No expression of magnetic compass orientation in *Clethrionomys glareolus* in total darkness (Rodentia: Cricetidae)

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Abstract. Our knowledge about magnetoreception in mammals remains limited. Among rodents, magnetic compass orientation has been documented in four subterranean mole-rats and four epigeic (i.e., active above ground) species. While it is well established that the magnetic compass of mole-rats is light-independent and magnetite-mediated, recent evidence suggests that a radical pair-based mechanism may underlie magnetic orientation in epigeic rodents. To determine whether the magnetic compass of epigeic rodents is light-dependent, which is thought to be an inherent feature of radical pair-based magnetoreception, we investigated the ability of the bank vole *Clethrionomys glareolus* (Cricetidae) to use magnetic compass orientation in total darkness. The voles, which were allowed to explore/build a nest overnight in a circular arena, did not exhibit any directional preference in the initial stage of the experiments; later they tended to prefer the southern sector of the arena irrespective of magnetic field direction, implying that they relied on nonmagnetic orientation cues. Previous tests (OLIVERIUSOVÁ et al. 2014) in the same environment, but under lighting, showed a significant reaction to a change in the orientation of the magnetic field. On the basis of the presented study in comparison with the previous one, it can be concluded that bank voles do not use the magnetic compass for near-space orientation in darkness and thus support evidence for light-dependent magnetoreception in a mammal.

Key words. Spatial orientation, magnetoreception, magnetite-based mechanism, radical pair-based mechanism, bank vole.

INTRODUCTION

Although it has been widely documented that diverse animals use the magnetic field of the Earth for orientation and navigation (for a review, see WILTSCHKO & WILTSCHKO 2005), the evidence for magnetoreception in mammals remains limited (for a review, see BEGALL et al. 2013, 2014). Besides bats (e. g. HOLLAND et al. 2006, 2010, WANG et al. 2007), magnetic compass orientation has been documented in several subterranean mole-rats (BURDA et al. 1990, KIMCHI & TERKEL 2001, OLIVERIUSOVÁ et al. 2012), as well as in small rodents active above ground: the Siberian hamster (DEUTSCHLANDER et al. 2003), the inbred C57BL/6J mouse (MUHEIM et al. 2006, PHILLIPS et al. 2013), the bank vole (OLIVERIUSOVÁ et al. 2014) and the wood mouse

(MALKEMPER et al. 2015). One mechanism underlyin magnetic compass orientation in rodents had been inferred from studies on strictly subterranean, congenitally microphthalmic mole-rats (for a review, see MORITZ et al. 2007). Behavioural experiments characterized the mole-rat magnetic compass as light-independent, polarity-based and magnetite-mediated (MARHOLD et al. 1997a, b, KIMCHI & TERKEL 2001, THALAU et al. 2006). More recent studies, however, suggest that the magnetic compass of epigeic rodents shares some important features with birds rather than with mole-rats. Most importantly, their compass seems to need blue light and is sensitive to magnetic fields oscillating in the MHz range (MUHEIM et al. 2006, PHILLIPS et al. 2013, MALKEMPER et al. 2015), a diagnostic property of a radical pair-based mechanism (RITZ et al. 2004). Whether the magnetic compass of epigeic rodents exhibits also other functional characteristics that are typical for the avian magnetic compass remains unknown.

Following the successful demonstration that the bank voles can orient using a magnetic compass under broad-spectrum light (400–700 nm) (OLIVERIUSOVÁ et al. 2014), here we investigated their ability of magnetic compass orientation in total darkness, to test the hypothesis, that the magnetic compass of epigeic rodents is light-dependent (MALKEMPER et al. 2015), as in birds (for review, see e. g. WILTSCHKO et al. 2010).

MATERIALS AND METHODS

Animals

The bank voles, *Clethrionomys glareolus* (Schreber, 1780) were caught in the vicinity of České Budějovice (Czech Republic), at the same locality as in our previous study: 48°58'40"N, 14°25'50"E, 415 m a. s. l. (OLIVERIUSOVÁ et al. 2014). In total, we tested 30 bank voles (13 females, 17 males). The animals were kept individually in plastic boxes (55×35×20 cm) at a temperature of 18±1 °C and under a 12L/12D light regime. Rodent pellets and carrots were provided *ad libitum*; wood shavings and hay were used as bedding and nesting material, respectively. All experiments were approved by the Institutional Animal Care and Use Committee at the University of South Bohemia and by the Ministry of Education, Youth and Sports (No. 7946/2010-30).

Experimental apparatus

Except for light conditions, we followed the exactly same protocol and use the same facility and equipment as in our previous study (OLIVERIUSOVÁ et al. 2014). The shifted fields were generated by a three-axis, double-wrapped coil system (four 200×200 cm square coils per axis with a coil spacing of 74.4/51.2/74.4 cm and coil winding ratio of 26:11:11:26; see MERRITT et al. 1983). This Merritt-coil system was powered by a Voltcraft DPS-8003 PFC current-regulated power supply (Conrad Electronic, Germany) located in a separated technical room. The magnetic fields were measured using a Mag-01 single axis fluxgate magnetometer (Bartington Instruments Ltd., Oxford, England) before and after each experiment. The total intensity (~47 μT) and the inclination (+66°) remained unchanged during the experiment.

Behavioral assay

We repeated the experiment conducted by OLIVERIUSOVÁ et al. (2014), however, with one key difference – all experiments were performed in total darkness. Individual bank voles were removed from their cages, placed in a light-tight container, transported to a testing room, and released in a circular arena (82 cm in diameter and 41 cm in height) placed in the centre of the Merritt's coil. During transport, the container was rotated slowly to prevent the voles from gathering information about the direction of displacement. The experimenter used a small red flashlight and left the testing room immediately after releasing the vole

in the arena. The arena was filled with a thin layer of hay as nesting material and randomly distributed pieces of carrot as food. The animal was allowed to explore the arena and build a nest there overnight (7.00 pm – 7.05 am). The nest position or, for individuals that did not build any nest, the resting position was taken as a proxy for determination of directional preference. The animals' positions were recorded automatically during the first five minutes of each hour by an overhead infrared-sensitive CCD video camera (see, OLIVERIUSOVÁ et al. 2014).

The bank voles were tested in four different magnetic fields, once in each condition: the local geomagnetic field (mN=0°) and three shifted fields with the magnetic north at the geographic east (mN=90°), south (mN=180°) or west (mN=270°). The sequence of the magnetic fields tested was randomized. The Rayleigh test was used to assess significant deviations from a random distribution of bearings; the Watson's U^2 test was used to compare the distribution of bearings between groups subjected to different magnetic conditions. All calculations were carried out in Oriana 4.0 (Kovach Computing). Pooled bearings were analysed by a permutation test for uniformity of repeated circular measurements (for details, see OLIVERIUSOVÁ et al. 2014).

RESULTS

Bank voles actively explored the arena, but only 13% of them gathered nesting material and built a nest; the majority (87%) of the animals exhibited no nesting activity and simply slept/rested alongside the arena wall. Moreover, many voles changed their nest/resting position during the night. Therefore, we analysed nest/resting positions observed in the evening and those observed in the morning separately.

The initial nest/resting positions observed in the evening were randomly distributed in the local geomagnetic field as well as in all three experimentally shifted magnetic fields (Rayleigh test, all p>0.3; Table 1). Even when the four data sets were pooled, the topographic distribution of the nest/resting positions remained indistinguishable from random (Table 1). The same was true when the evening nest/resting positions were pooled with respect to the magnetic north in the arena (standardized to 0°; Table 1). Thus, the bank voles did not exhibit any spontaneous directional preference in the evening.

The morning nest/resting positions, by contrast, were significantly clustered in the southern sector of the arena in the local geomagnetic field and, surprisingly, also in the magnetic field the north of which was shifted by 180° (Fig. 1a, b, Table 1). Animals tended to prefer the southern sector of the arena also in the experimental fields shifted by 90° and 270°, but the clustering of nest/resting positions was not significant (Fig. 1c, d, Table 1). The distributions of the morning

Table 1. Orientation of the bank voles in total darkness; μ – mean orientation; r – mean vector length; p – probability of the Rayleigh test; N – number of animals. * Note that the pooled data were analysed using a uniformity test for repeated circular measurements (see Figs. 2 and 3)

test conditions	evening nest / resting positions				morning nest / resting positions			
	μ (deg.)	r	p	N	μ (deg.)	r	p	N
N = 0°	217	0.083	0.850	24	192	0.441	0.008	24
N = 90°	81	0.201	0.383	24	159	0.318	0.088	24
N = 180°	161	0.121	0.720	23	173	0.438	0.011	23
N = 270°	85	0.190	0.425	24	176	0.224	0.304	24
topographic bearings*	109	0.100	0.590	95	176	0.346	0.020	95
magnetic bearings*	297	0.029	0.960	95	314	0.023	0.990	95

A   N = 0°

B   N = 90°

C   N = 180°

D   N = 270°

Topographic North

Magnetic North

E

F
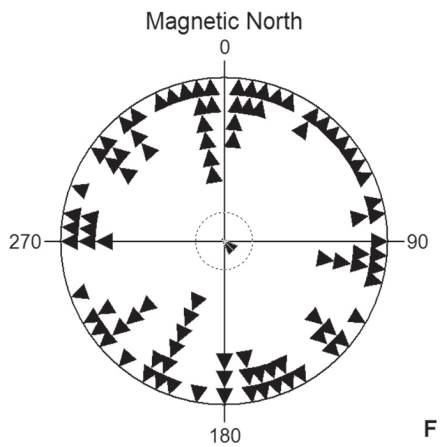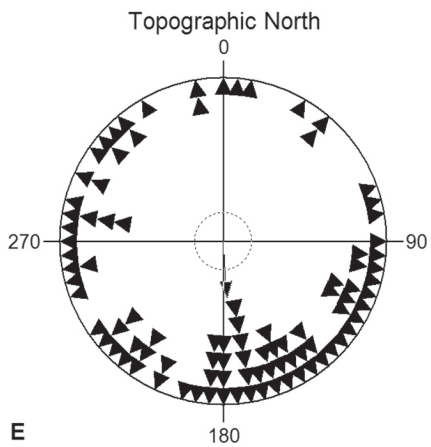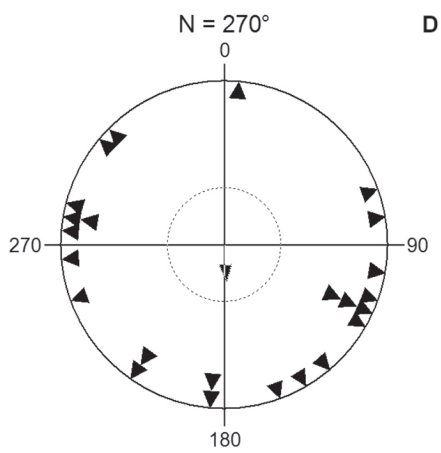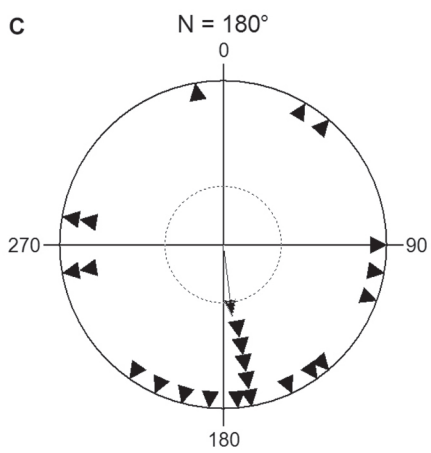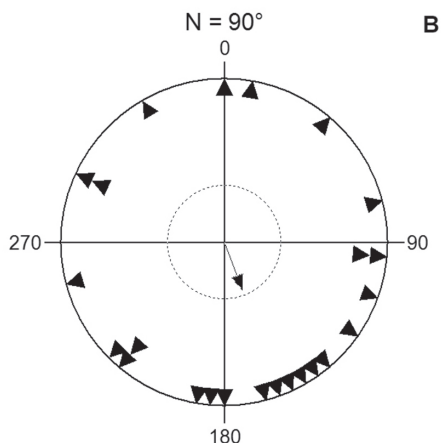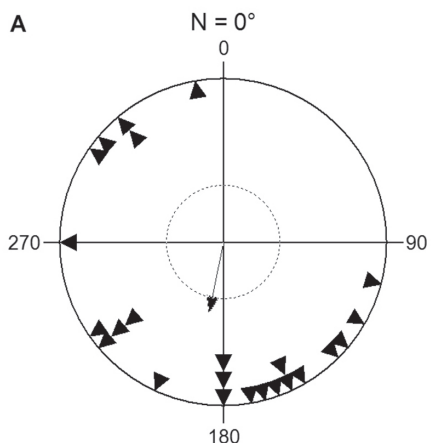
Fig. 1. Orientation of the bank voles in total darkness. Morning nest/resting positions. Each triangle represents a nest/resting position of an individual bank vole. Bearings of thirty bank voles under different magnetic conditions: the natural magnetic field (a) and three shifted fields with magnetic north at geographic east (b), south (c) or west (d). (e) Topographic distribution of all bearings in the arena plotted irrespective of the experimental magnetic field conditions. (f) All bearings plotted relative to the magnetic north in the arena (standardized to 0°). Arrows indicate the mean vector for the distribution of the bearings; the length of the mean vector provides a measure of the degree of clustering in the distribution of the bearings. The inner dashed circles mark the 5% significance border of the Rayleigh test. Note that in a–d it refers to the tabulated critical r values for the given sample size, whereas in e and f it is derived from a simulated null reference distribution of the mean vector lengths for the pooled data – see Fig. 2 (for the evening (first) nest/resting positions see Fig. 3).

nest/resting positions did not differ between tests performed in different magnetic fields (Watson U^2 test, all p>0.1; Table 2). When the four data sets were pooled, the topographic distribution of nest/resting positions remained significantly biased towards the southern sector of the arena (Fig. 1e, Table 1). In contrast, when the morning nest/resting bearings were pooled with respect to the magnetic north in the arena (standardized to 0°), the bearings were indistinguishable from random (Fig. 1f, Table 1), indicating that magnetic field azimuth did not significantly affect the orientation of the bank voles. The effect is not significant even in axial evaluation (μ=179°; r=0.141; p=0.153).

DISCUSSION

The arena assays performed in this study show that bank voles do not use the magnetic compass for near-space orientation when tested in total darkness. The animals did not exhibit any directional preference in the initial stage of the experiments; later they tended to prefer the southern
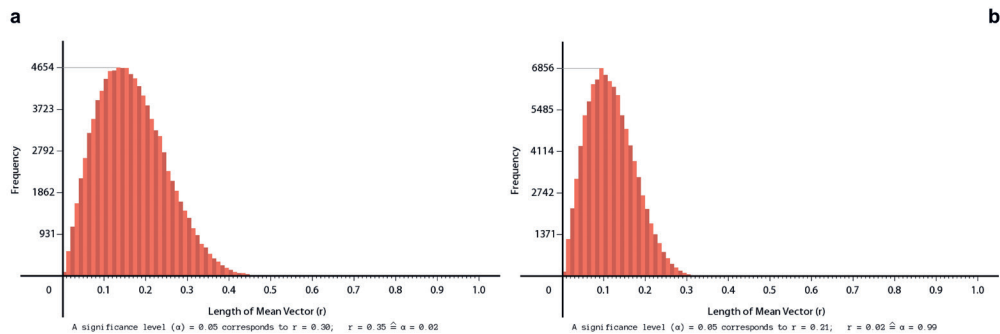

Fig. 2. Simulated null reference distributions (100,000 iterations) of the mean vector lengths – r (Rayleigh test) for the pooled data of morning (last) nest/resting positions. Bearings pooled with respect to the topographic north of the arena (a); bearings pooled with respect to magnetic north (b). A significance level α=0.05 corresponds to r=0.3 and r=0.21 in (a) and (b), respectively. For evaluation of Table 1 data: r=0.35 corresponds to α=0.02 and r=0.02 to α=0.99 in (a) and (b), respectively.
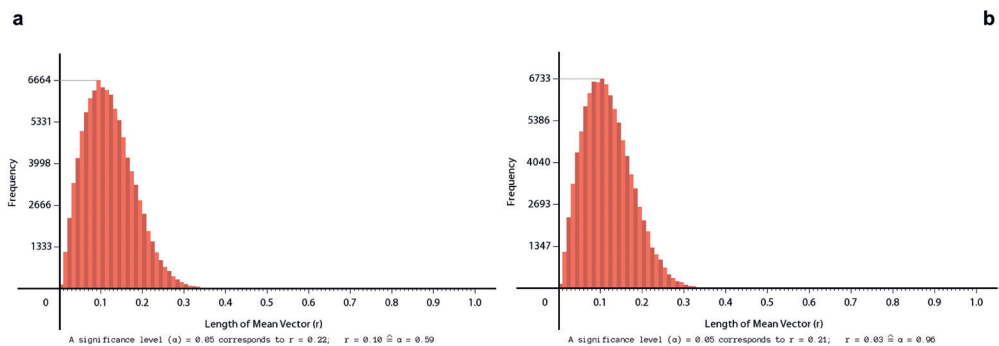
a

b

Fig. 3. Simulated null reference distributions (100,000 iterations) of the mean vector lengths – r (Rayleigh test) for the pooled data of evening (first) nest/resting positions. Bearings pooled with respect to the to-pographic north of the arena (a); bearings pooled with respect to the magnetic north (b). A significance level $\alpha=0.05$ corresponds to r=0.22 and r=0.21 in a and b, respectively. For evaluation of Table 1 data: r=0.1 corresponds to $\alpha=0.59$ and r=0.03 to $\alpha=0.96$ in (a) and (b), respectively.

sector of the circular arena irrespective of magnetic field azimuth, implying that they relied on unidentified nonmagnetic orientation cues. One of the possible sources of targeted orientation could have been the muffled sounds that could penetrate from the adjacent corridor sometimes. They could be effective only in the absence of any other cues. Other cues were highly unlikely. These results contrast starkly with our previous observations (OLIVERIUSOVÁ et al. 2014) that bank voles exhibit a robust bimodal magnetic compass orientation response when tested under white (full spectrum) light. Together, these findings suggest that the magnetic compass of the bank vole is light-dependent.

The current experiments represent a direct follow-up of the previous study (OLIVERIUSOVÁ et al. 2014). Except for different light conditions, both studies follow the same experimental paradigm: we performed the same nest building assay (BURDA et al. 1990, OLIVERIUSOVÁ et al. 2012, PAINTER et al. 2018) using the identical coil system, arena and testing room. Thus, the presence of light seems to be crucial for the manifestation of magnetic compass orientation in the bank vole. A comparison with other studies suggests that this may hold also for other epigeic rodents. Although conducted overnight, nest building experiments that demonstrated magnetic compass orientation in laboratory mice, hamsters and wood mice (see Introduction) invariably

Table 2. Values of Watson's U^2 test are presented in the upper part, the corresponding significance p values in the lower part; NN – magnetic north = 0°; NS – magnetic north = 180°; NE – magnetic north = 90°; NW – magnetic north = 270°

	NN	NS	NE	NW
NN		0.038	0.064	0.142
NS	>0.5		0.052	0.102
NE	>0.5	>0.5		0.047
NW	0.2>p>0.1	0.5>p>0.2	>0.5	

started with a period of time during which the experimental animals were allowed to explore the arena under natural (Malkemper et al. 2015) or artificial white light (Deutschlander et al. 2003, Muheim et al. 2006). Likewise, the water maze experiments in which mice were successfully trained to remember the magnetic compass direction of a submersed platform were performed under white light (Phillips et al. 2013).

For now, it remains unclear whether light influences rodents' motivation or primary magnetoreception events. Rodents exploring a novel environment in the presence of salient orientation cues typically establish nests/home bases, around which they centre their exploratory excursions (e.g., Clark et al. 2006). Therefore, the fact that bank voles exhibited high locomotor activity but seldom built a nest during the exploration of the arena hint at their inability to use magnetic field direction as an orientation cue. Experiments testing the effect of the wavelengths of light on magnetic compass orientation in epigeic rodents are needed to draw more definite conclusions (Phillips et al. 2022).

In sum, the findings that magnetic orientation of epigeic rodents is sensitive to weak radio frequency fields (see Introduction) and that its manifestation requires light (present study) suggest the involvement of a light-induced, radical pair-based magnetoreception mechanism (Ritz et al. 2000, Hore & Mouritsen 2016). Further research is needed to scrutinize this hypothesis and to provide a more thorough characterization of the underlying mechanism.

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