Journal of Insect Physiology 59 (2013) 345–350

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

In search of magnetosensitivity and ferromagnetic particles in *Rhodnius prolixus*: Behavioral studies and vibrating sample magnetometry

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ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised form 24 December 2012
Accepted 27 December 2012
Available online 3 January 2013

Keywords:
Magnetosensitivity
Magnetite
Rhodnius prolixus
Apis mellifera
Vibrating sample magnetometry

ABSTRACT

Magnetoreception is a sensory mechanism with wide phylogenetic distribution, which many organisms use for navigation and orientation. Radical pair reactions and the use of magnetic particles have been proposed as mechanisms for magnetosensitivity in terrestrial animals. Magnetosensitivity and the presence of a ferromagnetic material were tested in the hematophagous bug *Rhodnius prolixus* (Hemiptera: Reduviidae: Triatominae) vector of Chagas disease in Colombia and Venezuela. *R. prolixus* is well known in both countries for its active dispersal that allows flow of individuals from sylvatic to domestic environments.

Behavioral experiments quantifying the number of body rotations and quadrant changes in a Petri dish were carried out, applying 1 mT artificial field in a constant direction for 45 min and rotated 180° every 5 min for 45 min. In addition, magnetite presence in the abdomens of *Apis mellifera* (positive control) and the bodies of *R. prolixus* was tested using a vibrating sample magnetometer (VSM).

No differences in the number of body rotations and quadrant changes were found in *R. prolixus* with and without the presence of an artificial magnetic field. Results obtained with the VSM indicate presence of ferromagnetic material (hysteresis loop) in *A. mellifera* abdomens and absence of ferromagnetic material in *R. prolixus* bodies.

Both VSM and behavioral results suggest that magnetosensitivity by a ferromagnetic hypothesis is not present in *R. prolixus*. Finally, our results indicate that the VSM magnetometer is a sensitive technique for detecting ferromagnetic material in insect tissues.

1. Introduction

The Earth's magnetic field resembles the dipole field of a giant bar magnet (Johnsen and Lohmann, 2005). The Earth's field is a constant stimulus that has been present for 2 billion years, allowing the evolution of sensory structures in organisms that can potentially extract two distinct types of information: (a) directional or compass information and (b) latitudinal information (Johnsen and Lohmann, 2005).

The wide phylogenetic distribution of magnetoreception in animals suggests that this sensorial modality may have had its origin prior to the radiation of all the phyla and may have a common origin (Kirschvink et al., 2001).

In insects, magnetoreception has been reported in adults of Diptera, Orthoptera, Lepidoptera, Blattodea and Hymenoptera (Gegear et al., 2008; Riveros and Srygley, 2010; Vácha, 2006; Vácha et al., 2009; Wajnberg et al., 2010; Walker and Bitterman, 1985) and in pupal stages of some coleopterans (Vácha, 2007).

Information obtained from the magnetic field is used by different species of insects for body orientation, homing, short and long-distance migrations, and nest building (Riveros and Srygley, 2008, 2010; Walker and Bitterman, 1985). The effects on such behaviors by shifts of the natural magnetic field have been reported in several insect orders (Riveros and Srygley, 2008; Vácha, 2006).

Since magnetic field lines can pass through biological tissue, magnetoreceptors can plausibly be located almost anywhere in an animal's body (Johnsen and Lohmann, 2005, 2008). Two mechanisms have been proposed in terrestrial animals for detection of magnetic information: Radical pair reactions and the use of magnetite crystals (Johnsen and Lohmann, 2008; Riveros and Srygley, 2010; Wajnberg et al., 2010).

Magnetite based magnetoreception predicts that magnetic fields apply a torque on nanocrystals of magnetite (Fe_3O_4) anchored to ion channels via cytoskeletal filaments. The torque could cause the ion channel to open, leading to membrane depolarization (Kirschvink et al., 2001; Wajnberg et al., 2010).

Detection of ferromagnetic nanoparticles (like magnetite) in insect tissues could help confirm the hypothesis of magnetic field detection by magnetite crystals (Wajnberg et al., 2010). Two

Abbreviation: VSM, vibrating sample magnetometer.

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http://dx.doi.org/10.1016/j.jinsphys.2012.12.005

different approaches have been frequently used to detect magnetic material in insects: the SQUID (Superconducting Quantum Interference Device) (Ferreira et al., 2005; Wajnberg et al., 2004) and the extraction of magnetite particles with sodium hypochlorite (NaOCl) and further analysis with an electron microscope (Acosta-Avalos et al., 1999; de Oliveira et al., 2010). Another useful but as yet untested tool for measuring magnetic properties in insects is the vibrating sample magnetometer (VSM). The principle behind VSM allows for measuring low magnetic moments of small magnetic particles.

Rhodnius prolixus (Hemiptera: Reduviidae: Triatominae) is an important vector of *Trypanosoma cruzi* in Colombia and Venezuela (López et al., 2007). Chagas disease is a major public health issue in Colombia with 5% of the population infected and about 3.5 millions of people at risk of infection (López et al., 2007).

There is evidence that *R. prolixus* has active dispersion at night, and that it leaves its natural habitats in palm trees to infest and colonize domestic environments (Feliciangeli et al., 2007).

Local magnetic field anomalies due to concentrations of ferromagnetic materials in the Earth's crust produce fine-scale variations that can be used by animals to obtain information during short-distance migrations or in the course of dispersion involved in homing behaviors (Johnsen and Lohmann, 2005).

Field experiments of dispersive flight in *Rhodnius* have not been carried out, but in *Triatoma* results showed that bugs were capable of flights in excess of 100 m and up to 550 m (Lehane and Schofield, 1981; Schofield et al., 1992). In addition, no homing behavior has been described in triatomines, but there are reports of *Rhodnius* feeding in houses and leaving afterwards (Feliciangeli et al., 2002).

Finally, *R. prolixus* is an obligated haematophagous insect that after blood degradation leaves free Fe^{2+}, a well known precursor of magnetite biomineralization in microorganisms (Baumgartner and Faivre, 2011; Paiva-Silva et al., 2006).

To our knowledge, there is no evidence of magnetosensitivity in the order Hemiptera; nevertheless we decided to assess if *R. prolixus* responds behaviorally to changes in magnetic fields, and we complement our behavioral results with physical measurements to detect possible presence of magnetic material such as iron oxide or maybe magnetite as it is the most commonly observed in other animals.

2. Materials and methods

2.1. Insects

Adult females and males of *R. prolixus* captured from wild populations in San Juan de Arama, Meta (Northeast of Colombia) and reared since 1979 at 27 ± 2 °C, 75 ± 10% relative humidity and maintained under an artificial 6:00/18:00 h light/dark illumination regime, were used in the experiments. Insects were fed monthly *in vivo* with chicken blood, however all behavioral and anatomical experiments were carried out with adults starved for over a week.

Apis mellifera adults ($N = 8$) captured from natural populations in Guasca, Cundinamarca (central region of Colombia) were used as positive controls for detecting abdominal magnetic material.

2.2. Behavioral experiments

All experiments with *R. prolixus* were carried out exclusively during the photophase (between 7:00 h and 16:00 h) in an isolated room in complete darkness, to ensure that any movement recorded was not due to host searching. Adults were tested once individually (control ($N = 27$) or manipulation experiment ($N = 27$)) for two and a half hours inside a closed Petri dish (9 cm diameter) divided in 4 quadrants. The dish was placed on a wooden base in the middle of a Helmholtz coil (40 cm diameter) connected to an automated system supplying current to generate rotation of the horizontal component of the magnetic field at specific moments of the experiment. A current of 3.55 A and a voltage of 16 V were used to obtain an artificial magnetic field of 1 mT measured at the location of the Petri dish containing the insect.

Insect movement was recorded with a video camera (Sony Handycam DCR-SR200) using infrared illumination to avoid visual cues (Reisenman et al., 1998). All experiments were initiated after a 30 min acclimation period, after which all insect movements were recorded on three occasions (A–C). Period A consist of 45 min with a constant magnetic field of 1 mT in one direction (rotated 53° from the magnetic North). For period B the artificial magnetic field was rotated 180° every 5 min, and for 45 min. In period C, the artificial magnetic field was removed and movements were recorded for 30 min. Field shifts were confirmed with a compass and the natural local geomagnetic field was 27 μT (National Geophysical Data Center).

Control experiments were carried out always before the manipulation experiments and insect movements after 30 min of acclimation were recorded during 120 min without applying any artificial magnetic field.

Two different parameters were registered for each insect in both control and manipulation experiments: (i) number of body rotations above 15° (Vácha, 2006) using Image J (Rasband, 1997–2011) and (ii) number of quadrant changes. Both parameters were measured for the three periods described above (A–C).

2.3. Magnetic properties of the body

Bodies of *R. prolixus* were tested for presence of magnetic particles, and abdomens of *A. mellifera* were used as a positive control (Wajnberg et al., 2001). Individuals of both species were killed and preserved in ethanol up to the moment when experiments were conducted. Insect bodies were dried at 35 °C for 10 h followed by 39 °C for 21 h (Wajnberg et al., 2001), and were maintained inside hermetic recipients with silica gel until maceration. Dry bodies were macerated with an agate mortar, removing larger pieces of cuticle manually whenever possible. Plastic tweezers were used to manipulate the bodies to avoid contamination with metallic material.

Magnetic material was detected introducing macerated tissue in a cellulose capsule; magnetization hysteresis loops were measured as a function of the magnetic field at room temperature using a vibrating sample magnetometer with errors expected between 2 and 3 μemu (VSM; LakeShore model 7404). The loop consisted of a ramp between 0.5 T to −0.5 T and back to 0.5 T for *A. mellifera*, and from 0.6 T to −0.6 T and back to 0.6 T for *R. prolixus*. Three different magnetization hysteresis loops were measured in *R. prolixus*: (i) complete body ($N = 4$), (ii) abdomen only ($N = 8$), (iii) head, thorax, appendages and antennae ($N = 8$).

Magnetization hysteresis loops were measured in abdomens of *A. mellifera* ($N = 8$) as a positive control. Background measurements with empty cellulose capsules were conducted, and values obtained subtracted from all insect tissue measurements.

2.4. Statistical analysis

The number of body rotations and number of quadrant changes recorded were analyzed for normality with Shapiro–Wilk test. *t*-Tests (or a Wilcoxon sign ranked test when data were not normal) comparing control and trial individuals were calculated for all three tested periods (A–C). All statistical analyses were carried out with R software version 2.11.1.