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**Faculty of Science**

# Behavioural evidence for magnetic orientation in rodents

Ph.D. Thesis

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## Annotation

Magnetoreception in rodents was studied in this Ph.D. thesis. Behavioural evidence for compass magnetic orientation was found in two subterranean African rodents: the social giant mole-rat (*Fukomys mechowii*) and solitary silvery mole-rat (*Heliophobius argenteocinereus*) and epigeic rodent bank vole (*Clethrionomys glareolus*). The study is also focused on the role of magnetic orientation in solving the orientation task in Morris water maze in bank vole.

## Declaration [in Czech]

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## List of papers and author's contribution

The Ph.D. thesis is based on the following papers:

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($IF_{2012}$=3.236)
*Ludmila Oliveriusová participated in laboratory work, completing literature sources and writing manuscript.*

**Oliveriusová L**., Němec P., Pavelková Z., Sedláček F. (2014). Spontaneous expression of magnetic compass orientation in an epigeic rodent: the bank vole, *Clethrionomys glareolus*. Naturwissenschften 101: 557-563
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**Oliveriusová L**., Nováková M., Sedláček F. (in prep) Consistent repeat of the water maze method for magnetic orientation: unlike mice bank voles reacted weakly (original manuscript)
*Ludmila Oliveriusová participated in laboratory work, statistical analysis, completing literature sources and writing manuscript.*

# CONTENTS

# 1. INTRODUCTION

## Magnetic field of the Earth and its role in animal orientation

The ability to perceive magnetic field and use its cues for orientation was observed in some invertebrates (see e.g. Vácha 1997; Vidal-Gadea et al. 2015) and is widely spread in all major groups of vertebrates. Magnetic orientation was found in fish, amphibians, sea turtles, migratory birds and mammals (Wiltschko and Wiltschko 1995). In contrast to this evidence for magnetoreception across all major groups of vertebrates knowledge of the role of magnetic sense and underlining mechanism is still elusive. Animals are able to use different parameters of geomagnetic fields (polarity, inclination, intensity) and their use may also vary based on different orientation and navigation tasks. Whereas e.g. the magnetic compass does not respond to slight changes in the intensity of the magnetic field these changes and animal ability to perceive them are crucial for the function of the magnetic map sense.

- **Magnetic field of the Earth**

Magnetic field of the Earth resembles the dipole field of a huge magnet with poles situated near to the geographical poles. Magnetic lines arise from the north magnetic pole (close to the south geographic pole) and lead to the south magnetic pole. The inclination (angle between the magnetic line of force and the horizon) changes continuously form +90˚ to -90˚ at poles to 0˚ at the magnetic equator.

As mentioned above magnetic field provides three types of cues which can be used for orientation and navigation: polarity of the magnetic lines, inclination and total intensity (the strength of magnetic field). These cues can delivery directional (compass) or positional (map) information (Wiltschko and Wiltschko 1995; Wiltschko and Wiltschko 2005; Lohmann et al. 2007; Wiltschko and Wiltschko 2007).

- **Magnetic compass**

Magnetic compass which is widely distributed over all major groups of vertebrates was first time found in European robins (*Erithacus rubecula*) and then was confirmed also in others bird species. European robins changed the direction of flight when inclination was inverted but with no effect of reversing the horizontal component of earth's magnetic field (Wiltschko and Wiltschko 1995). Inclination compass was also described in sea turtles (Light et al. 1993; Lohmann and Lohmann 1992). Lately a different type of magnetic compass was described in mammals. Mole-rats changed the directional preference in a nest building assay only when horizontal component of the magnetic field was shifted (polarity of the magnetic lines) (Burda et al.1990). Inverting of inclination had no effect to directional preference (Marhold et al. 1997b, Kimchi and Terkel 2001). Inclination and polarity magnetic compass differ not only in type of cues which is used from the magnetic field of the Earth but also in their characters and mechanisms. While polarity compass distinguishes northward and southward directions (Fig. 1) inclination compass distinguishes only poleward and equatorward directions (Fig. 2). Bird’s inclination compass is tuned only for narrow range of magnetic field intensity. When intensity is increased or decreased birds are disoriented and the time is needed for habituation of birds to the new intensity of magnetic field to which they are exposed (Wiltschko and Wiltschko 2005). Polarity compass is not affected by intensity of the magnetic field. E.g. bats exhibited undisturbed directional preference in the magnetic field turned up 200% of natural intensity (Wang et al. 2007) and also in quite weak fields (Tian et al. 2015).

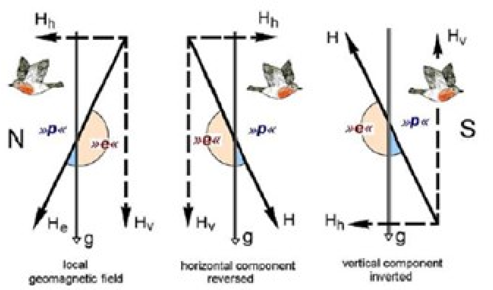

Fig. 1 Vertical section through the geomagnetic field to illustrate the functional mode of the inclination compass. N, S, magnetic North and South. H, magnetic vector, with He, the vector of the geomagnetic field; Hh, Hv, horizontal and vertical component respectively; g, gravity vector. »p«, »e« , ‘poleward’ and ‘equatorward’, the readings of the inclination compass. The bird flies ‘poleward’ (Wiltschko and Wiltschko 2005)

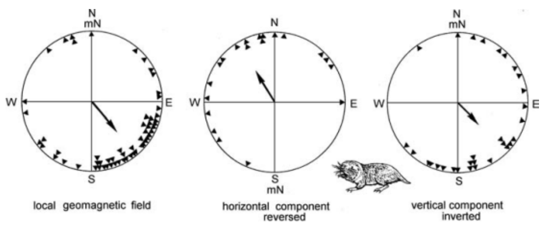

Fig. 2 Orientation of mole rats (Rodentia) in the geomagnetic field and in two experimental fields. The triangles at the periphery of the circle mark the direction of the nest position from the center of the arena; the arrow represents the mean vector proportional to the radius of the circle (data from Marhold et al. 1997, graphs form Wiltschko and Wiltschko 2005)

- **Magnetic map**

Only directional information which is provided by magnetic compass is insufficient for long distance travelling and navigation. Gradients in inclination and intensity of the magnetic field of the Earth are used for derivation of positional information which is necessary for the magnetic map sense.

Although an evidence for the magnetic map exists in several animals (lobsters, sea turtles, birds) the knowledge about structure, organization and function of these maps is still limited (Lohnmann et al. 2007).

## Mechanisms of the magnetic orientation

In terrestrial vertebrates two different mechanism of magnetic orientation are distinguished at the base of existing evidence.

- **Magnetite-based model**

Particles of biogenic magnetite have been found in a number of animals including insect, fish, sea turtles, birds and bats. Properties of the perception of directional information and the field intensity depend on the size and shape of the magnetite particles (Kirschvink and Gould 1981). Two particle sizes can be considered for use in magnetoreception.

Most magnetite isolated from animals has been in the form of large single-domain particles. These particles are permanently magnetized and they twist according to the alignment of the magnetic field. This rotation could then exert pressure on secondary receptors (such as stretch receptors, hair cells or mechanoreceptors), open ion channels, or act physically on the cell in some other fashion.

SPM (superparamagnetic) particles are smaller than single-domain particles and because of their size don't have a permanent magnetic moment. SPM particles can form clumps or chains in tissue. Particles or their clusters may attract or repel each other under the influence of the magnetic field of the Earth. Perception of magnetic field is based on these interactions.

- **Radical pair-based model**

Second type of magnetic orientation mechanism which is demonstrated in vertebrates is the light dependent model based on radical-pairs.

Radical–pairs mechanism model is based on set of biochemical electron transfer reactions induced by light. Strength of the magnetic field has effect on the singlet-triplet interconversion rate of a spin-correlated radical pair formed after photo-excitation (Fig. 3) (Ritz et al. 2000)

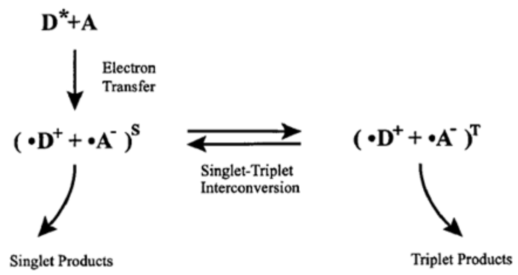


Fig. 3 Reaction scheme for a radical pair reaction with magnetic field-dependent reaction products. The radical pair is generated by an electron transfer from a donor molecule D to an acceptor molecule A. An external magnetic field affects interconversion between singlet and triplet states of the radical pair (Ritz et al. 2000).

One possible way how animals perceive magnetic field is the visualization of magnetic lines (Ritz et al. 2000). E.g. birds could theoretically see magnetic line as a special pattern caused by geomagnetic field. These patterns would differ depending on orientation of animal's head with respect to the direction of the geomagnetic field.

Presence of both magnetoreception mechanisms was found in migratory birds. Both types of magnetic senses are used in different orientation tasks. The right eye retina with photopigments associated with radical pair-based mechanism is used for determining direction – magnetic compass. Magnetite particles found in the upper beak are responsible for determining position – the magnetic map (Wiltschko and Wiltschko 2007).

- **Difference between magnetite and radical-pairs mechanisms**

It is possible to disturb the mentioned mechanisms with specific treatments according to their unique properties. Magnetic orientation in animals based on the radical-pairs mechanism is significantly disrupted by oscillating fields of specific frequencies in the MHz range which interfere with singlet-triplet interconversion. In contrast, the magnetite-based magnetoreception is not affected by these frequency fields. The mechanism based on single-domain magnetite particles which are bounded to tissue (not rotating freely) is sensitive to pulse remagnetization.

Due to specific reactions of animals exposed to these treatments it is possible to distinguish between the two mechanisms. Two frequency ranges are used as a diagnostic tool to identify the radical-pairs mechanism: broadband – 0,1-10 MHz and the Larmor frequency 1,315 MHz. Migratory birds were disoriented when broadband frequency fields were added to the static geomagnetic field (Ritz et al. 2004). During the time of spring and autumn migration European robins were also exposed to the Larmor frequency field. Bird's response depended on alignment of the frequency field to the static magnetic field. Non-parallel alignment of Larmor frequency field caused disorientation in birds in both seasons (Thalau et al. 2005). Similar effect of frequency magnetic field was observed in insect (Vácha et al. 2009)

Contrasting results were obtained when Ansell's mole-rats were exposed to the broadband and Larmor frequency fields. Orientation of mole-rats was not disrupted and the animals maintained their preference for south direction in nest building assay neither when the intensity of 1,315 MHz frequency field increased multiple (Thalau et al. 2006).

Concerning the magnetic compass in mole rats – the polarity compass independent on light and based on the magnetite mechanism (Marhold et al. 1997b) was considered as "rodent" model for many years. The cues suggesting different type of magnetoreception in aboveground rodents apperead over time. After all, shielding of the manmade frequency field noise was necessary for successful proof of the magnetic orientation in mice (Phillips et al. 2013). Last study in the wood mice (Malkemper et al. 2015) shed light on question if the magnetoreception of mole-rats is the same for all rodents or magnetoreceptions in aboveground and subterranean rodents are based on different mechanism. Results obtained when wood mice were exposed to frequency magnetic fields added to the static geomagnetic field identified in the aboveground rodent probably the radical-pairs mechanism.

## Behavioural evidence for magnetic orientation in mammals

However, the magnetic orientation in mammals has been studied more than three decades knowledge of mammal magnetoreception compared to the evidence in birds remains still very limited. In fact the true magnetic orientation was demonstrated only in two groups – rodents and bats.

For magnetic alignment in ungulates (cattle and deer) and carnivores (foxes and dogs) see chapter 6 Magnetic alignment.

- **Rodents**

Up todays magnetic orientation was found in several species of rodents. Underground rodents Ansell's mole-rat (*Fukomys anselli*) (Burda et al. 1990; Marhold et al. 1997a, b), the giant mole-rat (*Fukomys mechowii*), silvery mole-rat (*Heliophobius argenteocinereus*) (Oliveriusová et al. 2012), blind mole-rat (*Spalax ehrenbergi*) (Marhold et al. 2000; Kimchi and Terkel 2001) and aboveground rodents Siberian hamster (*Phodopus sungorus*) (Deutschlander et al. 2003), C57BL/J6 mouse (Muheim et al. 2006; Phillips et al. 2013), bank vole (*Clethrionomys glareolus*) (Oliveriusová et al. 2014), wood mouse (*Apodemus sylvaticus*) (Malkemper et al. 2015)

- Aboveground rodents

Mather and Baker (1981) published the first study about magnetic sense in rodents but their findings that wood mouse use magnetic orientation during homing was impeached because of failure to repeat the experiment with similar design (Sauvé 1988). Baker's studies were also associated with doubts about the experimental design and statistical analysis. Similarly experiment with *Peromyscus leucopus* did not shed light on the topic because the results were conflicting in different populations (August et al. 1989). However, it seems, magnetic orientation in the wood mouse was finally found (Malkemper et al. 2015). Wood mice were tested in a symmetrical circular arena and mice showed spontaneous directional preference with bimodal character for North-South axis of the magnetic field. The distribution of nests was disoriented when mice were exposed to artificial oscillating magnetic fields. This is the first clear evidence for radical pair-based mechanism in mammals.

Evidence for magnetic orientation was also found in laboratory experiment in the Siberian hamster, *Phodopus sungorus.* In the first study hamsters were not able to choose the right direction which led to food in a four-arm maze (Madden and Phillips 1987). However, sixteen years later magnetic orientation in the Siberian hamster was found using directional preference test in nest building (Deutschlander et al 2003). Siberian hamsters showed weak spontaneous bimodal preference in natural magnetic field. Then, after training in cage with light/dark gradient the animals showed robust unimodal preference for learned direction. Similar results were obtained from experiment with C57BL/6J mouse (Muheim el al. 2006). Mice were trained to build nest in four specific directions. Trained mice manifested robust unimodal preference for the learned direction. Both rodents, hamsters and mice, were breed and kept in laboratory condition. We decided to support the mentioned results by testing the magnetic orientation in a common wild living rodent - the bank vole (*Clethrionomys glareolus*). Bank voles were tested during night and at the beginning of the experiment they were exposure to the light. The position of nests in circular arena was recorded. Bank voles exhibited spontaneous directional preference for north-south axis of the magnetic field. Unlike Siberian hamsters and mice whose spontaneous directional preference was quite weak bank vole's spontaneous directional preference appeared to be more pronounced.

- Subterranean rodents

Impetus for the study of magnetic orientation in subterranean rodents was given by Hynek Burda (1987) who noticed that main axis of tunnels of underground systems in *Fukomy*s *anselli* are oriented according to N-S axis of the Earth magnetic field. Under laboratory conditions mole-rats were placed to a circular arena where they could build nests. In this assay mole-rats exhibited spontaneous directional preference to build nests in the south-eastern sector and this preferrence changed when magnetic field was shifted (Burda et al. 1990). The experimental design when animals build nest in a circular arena is widely used up to the present days. Magnetoreception in mole-rats was identified as light-independent polarity compass. The mole-rats changed distribution of nests if polarity of the field was shifted. Changing in inclination had no effect to the directional preference of mole-rats as well as presence or absence of the ilumination (Marhold et al. 1997b).

The evidence for magnetoreception was also found in the blind mole rat *Spalax ehrenbergi* (Kimchi and Terkel 2001). The study was divided into three parts. In the first and secon parts mole rats were tested in an eight-arm maze in earth's natural magnetic field and in a magnetic field shifted 180˚. On the beginning mole rats exhibited significant spontaneous preference for southern sector of the maze. Then magnetic field was alternated and mole rats shifted location of their nests and food stores to the northern sector. Second part of the experiment was run under total darknes which had no effect on mole rat directional preference, the indepenedent of light character of mole rat's magnetic compass was demonstrated. In the last part mole rats were placed into a complex labyrint. They were trained under natural magnetic field to find path from the start to the goal. Then mole rats were divided into two groups where one was tested under natural magnetic conditions and the second one in a shifted field. The number of errors was significantly higher in latter group. This finding suggests that mole rats use cues from the magnetic field during orientation task in the labyrinth. The study continued with the role of magnetic orientation and its participation in the path integration (Kimchi et al. 2004). Two different lenghts of the route were set up in the labyrinth. There was no diference between two goups trained and tested along a short route. In contrast the group of blind mole rats which was trained along the long route and then tested in shifted magnetic field exhibited significantly poorer results than the control group. This result probably indicates that mole rats use magnetoreception for a long distance orientation to reduce an accumulation of errors in the path integration.

However the question if there is a general direction preference or the preference is coupled with a unique environmental (laboratory) configuration was not answered so far. Therefore we tested two new species of African mole rats, the social giant mole-rat (*Fukomys mechowii*) and the solitery silvery mole-rat (*Heliophobius argenteocinereus*) again in a circular arena for spontaneous nest building. Their directional preference was shifted according to changes in magnetic field orientation. However, unlike to previous mole-rat studies both species preferred west direction. This suggested that the preferred direction is not innate for all mole-rat but more likely species/population specific or could be learned (Oliveriusová at al. 2012). For details see paper No. 1.

Attempt to find the magnetic orientation in the tuco-tuco (*Ctenomys talarum*) (Schleich and Antinuchi 2004) was unsuccessful.

- **Bats**

At the beginning of magnetoreception research in bats a dismissive attitude prevailed it is very unlikely that bats have the ability to use magnetic field for orientation (Davis 1966). Despite this idea magnetic compass has been reported in *Eptesicus fuscus* specimens (Holland et al. 2006). Their homing direction was shifted by shifting of magnetic field during homing. Really complex experiment was provided by Wang and his colleagues (Wang et al. 2007). They identified magnetoreception in *Nyctalus plancyi* as magnetic polarity-based compass. Bats were tested in artificial magnetic fields with changes in the horizontal and vertical components. Bats showed preference for northern sector in naturally oriented field with intensity twice stronger than the Earth's magnetic field. Their roosting position was shifted when both horizontal and vertical components were shifted. To distinguish polarity and inclination compass further tests were carried out with a change of only one of the components. If inclination was shifted the roosting position remains unchanged. In opposite when horizontal component was reversed bats moved to the southern sector of the basket. Relation between the magnetic compass and the sunset was described in the Great mouse-eared bat, *Myotis myotis* (Holland et al. 2010). Bats calibrated magnetic compass with the sunset. The bats were also exposed to the altered magnetic field at and after sunset. While exposure at sunset caused a change of orientation exposure after sunset had no effect on homing bats.

Very recent study presents that *Nyctalus plancyi* is able to perceive weak magnetic field (Tian et al. 2015). This finding is also inspirational for further research magnetic orientation in rodents. Comparative experiment with mole-rats which magnetic compass is polarity based and independent on light and aboveground rodent possessed likely with radical-pairs based magnetic sense should be very interesting.

Although, magnetic compass in bats has been identified as polarity-based magnetic compass (Wang et al. 2007) and also magnetite particles were found in bat's sensory cells (Holland et al. 2008) we still know little about the position (role) of magnetic orientation in bats.

## Magnetic alignment

Magnetic alignment is the simplest response to the magnetic field of the Earth in organisms. As well as other types of alignment also magnetic alignment is directional orientation of body of e.g. animals during resting, moving and other activities. In contrast to the true magnetic orientation magnetic alignment is not goal-directed - it is a type of fixed directional response not related to animal preferences. Magnetic alignment is spontaneous orientation of the body axis along the lines of force (direction) of the geomagnetic field (typical is bimodal or quadrimodal manifestation) (Begall et al. 2013).

Although the study which convinced scientific public about the existence of magnetic alignment was published in 1975 in Science (Blakemore 1975), first study mentioned this phenomenon in bacteria was published already in the sixties (Bellini 1963). This magnetotactic bacteria (*Magnetospirillum magnetotacticum)* presents the simplest way of the magnetic alignment. The body of the bacteria is passively orientated by the chains of magnetite crystals inside the bacterial cell. In the animal kingdom the magnetic alignment was observed first time in insect (termites) in 1958 (Roonwal 1958). Until the present the magnetic alignment was found in a wide range of species from insect up to the mammals (Begall et al. 2013).

- **Magnetic alignment in mammals**

- Ungulates

Magnetic alignment in mammals was first time reported in 2008 in cattle (Begall at al. 2008). With the use of Google Earth the pictures of cattle herds from 308 pastures were collected. Data analysis showed that animal's body orientation is significantly different from a random distribution. Their body axes were oriented along the lines of force of the magnetic field of the Earth (N-S). This orientation was disturbed on pastures which lay under or in close proximity of high-voltage lines (Burda et al. 2009). Extremely low frequency magnetic fields produced by electric power in high-voltage lines disrupt the geomagnetic field and thereby magnetic alignment. Magnetic alignment was also observed in two deer species (roe deer and red deer) based on direct observation of body orientation during grazing and resting as well as deer beds (body print of the resting deer in snow) (Begall et al. 2008).

- Carnivores

Červený and his colleagues (2011) found magnetic alignment in foxes. Foxes hunting in high vegetation or snow cover use special hunting tactic – the mousing - a jump to surprise their prey from above. Foxes do mousing more often along the North-South axis of the geomagnetic field than in others direction. These jumps in N-S direction are moreover successful despite the high vegetation or snow cover.

In study, which also attracted media and public interest, was found that dogs preferred to urinate having body orientated along the N-S axis of magnetic field of the Earth (Hart et al. 2013). A very interesting and important finding of this study is that this phenomenon is abolished when the magnetic field of Earth is unstable and disrupted by solar storms. It could be one of the possible explanations why repetitions of magnetic orientation experiments are often difficult and the results are often quite scattered.

- **Rodents – true magnetic orientation or magnetic alignment?**

In rodents magnetic orientation was found using nest building experiments in a circular arena or radial maze (Burda et al. 1990; Marhold et al. 1997a, b; Kimchi and Terkel 2001; Deutschlander et al. 2003; Muheim et al. 2006; Oliveriusová et al. 2012, 2014; Malkemper et al. 2015). Because this spontaneous behavior is not goal directed a question arose if this direction preference is more likely a magnetic alignment than true magnetic orientation.

Aboveground rodents – the Siberian hamster and mouse C57BL/6J (Deutschlander el al. 2003; Muheim et al. 2006) showed only weak spontaneous directional preference during nest building. But this preference became stronger when testing in a circular arena was preceded with training in which the direction of the magnetic field was associated with a cage illumination gradient. Aboveground rodents are able to learn specific magnetic field azimuth when it is associated with relevant stimulus. This ability of C57BL/6J mice to learn magnetic direction in relevant conditions was also showed in water maze (Phillips et al.2013). Learned magnetic direction preference is a clear proof that in the case of aboveground rodents it is the true magnetic compass orientation.

Maybe less clear is the situation in underground rodents. African mole-rats, in which magnetic response was found, exhibited spontaneous directional preference in nestling only (Burda et al. 1990,1991; Marhold et al. 1997a, b, 2000; Kimchi and Terkel 2001;

Oliveriusová et al. 2012). Ansell's mole-rats showed quite stabile directional preference for S-E sector of the circular arena in nest building experiments in two laboratories during two decades. Under this fact it was repeatedly proposed that this spontaneous preference is not true compass orientation but a type of the magnetic alignment. On the other hand we found in two species of African mole-rats spontaneous preference for west direction of the magnetic field (Oliveriusová et al. 2012). Also blind mole-rat *Spalax ehrenbergi* which was tested in three different laboratories preferred different directions (SE, EN and SW) (Marhold et al. 2000; Kimchi and Terkel 2001). Clear proof for true compass orientation in blind mole-rat was an increasing number of mistakes in path integration test when magnetic field was shifted (Kimchi et al. 2004). In conclusion magnetic orientation in rodents could be considered as true compass orientation.

# 2. Summary of contributions of Ph.D. thesis to the state of knowledge

In two presented publications and one manuscript we were focused on orientation in rodents based on information from magnetic field. We found behavioural evidence for magnetic compass orientation in three rodent species – two subterranean mole-rats *Fukomys mechowii*, *Heliophobius argenteocinereus* (Oliveriusová et al. 2012) and one epigeic rodent *Clethrionomys glareolus* (Oliveriusová et al. 2014). With regard to the total number of rodent species with evidence for magnetoreception our findings make a significant contribution to the current state of research.

However, magnetic orientation in mammals has been studied for decades the evidence remains still limited. Only in several species was magnetic orientation found without a doubt and its role is still obscure. Fist study was focused on magnetic orientation in subterranean African rodents. We tested two different mole-rats species the social giant mole-rat (*Fukomys mechowii*) and solitary silvery mole-rat (*Heliophobius argenteocinereus*) in nest building assay in circular arena. Both mole-rats showed strong unimodal spontaneous directional preference for west direction.

Previous study in mole-rats showed very uniform spontaneous preference for southeastern or southern direction. These findings led to the hypothesis that this preference is innate and common to all strictly subterranean rodents (Marhold et al. 1997b). In contrasts we found strong preference for west direction in two different mole-rats species which suggests that spontaneous directional preference is either species-specific or learned.

Our evidence for strong spontaneous directional preference in two mole-rats species for west direction in circular arena is supporting argument that rodents exhibited magnetic compass orientation rather than magnetic alignment.

We found behavioural evidence in wild living common epigeic bank vole (*Clethrionomys glareolus*). Bank voles showed spontaneous directional preference in circular arena for north-south axis of the magnetic field. Quite weak bimodal directional preference was observed also in previous studies in laboratory species. Spontaneous preference in wild living bank vole seems to be more pronounced.

Another major issue is still the repetition of the experiments. In the past, many attempts to repeat previous experiments failed. In case of wood mouse this failure of repetition (Sauvé 1988) leads to refusal of magnetoreception evidence although it was at last successfully found (Malkemper et al. 2015) In third paper we focused on repeating experiment in water maze. However, bank voles showed ability to learn direction of submerged platform in four-arm water maze the results in bank voles were more scatter than in previous study (Phillips et al. 2013). The possible reasons are discussed. We also suggested changes in experimental design.

# 3. CONCLUSIONS AND FURTHER CONTINUATION

Examples of hamster and woodmouse which both displayed different results in different experiment designs show how important is the role of experimental design. To find a suitable experimental design, which enables animals to show their natural behavior motivated by magnetoreception is necessary and crucial but also very difficult task. This was also proved in our replication (Oliveriusová unpublished data) of Phillip's experiment in plus water maze (Phillips et al. 2013).

Magnetic orientation of all eight rodent species tested to date was first time showed in a circular arena or radial eight-arm maze. It seems, they are both very useful tools – e.g. in two cases (Siberian hamster and wood mouse) the clear evidence for magnetoreception was found in circular arena even when previous attempts failed (Deutschlandet et al. 2003; Malkemper et al. 2015). After more than two decades it is still relevant to find evidence for magnetic orientation in new mammal species for better identification of factors which change the magnetic response - e.g. experimental device form, effect of light, frequency fields etc. However, it says still very little about the way how the magnetic orientation ability is used by animals and its role in solving orientation tasks. To answer these questions more complex experimental designs are needed where animals are conspicuously forced to solve orientation tasks like in a complex maze (Kimchi and Terkel 2001; Kimchi et al. 2004) or water maze (Phillips et al. 2013).

For continuation we suggest experimental test focused on food foraging behavior. The idea for the test linking magnetic orientation and food foraging comes from 80's (Madden and Phillips 1987). But in my opinion the repetitive switching of the magnetic field in quite short intervals is not suitable because it doesn't match the natural reality. I consider it important that the methodology should suits to the real situation with which the animal is commonly encountered and is able to respond to it adequately. Therefore experimental animals will be trained and tested again in a radial arm maze. However this time food reward will be placed in the end of one arm which will be correlated with e.g. the north direction of the magnetic field. The trajectory or time spent in each of arms will be analyzed. Of course, pilot tests will precedes, because our previous experience with testing bank voles in water maze (Oliveriusová unpublished data) suggests it is important to adjust the details of the experimental design - the proportions of maze, number of training trials etc. according to the characteristics of each tested species.

## A final essay on magnetoreception

One of possible reason why testing of magnetic orientation in mammals has showed to be difficult is that man himself doesn’t perceive at least consciously magnetic field. It is difficult for us to fully understand this phenomenon and identify the way how animal perceive earth’s magnetic field as we have in others senses like sight or hearing. This could be also one reason why replications of the experiment are so difficult. We try to manage all possible factors (light, frequency magnetic fields etc.) which are affecting the magnetic responce in animals with no possibility of personal experience. In fact we just can only tested the effect of each factor by trial and error, and thus step by step build a picture of the perception of the magnetic field in animals.

The entire situation also makes more problematic existence of more than one mechanisms of magnetoreception. The factors which affected radical pair-based mechanism are different from factors important for the magnetite-based mechanism and as I tried to indicate the related species (rodents: mole-rats and wood mouse, bank vole) could have different mechanism of magnetoreceptiong dependent likely on their evolution and ecology.

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# 5. ATTACHED PAPERS

# Paper I

With kind permission of Journal of Experimental Biology:

**Oliveriusová L**., Němec P., Králová Z., Sedláček F. (2012) Magnetic compass orientation in two strictly subterranean rodents: learned or species-specific innate directional preference? The Journal of Experimental Biology 215: 3649 – 3654

# Magnetic compass orientation in two strictly subterranean rodents: learned or species-specific innate directional preference?

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## Abstract

Evidence for magnetoreception in mammals remains limited. Magnetic compass orientation or magnetic alignment has been conclusively demonstrated in only a handful of mammalian species. The functional properties and underlying mechanisms have been most thoroughly characterized in Ansell's mole-rat, *Fukomys anselli*, which is the species of choice due to its spontaneous drive to construct nests in the southeastern sector of a circular arena using the magnetic field azimuth as the primary orientation cue. Because of the remarkable consistency between experiments, it is generally believed that this directional preference is innate. To test the hypothesis that spontaneous southeastern directional preference is a shared, ancestral feature of all African mole-rats (Bathyergidae, Rodentia), we employed the same arena assay to study magnetic orientation in two other mole-rat species, the social giant mole-rat, *Fukomys mechowii*, and the solitary silvery mole-rat, *Heliophobius argenteocinereus*. Both species exhibited spontaneous western directional preference and deflected their directional preference according to shifts in the direction of magnetic north, clearly indicating that they were deriving directional information from the magnetic field. Because all of the experiments were performed in total darkness, our results strongly suggest that all African mole-rats use a lighting dependent magnetic compass for near-space orientation. However, the spontaneous directional preference is not common and may be either innate (but species-specific) or learned. We propose an experiment that should be performed to distinguish between these two alternatives.



**Keywords:** spatial orientation, magnetic sense, magnetoreception, mole-rat, Bathyergidae, *Fukomys*, *Heliophobius*.

# Paper II

With kind permission of Springer Science and Business Media:

**Oliveriusová L**., Němec P., Pavelková Z., Sedláček F. (2014). Spontaneous expression of magnetic compass orientation in an epigeic rodent: the bank vole, *Clethrionomys glareolus*. Naturwissenschften 101: 557-563

# Spontaneous expression of magnetic compass orientation in an epigeic rodent: the bank vole, *Clethrionomys glareolus*

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## Abstract

Magnetoreception has been convincingly demonstrated in only a few mammalian species. Among rodents, magnetic compass orientation has been documented in four species of subterranean mole rats and two epigeic (i.e. active above ground) species—the Siberian hamster and the C57BL/6J mouse. The mole rats use the magnetic field azimuth to determine compass heading; their directional preference is spontaneous and unimodal, and their magnetic compass is magnetite-mediated. By contrast, the primary component of orientation response is learned in the hamster and the mouse, but both species also exhibit a weak spontaneous bimodal preference in the natural magnetic field. To determine whether the magnetic compass of wild epigeic rodents features the same functional properties as that of laboratory rodents, we investigated magnetic compass orientation in the bank vole *Clethrionomys glareolus* (Cricetidae, Rodentia). The voles exhibited a robust spontaneous bimodal directional preference, i.e. built nests and slept preferentially along the north-south axis, and deflected their directional preference according to a shift in the direction of magnetic north, clearly indicating that they were deriving directional information from the magnetic field. Thus, bimodal, axially symmetrical directional choice seems to be a common feature shared by epigeic rodents. However, spontaneous directional preference in the bank vole appeared to be more pronounced than that reported in the hamster and the mouse. These findings suggest that bank voles are well suited for future studies investigating the adaptive significance and mechanisms of magnetic orientation in epigeic rodents.

**Keywords:** Spatial orientation, magnetoreception, magnetite-based mechanism, radical pair-based mechanism, bank vole

# Paper III

**Oliveriusová L.**, Nováková M., Sedláček F. (in prep) Consistent repeat of the water maze method for magnetic orientation: unlike mice bank voles reacted weakly (original manuscript)

# Consistent repeat of the water maze method for magnetic orientation: unlike mice bank voles reacted weakly

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## Abstract

One of the long-term issues in study of the magnetic orientation is the repeating of the experiments in different laboratories. Attempt to repeat experiments failed many times. After our previous study where we successfully found magnetoreception in the bank vole (*Clethrionomys glareolus*) we decided to replicate water maze experiment. The bank voles were trained and tested in “plus” maze in different magnetic conditions – natural magnetic field and three magnetic fields with shifted position of magnetic north (+90˚, +180˚ and +270˚). Although the bank voles showed learned directional preference in water maze the results were more scatter than in study with C57BL/6J mice.

### Keywords

Learned magnetic orientation, magnetoreception, Morris water maze, rodents, bank vole

## Introduction

Despite the fact, that magnetic orientation has been studied since 70. of the last century and magnetoreception was found in all main groups of vertebrates (Wiltschko and Wiltschko 1995) evidence for magnetoreception in mammals remains, compared with e.g. birds, rather limited. Magnetic compass orientation has been convincingly demonstrated in only several species of subterranean rodents (Burda et al. 1990; Kimchi and Terkel 2001; Oliveriusová et al. 2012) and epigeic rodents (Deutschlander et al. 2003; Muheim et al. 2006; Oliveriusová et al. 2014; Malkemper et al. 2015) and three echolocating bats (Holland et al. 2006, 2010; Wang et al. 2007). Magnetic alignment (non-compass magnetic respond) has been recently demonstrated in grazing and resting cattle and deer, hunting foxes, carps (Begal et al. 2013) and dogs (Hart et al. 2013).

Magnetic orientation in subterranean rodents was recognized as a polarity compass independent on light (Marhod et al. 1997a) and has been found in four species: *Fukomys anselli* (Burda et al. 1990; Marhold et al. 1997a, b), *Spalax ehrenbergii* (Kimchi and Terkel 2001; Kimchi et al. 2004), *Fukomys mechowii* and *Heliophobius argenteocinereus* (Oliveriusová et al. 2012).

Initial studies in aboveground rodents, mainly from eighties, brought controversial results. First study on the wood mouse (*Apodemus sylvaticus*) showed that magnetic orientation is used during homing (Mather and Baker 1981) but effort to repeat this experiment failed (Sauvé 1988). Recently the evidence for magnetic orientation in wood mouse was found (Malkemper at al. 2015). For first time also no evidence for magnetic orientation was found in an experiment with the Siberian hamster (*Phodopus sungorus*). Hamsters were not able to choose the right arm with food in a four-arm maze (Madden and Phillips 1987). Sixteen years later magnetic orientation in the Siberian hamster was found in a directional preference test of nest building (Deutschlander et al 2003). Conflicting results brought also study in two populations of the white-footed mouse (*Peromyscus leucopus*) (August et al. 1989).

Until today magnetic orientation was undoubtedly shown in four species of aboveground rodents only: the Siberian hamster (Deutschlander et al. 2003), the C57BL/6J mouse (Muheim el al. 2006), the bank vole (Oliveriusová et al. 2014) and the wood mouse (Malkemper et al. 2015). Siberian hamsters showed weak spontaneous bimodal preference in the natural magnetic field. After training in a cage with light/dark gradient, animals exhibited unimodal preference for the learned direction. Similar results were obtained in an experiment with the C57BL/6J mouse. Mice were trained to build nests in four specific directions. Trained mice manifested robust unimodal preference for the learned direction. In the last mentioned species, bank voles showed spontaneous bimodal direction preference of the nest building in a circular arena under natural magnetic conditions as well as under magnetic field shifted by 90˚.

Recently a new conception has appeared to study the magnetic compass orientation more efficiently (Phillips et al. 2013). A four-arm "plus" water maze was used for forced learning. C57BL/6J mice were trained to find the right arm with a submerged platform according to the magnetic field orientation. After that each mouse was tested in one of four magnetic field directions. After just only two training trials the mice were able to learn the magnetic compass direction of a submerged platform.

However, one principal problem of the magnetic orientation studies still remains - the replication of experiments in different laboratories and/or under different conditions. After successful proof of magnetic orientation in the bank vole we decided to try to replicate the water maze study (Phillips et al. 2013) by using this species.

## Materials and methods

### Animals

The bank vole (*Clethrionomys glareolus*) is a small rodent common in Europe and Western Siberia. Bank voles live in the understory of more or less extensive forests, shrublands, and dry reedbeds. They build simple underground tunnel systems which contains nests and food stores.

The 24 bank voles of both sexes used in the experiment were caught in a forest in the vicinity of Ceske Budejovice, Czech Republic (N48° 58´ 45´´, E14° 25´ 22´´, 415 m a. s. l.). The animals were kept in a breeding room with moderate temperature (18±1ºC) and a 12L/12D light regime at the breeding facility of the University of South Bohemia in Ceske Budejovice, CR. The bank voles were housed individually in plastic boxes (55 x 35 x 20 cm). They were fed with carrots and rodent pellets *ad libitum* and provided with bedding (wood shavings) and nest material (hay). 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).

## Behavioural assay

The behavioural assay designed to test magnetic orientation in water maze has been described in detail previously (Phillips et al. 2013). The assay was divided into two parts – i) training of directional preference and ii) subsequent testing.

The experimental apparatus was set up from several parts. A four-arm water maze was placed in a circular arena in the center of triaxial Merritt´s coil. The axially symmetric water maze with four arms and central octagonal area was made from opaque white plastic (Fig. 1). Each arm could be separated by movable transparent plexiglass door. The temperature of water was maintained between 27-29˚C and colored white by non-toxic water soluble color.

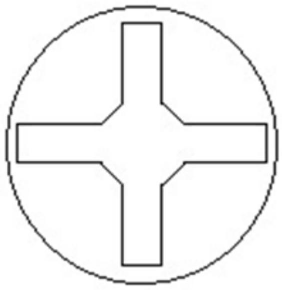

Fig. 1 Schema of the four arm water maze in circular arena

Four arms of the maze were aligned according to main magnetic field directions (north-south, east-west). The coil was powered by a Voltcraft DPS-8003 PFC current-regulated power supplies (Conrad Electronic, Germany) located in a separated technical room. Four magnetic alignments were used – natural magnetic field, magnetic north shifted by 90˚, 180˚ and 270˚. Total intensity and inclination remained unchanged.

Complex of coils were surrounded by a white curtain in order to create a stable and uniform environment and minimize the effect of visual cues in the nearby area of experimental room.

### i) Training

The first training trial started between 3 p.m. – 4 p.m. For both training trials the north arm of the maze was closed by plexiglass door so bank voles were able to swim only inside the arm but they also could see the rest of labyrinth. At the end of the arm a submerged platform from clear plexiglass was placed. In each training trial bank voles were released into the closed arm facing the center of the water maze. When the bank voles reached the submerged platform and climb up to the wall of the water maze they were captured with short delay (the animals could obtain the spatial context) and placed back in a small plexiglass rest box. During both testing trials experimenter stayed quietly in the room on the same place. The rest between the first and second training trials lasted 45 min at least. The resting box was filled with strips of filter paper and warmed up by a red heat lamp. Carrot and sunflower seeds were also provided to bank voles *ad libitum*. Before the second training trial, alignment of the magnetic field was shifted by 180˚.

### ii) Testing

Each bank vole was tested only once in one of the four magnetic field alignments (natural magnetic field, magnetic north shifted by 90˚, 180˚ and 270˚). Testing trials started the second day morning approximately 18 hours after the training trials. The animal was placed into a releasing device which was made from an opaque plastic and transported to the testing room. The releasing device was placed into the center of the octagon of the water maze and started to sink slowly while the lid opened slowly so the animal could swim out. At the end of sinking the releasing device was completely submerged under water level. Experimenter left the testing room during the first phase of sinking when the lid was still closed. The swimming trajectory of the animal in the water maze was recorded with a digital camera placed at the ceiling above the center of the circular arena. At the end of experiment the bank vole was gently captured and placed into the resting box. The testing interval during which the animal swam and looked for hidden islet was shortened from the original 60 s to the 30 s (see Phillips et al. 2013).

### Analysis

The swimming trajectory of each animal was evaluated within a 30 s interval after leaving the releasing device. The time spent in each arm was calculated from video record using the software EthoWatcher. The angle of deviation from the trained direction (north) for each bank vole and vector lengths were calculated as the vector sum of the time spent in all four arms. For these calculations CirkStat software was used.

The distribution of bearings presented by topographic or magnetic way was analyzed by means of the Rayleigh test (significance level of $\alpha = 0.05$). The two distributions of bearings were compared by the Mardia-Watson-Wheeler test. Both these statistical analyses were calculated and the circular diagrams were plotted using the Oriana ver.4 software (Kowach Computing).

## Results

24 bank voles of both sexes were trained for north direction and tested in one of four magnetic field alignments (natural magnetic field and magnetic fields with north shifted by 90˚, 180˚and 270˚). Four individuals had to be excluded from analysis because they did not complete the testing assay. These bank voles escaped the water maze during diving of the releasing device by jumping over the gap between the releasing device and the wall of octagonal central part of the maze. If the animals once found only one moment when it is possible to escape from the labyrinth by this way without swimming they were able to repeat it every time.

Data from the four magnetic field alignments were pooled into one figure (Fig. 2) presenting distributions of topographic and magnetic bearings. The graph A shows distribution of the bearings after a topographic arrangement (distribution of deviations from the trained direction according to the topographic 0˚ position regardless to the magnetic field alignment). The distribution of topographic bearings is not statistically different from a random distribution (Rayleigh test p=0.06). The distribution of magnetic bearings is shown in the graph B – distribution of deviations from the trained direction when the trained direction to the magnetic north is fitted to 0˚. The magnetic bearings distribution significantly differs from a random distribution (Rayleigh test p=0.024, mean vector 60˚). The two distributions of bearings were significantly different (Mardia-Watson-Wheeler test W=13,7; p=0,001).

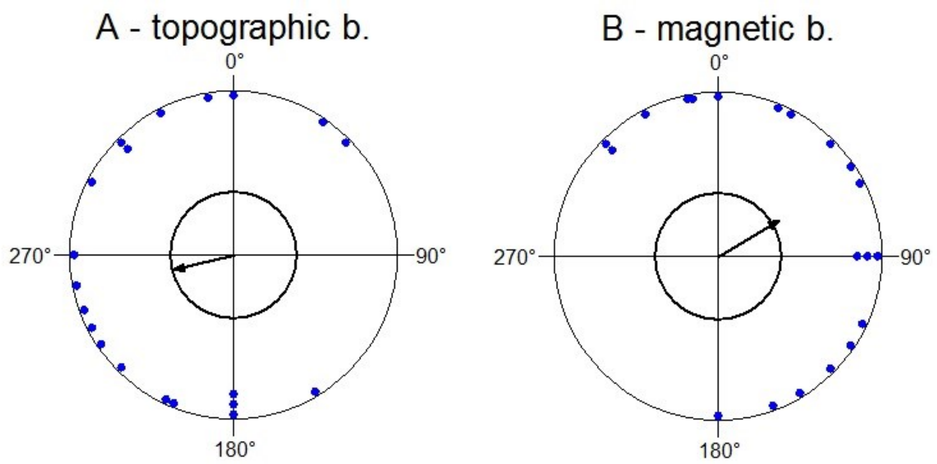


Fig. 2 The distributions of topographic and magnetic bearings. Dots represent the deviation from trained direction of each bank voles. Inner circle is Rayleigh test critical value p=0,05 and the direction and length of the arrow represents mean vector of the bearings.

## Discussion

However, we had effort to replicate previous “plus” water maze experiment (Phillips et al. 2013) very carefully including details, the results are quite sheepish. Bank voles showed altogether weak preference for the trained direction in the four magnetic field alignments. The results are very scattered.

Low success rate of repetition of experiments is still one of the biggest problems in magnetic orientation research nowadays. There are many factors which can affect perception of the magnetic field as well as response to this field in animals and it is still difficult to deal with them. However, the influence of many factors is known - e.g. different light wavelengths or low frequency magnetic fields (Wiltschko et al. 1993; Burda et al. 2009). It is likely that can exist other cue whose ability to affect perception of the magnetic field or response to the magnetic field is unclear so far.

However, the planned simple repetition of previous water maze experiment with mice (Phillips et al. 2013) was not successful completely because of probably the tested animal itself. The bank voles used in this study came from trapping in the wild nature in contrast to the C57BL/6 mouse which is a typical laboratory animal kept and bred in captivity for easy handling above all. There are a lot of essential differences e.g. in locomotor rate and swiftness, learning ability, and anxiety to mention the most visible of them. In the bank vole e.g. we could see beside a quick systematic exploratory behavior also quite frequent escape attempts.

As a consequence of several pilot experiments we decided to shorten the test interval (the time of swimming after leaving the releasing device to the end of experiment) from 60 s to 30 s. After a relatively short time the bank voles leaved searching strategy for the hidden platform and they used an alternative strategy to escape from the water maze completely.

A need of different conditions and forms of water maze to show similar responses to magnetic fields in mice and bank voles can be related to different life history (climbing omnivore vs. surface-dwelling herbivore) (Niethammer and Krapp 1982). Also a different extent of behavioral variation or (on the other hand) uniformity of individuals is an important factor. Different performance in the radial maze based on individual differences was observed in the meadow vole (Teskey et al. 1998). In our previous studies (Oliveriusová et al. 2014, Oliveriusová unpublished data) bank voles showed higher variation in nest building inside a circular arena then different species of mole rats whose preference seems to be more uniform across populations (Burda et al. 1990; Marhold et al. 1997a, b; Kimchi and Terkel 2001; Oliveriusová et al. 2012). Also effects of age, sex and animal species on the performance in the water maze was described (D'Hooge and De Deyn 2001). While laboratory mice tested previously were individuals of the same sex and age the bank voles in our study were males as well as females at different ages.

Exposure of animal to water maze experiment is a very stressful situation (D'Hooge and De Deyn 2001). The animal is situated in a direct threat of life, from its point of view, because it is forced to swim in an unknown environment without view of a safe shore. Reaction to this situation can be quite different in laboratory and wild animals. But we still do not know what species/specimens (bold or shy) are able to master this situation better, because e.g. bank voles swim very well (Niethammer and Krapp 1982). In any case the simplest way to overcome the above mentioned sources of variation is to use an appropriate number individuals maybe increased by a factor of two. Another possibility is to enhance the number (maybe to double) of swims during the learning phase.

In conclusion we can say, presumably, that changes of the maze form and test conditions are needed to show in the bank vole strong and clear preference like in the laboratory mouse. In particular, we propose following changes: i) in water maze construction to avoid ability of bank voles to escape the experiment assay by jumping over the gap between releasing device and the wall of the labyrinth; ii) an increase in the number of training trials to overcome low concentration on the task above all in shy animals.

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