Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal

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Summary

The mechanism of signal transduction during magnetic compass orientation is rarely evident in vertebrates and is as yet unknown in mammals. This transmission has been associated with magnetite-based receptors innervated by the ophthalmic nerve or with the involvement of the eye, particularly the retina. We provide the first behavioural support for the cornea carrying the respective primary sensors in mole-rats (*Fukomys anselli*) by showing that local anaesthesia disrupts their normal directional magnetic orientation. During corneal anaesthesia in normal geomagnetic conditions, mole-rats did not maintain their preferred nesting direction, but displayed a random orientation pattern. A second experiment showed that the ability of the mole-rat to discriminate between light and dark was not impeded by the same anaesthetic treatment, suggesting no retinal involvement in mole-rat magnetic orientation. Our study restricts the peripheral primary sensors in mole-rats to the ophthalmic region, probably the cornea and indicates magnetite as the responsible signal mediator.

Key words: magnetic compass orientation, sensory transduction, mole-rat, cornea, magnetite, Bathyergidae.

Introduction

Magnetic compass orientation has been demonstrated in a number of vertebrate model groups including salmonids, newts, sea turtles, birds and rodents (Deutschlander et al., 2003; Gudmunsson and Sandberg, 2000; Wiltschko and Wiltschko, 1995). Both magnetite-based receptors and retinal biochemical processes have been shown to play roles in the biophysical magnetic signal transduction in birds (Wiltschko and Wiltschko, 2005). In mammalian compass orientation, Zambian Ansell's mole-rats of the genus *Fukomys* (Bathyergidae) have been the model species of choice. These subterranean, microphthalmic rodents orientate themselves in their constantly dark ecotope with the help of the Earth's magnetic field (Burda et al., 1990). However, the primary biophysical signal transduction for their light-independent polarity compass (Marhold et al., 1997a) has remained unclear.

Two hypotheses are currently considered to explain the sensory mechanism of magnetic compass orientation. The first is based on retinal chemo-physical radical-pair reactions (Ritz et al., 2000), a system suggested as the primary signal transduction process for migratory birds (Ritz et al., 2004). This obviously light-dependent mechanism seems unlikely, however, for the rodent genus *Fukomys*, whose members spend the majority of their life underground in sealed burrow systems.

A recent study has ruled out the radical-pair mechanism for this rodent (Thalau et al., 2006). The second principal hypothesis, based on a primary involvement of magnetite, seems much more reasonable for mole-rats, particularly as this mechanism is light-independent. Magnetite (Fe_3O_4) has been considered a possible basis for magnetic compass orientation in diverse species (Fleissner et al., 2003; Kirschvink and Gould, 1981; Kirschvink et al., 2001; Presti and Pettigrew, 1980; Winklhofer et al., 2001). In trouts (Walker et al., 1997) and some bird species (Fleissner et al., 2003; Hanzlik et al., 2000; Williams and Wild, 2001; Winklhofer et al., 2001), clusters of tiny magnetite crystals (diameter ~1–3 μm) were found in regions innervated by the ophthalmic branch of the trigeminal nerve. Physiological studies indicated that this nerve may carry magnetic field information to the brain (Beason and Semm, 1996; Mora et al., 2004). The immediate and long-term impairment of mole-rat nesting orientation induced by a magnetic pulse designed to change the magnetisation of magnetite (Marhold et al., 1997b), implicates magnetite in the signal transduction mechanism in these rodents. The highly mechano-sensitive structure of the cornea makes it a prime candidate for the location of receptors translating magnetic field information into mechanical signals.

In addition to these considerations, our preliminary findings of ferrous inclusions in the corneal epithelium (Fig. 1)
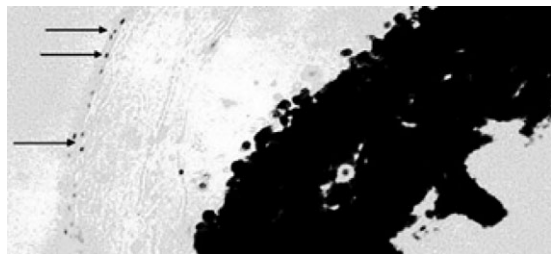
Fig. 1. Ferrous inclusions as indicated by arrows in the corneal epithelium of *Fukomys anselli* after Prussian blue staining.

motivated behavioural tests on a possible corneal involvement in magnetic compass orientation in mole-rats, i.e. on a prominent source of ophthalmic nerve-mediated mechano-sensibility. We made use of the spontaneous nest-building drive of mole-rats to examine whether mechano-sensitive desensitisation due to local anaesthesia of the corneal region affected their magnetic compass orientation. In our established experimental design, mole-rats place their nests predominantly in the southern sector of a circular arena under control conditions (Burda et al., 1990). We expected that any direct impairment of primary magneto-receptors would result in random nest placement rather than the usual directional behaviour.

Materials and methods
Study mole-rats

The adult mole-rats tested were from two closely related sibling *Fukomys* species, *Fukomys anselli* and *Fukomys kafuensis* (Burda et al., 1999), and hybrids between them [*Fukomys* were formerly known as *Cryptomys* (Kock et al., 2006)]. Importantly, these species do not differ in their directional preferences. Animals were tested in pairs, since testing these highly social animals in nesting experiments has proved more efficient when done with at least two individuals (personal observations). Six pairs of mole-rats were tested repeatedly, and other pairs were tested once in each condition: control ($N=40$) and treatment ($N=42$) with n being the number of pairs. This difference in sample size resulted from two pairs that were split by natural death of one partner during the study and could not be used for the respective control experiment. Most pairs used in this study consisted of a male and a female breeder; a few were sibling pairs. The animals derived from our breeding stock at the University of Duisburg-Essen and were either born in the laboratory or captured in the field, but had been living under laboratory conditions for at least 18 months prior to experiments. Each animal carried a tissue compatible, subcutaneous transponder (bio-capsule, 12×2.1 mm, ISO-standard 11784) with a unique number code (ALVIC-transponder, ALVETRA GmbH, Neumünster, Germany), to ensure individual identification. Mole-rats were housed at ambient room temperature under natural daylight in glass cages filled with

a layer of horticultural peat and were fed *ad libitum* with carrots, potatoes, salad and apples.

Experimental set-up

We initially tested orientation in six adult pairs of mole-rats under the local geomagnetic field of Essen, Germany (45 μT; 66° inclination) with four replications in each condition. Then, we tested all available pairs of mole-rats from our breeding stock once per condition ($N=40$ in controls; $N=42$ in treatments) thus obtaining a large data set without replicates (Batschelet, 1981). In addition to the treatment conditions with local corneal anaesthesia, we performed control experiments in which the cornea was treated with sodium chloride solution.

Testing followed a previously used procedure (Burda et al., 1990; Marhold et al., 1997a): mole-rat pairs were tested on warm days in an opaque plastic arena (80 cm diameter, 30 cm height) in silent, outside premises of the University campus in Essen, in the undisturbed local geomagnetic field. The arena was covered with a thin layer of peat; tissue paper stripes and carrot pieces were spread radially across the surface. A bucket was placed in the middle of the arena to prevent undirected, central nesting. Animals were introduced into the arena at random directional segments. During testing, the arena was covered with a light impervious lid to exclude possible visual orientation. Mole-rat pairs collected the tissue paper and built a nest; the exact nest position was then recorded with reference to geographic north.

To exclude order effects, half of the subjects were tested first in the sodium chloride condition, and the others were tested first in the corneal anaesthesia condition. In the repeated tests group, the mole-rat pairs were tested alternately in the sodium chloride and corneal treatment conditions with at least a day between tests. Tests lasted from 30 min to 1 h. In tests lasting more than 30 min, the anaesthetic was re-applied to ensure that the cornea remained anaesthetised.

Although the magnetic compass of these rodents has been described as light-independent (Marhold et al., 1997a), the possibility that corneal anaesthesia disrupted a photoreceptor-based magneto-sensory system in the mole-rats' eyes had to be ruled out. Therefore in this study, we also tested the effect of the same anaesthetic treatment on the animals' ability to discriminate light from dark and to nest preferentially in darkness with a two-armed maze preference test (see Wegner et al., 2006). Mole-rat pairs had to make a choice between a dark and an illuminated chamber for nesting; their choice was recorded. Controls and anaesthetic treatment were also performed in the same alternating manner detailed above.

Study treatment

In the controls, mole-rat eyes were treated with sterile sodium chloride solution used for medical and physiological purposes. For application, animals were motivated to crawl into a paper roll (4.5 cm diameter; 9.5 cm length). When inside this roll, the mole-rats were immobilized by gently gripping a skin fold above the tail. Sodium chloride solution was then gently dropped into the animals' eyes. In the same way, the animals'

eyes were anaesthetised with 2% Xylocain® solution (active substance: Lidocain hydrochloride; Astra GmbH, Wedel, Germany), a surface anaesthetic used routinely in medical practice for mucous membrane anaesthesia. The viscous liquid was applied generously to the opened eyes with a soft brush. During this procedure the animals did not show any adverse behaviour such as teeth chattering, distress or aggression vocalisations. No efforts to clean their eyes were observed. Experiments and Xylocain®-treatment conformed to the relevant regulatory standards and were approved by the authorities of the University of Duisburg-Essen and the District Government, Düsseldorf (50.05-230-37/06).

Statistical analysis

From the nest positions of each pair, we calculated the mean vectors for both test conditions, with direction (α_p) and length (r_p). The mean directions (α_p) of the six repeatedly tested pairs were pooled in grand mean vectors for each testing condition, with direction (α_M) and length (r_M). From the nesting data of the 'once per condition' group, we calculated the overall mean vector with direction (α_A) and length (r_A). The group mean vectors (α_p) as well as the mean vector of the once per condition group (α_A) were examined for significant directional preferences with the Rayleigh-test of uniformity (Batschelet, 1981; ORIANA 2.02, Kovach Computing Services, Anglesey, UK); grand mean vectors α_M and the two mean vectors of the once per condition group were tested for differences in distribution between the study conditions with the Watson's U^2 test (Batschelet, 1981; ORIANA 2.02).

The data from the two-armed maze preference tests which examined the possible influence of the anaesthetic on the animals' retinal performance was analyzed for a preferential choice using χ^2 tests (SPSS® 12.0 for Windows).

Results

Under control conditions, the mole-rats retained to their preference for nesting in a southern sector of the arena both in the repeated tests group (Fig. 2A) and the once per condition group (Fig. 2C). With corneal anaesthesia, the mole-rats still built nests, but without any directional preferences, showing a random distribution (Fig. 2B,D). This difference between corneal anaesthesia and control groups was significant for both the repeated tests group (U^2=0.206, $P<0.05$) and for the once per condition group (U^2=0.218, $P<0.05$; Table 1).

In the experiment examining a possible retinal disturbance from the Xylocain®, the mole-rats' behavioural response clearly showed that corneal anaesthesia did not affect photoreceptor performance; their ability to perceive light and prefer darkness for nesting was not disturbed (N=11, χ^2=7.4, P=0.007).

Discussion

Our findings of disrupted directional compass orientation after corneal anaesthesia suggest that the ocular region might
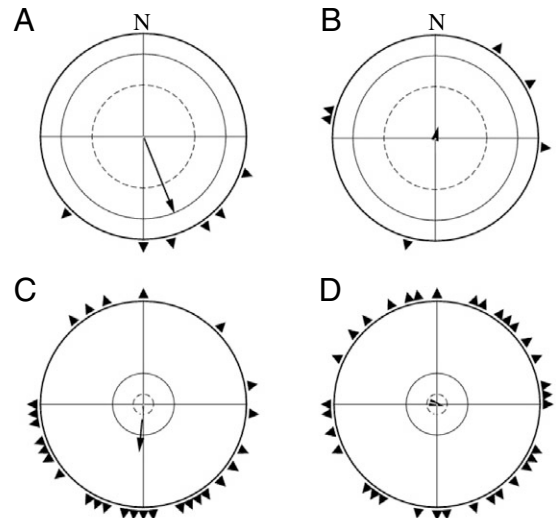

Fig. 2. Random nest distribution of mole-rats under corneal anaesthesia. Triangles indicate mean nesting directions of the repeatedly tested six pairs in control (A) and under anaesthesia (B), and nesting directions of the singular tested group in control (C; N=42) and treatments (D; N=40). The arrows, proportional to the outside radius (=1), mark the grand mean vector based on the pairs' mean directions (A,B) or the mean vector of all single decisions (C,D). The inner circles mark the Rayleigh significance thresholds: 5% (broken) and 1% (solid) in A, B; 1% (broken) and 0.1% (solid) in C, D. Table 1 gives numerical values.

Table 1. *Orientation of nest position of Zambian mole-rats without and with corneal anaesthesia*

Repeatedly tested pairs	Control		Corneal anaesthesia	
	α_p (deg.)	r_p	α_p (deg.)	r_p
P1	143	0.77	283	0.48
P2	108	0.57	279	0.21
P3	180	0.88	35	0.33
P4	225	0.86	197	0.47
P5	163	0.74	59	0.22
P6	137	0.45	94	0.30
Grand mean vector	158°, 0.81*		14°, 0.12	
Median vector length	0.76		0.32	
Direction deviation		144°, $P<0.05$		
Singular tested pairs	α_A	r_A	α_A	r_A
Mean vector	185°, 0.47***		105°, 0.07	
Direction deviation		80°, $P<0.05$		

α_P and r_P values indicate direction and length of the six pairs' mean vectors based on four trials; α_A and r_A give direction and length of the mean vectors of all single directions in the singular tested group.

P1–P6, indicate the six repeatedly tested pairs.

Grand mean vectors and mean vectors are given with significance (Rayleigh-test) marked by asterisks:*P=0.05; ***P=0.001; median individual vector lengths are given for the repeated testing condition.

accommodate the primary magnetic receptors in mole-rats. Contrary to the putative connection of magnetite-based receptors with the ophthalmic nerve, Cernuda-Cernuda et al. (Cernuda-Cernuda et al., 2003) reported findings of retinal crystalloid bodies in the inner photoreceptor segment of the Ansell's mole-rat. The authors interpreted these structures as potential magnetite grains, and suggested the retinal photoreceptors as the magnetite-based structure. However, the ability of the mole-rats to discriminate between light and dark (Wegner et al., 2006) during corneal anaesthesia suggests that magnetic compass orientation in *Fukomys* is not photoreceptor-based because the application of anaesthetics did not influence retinal performance or differential orientation behaviour per se in our study. Our results point instead to a peripheral ophthalmic locus as the stimulus mediator in these mammals. Given the behavioural evidence presented here, previous neuroanatomical findings of magnetoresponsive neurons in *F. anselli* identified within the inner sublayer of the intermediate grey layer of the superior colliculus (Němec et al., 2001), i.e. in a layer dominated by trigeminal input in other mammals (Huerta and Harting, 1984), could be interpreted as supporting the hypothesis that the putative primary magnetoreceptors are located in the cornea. Systematic impairment experiments involving specific bilateral section of the ophthalmic branch of the trigeminal nerve and enucleation will provide definitive evidence for or against the corneal magneto-receptor location.

We further hypothesized that in Zambian mole-rats, the mechano-sensors mediating signals during magnetic orientation are magnetite-based. Along with the previous findings that ruled out retinal chemo-physical radical-pair reactions as the underlying signal mediating mechanism (Marhold 1997b; Thalau et al., 2006), our results support innervated magnetite as the responsible sensory structure. This is because desensitisation of the cornea significantly affected mechano-sensibility and therefore magnetic stimulus transmission. Further histological, histochemical, and ultra-microscopic examination of the mole-rat cornea is clearly warranted. Given the accessibility of the cornea, our finding may open new vistas for detailed studies of the primary transduction mechanisms of magnetite-based magneto-reception in mammals.

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