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Evidence for magnetic orientation in *Clethrionomys glareolus* in a water maze assay (Rodentia: Cricetidae)

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Abstract. A long-term issue when studying magnetic orientation is the replicability of the experiments conducted in different laboratories. Attempts to replicate experiments have failed many times. After our previous study where we successfully found magnetoreception in the bank vole (*Clethrionomys glareolus*) we decided to replicate the water maze experiment. The bank voles were trained and tested in a four-arm "plus" maze in different magnetic conditions – natural magnetic field and three magnetic fields with shifted position of magnetic north (+90°, +180°, and +270°). The tested bank voles showed learned directional preference in the water maze therefore we can consider this species magnetoreceptive, able to use magnetic field for orientation. However, the results were more scattered than in the study with C57BL/6J mice. This difference probably corresponds with the absence of the protection against disturbing radio frequency magnetic fields during experiment, as well as, with the behavioural differences of free-living voles and laboratory mice.

Key words. Learned magnetic orientation, magnetoreception, Morris' water maze, rodents, bank vole.

INTRODUCTION

Magnetic orientation has been studied since the 1970s and magnetoreception was found in all main groups of vertebrates (Wiltschko & Wiltschko 1995, 2005). Yet, compared with for example, birds, evidence for magnetoreception in mammals remains rather limited. Magnetic compass orientation has been convincingly demonstrated in only several species: in bats (e.g. Holland et al. 2006, 2010, Wang et al. 2007), subterranean rodents (Burda et al. 1990, Kimchi & Terkel 2001, Oliveriusová et al. 2012) and epigeic rodents: 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). But in the naked mole-rats, the results could be considered display of magnetosensitivity only (Malewski et al. 2018).

Magnetic orientation in subterranean rodents was recognized as a polarity compass independent of light (Marhold et al. 1997) and has been found in several species: *Cryptomys* sp. (Burda et al. 1990), *Spalax ehrenbergii* (Kimchi & Terkel 2001, Kimchi et al. 2004), *Fukomys mechowii* and *Heliophobius argenteocinereus* (Oliveriusová et al. 2012). Initial studies in aboveground rodents had controversial results. The first study on the wood mouse (*Apodemus sylvaticus*) showed that magnetic orientation is used during homing (Mather & Baker 1981)

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but efforts to repeat this experiment failed (Sauvé 1988). Three decades later, Malkemper et al. (2015) showed that magnetoreception in wood mice is sensitive to disturbing radio frequency magnetic fields and provided so the first evidence for a magnetic compass in mammals based on a radical-pair mechanism.

Initially, no evidence for magnetic orientation was found in an experiment with the Siberian hamster (*Phodopus sungorus*). Hamsters were not able to choose the correct arm with food in a four-arm maze (Madden & Phillips 1987). Later magnetic orientation in the Siberian hamster was found in a directional preference test of nest building (Deutschlander et al. 2003). 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 showed robust unimodal preference for the learned direction (Muheim et al. 2006). In our previous test, the bank voles showed spontaneous bimodal directional preference of nest building in a circular arena under natural magnetic conditions as well as under magnetic field shifted by 90° (Oliveriusová et al. 2014).

Recently a new method has appeared to study 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 correct arm with a submerged platform according to the magnetic field orientation. Each mouse was tested in one of four magnetic field directions. After only two training trials, the mice were able to learn the magnetic compass direction of a submerged platform. However, a principal problem of magnetic orientation studies still remains – interlaboratory experimental replicability and/or under different conditions.

After successful test of magnetic orientation in the bank vole (Oliveriusová et al. 2014), we decided to use for a new orientation test the water maze according to Phillips et al. (2013). For our studies, the previous (Oliveriusová et al. 2014), as well as the just presented, we

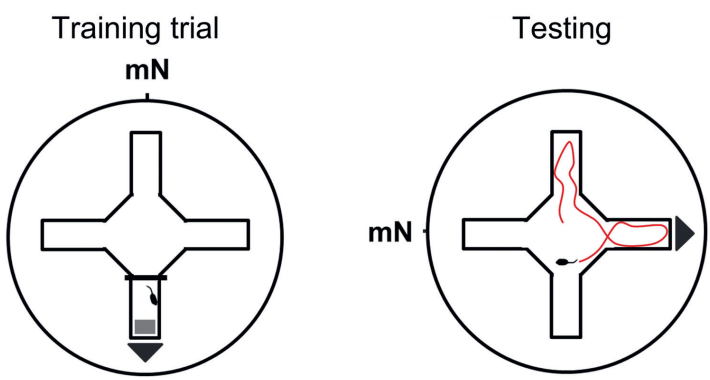

Fig. 1. Training and testing schema according to Phillips et al. (2013). Bank voles were given two training trials in different arms of the water maze with the submerged platform. Here, the mouse is being trained to orient to magnetic south. For testing the submerged platform was removed and the voles were released from the central point. Orientation direction was calculated by the tracking software as the vector sum of the times spent in the four arms during the 30 s testing trial.

have chosen the bank vole, *Clethrionomys glareolus* (Schreber 1780), a rodent with different phylogeny, but similar locomotor ability like murids. Bank voles live in the understory of forests, shrublands, and dry reedbeds, i.e. in rich-structured habitats, demanding for orientation (Niethammer & Krapp 1982).

MATERIALS AND METHODS

Animals

The 24 bank voles of both sexes (12 males and 12 females) used in the experiment were caught in a forest in the vicinity of České Budějovice, Czech Republic, at the same locality as in our previous study (Oliveriusová et al. 2014; 48°58’40”N, 14°25’50”E, 415 m a. s. l.). The animals were kept in a breeding room with moderate temperature (18±1 °C) and a 12L:12D light regime at the breeding facility of the University of South Bohemia in České Budějovice, Czech Republic. The bank voles were housed individually in plastic boxes (55×35×20 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).

Experimental apparatus

The experimental setup was the same 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’s coil 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 during the experiment unchanged. During training and testing, the testing room was illuminated by four fluorescent light tubes covered with Plexiglas diffusers. In the spectral range of 400–700 nm, the emitted light exhibited five intense spectral lines likely associated with excited atoms/ions of noble gases (Ar I – 404.5 nm; Ar II – 435.0 nm; Xe II – 545.9 nm) and mercury (Hg I – 578 nm) and two peaks with maxima located at 470 and 571 nm (see Oliveriusová et al. 2014). Concerning the magnetic fields around the facility, neither the animal breeding room nor the testing room were shielded to minimize electromagnetic interference. Measurements were carried out in the frequency range of 20 Hz to 2.5 GHz. Peak magnetic induction of about 32 nT was reached at 50 Hz (local AC power distribution), maximum levels of radio-frequency interference in the arena reached about 0.72 nT (for detailed measurements see Oliveriusová et al. 2014).

Behavioral assay

The behavioural assay designed to test magnetic orientation in a water maze has been described in detail by Phillips et al. (2013). The assay was divided into two parts – (i) training of directional preference, and (ii) subsequent testing. The experimental apparatus was comprised of several parts. A four-arm water maze was placed in a circular arena in the centre of the 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 a movable transparent plexiglass door. The temperature of the water was maintained between 27–29 °C and coloured white by non-toxic water-soluble colour.

Training
Two training trials started between 3 p.m. and 4 p.m. For both training trials, one arm of the maze was closed by a plexiglass door so bank voles were able to swim only inside this arm but they could see the

rest of labyrinth. At the end of the arm a submerged clear plexiglass platform was placed. In each training trial bank voles were released into the closed arm facing the centre of the water maze. When the bank voles reached the submerged platform and climbed up the wall of the water-maze they were captured quickly (the animals learn the spatial context) and placed back in a small plexiglass rest box. During both testing trials the experimenter stayed quietly in the room at 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 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°.

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 on the second day in the morning, approximately 18 hours after the training trials. The animal was placed into a releasing device, which was made from opaque plastic and transported to the testing room. The releasing device was placed into the centre of the octagon of the water maze and slowly sank while the lid opened so the animal could swim out. After sinking the releasing device was completely submerged. The experimenter left the testing room during the first phase when the lid was still closed. The swimming trajectory of the animal in the water maze was recorded with a digital camera placed on the ceiling above the centre of the circular arena. At the end of the experiment the bank vole was gently captured and placed into the resting box. The testing interval during which the animal swam and looked for the hidden islet was shortened from the original 60 s to 30 s (see Phillips et al. 2013).

Analysis

The swimming trajectory of each animal was evaluated within the 30 s interval after leaving the releasing device. The time spent in each arm was calculated from the 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 (Masaryk University Brno, CZ) was used. To be unbiased, the data were analysed without knowledge of the context.

The distribution of bearings presented by topographic or magnetic direction was analysed by means of the Rayleigh test (significance level of $\alpha=0.05$). The two distributions of bearings were compared by the Watson U^2 test. Statistical analyses were conducted, and circular diagrams were plotted using the Oriana ver. 4 software (Kovach Computing).

RESULTS

Twenty-four bank voles of both sexes were trained for north direction and tested in one of four magnetic field alignments (magnetic north shifted by 0°, 90°, 180°, and 270°). Four individuals had to be excluded from analyses because they did not complete the testing assay. These bank voles escaped the water maze during sinking of the releasing device by jumping over the gap between the releasing device and the wall of the octagonal central part of the maze. Once the animals had found out they could escape from the labyrinth without swimming, they consistently repeated it every time.

Data from the four magnetic field alignments (Table 1) were pooled into one polar coordinate scheme presenting distributions of topographic and magnetic bearings (Fig. 2). Graph A shows the distribution of bearings after topographic arrangement (distribution of deviations from the trained direction according to the topographic 0° position regardless of the magnetic field alignment). The distribution of topographic bearings is not statistically different from a random distribution ($n=20$, Rayleigh test $Z=2.79$, $r=0.373$, $p=0.06$). The distribution of magnetic bearings is shown in graph B – distribution of deviations from the trained direction when the

Table 1. Swimming time (s) in the arms under four magnetic field alignments and the resulting orientation of the animals in topographic as well as magnetic representation. Abbreviations: NN – magnetic north = 0°, NS – magnetic north = 180°, NE – magnetic north = 90°, NW – magnetic north = 270°

ID	field	arms 0	90	180	270	topo (deg.)	vector	magnetic (deg.)
1	NN	0	11	5	8	149	0.243	149
2	NN	2	4	4	4	180	0.143	180
3	NW	7	4	6	4	0	0.048	90
4	NW	7	0	8	5	259	0.255	349
5	NS	3	5	5	7	225	0.141	45
6	NE	0	0	27	0	180	1.000	90
7	NW	7	6	4	4	34	0.172	124
8	NN	8	1	2	2	351	0.468	351
9	NN	6	3	0	6	333	0.447	333
10	NN	8	3	6	5	315	0.129	315
11	NS	6	0	0	6	315	0.707	135
12	NS	5	2	10	4	202	0.256	22
13	NW	7	1	6	3	297	0.132	27
14	NS	0	0	2	4	243	0.745	63
15	NE	6	6	3	3	45	0.236	315
16	NE	6	3	7	3	180	0.053	90
17	NE	0	0	4	11	250	0.780	160
18	NE	0	0	17	8	205	0.752	115
19	NS	4	2	6	5	236	0.212	56
20	NW	0	7	0	9	270	0.125	0

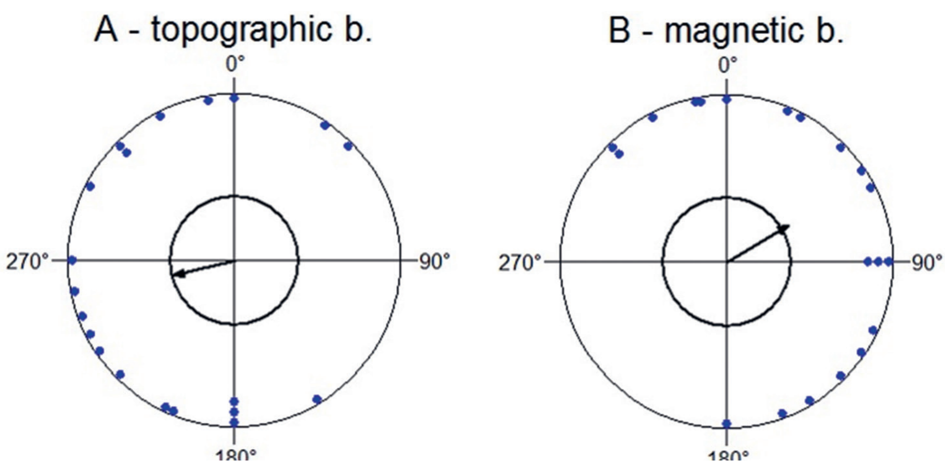

Fig. 2. The distributions of topographic (A) and magnetic bearings (B). Dots represent the deviation from trained direction of each bank vole. Inner circle is Rayleigh test critical value p=0.05 and the direction and length of the arrow represents mean vector of the bearings.

trained direction to magnetic north is fitted to 0°. The magnetic bearings distribution significantly differs from a random distribution ($n=20$, $Z=3.63$, $r=0.426$, $p=0.024$).

DISCUSSION

Our attempt to explicitly repeat the previous "plus" experiment with a water maze (Phillips et al. 2013) brought a low but still statistically significant response to the change in the magnetic field. Bank voles showed a 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 difficulties to deal with factors which can affect perception of the magnetic field, as well as an animal's response to the latter. However, the influence of many factors is known – e.g. different light wave lengths or radio frequency fields (Wiltschko et al. 1993, Burda et al. 2009, Phillips et al. 2022). The measured RF levels (0.72 nT; see Oliveriusová et al. 2014) are still more than 1 order of magnitude lower than those reported to disrupt the radical pair-based compass of e.g. birds (Ritz et al. 2009). Thus, the data presented here does not rule out the radical pair-based mechanism. However, bank vole orientation could be affected by extremely low-frequency electromagnetic field (32 nT), because e.g. mice seem to be able to perceive such weak extremely low-frequency fields (Prato et al. 2013). In these considerations, the magnetite-based mechanism must be mentioned too and it should be verified in voles in further tests (see Kirschvink 1982, Holland & Helm 2013).

The bank voles used in this study were trapped in the wild in contrast to the C57BL/6 mouse, which is a typical laboratory animal kept and bred in captivity for easy handling. There are a lot of essential differences, for example, in locomotion rate and swiftness, learning ability, and anxiety to mention the most obvious (Painter et al. 2018). For instance, in the bank vole we saw quick systematic exploratory behaviour and 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 the experiment) from 60 s to 30 s. After a relatively short time the bank voles stopped searching for the hidden platform and used an alternative strategy to escape from the water maze completely.

The need for 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 traits (climbing omnivore vs. surface-dwelling herbivore; Niethammer & Krapp 1982). Differing extent of behavioural variation could also play a role. Conversely, uniformity of individuals could be any other 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 unpubl. data), bank voles showed higher variation in nest building inside a circular arena than different species of mole rats whose preference seems to be more uniform across populations (Burda et al. 1990, Marhold et al. 1997, Kimchi & Terkel 2001, Oliveriusová et al. 2012). The effects of age, sex and animal species on performance in the water maze was described by D'Hooge & 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 an animal to the water maze experiment is a very stressful situation (D'Hooge & De Deyn 2001). From its point of view, the animal is experiencing a direct threat to its life, 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 bank voles can swim very well (Niethammer & Krapp 1982). In any case, the simplest way to overcome the above-mentioned sources of variation is to use an appropriate number of individuals, maybe increased by a factor of two. Another possibility is to increase (double) the number of swims during the learning phase.

In conclusion, we can presumably say that changes of the maze form and test conditions are needed to demonstrate a strong and clear preference in the bank vole as those observed in the laboratory mouse. In particular, we propose the following changes: (i) the water maze construction needs to eliminate the possibility of bank voles escaping the experimental 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, especially in shy animals.

REFERENCES

Burda H., Marhold S., Westenberger T., Wiltschko R. & Wiltschko W., 1990: Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). *Experientia*, **46**: 528–530.

Burda H., Begall S., Červený J., Neef J. & Němec P., 2009: Extremely low-frequency electromagnetic fields disrupt magnetic alignment of ruminants. *Proceedings of the National Academy of Science of the United States of America*, **106**: 5708–5713.

D'Hooge R. & De Deyn P. P., 2001: Applications of the Morris' water maze in the study of learning and memory. *Brain Research Reviews*, **36**: 60–90.

Deutschlander M. E., Freake M. J., Borland S. C., Philips J. B., Madlen R. C., Anderson L. E. & Wilson B. W., 2003: Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus*. *Animal Behaviour*, **65**: 779–786.

Holland R. A. & Helm B., 2013: A strong magnetic pulse affects the precision of departure direction of naturally migrating adult but not juvenile birds. *Journal of the Royal Society Interface*, **10**(20121047): 1–8.

Holland R. A., Thorup K., Vonhof M. J., Cochran W. W. & Wikelski M., 2006: Bat orientation using Earth's magnetic field. *Nature*, **444**: 702.

Holland R. A., Borissov I. & Siemers B. M., 2010: A nocturnal mammal, the greater mouse-eared bat, calibrates a magnetic compass by the sun. *Proceedings of the National Academy of Science of the United States of America*, **107**: 6941–6945.

Kimchi T. & Terkel J., 2001: Magnetic compass orientation in the blind mole rat *Spalax ehrenbergi*. *Journal of Experimental Biology*, **204**: 751–858.

Kimchi T., Etienne A. S. & Terkel J., 2004: A subterranean mammal uses the magnetic compass for path integration. *Proceedings of the National Academy of Science of the United States of America*, **101**: 1105–1109.

Kirschvink J. L., 1982: Birds, bees and magnetism: a new look at the old problem of magnetoreception. *Trends in Neurosciences*, **5**: 160–167.

Madden R. C. & Phillips J. B., 1987: An attempt to demonstrate magnetic compass orientation in two species of mammals. *Animal Learning & Behavior*, **15**: 130–134.

Malewski S., Malkemper E. P., Sedláček F., Šumbera R., Caspar K. R., Burda H. & Begall S., 2018: Attracted by a magnet: Exploration behaviour of rodents in the presence of magnetic objects. *Behavioural Processes*, **151**: 11–15.

Malkemper E. P., Eder S. H. K., Begall S., Phillips J. B., Winklhofer M., Hart V. & Burda H., 2015: Magnetoreception in the wood mouse (*Apodemus sylvaticus*): influence of weak frequency-modulated radio frequency fields. *Scientific Report*, **5**(9917): 1–6.

Marhold S., Wiltschko W. & Burda H., 1997: A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften*, **84**: 421–423.

Mather J. G. & Baker R. R., 1981: Magnetic sense of direction in wood mice for route-based navigation. *Nature*, **291**: 152–155.
Muheim R., Edgar N. M., Sloan K. A. & Phillips J. B., 2006: Magnetic compass orientation in C57BL/6J mice. *Learning & Behavior*, **34**: 366–373.
Niethammer J. & Krapp F., 1982: *Handbuch der Säugetiere Europas*. *Band 2/I Rodentia 2*: *Cricetidae*, *Arvicolidae*, *Zapodidae*, *Spalacidae*, *Hystricidae*, *Capromyidae*. Akademische Verlagsgesellschaft, Wiesbaden, 649 pp.
Oliveriusová L., Němec P., Králová Z. & Sedláček F., 2012: Magnetic compass orientation in two strictly subterranean rodents: learned or species-specific innate directional preference? *Journal of Experimental Biology*, **215**: 3649–3654.
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.
Painter M. S., Davis M., Ganesh S., Rak E., Brumet K., Bayne H., Malkemper P. & Phillips J. P., 2018: Evidence for plasticity in magnetic nest-building orientation in laboratory mice. *Animal Behaviour*, **138**: 93–100.
Phillips J. B., Youmans P. W., Muheim R., Sloan K. A., Landler L., Painter M. S. & Anderson C. R., 2013: Rapid learning of magnetic compass direction by C57BL/6 mice in a 4-Armed ‘plus’ water maze. *Public Library of Science One*, **8**(8; e73112): 1–11.
Phillips J., Muheim R., Painter M., Raines J., Anderson C., Landler L., Dommer D., Raines A., Deutschlander M., Whitehead J., Fitzpatrick N. E., Youmans P., Borland C., Sloan K., McKenna K., 2022: Why is it so difficult to study magnetic compass orientation in murine rodents? *Journal of Comparative Physiology A*, **208**: 197–212.
Prato F. S., Desjardins-Holmes D., Keenliside L. D., DeMoor J. M., Robertson J. A. & Thomas A. W., 2013: Magnetoreception in laboratory mice: sensitivity to extremely low-frequency fields exceed 33 nT at 30 Hz. *Journal of the Royal Society Interface*, **10**(81; 20121046): 1–8.
Ritz T., Wiltschko R., Hore P. J., Rodgers C. T., Stapput K., Thalau P., Timmel C. R. & Wiltschko W., 2009: Magnetic compass of birds is based on a molecule with optimal directional sensitivity. *Biophysical Journal*, **96**: 3451–3457.
Sauvé J. P., 1988: Analyse de l’orientation initiale dans une experience de retour au gite ches le mulot, *Apodemus sylvaticus*. *Science et Techniques de l’Animal de Laboratoire*, **13**: 89–91.
Teskey G. C., Ossenkopp K. P., Kavaliers M., Innis N. K. & Boon F. H., 1998: Individual differences in radial maze performance and locomotor activity in the meadow vole, *Microtus pennsylvanicus*. *Physiology & Behavior*, **65**: 555–561.
Wang Y., Pan Y., Parsons S., Walker M. & Zhang S., 2007: Bats respond to polarity of a magnetic field. *Proceedings of the Royal Society B*, **274**: 2901–2905.
Wiltschko R. & Wiltschko W., 1995: *Magnetic Orientation in Animals*. Springer Verlag, Berlin, Heidelberg & New York, 297 pp.
Wiltschko W. & Wiltschko R., 2005: Magnetic orientation and magnetoreception in birds and other animals. *Journal of Comparative Physiology A*, **191**: 675–693.
Wiltschko W., Munro U., Ford H. & Wiltschko R., 1993: Red light disrupts magnetic orientation of migratory birds. *Nature*, **364**: 525–527.