turn against the rotating direction (*avoided* e-vector). Preferred and avoided e-vectors, determined in 82 animals, were broadly distributed from 0 to 180° (Fig. 5A). Statistical evaluation (χ^2 -test) shows no significant difference of both histograms from random distributions ($P < 0.5$). For all animals, however, the difference between preferred and avoided e-vector is approximately 90° (Fig. 5B).

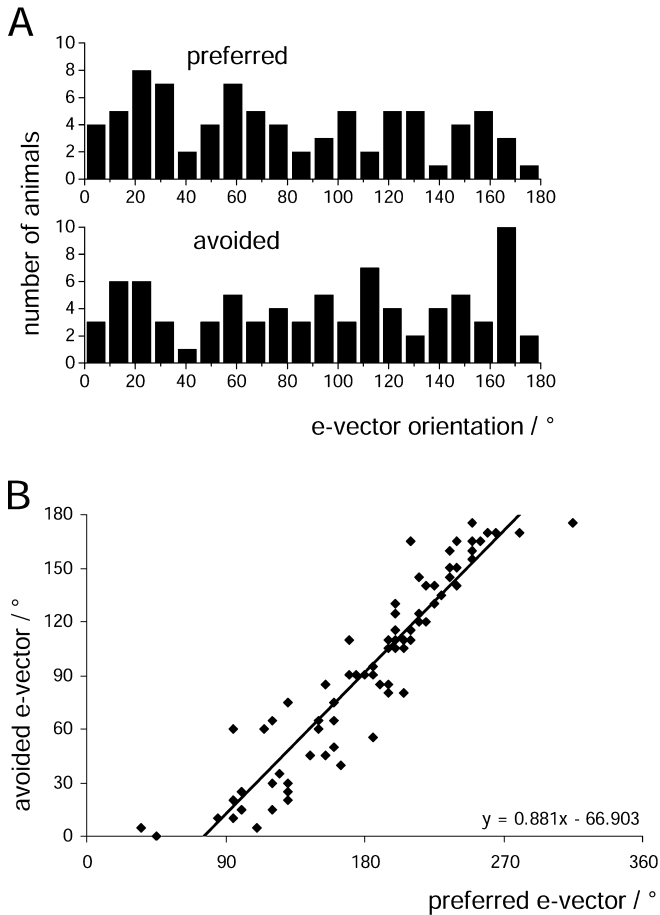


Fig. 5 **A** Distribution of preferred and avoided e-vector orientations of 82 animals under the rotating polarizer. Both histograms are not significantly different from a uniform distribution (χ^2 -test, $P > 0.5$). **B** Preferred versus avoided e-vector orientations from 82 animals

considerable overlap between the periodicity histograms for polarized light and those for control experiments. Possible reasons for this may lie in the choice and balance of the different parameters that contribute to evaluation of the periodicity scores. Flying locusts showed occasional brief but strong turning movements during tethered flight, apparently irrespective of sensory stimulation. These led to considerable increases in the periodicity score, particularly when they occurred against an overall low yaw-torque background. Addition of a low-pass filter in the averaging procedure, as done by Brunner and Labhart (1987) and von Philipsborn and Labhart (1990), had no consistent effect on the statistical evaluation of the data and was therefore not included in the present study.

A difference between this study and the work on crickets and flies is the lack of general preferred and avoided e-vector orientations in locust polarotaxis. Crickets and flies showed a slight preference for e-vector orientations perpendicular to their longitudinal axis (Brunner and Labhart 1987; von Philipsborn and Labhart 1990), but the reason for this behavior is unclear.

While a general preferred e-vector orientation might be expected for genetically programmed compass directions, as found in seasonal migrants such as the monarch butterfly (Mouritsen and Frost 2002), laboratory-reared insects used here or central place foragers which change their navigational directions depending on the availability of a food source would be expected to lack generally preferred directions.

Neurobiology of polarization vision in the locust

The present study demonstrates that polarotactic yaw-torque responses of tethered flying locusts are mediated exclusively through photoreceptors of the DRA of the compound eye. Similar results were obtained in behavioral studies on larval locusts (Eggers and Weber 1993), honeybees, ants, crickets, and flies (reviewed by Labhart and Meyer 1999, 2002). The data strongly suggest that the DRA of these insects serves a role in sky compass orientation. As in many other insect species (Labhart and Meyer 1999) the DRA of the locust shows a number of morphological specializations favoring a role in polarization vision (Homberg and Paech 2002), and single cell recordings demonstrated high polarization sensitivity of DRA photoreceptors with maximum spectral sensitivity in the blue range (Eggers and Gewecke 1993). Several central processing stages involved in polarization vision have recently been identified in the locust. Tracing studies showed that visual pathways originating in the DRA of the compound eye include specially modified dorsal rim areas in the lamina and medulla, a ventral layer of the anterior lobe of the lobula, the lower unit of the anterior optic tubercle, certain subunits of the lateral accessory lobe, and the lower division of the central body (Homberg and Paech 2002; Vitzthum et al. 2002; Homberg et al. 2003). Polarization-sensitive interneurons have been characterized especially in the optic tubercle, lateral accessory lobe, and central complex (Vitzthum et al. 2002; Pfeiffer and Homberg 2003), but have also been found at the level of the medulla (Homberg and Würden 1997). All neurons studied so far show polarization opponency, i.e., they are maximally excited at a particular e-vector orientation (Φ_{\max}) and are maximally inhibited at an e-vector orientation perpendicular to Φ_{\max} . The central complex appears to serve an important role in motor control and Vitzthum et al. (2002) specifically proposed a function of the central complex as an internal compass for spatial orientation.

Functional significance

Although locusts perform long-range migratory flights, both in swarms and as solitary animals (reviewed by Uvarov 1977; Baker 1978; Farrow 1990), the potential involvement of a polarization compass in spatial ori-

entation of these insects has not been studied. Even more surprising, the necessity for sky compass orientation in migrating gregarious locusts has been denied by several investigators (Draper 1980; Baker et al. 1984). On the other hand, most studies agree that wind speed and wind direction plays the dominant role in controlling flight direction (Kennedy 1951; Baker 1978; Riley and Reynolds 1986). At low wind speeds locusts often fly upwind, but may turn into the wind as wind speed increases (Kennedy 1951). Interestingly, however, many studies both on solitary animals and on migrating swarms note that the mean course angle in relation to wind direction can be significantly different from zero suggesting active orientation mechanisms (Kennedy 1951; Riley and Reynolds 1986; Baker et al. 1984; reviewed by Farrow 1990). Moreover, flying swarms as well as migrating walking larvae ("marching hopper bands") usually maintain a particular migratory direction for several hours irrespective of temporary wind shifts (Kennedy 1945, 1951; Baker et al. 1984). Although sky compass orientation has been suspected to play a role in maintaining these course directions (Baker 1978), only two field studies include experiments to address this question (Kennedy 1945, 1951). In observations on marching hopper bands, Kennedy (1945) used a blanket and a mirror to change the sun's position artificially by 180° (from left to right). As a result of this, all marching locusts stopped and marched off in the opposite direction. Kennedy even noted that the angle of orientation to the sun shifted gradually during the course of the day, as expected for time-compensated sun compass orientation. While these experiments on walking locusts were quite striking, similar experiments on flying individuals were more difficult. In one fortuitous attempt, however, five animals coming out of the shadow of a tree instantly changed flight directions in the predicted way, when experiencing the altered sun position (Kennedy 1951). Although these experiments are anecdotal, they clearly suggest the presence of sun compass orientation in migratory locusts, but studies over the last 50 years have not investigated this aspect of spatial orientation any further.

In view of our behavioral evidence in tethered flight as well as the neurobiological evidence for polarization vision in the desert locust, field studies concluding a predominant role of wind directions on flight orientation in migratory locusts may need serious reconsideration. We propose that the locust, like many other insect species, has a well developed sense for spatial navigation based on a sun/polarization compass. Field studies will be essential to reveal when and how this compass is used during spatial orientation and migration.

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Appendix

Calculation of periodicity score P

The periodicity in the locusts yaw-torque response is quantified by the periodicity score P (for further details see von Philipsborn and Labhart 1990). It takes into account several parameters from the yaw torque histograms:

$$P = x \cdot q^z$$

$$x = \frac{(a+b)^2}{(a-18)^2 + (b-18)^2 + |b-a| + c + m + n}$$

$$q = \frac{\sum_A RG_i + \sum_B |RG_i|}{\sum_{i=1}^{36} |RG_i|}, \quad 0 \leq q \leq 1$$

$$z = \frac{36 - (a+b)}{6}$$

where a is the maximum number of positive yaw-torque responses (RG_i) following each other in one string (A) of the histogram, b is the maximum number of negative yaw-torque responses following each other in one string (B) of the histogram, c is the number of sign alterations (+/-) in the histogram, m is the number of maxima in string A, n is the number of minima in string B, $\sum_A RG_i$ the sum of positive RG_i values in string A, $\sum_B |RG_i|$ the overall sum of negative RG_i values in string B, and $\sum_{i=1}^{36} |RG_i|$ the overall sum of all 36 RG_i values.

References

- Baker RR (1978) The evolutionary ecology of animal migration. Hodder and Stoughton, London
- Baker PS, Gewecke M, Cooter RJ (1984) Flight orientation of swarming *Locusta migratoria*. *Physiol Entomol* 9:247–252
- Brunner D, Labhart T (1987) Behavioural evidence for polarization vision in crickets. *Physiol Entomol* 12:1–10
- Burghause FMHR (1979) Die strukturelle Spezialisierung des dorsalen Augenteils der Grillen (Orthoptera, Grylloidea). *Zool Jahrb Physiol* 83:502–525
- Coulson KL (1988) Polarization and intensity of light in the atmosphere. Deepak, Hampton, VA
- Draper J (1980) The direction of desert locust migration. *J Animal Ecol* 49:959–974
- 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 FG, Popov AV, Renninger G (eds) Sensory systems of arthropods. Birkhäuser, Basel, pp 101–109
- Eggers A, Weber T (1993) Behavioural evidence for polarization vision in locusts. In: Elsner N, Heisenberg M (eds) Gene-brain-behaviour. Thieme, Stuttgart, p 336
- Farrow RA (1990) Flight and migration in acridoids. In: Chapman RF, Joern A (eds) Biology of grasshoppers. Wiley, New York, pp 227–314
- Frisch K von (1949) Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzern der Bienen. *Experientia* 5:142–148

- Gewecke M (1975) The influence of the air-current sense organs on the flight behaviour of *Locusta migratoria*. *J Comp Physiol* 103:79–95
- 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
- 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
- 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
- Kennedy JS (1945) Observations on the mass migration of desert locust hoppers. *Trans R Entomol Soc Lond* 95:247–262
- Kennedy JS (1951) The migration of the desert locust (*Schistocerca gregaria* FORSK.). I. The behaviour of swarms. II. A theory of long-range migrations. *Philos Trans R Soc Lond Ser B* 235:163–290
- Labhart T, Meyer EP (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
- Labhart T, Meyer EP (2002) Neural mechanisms in insect navigation: polarization compass and odometer. *Curr Opin Neurobiol* 12:707–714
- Mappes M, Homberg U (2003) Behavioral evidence of polarization vision in the locust *Schistocerca gregaria*. In: Elsner N, Zimmermann H (eds) *The neurosciences from basic research to therapy*. Thieme, Stuttgart, p 567
- Mouritsen H, Frost BJ (2002) Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. *Proc Natl Acad Sci USA* 99:10162–10166
- 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, p 567–568
- Philipsborn A von, Labhart T (1990) A behavioural study of polarization vision in the fly, *Musca domestica*. *J Comp Physiol A* 167:737–743
- 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
- Riley JR, Reynolds DR (1986) Orientation at night by high-flying insects. In: Danthanarayana W (ed) *Insect flight: dispersal and migration*. Springer, Berlin Heidelberg New York, pp 71–87
- Rossel S, Wehner R (1986) Polarization vision in bees. *Nature* 323:128–131
- Uvarov BP (1977) *Grasshoppers and locusts*, vol 2. Centre of Overseas Pest Research, London
- 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
- Waterman TH (1981) Polarization sensitivity. In: Autrum H (ed) *Handbook of sensory physiology*, vol VII, part 6B. Springer, Berlin Heidelberg New York, pp 281–461
- Wehner R (1982) Himmelsnavigation bei Insekten: Neurophysiologie und Verhalten. *Neujahrsbl Naturforsch Ges Zürich* 182: 1–132
- Wehner R (1984) Astronavigation in insects. *Annu Rev Entomol* 29:277–298
- Wehner R, Michel B, Antonsen P (1996) Visual navigation in insects: coupling of egocentric and geocentric information. *J Exp Biol* 199:129–140

CHAPTER II:

Surgical lesion of the anterior optic tract abolishes polarotaxis in tethered flying locusts, *Schistocerca gregaria*

Surgical lesion of the anterior optic tract abolishes polarotaxis in tethered flying locusts, *Schistocerca gregaria*

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Running title: Lesions of the anterior optic tract of the locust

Abbreviations: AOT: anterior optic tract; AOTu: anterior optic tubercle; DRA: dorsal rim area; FFT: fast Fourier transform; PAP: peroxidase-antiperoxidase; POT: posterior optic tract; POTu: posterior optic tubercle

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ABSTRACT

Many insects can detect the polarization pattern of the blue sky and rely on polarization vision for sky compass orientation. In laboratory experiments, tethered flying locusts perform periodic changes in flight behavior under a slowly rotating polarizer even if one eye is painted black. Anatomical tracing studies and intracellular recordings have suggested that the polarization vision pathway in the locust brain involves the anterior optic tract and tubercle, the lateral accessory lobe, and the central complex of the brain. To investigate whether visual pathways through the anterior optic tract mediate polarotaxis in the desert locust, we transected the tract on one side and tested polarotaxis (i) with both eyes unoccluded and (ii) with the eye of the intact hemisphere painted black. In the second group of animals, but not in the first group, polarotaxis was abolished. Sham operations did not impair polarotaxis. The experiments show that the anterior optic tract is an indispensable part of visual pathways mediating polarotaxis in the desert locust.

Keywords Polarization vision · polarotaxis · visual system · compass navigation · *Schistocerca gregaria*

Introduction

Many insect species share the ability to detect the polarization pattern of the blue sky and use it for spatial orientation. While most work has focused on honeybees and ants (v. Frisch 1949; Rossel and Wehner 1986; Wehner 2003), polarization-dependent orientation behavior has also been demonstrated in crickets (Brunner and Labhart 1987), flies (v. Philipsborn and Labhart 1990), beetles (Dacke et al. 2003), locusts (Mappes and Homberg 2004), and monarch butterflies (Sauman et al. 2005; see however Stalleicken et al. 2005). Detection of sky polarization is achieved with a small dorsal part of the compound eye, the dorsal rim area (DRA). It contains ommatidia with homochromatic photoreceptors that respond differentially to the *E*-vector orientation of polarized light (reviewed by Labhart and Meyer 1999). Photoreceptors from the DRA project to dorsal areas in the lamina and medulla of the optic lobe, but further processing stages for polarized light signals have been studied only in the locust *Schistocerca gregaria* and in the cricket *Gryllus campestris* (reviewed by Labhart and Meyer 2002; Homberg 2004). Most studies in crickets have focused on a small group of polarization-sensitive interneurons in the optic lobe (POL1-neurons). These neurons connect the right and left medulla through a posterior commissure, but have no ramifications in the midbrain (Labhart and Meyer 2002). More recently, Sakura and Labhart (2005) demonstrated polarization sensitivity in tangential neurons of the cricket central complex. In locusts, tracing studies revealed the polarization-vision pathway from the DRA to the central complex (Homberg et al. 2003a). Medulla line tangentials with ramifications in the dorsal rim area of the medulla project through the anterior optic tract (AOT) to the lower unit of the anterior optic tubercle (AOTu). Second-order interneurons project from the AOTu to the lateral accessory lobe and make contact with tangential neurons that innervate the lower division of the central body (Homberg et al. 2003a; Fig. 1). Intracellular recordings showed that all types of interneurons encountered in the lower unit of the AOTu and the majority of neurons in the lower division of the central body are polarization-sensitive (Vitzthum et al. 2002; Pfeiffer et al. 2005). The central body is an integral part of the central complex in the locust brain. The central complex consists of the upper and lower divisions of the central body, a pair of spheroidal noduli and the protocerebral bridge. While neurons with arborizations in the AOTu receive ipsilateral monocular input (Pfeiffer et al. 2005), many neurons

of the central complex receive input from both eyes (Vitzthum et al. 2002; Homberg 2004). These data strongly support recent evidence from *Drosophila melanogaster* (Strauss 2002; Liu et al. 2006) that point to a role of the central complex in spatial recognition of visual patterns, spatial orientation, and navigational control of locomotion.

Although these studies showed that parts of the AOT carry polarization vision signals to the median protocerebrum that are suited for sky compass orientation, it remains unclear whether the AOT is the only visual pathway relevant for polarotactic behavior. We, therefore, studied the effects of AOT lesions on polarotactic orientation of locusts and show that polarotaxis in tethered flight is abolished following AOT lesions. This study demonstrates that the AOT is an indispensable part of visual pathways involved in polarotaxis behavior of the desert locust.

Material and Methods

Animals

Experiments were performed on adult desert locusts (*Schistocerca gregaria*) of both sexes obtained from a crowded laboratory colony at the University of Marburg. Animals were kept under LD 12:12h conditions at 28°C and 50% relative humidity. Only animals with intact wings, legs, and antennae were used for experiments.

Experimental setup

Tethered locusts were flown in front of a horizontal wind tunnel (Gewecke 1975) inside a flight chamber (34 cm x 25.5 cm x 26 cm) made of matt-black plastic material. The animal's head was positioned centrally under a rotating polarization filter (Polaroid HN 38S; visual angle 87.3°, speed 5°/s) in the ceiling of the flight chamber. Light was produced by a fluorescent lamp (Osram Dulux EL16W; irradiance 25.8 $\mu\text{W cm}^{-2}$). A horizontal diffuser was inserted between the light source and the polarization filter to guarantee even illumination. Yaw-torques produced by the flying locusts were measured by a yaw-torque meter (Preiss and Gewecke 1991; Mappes and Homberg 2004, see below).

Polarotaxis following occlusion of one eye

To assess the effects of unilateral exclusion of input on polarotaxis, one eye was painted with black paint (Marabu Decorlack matt, water-based). Animals were first flown with both eyes open and, after painting their right eye black, they were tested again (see Behavioral experiments). In unilaterally operated locusts, the eye of the intact (right) side was painted.

Surgery

Animals were cooled to 5°C in a Peltier control unit to minimize hemolymph pumping during surgery. The left side of the head capsule was opened and the left AOT near the AOTu was transected with a fine razorblade (Fig. 1). Fat tissue and tracheae were carefully pushed aside. Afterwards the head capsule was closed with wax. For recovery and increased flight motivation, animals were kept isolated and without food for about 1-2 hours under a heat lamp prior to behavioral testing. Two types of control experiments were performed: (i) surgery as described above, but without transection of the AOT and (ii) surgery and transection in the antennal lobe.

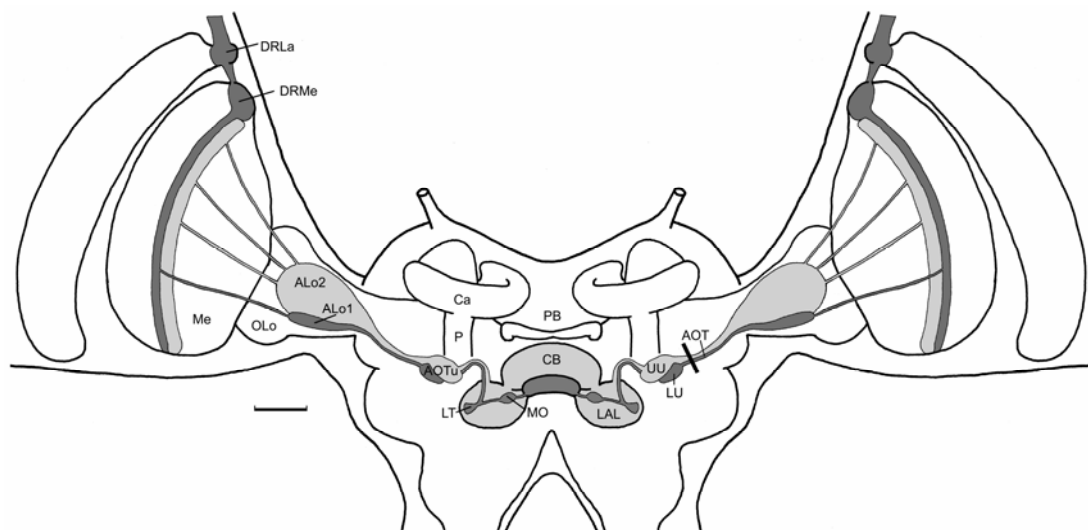


Fig. 1 Schematic diagram of visual pathways through the anterior optic tract and tubercle (AOT; AOTu) in the brain of the locust *Schistocerca gregaria*. The polarization-vision pathway to the lower division of the central body (CB) is shown in dark grey; a parallel visual pathway to the upper division of the central body is shown in light grey. ALo1, ALo2 layers 1 and 2 of the anterior lobula; Ca calyces of the mushroom body; DRLa, DRMe dorsal rim of the lobula and the medulla; LAL lateral accessory lobe; LT lateral triangle; LU, UU lower and upper unit of the AOTu; Me medulla; MO median olive; OLo outer lobe of the lobula; P pedunculus of the mushroom body; PB protocerebral bridge. Black bar indicates site of lesion. Scale bar: 200 μ m. Modified after Homberg et al. (2003a).

Behavioral experiments

After surgery the animals were attached ventrally to a torque gauge (Preiss and Gewecke 1991; Mappes and Homberg 2004) and were tested for polarotaxis in the flight chamber. Each experiment consisted of three full rotations of the polarizer, so that each *E*-vector position relative to the animal's longitudinal axis occurred six times. Experiments were performed on animals treated in the following ways: (i) intact (untreated) animals with both eyes free or the right eye painted; (ii) left AOT lesioned with both eyes free or the right eye painted; (iii) sham operated locusts with both eyes free or the right eye painted, and (iv) antennal-lobe lesioned animals with right eye free or painted. In all groups, animals were first tested with both eyes open, and a second time after the right eye (eye of the intact side of lesioned animals) was painted black. Yaw torque responses of the animals were analyzed with the fast Fourier transform (FFT; Origin 6.0, Microcal Inc., Northampton, MA, USA). Amplitude values of the Fourier graphs at 180° from flights before and after one eye was painted black were taken and compared using the non-parametric Mann-Whitney U test (Clampfit 9.0, Axon Instruments, Union City, CA, USA).

Immunocytochemistry

Many neurons of the AOT are immunoreactive with an antiserum against the neuropeptide locustatachykinin II (Lom-TK II; Homberg et al. 2003a). To selectively analyze the extent of transection, the brains of the experimental animals were processed for Lom-TK II immunostaining immediately after the flight experiments. The primary antiserum against Lom-TK II was generously provided by Dr. H. Agricola (University of Jena, Germany) and was diluted at 1:80,000. The indirect peroxidase-antiperoxidase (PAP) technique of Sternberger (1979) for free-floating sections was used for staining. Fixation, tissue embedding, and sectioning have been described by Vitzthum and Homberg (1998) and details of the immunostaining protocol by Hofer et al. (2005).

Results

In this study we tested yaw-torque responses of tethered flying locusts under a rotating polarizer after unilateral surgical lesion of the AOT. In all experiments the

readiness of the locusts to fly was highly variable and did not depend on sex. No significant difference in periodicity and amplitude occurred between flights under the polarizer rotating clockwise or counterclockwise (Mappes and Homberg, 2004). Only flights in which animals flew constantly for three full rotations of the polarizer were evaluated and analyzed.

Polarotaxis of intact locusts

Since polarotactic yaw torque responses in the tethered locusts were highly variable between individuals, it was essential to compare polarotaxis with intact and lesioned AOT in the same animals. Intact animals generally tried to turn with the rotating polarizer, but when yaw-torque reached a certain level, they turned against the rotating direction and then followed again. Since the rotating polarizer has a periodicity of 180° , the locusts usually changed their flight direction twice during a 180° -rotation of the polarizer. Between animals there was no particular relationship between the preferred orientation of the animal with respect to polarizer orientation (Mappes and Homberg 2004). In a first series of experiments, we analyzed polarotaxis in 48 flights of 20 “one-eyed” locusts. Yaw-torque was recorded during rotation of the polarizer in untreated animals and a second time after their right eye was painted black (Fig. 2A, C). FFT graphs showed peaks of similar height at 180° (Fig. 2B, D). Amplitudes at 180° were taken as a measure for the strength of periodicity; they were not significantly different before and after painting the right eye black (Fig. 2, Fig. 6). In contrast, flights with occluded right eye and flights with both eyes painted black (not shown) showed a highly significant difference in periodicity ($p < 0.01$).

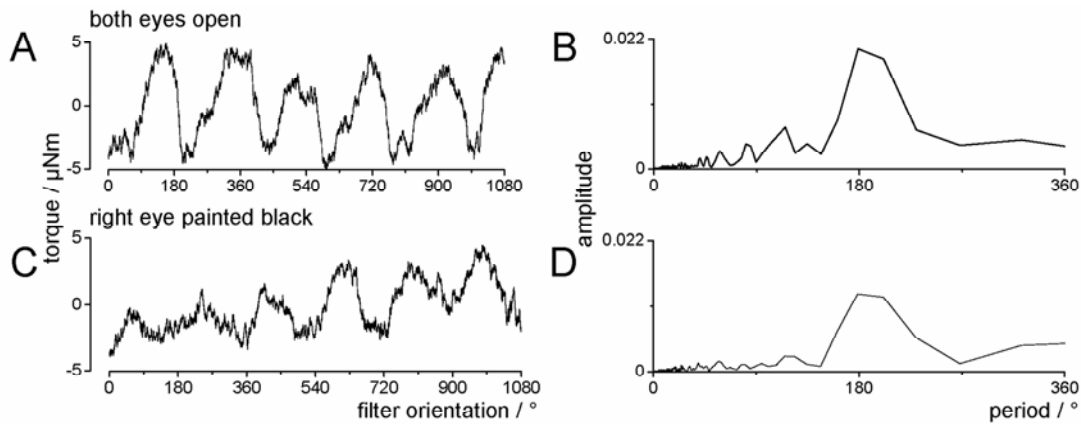


Fig. 2A-D Yaw torque responses and Fourier graphs from flight traces of a tethered flying untreated locust under a rotating polarizer. For the analysis of periodicity, in this and all other figures, the amplitude of the Fourier graphs is plotted against the period of rotation. **A** Flight with both eyes open under a polarizer turning clockwise. Positive yaw torque in this and all other graphs corresponds to right turns of the locust and negative yaw-torque, to left turns. At 0° and 180° the *E*-vector of the rotating polarizer is parallel to the animals' longitudinal axis. Yaw torque responses show a 180° periodicity. **B** Fourier graph from a fast Fourier transform (FFT) of the flight trace in **A**. **C** Flight trace to counterclockwise rotation of the polarizer after painting the right eye of the animal black. A 180° periodicity is still present. **D** Fourier graph of flight in **C**.

Polarotaxis of locusts with transected AOTs

Data from ten operated animals were evaluated for polarotactic flight behavior. Immunocytochemical staining showed that in all of these animals, transection of the the AOT was complete. Adjacent tissue in the inferior lateral protocerebrum was slightly affected in some animals, but all other tracts from the optic lobe to the median protocerebrum remained intact.

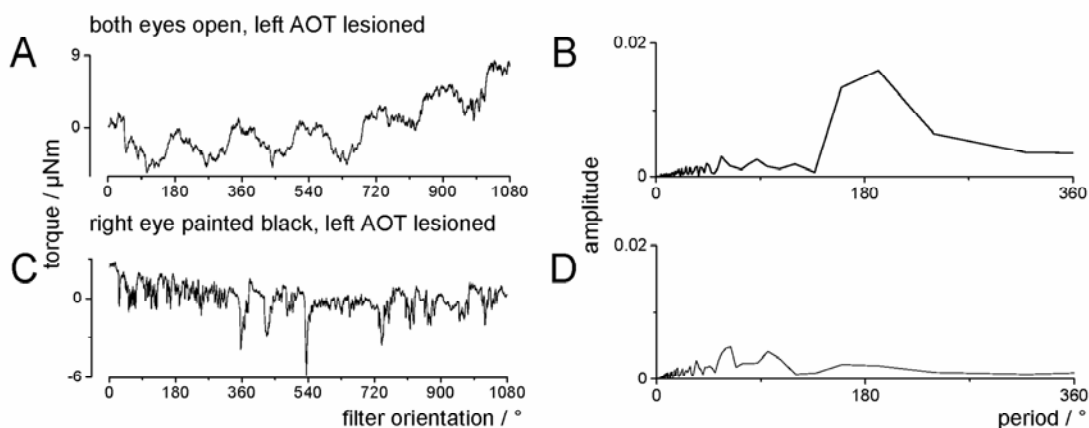


Fig. 3A-D Flight traces and Fourier graphs of tethered flying locusts with lesioned left AOT under a rotating polarizer. **A** Flight of an animal with left AOT lesioned and both eyes unoccluded. **B** Fourier graph of flight in **A**, showing strong periodicity. **C** Flight with the eye of the intact brain hemisphere painted black. Flight behavior becomes highly irregular and lacks periodicity. **D** Fourier graph of flight in **C**; no periodicity is visible.

With both eyes left open (16 flights), all animals showed strong 180° periodicity (Fig. 3A), i.e. they changed their yaw-torque direction two times within a 180° rotation of the polarizer. When torque is zero, the animals fly straight ahead. Flights under this situation had a clear peak at 180° in the Fourier graph (Fig. 3B, Fig. 6). After painting the eye of the intact brain hemisphere black, polarotaxis vanished (20 flights, Fig. 3C, D). Flights became irregular and yaw torque peaks during the experiment did not correspond to a consistent orientation of the polarizer, but occurred spontaneously and unidirectional. The mean of all Fourier graphs from 16 flights with both eyes open showed 180° periodicity indicating polarotaxis (Fig. 4A) but revealed lack of periodicity in flights in which the intact eye was painted black ($n = 20$; Fig. 4B). Amplitudes of the flights of the lesioned animals with contralateral eye painted black were not significantly different from flights of unlesioned animals with both DRAs painted black ($p > 0.5$, not shown).

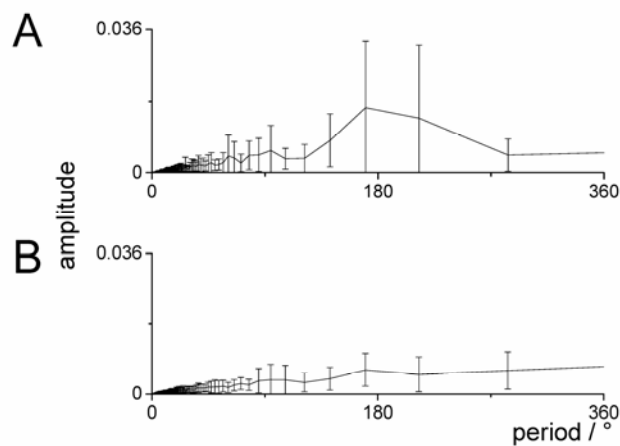


Fig. 4A, B Average Fourier graphs (\pm SD) of flights with left AOT lesioned. **A** When both eyes are unoccluded, the average of all Fourier graphs ($n = 16$) shows a 180° periodicity. **B** After painting the right eye black, the average of all Fourier graphs ($n = 20$) lacks periodicity.

Polarotaxis of locusts after control surgery

In order to demonstrate that lack of polarotaxis in tests with AOT lesioned animals was specifically induced by AOT lesion, sham operations were performed. In six animals the left side of the head capsule was opened and closed again without lesioning any part of the brain. Nine flights with covered right eye and ten flights with both eyes open showed no difference in strength of periodicity (Fig. 5, Fig. 6). In the second control, seven animals were operated as described above and a lesion

was made in the antennal lobe. Results were similar to those described for the first control, i.e. polarotaxis and 180°- periodicity were present in both test series and no significant difference in periodicity was found (Fig.5, Fig. 6).

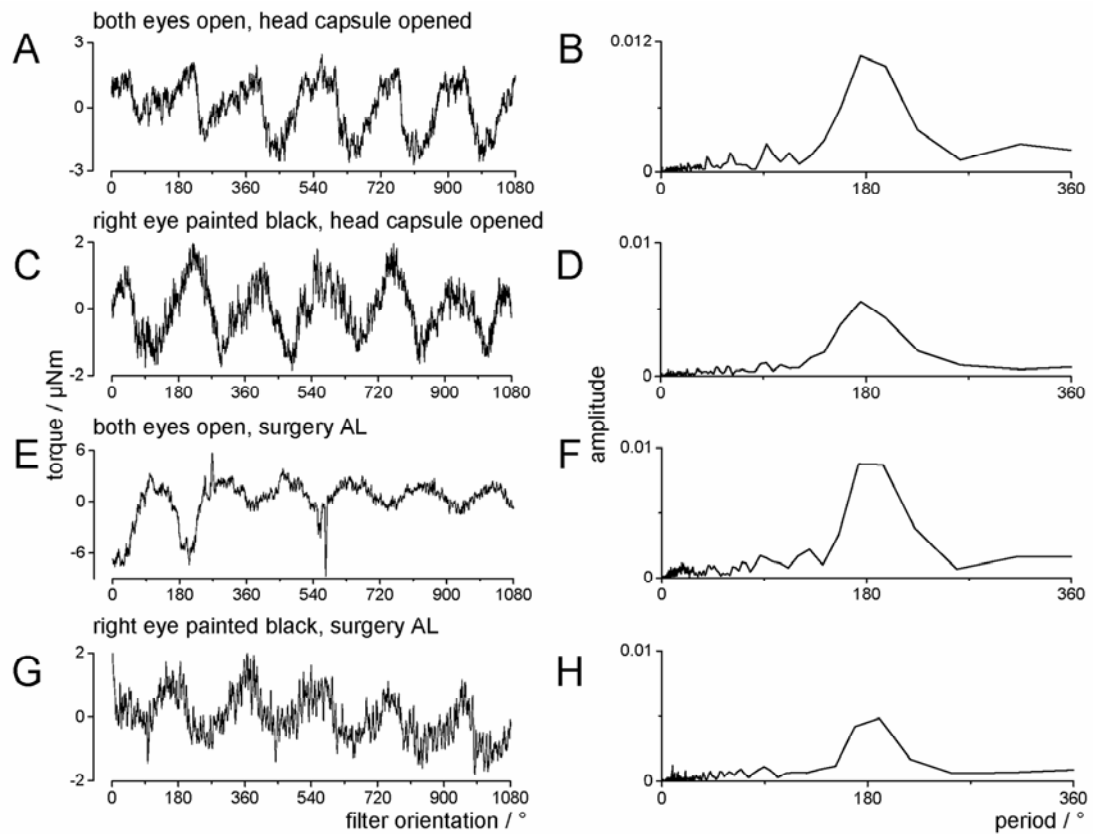


Fig. 5A-H Flight traces and Fourier graphs of two control experiments. **A-D** The head capsule was opened and closed again without a lesion. **E-H** Lesion was done in the antennal lobe. **A, E** Both eyes were left open; animals show a 180° periodicity in yaw torque. **B, F** Fourier graphs of flights in A and E. **C, G** After the eye of the intact brain hemisphere was painted over, polarotaxis is still present. **D, H** Fourier graphs of flights in C and G.

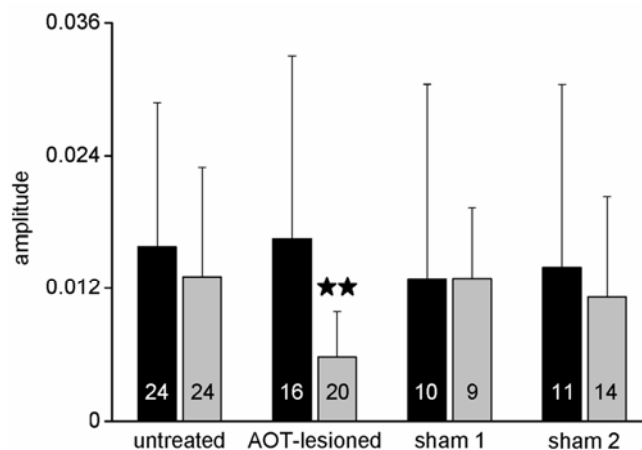


Fig. 6: Mean amplitudes (+ SD) of Fourier graphs from all flights at 180° . *Black bars* both eyes open, *grey bars* right eye painted black. Numbers of flights are shown in the bars. *Sham 1* Control experiments with head capsule opened; *sham 2* control experiments with transected antennal lobe. *Asterisks* indicate significant reduction in amplitude in flights with AOT transected after painting the right eye black ($p < 0.01$). In all other test series, flights with both eyes open and right eye painted black are not significantly different ($p > 0.5$ for both controls, $p > 0.1$ for flights with untreated animals).

Discussion

Spatial orientation of insects to polarized light of the blue sky has been shown in behavioral studies in bees, ants, and beetles (Rossel and Wehner 1986, Dacke et al. 2003, Wehner 2003). Sauman et al. (2005) also reported sky polarization dependent flight orientation in monarch butterflies, but these data were questioned by Stalleicken et al. (2005). Tethered flying locusts show periodic changes in yaw-torque corresponding to the orientation of a rotating polarizer presented from above. This behavior is induced by photoreceptors of the DRA of the compound eye (Mappes and Homberg 2004). What are the neural mechanisms underlying polarotactic flight orientation and which pathways are involved from the DRA to motor centers controlling flight performance? Considerable progress has been made in recent years in analyzing the polarization vision pathway in the locust brain (Homberg et al. 2003a; Pfeiffer et al. 2005). Among several processing stages, the AOTu appears to be a particularly important relay center for polarized light signals since it connects projections from the medulla to postsynaptic neurons with axons toward the locust midbrain and central complex (Homberg et al. 2003a; Pfeiffer et al. 2005). The present study shows that polarotactic flight behavior of the locust is critically controlled by neurons in the AOT.

Microsurgical lesions of insect brain regions have been used in several instances to demonstrate loss or change of behavior. They include studies supporting the role of horizontal cells in optomotor yaw torque responses in the blowfly *Calliphora erythrocephala* (Hausen and Wehrhahn 1983) and the role of the accessory medulla as the site of the circadian clock in the cockroach *Leucophaea maderae* (Homberg et al. 2003b). To investigate the importance of the AOT for locust polarization vision, we performed lesions of that tract and demonstrated that polarotaxis of tethered flying locusts with transected AOT disappeared, while sham operations with and without transecting other parts of the brain were without effect (Fig. 6). Although the presumed navigation center, the central complex, receives bilateral polarization-vision input, polarotactic behavior, at least to the extent and detail studied here, was unaffected after polarization-vision input from one side had been interrupted by eye painting. This indicates that locusts do not have to compare polarization information from both eyes for the polarotaxis behavior studied here.

Functional significance

Conspicuously, polarization-vision pathways mentioned so far are located anteriorly in the locust brain (Fig. 1). More prominent connections of the optic lobe with the midbrain, however, occur posteriorly in the brain, especially through the massive posterior optic tract (POT). Among those, a small fiber bundle connects the accessory medulla, the site of the circadian clock in cockroaches and flies, to a small posterior brain neuropil, the posterior optic tubercle (POTu) (Homberg and Würden 1997). The POTu, in turn, is closely connected with the protocerebral bridge of the central complex (Homberg 1991; Heinze and Homberg 2005; Homberg and Heinze 2006). Evidence for a timekeeping function of the accessory medulla, at least in cockroaches and flies, suggests that this posterior connection to the central complex may serve a role for time compensation in celestial navigation (Homberg 2004). On the other hand, certain neurons of the accessory medulla and neurons connecting the POTu and protocerebral bridge are, like neurons of the anterior pathway to the central complex, polarization-sensitive (Homberg and Würden 1997; Heinze and Homberg 2005; Homberg and Heinze 2006). Whether this posterior visual pathway to the central complex is, indeed, a second way of polarized light signals to enter the central complex, or whether the POTu rather acts as a convergence site of outputs of the central complex and a time signal from the accessory medulla, as proposed by Heinze and Homberg (2005), will have to await further studies. At least in the context of the behavioral assay used here, this posterior pathway alone does not support polarization-vision signaling sufficient for maintaining polarotaxis.

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References

- Brunner D, Labhart T (1987) Behavioural evidence for polarization vision in crickets. *Physiol Entomol* 12:1-10
- Dacke M, Nordström P, Scholtz C (2003) Twilight orientation to polarised light in the crepuscular dung beetle *Scarabaeus zambesianus*. *J Exp Biol* 206:1535-1543
- Frisch K von (1949) Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzen der Bienen. *Experientia* 5:142-148
- Gewecke M (1975) The influence of the air-current sense organs on the flight behaviour of *Locusta migratoria*. *J Comp Physiol* 103:79-95
- Hausen K, Wehrhahn C (1983) Microsurgical lesion of horizontal cells changes optomotor yaw responses in the blowfly *Calliphora erythrocephala*. *Proc R Soc Lond B* 219:211-216
- Heinze S, Homberg U (2005) A new set of tangential neurons of the protocerebral bridge in the desert locust *Schistocerca gregaria* is sensitive to polarized light. 98. Annual meeting of the DZG, Bayreuth, <http://www.uni-bayreuth.de/dzg-gebin2005>, p. 73
- Hofer S, Dircks H, Tollbäck P, Homberg U (2005) Novel insect orcokinin: characterization and neuronal distribution in the brains of selected dicondylarian insects. *J Comp Neurol* 490:57-71
- Homberg U (1991) Neuroarchitecture of the central complex in the brain of the locust *Schistocerca gregaria* and *S. americana* as revealed by serotonin immunocytochemistry. *J Comp Neurol* 303:245-254
- Homberg U (2004) In search of the sky compass in the insect brain. *Naturwissenschaften* 91:199-208
- 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
- Homberg U, Heinze S (2006) A computational map of *e*-vector orientations in the central complex of the desert locust (*Schistocerca gregaria*). *FENS Abstr.* 3: A129.9
- Homberg U, Hofer S, Pfeiffer K, Gebhardt S (2003a) Organization and neural connections of the anterior optic tubercle in the brain of the locust, *Schistocerca gregaria*. *J Comp Neurol* 462:415-430

- Homberg U, Reischig T, Stengl M (2003b) Neural organization of the circadian system of the cockroach *Leucophaea maderae*. *Chronobiol Int* 20:577-591
- Labhart T, Meyer EP (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
- Labhart T, Meyer EP (2002) Neural mechanisms in insect navigation: polarization compass and odometer. *Curr Opin Neurobiol* 12:707-714
- Liu G, Seiler H, Wen A, Zars T, Ito K, Wolf R, Heisenberg M, Liu L (2006) Distinct memory traces for two visual features in the *Drosophila* brain. *Nature* 439:551-556
- Mappes M, Homberg U (2004) Behavioral analysis of polarization vision in tethered flying locusts. *J Comp Physiol A* 190:61-68
- Pfeiffer K, Kinoshita M, Homberg U (2005) Polarization-sensitive and light-sensitive neurons in two parallel pathways passing through the anterior optic tubercle in the locust brain. *J Neurophysiol* 94:3903-3915
- Philipsborn A von, Labhart T (1990) A behavioral study of polarization vision in the fly, *Musca domestica*. *J Comp Physiol A* 167:737-743
- 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
- Rossel S, Wehner R (1986): Polarization vision in bees. *Nature* 323:128-131
- Sakura M, Labhart T (2005) Polarization-sensitive neurons in the central complex of the cricket, *Gryllus bimaculatus*. *Neuroforum* 2005 Suppl:154B
- Sauman I, Briscoe AD, Zhu H, Shi D, Froy O, Stalleicken J, Yuan Q, Casselman A, Reppert SM (2005) Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* 46:457-467
- Stalleicken J, Mukhida M, Labhart T, Wehner R, Frost B, Mouritsen M (2005) Do monarch butterflies use polarized skylight for migratory orientation? *J Exp Biol* 208:2399-2408
- Sternberger LA (1979) *Immunocytochemistry*. Wiley, New York
- Strauss R (2002) The central complex and the genetic dissection of locomotor behaviour. *Curr Opin Neurobiol* 12:633-638

- Vitzthum H, Homberg U (1998) Immunocytochemical demonstration of locustatachykinin-related peptides in the central complex of the locust brain. *J Comp Neurol* 390:455-469
- 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
- Wehner R (2003): Desert ant navigation: how miniature brains solve complex tasks. *J Comp Physiol A* 189:579-588

CHAPTER III:

Sky compass cues in desert locusts:

Field studies underneath natural sky light

**Sky compass cues in desert locusts:
Field studies underneath natural skylight**

Martina Mappes and Uwe Homberg

ABSTRACT

Bees and ants use compass information from the position of the sun and the polarization pattern of the blue sky for spatial navigation and long-range migration. In laboratory experiments, tethered flying desert locusts, *Schistocerca gregaria*, show *E*-vector-dependent yaw-torque responses under a slowly rotating polarizer. To investigate whether locusts indeed use sky signals as guiding cues for orientation, flight directions in tethered flying locusts were analyzed in an outdoor flight simulator under natural blue or cloudy skies. The experiments show that locusts maintain flight directions when the sun is visible. Uni-directional flight vanishes when the sky is cloudy or both dorsal rim areas of the compound eyes are painted black in the absence of the sun. The experiments suggest that menotactic orientation to direct sunlight contributes to the navigational system of the locust, as demonstrated for honeybees and desert ants. Orientation to polarized light could not be demonstrated by the small number of flights performed in the present study. For this purpose, further experiments must be performed. Mean flight directions were constantly opposite to the sun's position through the course of the day, suggesting that desert locusts do not use a time-compensated navigation compass as it has been demonstrated for monarch butterflies.

Introduction

For many animals, sun compass orientation is a common strategy for maintaining navigational directions during migrations and long-range excursions. Many insect species, in addition, can detect the polarization pattern of the blue sky and use it for navigation. The pattern of *E*-vector orientations in the sky is a reliable source of information for navigation, even if the sun is not visible and only a small patch of the blue sky is available. *E*-vectors in the sky are oriented in concentric circles around the sun and do not change their relative position to the sun in the course of the day. During flight in great height or in landscapes with few landmarks, the ability of polarization vision is important for orientation.

Polarotaxis under laboratory conditions has been demonstrated in crickets (Brunner and Labhart 1987) and flies (Wolf et al. 1980; von Philipsborn and Labhart 1990) and was shown in walking locust larvae by Eggers and Gewecke (1993). In tethered desert locusts, we demonstrated polarotactic flight behavior under a slowly rotating polarizer (Mappes and Homberg 2004). Polarization sensitivity under the natural sky was hitherto studied in ants, bees, monarch butterflies, and dung beetles (Wehner 2003; von Frisch 1949, Rossel and Wehner 1987; Dacke et al. 2003; Sauman et al. 2005; Stalleicken et al. 2005). Ants perform long walking tours during foraging with often very intricate paths. By measuring all rotatory and translatory components of movement during their walk, they are able to return to their nest on a direct path by updating continuously their spatial information with the assistance of the sky polarization pattern (Wehner 2003). Karl von Frisch (1949) was the first to show that polarized light is the most important orientation cue for bees if the sun is not visible, and Rossel and Wehner (1987) confirmed and extended his findings. Experiments with the dung beetle *Scarabaeus zambesianus* showed that these insects use the polarization pattern of the clear evening sky to roll their dung ball in a straight line away from the food source to avoid aggressive competitors (Dacke et al. 2003). Monarch butterflies, like desert locusts, perform long-range migrations, which are highly oriented especially in the fall. Sauman et al. (2005) demonstrated that monarchs use the sky polarization pattern for compass orientation, whereas Stalleicken et al. (2005) showed that they cannot navigate based on polarized light alone.

Orientation behavior dependent on the detection of the sky polarization pattern is mediated by a small dorsal part of the compound eye, the dorsal rim area (DRA, Labhart and Meyer 1999). Desert locusts have a particularly prominent DRA with a number of structural adaptations for high polarization sensitivity (Homberg and Paech 2002). In tethered flying locusts, polarotaxis vanishes if the DRAs are painted black (Mappes and Homberg 2004).

To examine whether desert locusts show polarotactic flight behavior under the open sky, we performed outdoor flight experiments under natural light conditions. We found that locust flight is strongly unidirectional with and without visibility of the sun and that it becomes undirected under cloudy sky or when the DRAs are painted black and the sun is not visible.

Material and methods

Behavioral experiments were carried out in an open field near the Biology Department of the University of Marburg, Germany, during the summer of 2005. Locusts were flown at different times of day from 8:00 a.m. to 8:00 p.m.

Animals

Adult desert locusts (*Schistocerca gregaria*) were used for all experiments. They were kept under natural light conditions from May to September 2005. Larvae from the 3rd to last larval stage were taken from a crowded indoor colony of the University of Marburg and were transferred into a greenhouse without additional air conditioning. Animals had free sight on the sky at any time and at least ten days after adult molt, they were taken for experiments.

Experimental setup

We used two identical flight simulators (kindly provided by H. Mouritsen, University of Oldenburg, Germany) made of white and translucent plastic material (diameter: 54cm, height: 64cm), offering free sight on the natural sky. Laminar air flow was obtained by blowing air through drinking straws in the bottom of the stimulators to ensure continuous flight (Fig. 1; for a detailed description of the flight simulator see

Mouritsen and Frost 2002; Stalleicken et al. 2005). Locusts were tethered in the center of the cylinder at a height providing a 100° visual field of the sky. An 8 cm mounting rod (tungsten wire, diameter: 0.4 mm) was attached dorsally to the animal's pronotum. This wire was connected through a small piece of rubber tube and a 15 cm tungsten wire to a miniature optical encoder (E4, US Digital, Vancouver, WA 98684 USA). Thus, the distance between the horizontally oriented body axis of the locust and the encoder was 23 cm.

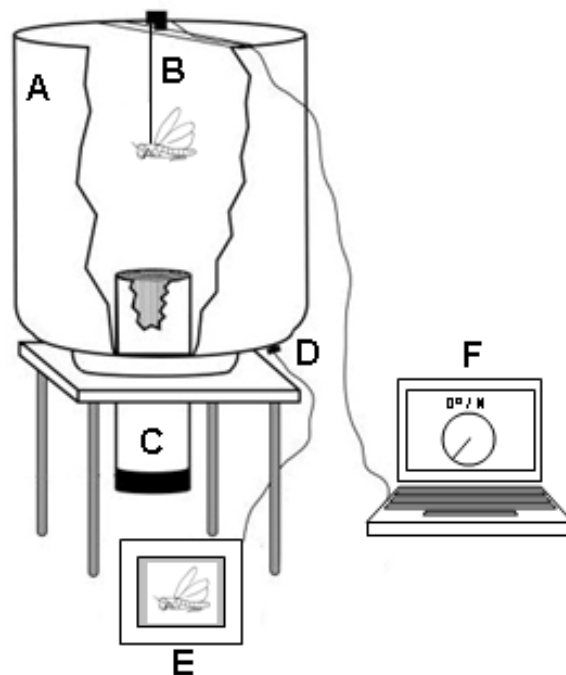


Fig. 1: Schematic drawing of the flight simulator. *A* plastic barrel for preventing the animals from non-celestial visual orientation cues; *B* mounting rod connected to the optical encoder; *C* fan producing laminar air flow through parallel drinking straws; *D* miniature camera; *E* video monitor; *F* recording computer. Modified after Mouritsen and Frost (2002).

Within the setup the animals were able to maintain or change flight directions freely within the whole range of 360°. Instantaneous flight directions, recorded by the optical encoder were digitized (US digital box) and stored on a personal computer (Acer TravelMate 8101WLMi). Two miniature cameras, positioned at the bottom of the simulator, were connected to two video monitors (model DM 2212, Sanyo Electronic Co., Ltd, Korea, 50Hz) to allow constant observation of flight activity.

Behavioral experiments

Experiments were performed from 12 July to 8 September 2005 under the open sky, which was either cloudless blue, partly clouded, or completely clouded. In addition, some experiments were done during increasing cloudiness up to a complete cloudy sky to study the locusts' flight motivation and orientation when no celestial orientation cue was present. Each flight took 10 minutes and only recordings without flight interruption or gliding flight were used. At the beginning of the experiment, locusts were aligned northwards using a compass for calibration of the recording software, so that north was equal to 0° flight direction relative to the animals' longitudinal axis. Each animal was flown as long as possible to get results under different light conditions. We discriminated between 4 testing procedures: (1) animals had free sight on the sun and blue sky; (2) the blue sky was visible, but not the sun, and the barrel side facing the sun was illuminated through the barrel walls and the opposite side showed an oval-shaped bright light spot reflected by the barrel walls (brightness artifacts, see also Stalleicken et al. 2005); (3) the experimental setup was completely shadowed, so that the sun was not visible for the locusts and the brightness artifacts bearing possibly remaining orientation information were eliminated; (4) the DRAs of both compound eyes were painted black and experiments were performed under light conditions as described in 1-3.

Data analysis

All flight experiments were recorded with US Digital software supplied with the optical encoders. Flight direction of the locusts relative to north ($0^\circ / 360^\circ$) was registered every 200 ms. Data were transferred to Spike2 (Cambridge Electronic Design, version 4.1.2, Cambridge, England) and Oriana (Kovach Computing Services, Anglesey, Wales, version 2.02a) for further analysis. For each flight, a circular chart was created and statistical analysis was performed. Rayleigh tests were performed to test the distribution of circular data around the mean. Mann-Whitney test and student's t-test were performed with Clampfit 9.0 (Axon Instruments, Union City, CA, USA).

Results

In this study we analyzed the directedness of flight of tethered flying locusts under the natural sky. Only animals with intact wings, legs, and antennae were used for the experiments. No differences in flight behavior between male and female locusts were observed.

Flight orientation under blue sky

To gain further insight on sky compass cues used by desert locusts for active orientation, we tested the directedness of flight under different sky conditions. 76 flights of 21 animals with direct view of the sun and/or the blue sky were analyzed. These animals usually maintained a particular flight direction throughout the test period (Fig. 2A). Length of mean vectors (r) was obtained for each flight and ranged from 0.173 to 0.999 (Fig. 3A). Therefore, flights are directed and the mean flight directions differ significantly from a uniform distribution (Rayleigh test, $p < 0.001$, respectively).

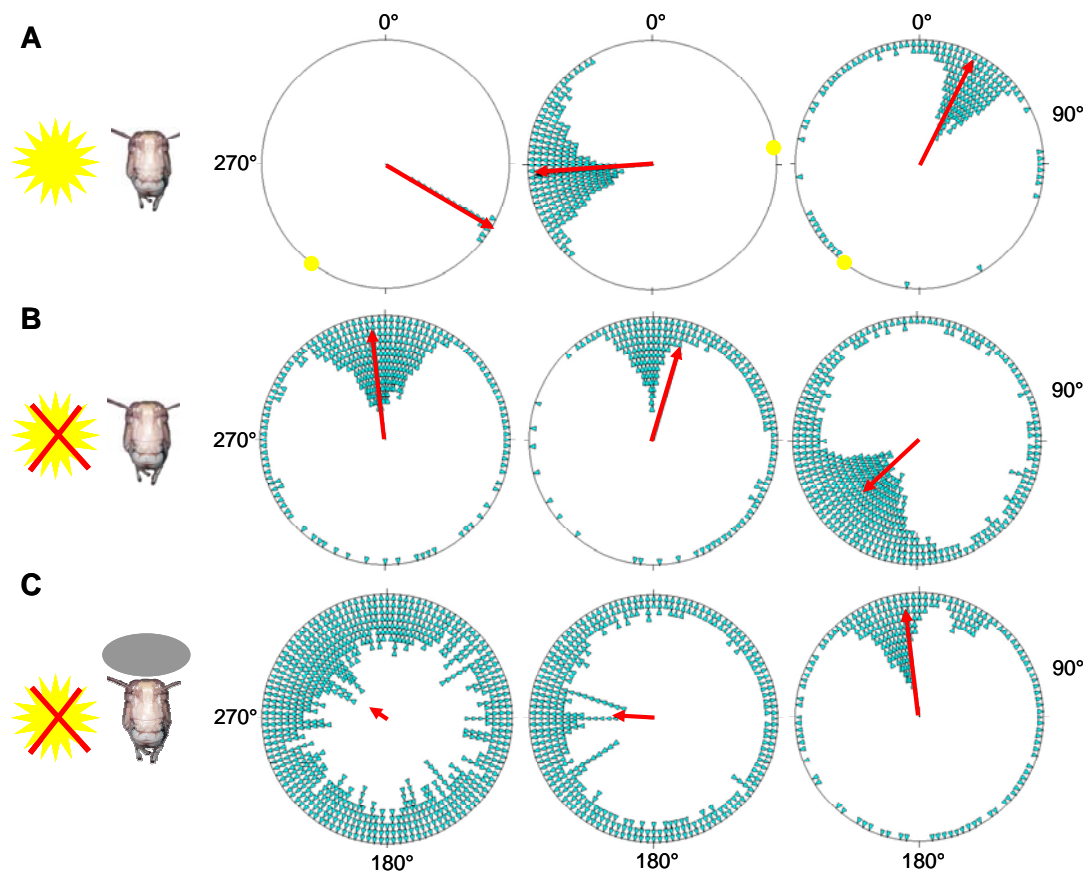


Fig. 2: Circular flight histograms of 10-minutes-flights under the blue sky with the sun visible (**A**), sun not visible, but brightness artifacts are present (**B**), and when setup was completely shadowed (**C**). Red arrows indicate mean vector directions (μ) and lengths (r). Yellow dots represent the sun. 0° = north; 90° = east. **A** Flights of three different animals with direct view of the sun. Locusts show highly directed flight. Left: sun elevation = 54° ; sun azimuth = 207° ; $r = 0.999$; $\mu = 121^\circ$. Middle: sun elevation = 21° ; sun azimuth = 82° ; $r = 0.945$; $\mu = 265^\circ$. Right: sun elevation = 54° ; sun azimuth = 207° ; $r = 0.933$; $\mu = 27^\circ$. **B** Flights of three different animals with direct view of the sky but not the sun. Flight is still clearly directed. Left: $r = 0.869$, $\mu = 354^\circ$. Middle: $r = 0.773$; $\mu = 16^\circ$. Right: $r = 0.591$; $\mu = 226^\circ$. **C** Flights of two different animals (middle and right histograms are from the same animal) with direct view of the sky, but setup in complete shadow. Flights are undirected. Left: $r = 0.15$; $\mu = 302^\circ$. Middle: $r = 0.317$; $\mu = 273^\circ$. Right: $r = 0.866$; $\mu = 352^\circ$.

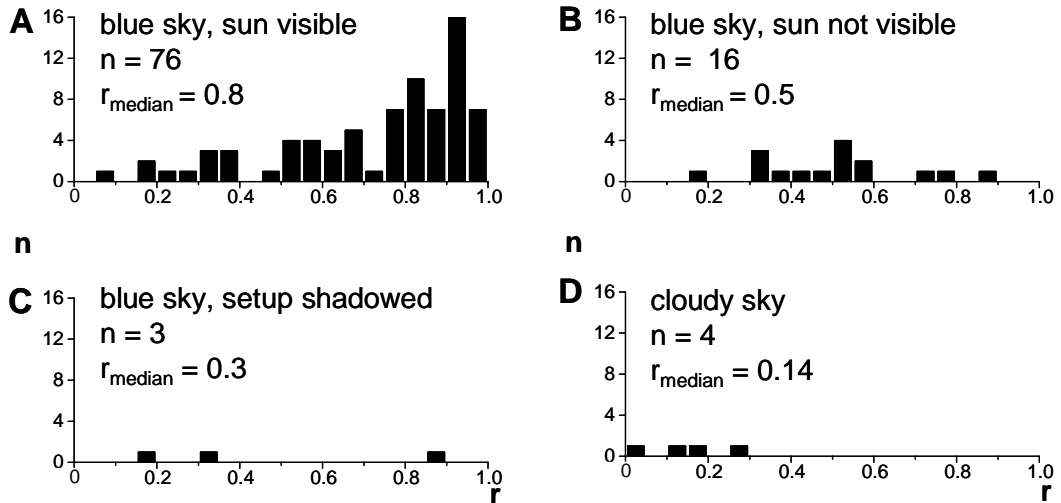


Fig. 3: Distribution of length of mean vectors (r) of flight direction at different light stimuli of all flights with unpainted eyes. Number of flights and median of r are given for each histogram. **A** blue sky, sun is visible for the animals; **B** blue sky, sun is invisible, but setup is partly illuminated and brightness artifacts are present; **C** blue sky, setup is completely shadowed; **D** cloudy sky, sun and patches of blue sky are not visible at any time.

In 16 flights from 5 animals from, only the blue sky and the brightness artifacts on the barrel walls were visible, but not the sun (Fig. 2B). R values ranged from 0.188 to 0.869 (Fig. 3B) and differed significantly from r -values under direct sunlight (Fig. 4, non-parametric Mann-Whitney test, $p < 0.001$). However, analysis of flights from 5 animals which performed flights at both stimuli (sun visible and not visible; 13 flights, respectively), no significant difference in the length of mean vectors occurred (Fig. 5; student's t -test for paired groups, $p = 0.89$). In all flights, p was < 0.001 (Rayleigh test). Three flights of two animals were performed, when the entire setup was shadowed. Two flights showed strongly reduced directedness, one flight was clearly directed (Fig. 2C). R values ranged from 0.15 to 0.866 (Fig. 3C) but were still significantly different from randomness (Rayleigh test, $p < 0.001$).

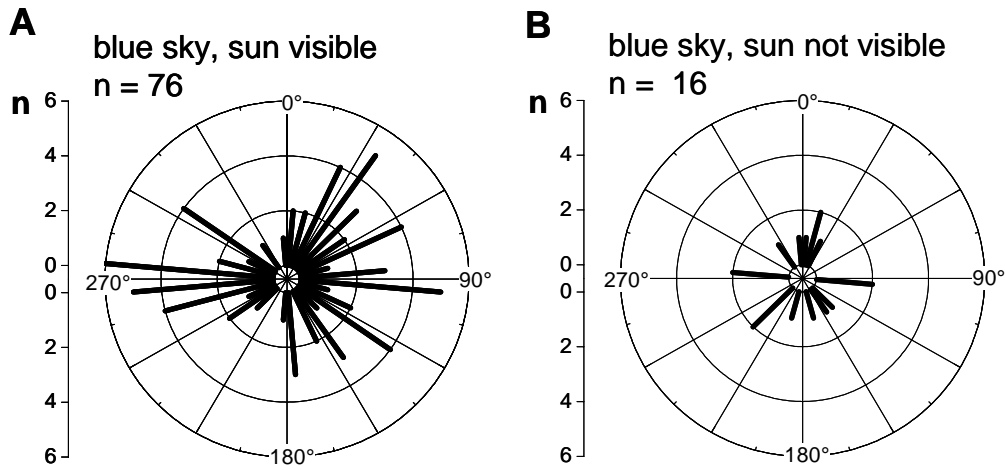


Fig. 4: Circular distribution of mean vectors of all flights with visible and invisible sun under blue sky. No preferred flight direction occurs, means are uniformly distributed.

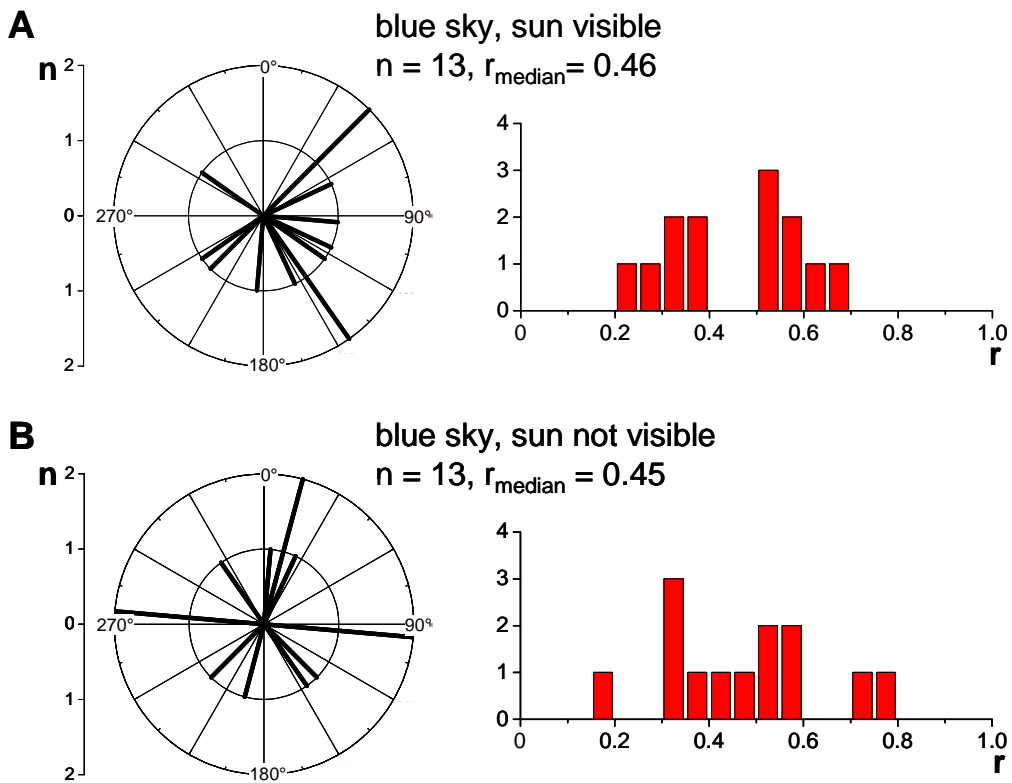


Fig. 5: Comparison between flights of the same animals with and without direct view of the sun. Circular distribution of mean flight directions and distribution of r is given for each testing situation. **A** Sun is visible, blue sky. **B** Sun is not visible, blue sky. Both circular histograms show a uniform distribution of means. There is no significant difference in r ($p = 0.89$, student's t -test, Clampfit 9.0).

Flight orientation under cloudy sky

In most cases, the locusts' flight motivation was strongly reduced under cloudy skies, and several animals did not start to fly at all. In three locusts, we succeeded in recording four flights under 100% cloudy sky (Fig. 6). R values ranged from 0.017 to 0.265 (Fig. 3D). The Rayleigh test demonstrates significant difference from uniform distribution in three of four cases ($p < 0.001$, respectively), for one flight, p was 0.4.

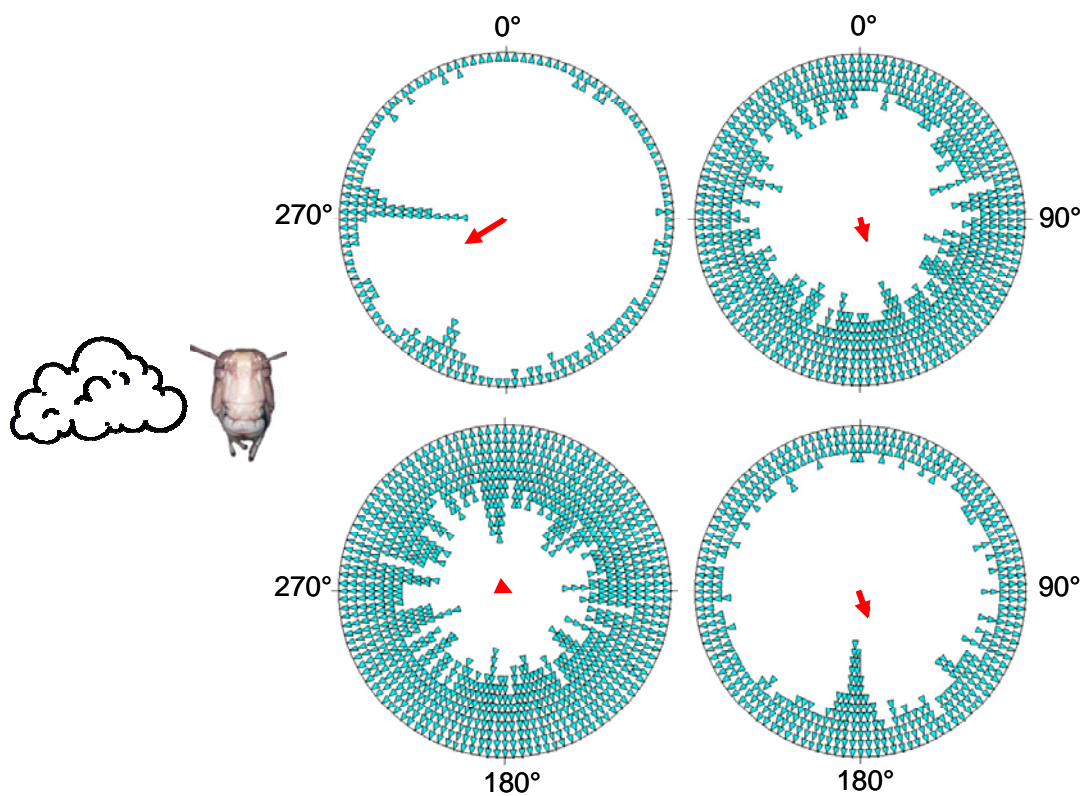


Fig. 6: Circular histograms of 10-minute flights under cloudy sky. *Upper* histograms show non-directed flight behavior of two animals. Left: $r = 0.265$; $\mu = 240^\circ$. Right: $r = 0.135$; $\mu = 162^\circ$. *Lower* histograms demonstrate two flights of the same animal at different days. Flight is again non-directed and has no definite direction. Left: $r = 0.017$; $\mu = 111^\circ$. Right: $r = 0.154$; $\mu = 163^\circ$.

Flight orientation under blue sky with both DRAs painted black

We painted the DRAs of the locusts with black paint, to eliminate the eye region essential for polarotaxis in the laboratory and investigated their flight behavior under the blue sky. Animals showed highly directed flight when sun was visible (Fig. 7A). We tested these conditions in eleven flights of seven animals and r ranged from 0.03 to 0.826 (Fig. 8A). Except for one flight ($p = 0.06$), the Rayleigh test shows

significant directedness with $p < 0.001$ for all flights. In 6 experiments (3 animals) when the sun was not visible, locusts showed rotating flight and were no longer uni-directional (Fig. 7B), although the Rayleigh test showed a significant difference from a uniform distribution ($p < 0.001$, respectively). However, r values only range from 0.057 to 0.487 and are, thus, strongly reduced as compared to unimpaired animals (Fig. 8B). Two animals showed strongly reduced directedness when the complete setup was shadowed (Fig 7C). R values were 0.091 and 0.128 (Fig. 8C).

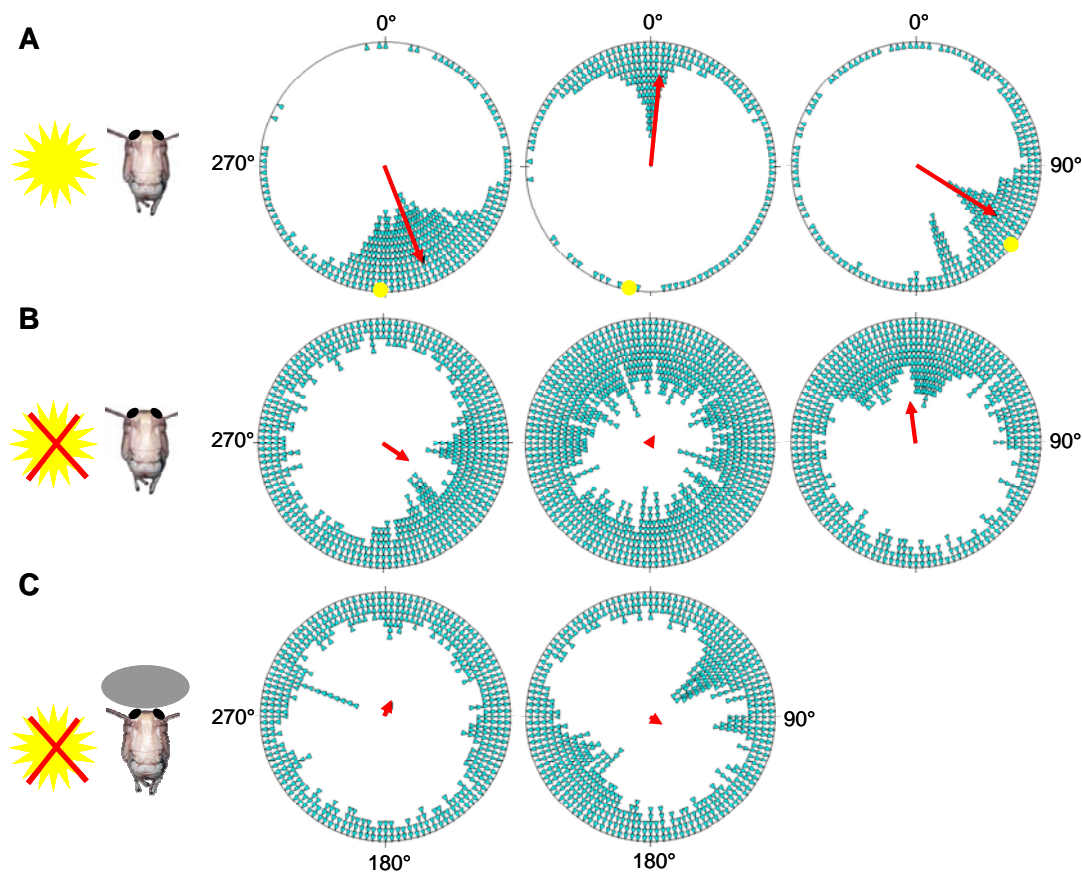


Fig. 7: Circular histograms of 10-minutes flights under the blue sky with the sun visible (**A**), sun not visible, but brightness artifacts are present (**B**), and when setup was completely shadowed (**C**). Both DRAs are painted black. **A** Histograms of three different animals, flight is clearly directed. Left: sun elevation = 48° ; sun azimuth = 182° ; $r = 0.826$; $\mu = 158^\circ$. Middle: sun elevation = 45° ; sun azimuth = 190° ; $r = 0.736$; $\mu = 6^\circ$. Right: sun elevation = 43° ; sun azimuth = 131° ; $r = 0.757$; $\mu = 121^\circ$. **B** Histograms of three different animals. Directed flight behavior is clearly reduced. Left: $r = 0.236$; $\mu = 124^\circ$. Middle: $r = 0.057$; $\mu = 28^\circ$. Right: $r = 0.323$; $\mu = 352^\circ$. **C** Histograms of two different animals. Flights do not show directedness. Left: $r = 0.128$; $\mu = 22^\circ$. Right: $r = 0.091$; $\mu = 125^\circ$.

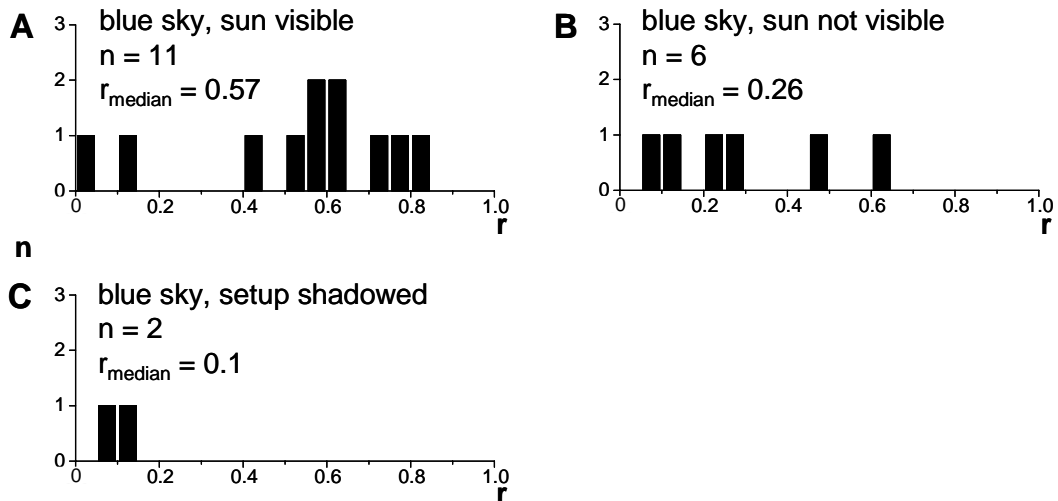


Fig. 8: Distribution of r at different light stimuli of all flights with both DRAs painted black. Number of flights and median of r are given for each histogram. **A** blue sky, sun is visible; **B** blue sky, sun is invisible, but setup is partly illuminated and brightness artifacts are present; **C** blue sky, setup completely shadowed.

Preferred flight direction with both eyes free or DRAs painted black

Circular distribution of mean flight directions when sun was visible or not and both eyes are free did not show a significant difference from a uniform distribution (Fig. 4; Rayleigh test; sun visible: $p = 0.4$; sun not visible: $p = 0.9$). By setting the sun as north and setting the mean vectors r in relation to the sun, a general flight direction away from the sun becomes apparent (Fig. 9A). Separate evaluation of flights in the morning, at noon, and in the afternoon, likewise shows that independent of time of day the mean flight direction is around 180° (Fig. 9B-D). No difference from a uniform distribution occurred for the ensemble of all flights and for the flights at noon (Rayleigh test; $p = 0.38$ and $p = 0.5$), while flights in the morning and in the afternoon differed significantly from a uniform distribution (Rayleigh test; $p < 0.001$, respectively). In experiments with both DRAs painted black, the mean flight directions have a bimodal distribution (Fig. 10).

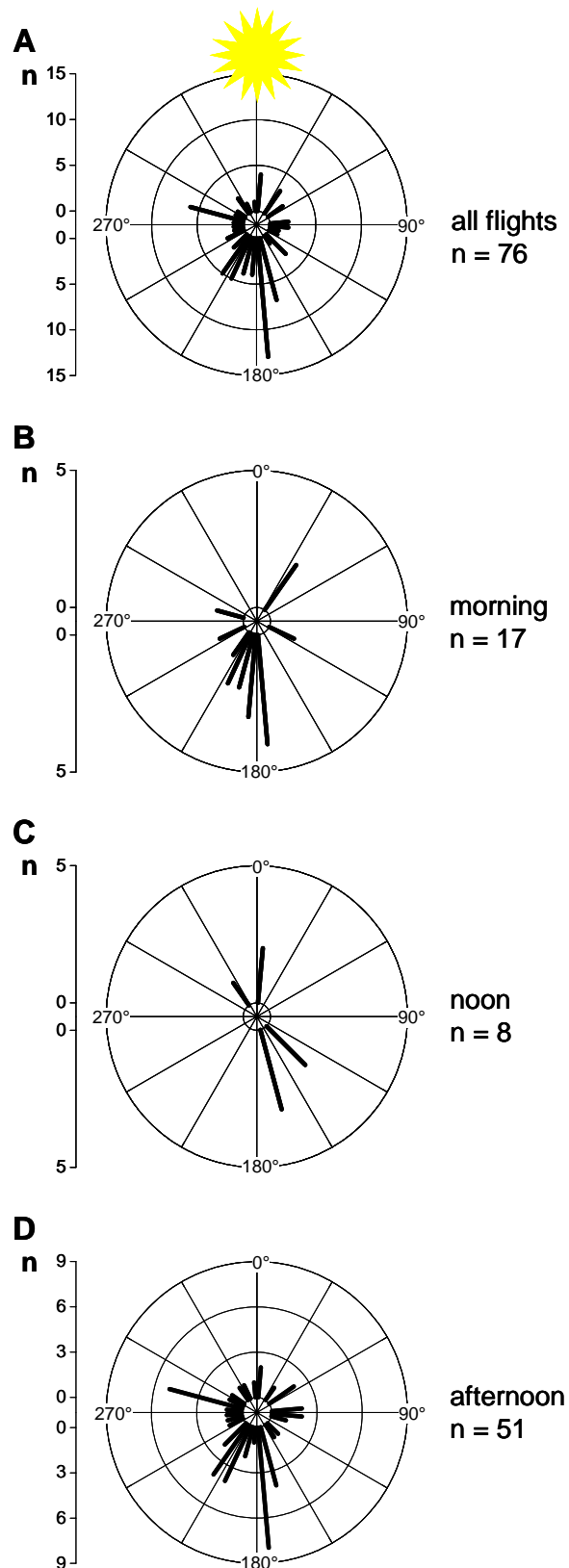


Fig. 9: Circular plots of mean flight vectors relative to the sun. Sun position was set as 0°. Animals fly away from the sun. **A** Distribution of all 76 flights, median of mean vectors = 179°. **B** Morning flights (8 a.m. - 12 a.m.), n = 17, median of means = 184° **C** Flights at noon (12:00a.m. - 1:30p.m.). n = 8, median of means = 148°. **D** Flights in the afternoon (1:30p.m. - 8p.m.); n = 51, median of means = 190°.

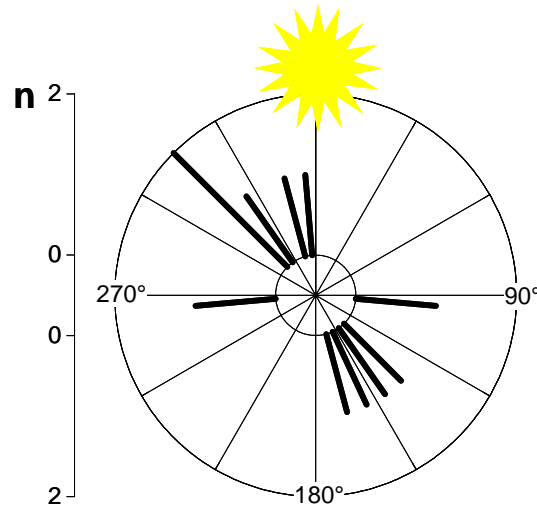


Fig. 10: Circular plot of 11 mean vectors relative to the sun when both DRAs are painted black. Sun position is set as 0°. Flight directions have a bimodal distribution and differ by nearly 180°. Median of means = 248°.

Preferred flight direction of the same animals on different days

In two animals flying under a clear blue sky a nearly 180° change in mean flight direction was observed in flights on two different days. This was the case when sun was not visible (Fig. 11A, B) as well as in experiments when the sun was visible (Fig. 11C). The time intervals between the experiments were 12, 13, and 18 days. Recording times on the first and the second day were at the same time of day (maximum difference 35 minutes).

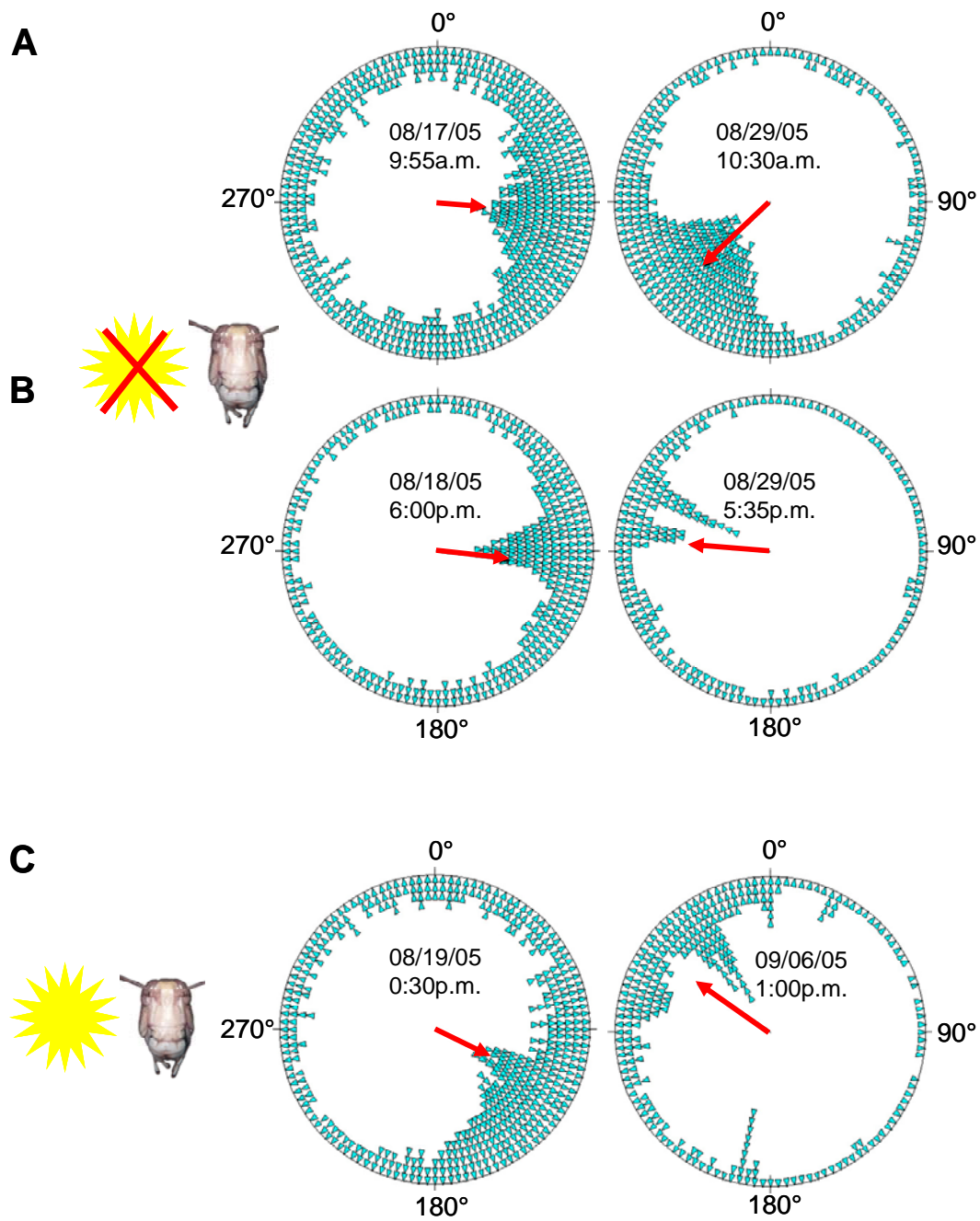


Fig. 11: Circular histograms of two animals at different days and approximately the same time. **A, B** Sun is not visible. **C** Same animal as in B, but sun is visible. In all cases an almost 180° difference in mean flight direction is observed. **A** Left: $r = 0.302$; $\mu = 96^\circ$. Right: $r = 0.591$; $\mu = 226^\circ$. **B** Left: $r = 0.459$; $\mu = 97^\circ$. Right: $r = 0.514$; $\mu = 275^\circ$. **C** Left: $r = 0.383$; $\mu = 116^\circ$. Right: $r = 0.562$; $\mu = 304^\circ$.

Discussion

In the present study we analyzed polarotactic flight behavior in the desert locust at natural sky light conditions. Without landmarks, locusts show oriented, unidirectional flight for extended periods when the sun and the polarization pattern of the sky are available as orientation cues. This result was obtained in unimpaired animals, in animals with DRAs painted black, and in unimpaired animals which only saw parts of the blue sky. Directed flight vanished when locusts flew under a completely cloudy sky and when the sun was not visible and both DRAs were painted black.

In bees and ants the role of polarized sky-light for homing and foraging has been demonstrated in field experiments by von Frisch (1948) and Fent (1986). In locusts, polarotactic yaw-torque responses under a rotating polarizer were hitherto only shown in laboratory experiments (Mappes and Homberg 2004). It still remained to be demonstrated, whether locusts also use the polarization pattern of the blue sky as an orientation cue. Generally, the sun has been regarded as the most prominent celestial orientation cue, as shown for honey bees and desert ants (von Frisch 1949, Fent 1986), but if the sun is covered by clouds, these animals can still navigate correctly. Recent findings, however, show that desert ants predominantly use the polarization compass for navigation (Wehner and Müller 2006).

The results of the present study show that the degree of directedness expressed in the r value is continuously reduced, the fewer celestial cues are available for the animals. But still the importance and priority of the different orientation cues to the locusts remain unclear. Therefore, critical experiments must necessarily be performed in the future, when the sun is exclusively presented to the locusts (e.g. by using a diffuser to eliminate the light information of the polarization pattern) to investigate directed flight performance.

For flight experiments with the sun not visible and the brightness artifacts present, no clear conclusion concerning the usage of the polarization pattern can be drawn. Hence, the number of experiments with the setup completely shadowed needs to be increased to study locust behavior exclusively under the polarization pattern. Therefore, the usage of sun shades (as described by Stalleicken et al. 2005) is advisable so that flight experiments with the sun visible and in complete shadow can be performed consecutively and independently of the day time.

We show here that locusts fly unidirectional when the sun is visible, but also when the animals were exposed to the blue sky with brightness artifacts remaining. Only after both DRAs were painted with black paint and the sun was not visible, so that direct sun light and polarized light information is eliminated, flight behavior becomes very undirected. Hence, we conclude that locusts, like honey bees, use the polarization pattern as an alternative navigation cue if the sun is not present. In addition, the difficulties to initiate flight under completely clouded sky, and, if successful, their frequent change in flight directions with constant rotations in some animals, suggests that either the sun or the polarization pattern of the sky are needed for orientation.

Polarization vision plays an important role in homing for bees and ants (von Frisch 1949; Wehner 2003), but it is still unknown, what exact role polarized light plays in locusts. Our results give clear evidence, that locusts use polarized light to ensure unidirectional flight, but especially for the tests in the shadowed setup, the number of experiments needs to be increased for clearcut conclusions of locust behavior. The crepuscular dung beetle *S. zambesianus* uses polarized light to move dung pills straight away from the food source to avoid possibly aggressive conspecifics. Therefore, they choose a certain *E*-vector and then orient along a straight line (Dacke et al. 2003). The observation that locust flight under natural sunlight conditions shows high directedness suggests that the animals set their longitudinal body axis at a particular angle of sky *E*-vector orientation and keep that angle for the time of flight. But the reason for the observed behavior cannot be explained yet and therefore has to be further studied.

Time compensation

In 1954, von Frisch and Lindauer showed that honeybees are able to use the sun for time compensated compass navigation. The bees found their homes after extended foraging trips, although meanwhile the sun had moved over the sky and gave different orientation information. Thus, the bees must be able to adjust permanently their compass bearings relative to solar azimuth with time of day (Homberg 2004). Monarch butterflies use a time-compensated sun compass during their annual fall migration from North America to Mexico (Mouritsen and Frost 2002). Therefore, an internal circadian clock is required to maintain a constant update and angular shift of flight orientation relative to the celestial signals (Homberg 2004). In cockroaches the

circadian clock has been located in the accessory medulla (Homberg et al. 2003). Until today a circadian center has not been identified in locusts, but the organization and connections of the accessory medulla of *S. gregaria* is highly similar to that in the cockroach (Homberg and Würden 1997) and might, therefore, also serve an internal clock function.

To investigate if locusts use a time-compensated navigation compass, we performed experiments with the same animals at different times of day. The results show a constant flight direction over the whole day away from the sun (negative phototaxis). This is similar to the observed behavior under laboratory conditions where light emitting diodes (LEDs) were used to represent the sun (not published). When both DRAs of the locusts were painted black, a bimodal distribution occurred, with flights concentrated around directions towards or away from the sun. Our findings, therefore, provide no evidence for a time-compensation in locust flight directions, but for sound conclusion, the number of experiments needs to be increased.

Mean flight directions at different days

We found a 180° difference in flight direction of two animals on different days. Both were studied when sun was not visible. The sun is always located at a 90° angle to each *E*-vector orientation, so that animals can expect the sun either in the solar or the antisolar half of the sky. Rossel et al. (1978) showed that bees orient bimodally when only small patches of the sky were visible. Hence, the bimodal distribution of flight directions in these experiments may reflect the ambiguity of sky polarization alone as a directional cue.

One of the locusts was also tested when the sun was visible and showed the same behavior. Sun position was almost the same at both days, but flight directions differed at 188°. No reason could be found for this behavior and so further experiments will be necessary to clarify whether this behavior is significant.

Outlook

The sun and the polarization pattern are meanwhile known to represent two orientation cues for insect spatial orientation. But also the chromatic gradient of the sky can be used a direction instruction. Spectral intensity gradients were shown to

bear orientation information for bees and ants when their DRAs were occluded and the sun was not visible (see Wehner and Labhart 2006). The testing procedure in the present study where both DRAs of the locusts were painted black was similar to the one described for bees. We presume that locusts are able to orient themselves even if sun and polarization input are not available as orientation guides. Recently, electrophysiological experiments performed by Kinoshita et al. (2006) demonstrated that polarization-sensitive neurons in the locust respond to unpolarized light dependent on stimulus position and wavelength. But how far these results give evidence for according findings in orientation behavior is still open to speculation and will have to await further behavioral experiments.

To test if the mean flight directions of all performed flight experiments are significantly different from a uniform distribution, the Rayleigh test was performed for statistical analysis. In cases when flights are very uni-directional, the test gives a clear significant difference from a uniform distribution. But also for flights where the directedness was obviously strongly reduced (three of four flights under cloudy sky; flights with both DRAs painted black and sun not visible) where nearly no directedness is observable in the circular histograms the Rayleigh test found a significant difference from a uniform distribution. Therefore, a significant result of this test is only a first reference for directed flight, but gives no clear information about the strength of directedness.

The results of this study give first evidence for uni-directed locust flight orientation underneath natural sky light. But to confirm our findings, the number of experiments has to be increased. Furthermore, additional experiments (e.g. the observation of flight behavior when a diffuser is used to eliminate the polarization pattern but the sun is visible) need to be performed to gain further insight in the locusts' navigational system.

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References

- Brunner D, Labhart T (1987): Behavioural evidence for polarization vision in crickets. *Physiol Entomol* 12:1-10
- Dacke M, Nordström P, Scholtz C (2003): Twilight orientation to polarised light in the crepuscular dung beetle *Scarabaeus zambesianus*. *J Exp Biol* 206:1535-1543
- Eggers A, Gewecke M (1993): Behavioural evidence for polarization vision in locusts. In: Elsner N, Heisenberg M (eds) *Gene-brain-behaviour*. Thieme, Stuttgart, p 336
- Fent K (1986): Polarized skylight orientation in the desert ant *Cataglyphis*. *J Comp Physiol A* 158:145-150
- Frisch, K von (1948): Gelöste und ungelöste Rätsel der Bienensprache. *Naturwissenschaften* 1:12-23; 38-43
- Frisch, K von (1949): Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzen der Bienen. *Experientia* 5:142-148
- Frisch, K von, Lindauer M (1954): Himmel und Erde in Konkurrenz bei der Orientierung der Bienen. *Naturwissenschaften* 41:245-253
- Homberg U (2004): In search of the sky compass in the insect brain. *Naturwissenschaften* 91:199-208
- 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
- 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
- Homberg U, Reischig T, Stengl M (2003): Neural organization of the circadian system of the cockroach *Leucophaea maderae*. *Chronobiol Int* 20:577-591

- Kinoshita M, Pfeiffer K, Homberg U (2006): Spectral properties of identified polarized-light sensitive interneurons in the brain of the desert locust, *Schistocerca gregaria*. J Comp Physiol A (submitted)
- Labhart T, Meyer EP (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
- Mappes M, Homberg U (2004): Behavioral analysis of polarization vision in tethered flying locusts. J Comp Physiol A 190:61-68
- Mouritsen H, Frost B (2002): Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. Proc Natl Acad Sci USA 99:10162-10166
- Philipsborn A von, Labhart T (1990): A behavioural study of polarization vision in the fly, *Musca domestica*. J Comp Physiol A 167:737-743
- Rossel S, Wehner R (1987): The bee's e-vector compass. In: Menzel R, Mercer A (eds) Neurobiology and behaviour of honeybees. Springer, Berlin Heidelberg New York, pp 76-93
- Rossel S, Wehner R, Lindauer M (1978): E-vector orientation in bees. J Comp Physiol A 125:1-12
- Sauman I, Briscoe AD, Zhu H, Shi D, Froy O, Stalleicken J, Yuan Q, Casselman A, Reppert SM (2005): Connecting the navigational clock to sun compass input in monarch butterfly brain. Neuron 46:457-467
- Stalleicken J, Mukhida M, Labhart T, Wehner R, Frost B, Mouritsen M (2005): Do monarch butterflies use polarized skylight for migratory orientation? J Exp Biol 208:2399-2408
- Wehner R (2003): Desert ant navigation: how miniature brains solve complex tasks. J Comp Physiol A 189:579-588
- Wehner R, Labhart T (2006): Polarization vision. In: Warrant E, Nilsson D-E (eds) Invertebrate vision. Cambridge University Press (in press)
- Wehner R, Müller M (2006): The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. Proc Natl Acad Sci 103:12575-12579
- Wolf R, Gebhardt B, Gademann R, Heisenberg M (1980) Polarization sensitivity of course control in *Drosophila melanogaster*. J Comp Physiol A 139:177-191

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ERKLÄRUNG

Ich versichere, dass ich meine Dissertation

“Polarization vision: Behavioral studies in tethered flying desert locusts,
Schistocerca gregaria”

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen Form oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg, 11.09.06

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