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Testing Magnetic Orientation in a Solitary Subterranean Rodent *Ctenomys talarum* (Rodentia: Octodontidae)

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Abstract

To test for the hypothesis that *Ctenomys talarum* can use the earth's magnetic field for spatial orientation, we carried out field and laboratory experiments to analyse if *C. talarum* burrows present any geomagnetic orientation in their natural habitat, if *C. talarum* show any spontaneous directional preference when starting to excavate their burrows and if this subterranean rodent is capable to use the earth's magnetic field to orient towards a goal in a complex maze. No correlation between the burrowing direction and the earth's magnetic field was found. We could not find any evidence for any spontaneous directional preference when starting to excavate the burrows in *C. talarum*. The change of the horizontal vector of the geomagnetic field did not affect the ability of this rodent to orient towards a goal in an artificial labyrinth. Explanations for these results and other possible mechanisms of orientation that could be used by *C. talarum* are discussed.

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Introduction

An accurate spatial orientation and localization of food patches, mates, escape routes and neighbour's locations are essential for survival and reproduction in subterranean rodents (Kimchi & Terkel 2001). Several cues can be used by animals for spatial orientation. These cues may be provided by visual, olfactory and/or acoustic signals, by intrinsic cues and/or by cues provided by the earth's magnetic field in general and by a magnetic compass in particular.

Magnetic compass orientation has been described in several species of insects, fishes, reptiles and birds (Kirschvink et al. 1985). Recently, various

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studies have been focused on magnetic field perception in mammals (Mather & Baker 1981; August et al. 1989; Deutschlander et al. 2003), and specially, on magnetic orientation in subterranean mammals, because of the unique characteristics of the subterranean habitat, which differs from surface environments in the variety of sensory stimuli (Burda et al. 1990; Lovegrove et al. 1992; Marhold et al. 1997, 2000; Kimchi & Terkel 2001). Although some evidence from these works is still controversial, the experiments carried out in these studies proved that *Cryptomys hottentotus* (then considered as *Cryptomys anselli*) and *Spalax ehrenbergi*, two subterranean rodents, perceive and use the earth's magnetic field to orient in space (Burda et al. 1990; Marhold et al. 1997; Kimchi & Terkel 2001).

The tuco-tucos (*Ctenomys*) are considered members of the family Ctenomyidae (Caviomorpha), and are distributed in the south cone of South America (Reig et al. 1990). *Ctenomys talarum* is a solitary subterranean rodent that inhabits a system of closed galleries parallel to the soil surface (Busch et al. 1989). The burrow system of *C. talarum* has a branching structure, consisting of a main axial tunnel and a variable number of lateral branches and feeding tunnels, all of them plugged (Antinuchi & Busch 1992).

Because of its subterranean way of life, optical cues cannot be used by *C. talarum* for spatial orientation within the subterranean tunnels. Although *C. talarum* usually marks several parts of its tunnel with urine and is able to discriminate individual and sexual odours (Fanjul 2001), the use of olfactory cues for directional orientation is not effective when steering a course to a new area (Burda et al. 1990). Moreover, olfactory signalling in different parts of the tunnel presents the problem of transferring olfactory cues in a non-selective manner to another parts of the burrow system while walking through the tunnels (Kimchi & Terkel 2001). All subterranean rodents yet studied to present a poor hearing sensitivity and a rudimentary ability to localize brief sounds (Heffner & Heffner 1990, 1992, 1993). Although hearing sensitivity has not been tested in *C. talarum*, the one-dimensional underground habitat in which *C. talarum* lives together with the reduced ear pinnae, limits any ability to localize sounds. Consequently, the use of acoustic signals to obtain spatial information for directional orientation seems to be limited for *C. talarum*.

Therefore, the restricted number and variety of sensory cues present in the underground habitat, together with the necessity of orienting efficiently within the complex tunnel system and with the existence of magnetic compass orientation in other species of subterranean rodents suggest the possibility that *C. talarum* also use the signals of the earth's magnetic field to orient in their subterranean environment. To test for this hypothesis, we carried out field and laboratory experiments to analyse if *C. talarum* burrows present any geomagnetic orientation in their natural habitat, if *C. talarum* show any spontaneous directional preference when starting to excavate their burrows and if this subterranean rodent is capable to use the earth's magnetic field to orient towards a goal in a complex maze.

Materials and Methods

Animals

Adult tuco-tuco (*C. talarum*) of both sexes, were trapped in Mar de Cobo (Buenos Aires Province, Argentina; 37°45′S, 57°56′W; natural magnetic inclination −39°54′, total natural magnetic field intensity 24.2 nanoteslas). *Ctenomys talarum* is a small size rodent (120 and 160 g body mass for females and males, respectively), associated with different type of friable soils (Busch et al. 2000). This species is found along the coast of Buenos Aires province, Argentina, possibly extending into Santa Fe province (Redford & Eisenberg 1992).

Captured individuals were carried to the laboratory and housed in individual plastic cages (25 × 32 × 42 cm) and maintained in an animal room with photoperiod and temperature automatically controlled (12L:12D, 25 ± 1°C). Relative ambient humidity ranged from 50 to 70%. Animals were feed with mixed grasses, sweet potatoes, lettuces, sunflower seeds, corn, carrots and alfalfa ad libitum. As *C. talarum* do not drink free water, this source was not provided to the animals. The experiments were carried just after the animals were collected in the field.

Burrow Orientation

Nineteen burrow systems were fully excavated with shovel. Then, tunnels were mapped on graph paper. Total area covered by each burrow was drawn using the minimum convex polygon method. The major axis of the ellipse including this polygon was used to determine burrow magnetic polarity orientation.

Directional Preference

To test if *C. talarum* show any spontaneous directional preference when starting to excavate their burrows, the individual tuco-tuco (n = 10; five males and five females) was released in a circular plastic arena (80 cm diameter) filled with sand and covered with a wooden plate. After 90 min the test was terminated and the position of the burrow entrances recorded. One trial was performed with each animal. After each trial, the sand was replaced and the circular arena washed.

Learning Experiments

To test the null hypothesis that tuco-tucos are not able to learn a pathway trough of a burrow system, we used eight individuals (four males and four females). To perform tests, we built a labyrinth with opaque polyvinyl chloride (PVC) tubes (10 cm in diameter) with small windows on their top to register the movements of the tuco-tucos. The labyrinth consisted of eight dead-end paths and one correct path starting in an opening with 12 turns leading to a sealed resource cage in the distal end (Fig. 1). To perform learning tests, animals were maintained among 75 and 80% of initial body weight in order to increase animal motivation to explore and learn the complex maze. Individuals were introduced into the labyrinth at the

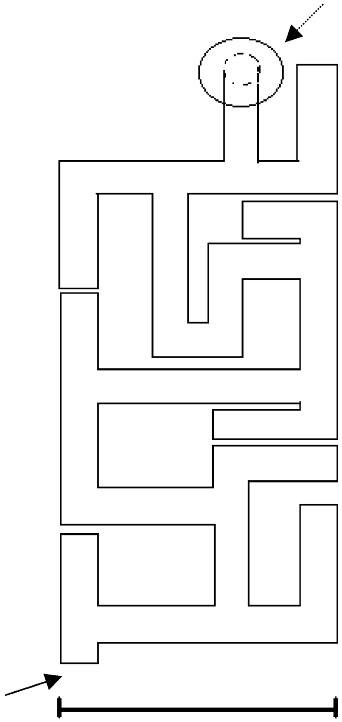

Fig. 1: Complex labyrinth used for testing magnetic polarity compass orientation in *Ctenomys talarum*. The solid arrow indicates the entrance and the dotted arrow the resource goal. The line below indicates 70 cm of longitude

entrance (under semidark light conditions), which was closed once the individual went into the system. Sweet potato and lettuce were put into resource cage, which entry was closed with a removable paper lid. Time spent by individuals to reach the resource cage and number of errors made were recorded for each individual during five consecutive daily trials (at 5 d trial, individuals showed no improvement in their performance after two consecutive trials) under earth's natural magnetic field. At the end of each trial, the labyrinth was thoroughly washed with tap water and odourless glassware cleaner, wiped with alcohol (70%) and then allowed to air dry to ensure that no odours from the previous trial remained.

Magnetic Orientation

To test the null hypothesis that tuco-tuco are not able to use the earth's magnetic field for spatial orientation, we built a Helmholtz coils system as the one developed by Wiltschko & Wiltschko (1975 and personal commentaries). Helmholtz coils were used to establish experimentally a new local south–north magnetic field. The system consisted of a pair of round coils (2 m diameter) wrapped with a 1 mm section copper wire calculated according to $Hi = 899 \times nI/r$ equation, where Hi is the generated magnetic field (in nT), 'n' the number of wire turns, 'I' the electric current (in Amperes) and 'r' the radius of the coils (in meters) (Wiltschko & Wiltschko 1995). The coils were placed vertically and parallel, 1 m apart, inducing a new horizontal magnetic field. The labyrinth was placed between coils and the coils were connected to a continuous current power supply (3 A). The horizontal axis of the pair of coils was placed in order to shift the polarity of the earth's natural magnetic field by 180, without changing its intensity and inclination. Animals were put into labyrinth as explained above. At the sixth trial, trained individuals were tested under the altered magnetic field. Again, time spent by individuals to reach the resource cage and number of errors made were recorded for each individual at the sixth trial.

At the end of the experiments, animals were fed ad libitum and recovered initial body weight after a few days, remaining in good physical conditions. Then they were returned to their site of capture.

Statistics

To test the existence of relationship between burrows and magnetic field orientation burrows were classified into four groups (resolution 45°: 0–45, 45–90, 90–135, 135–180). A chi-square test was used to establish the existence of relationships between burrow system and magnetic field orientation.

We used a chi-square test to evaluate the null hypothesis that animals did not have any preference in spatial orientation when starting to excavate a burrow. For analysis, we classified the orientation in which animals excavate into four groups: ≥ 0 to $< 90°$, $\geq 90°$ to $< 180°$, $\geq 180°$ to $< 270°$ and $\geq 270°$ to $< 360°$. Because some individuals excavated more than one hole in the trial, we used the data from animals excavating a unique entrance together with data randomly selected from those animals excavating more than a burrow entrance. Then, we performed a chi-square test for each data combination. We repeat the last procedure 1000 times using Monte Carlo iteration method.

A one-way repeated measures ANOVA was used to test the hypothesis of no differences in time spent to reach the food resource cage and number of errors made till reaching the resource cage among consecutive trials of the learning experiments. A paired t-test was used to test the null hypothesis of no differences in both time spent to reach the food resource cage and number of errors made between natural and altered magnetic field orientation.

Results

Burrow Orientation

No relationship between burrow orientation and natural geomagnetic field was detected (resolution 45°, $df = 3$, $n = 19$, $p > 0.05$; Fig. 2).

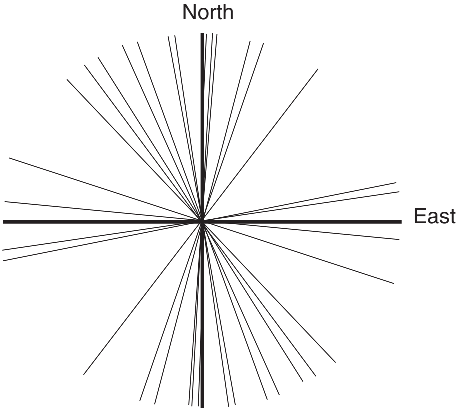

Fig. 2: Axial magnetic polarity orientation of fully excavated burrows of *Ctenomys talarum* in the open field

Directional Preference

The results obtained after using the Monte Carlo iteration method showed that the positions of the burrow entrances were not significantly different from random distribution (Fig. 3; $p > 0.05$), indicating the absence of any spontaneous directional preference when starting to excavate the burrows.

Learning Experiments

After the first trial, the time spend by animals to reach the resource cage started to drop until day 4, where it remained unchanged until the end of the

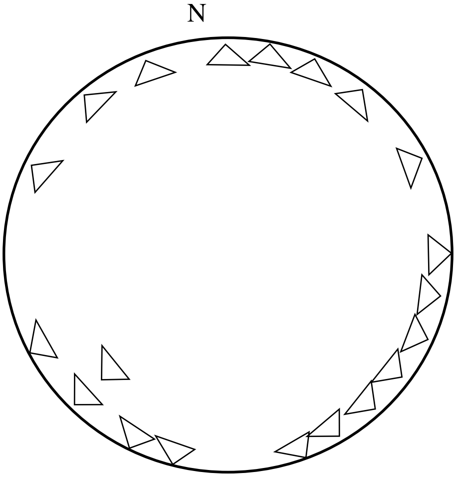

Fig. 3: Directional preferences of *Ctenomys talarum* in a circular arena under natural magnetic field. Triangle indicates the position of burrow entrances

Table 1: Time that animals spent traveling trough the labyrinth to reach the resource cage (time) and number of errors made to reach the resource cage (errors) for all the days of the learning trial

Day	Time (s)	Errors
1	182.1 ± 20.4^{a}	13.6 ± 2.3^{a}
2	120.3 ± 15.6^{b}	10.7 ± 2^{a}
3	96.5 ± 18.1^{bc}	8.1 ± 1.6^{ab}
4	61.6 ± 14.3^{cd}	4.1 ± 1.4^{b}
5	42 ± 8^{d}	2 ± 0.8^{b}

Values are showed as $\bar{x}$ ± SEM. Subscript letters represents statistical comparisons for learning trials. Different letters showed statistical differences [one-way repeated measures (RM) ANOVA].

learning experiment (Table 1; Fig. 4). The number of errors made by the tuco-tucos before reaching the resource cage started to drop after the second trial until the third one, maintaining then similar among consecutive trials (Table 1; Fig. 4).

Magnetic Orientation

No statistical differences in both time that animals spent traveling trough the labyrinth to reach the resource cage and number of errors made before reaching the resource cage were detected between animals tested under natural magnetic field (42 ± 8.07 s and 2 ± 0.86 errors) and under changed magnetic field (32 ± 3.52 s and 0.62 ± 0.26 errors) (paired t-test: $n = 8$, $p > 0.05$).

Discussion

Magnetic compass orientation has been subject of study in several species of animals (Wiltschko & Wiltschko 1995) and the use of geomagnetic cues for spatial orientation seems to be a ubiquitous trait in animals (Deutschlander et al. 1999).

Comparatively, little research has been undergone on magnetic field perception in mammals. The first report describing the use of the earth's magnetic field as a compass orientation cue came from the European woodmice, *Apodaemus sylvaticus* (Mather & Baker 1981), although later, Sauve (1985), following Mather & Baker's (1981) experimental protocol, was unable to obtain evidence of a home directed response in *A. sylvaticus* when displaced in unaltered magnetic field condition. August et al. (1989) showed that, when displaced 40 m away from their home area and released in a circular arena, the white-footed mice (*Peromyscus leucopus*) concentrated their exploratory activity in the portion of the arena corresponding to home direction, and when the horizontal component of the geomagnetic field was reversed, these individuals concentrated their activity in areas opposite to home place. Recently, Deutschlander et al. (2003) showed that laboratory raised Siberian hamsters, also use

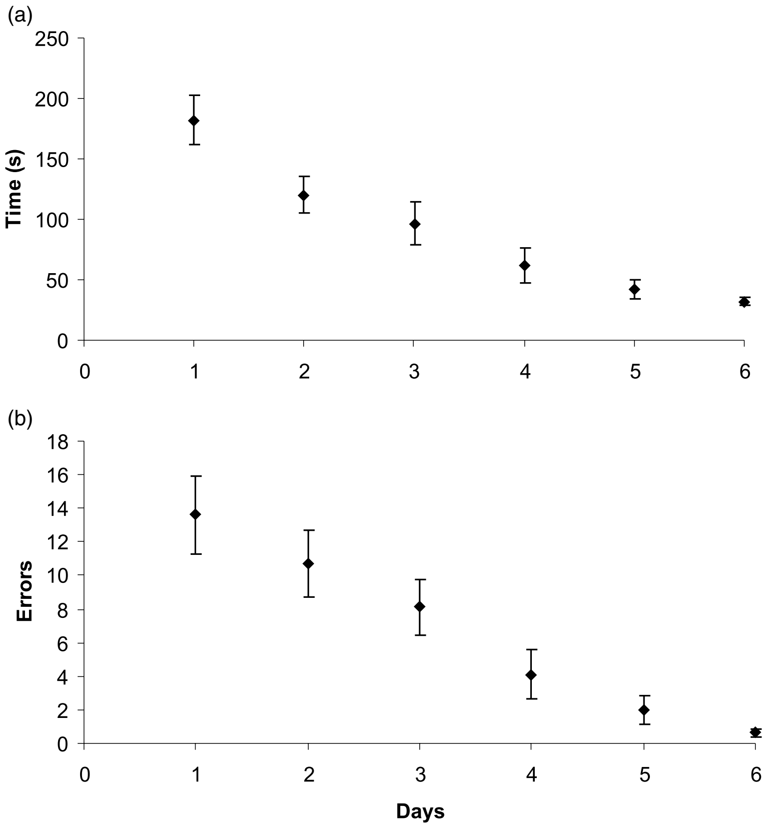

Fig. 4: Time ± SEM spent (a) and number of errors ± SEM (b) made by *Ctenomys talarum* to reach the resource cage in the complex labyrinth during six consecutive daily trials (n = 8). At the six trial the animals were tested under the altered magnetic field

directional information from the magnetic field to position their nests. However, the directional preference for nest position shown by Siberian hamsters appears to be learned.

Regarding subterranean rodents, Burda et al. (1990) showed that *C. hottentotus* (later renamed *C. anselli*) had a behavioural tendency to build their nests in a circular arena in the SE sector of the local geomagnetic field, and when magnetic north was turned by 120° or by 180°, the mole-rats changed their nest positions concomitantly. This magnetic compass of *C. anselli* was later shown to be a polarity compass and not an inclination compass as described for birds (Marhold & Wiltschko 1997). Moreover, Burda et al. (1990) also stated that the main axis of the natural burrows of this subterranean rodent extended roughly southward. However, this last claim was later refused by Lovergrove et al. (1992), who showed that burrow orientation was not significantly different from the expected one for a random distribution.

Recently, Kimchi & Terkel (2001) proved that *S. ehrenbergi* built their sleeping nest and food store in the southern sector of an eight-arm maze, and this location varied accordingly to the shift in the magnetic field. Moreover, these authors also demonstrated that this subterranean rodent used the magnetic field to reach a goal in a complex labyrinth, and this magnetic compass orientation was independent of light stimulation.

The results presented in this work showed that there is no correlation between the burrowing direction and the earth's magnetic field, that *C. talarum* did not posses any spontaneous directional preference when starting to dig their tunnels and, finally, we could not find any evidence that this species rely on the polarity compass of the magnetic field to obtain directional information to reach a goal in a complex labyrinth.

When analysing the results obtained in the field and laboratory burrow experiments, we did not find evidence that *C. talarum* have any geomagnetic direction preference when building their burrow systems. Although this result could give a first indication of the absence of a magnetic compass orientation in this species of subterranean rodent, it is certainly not enough to make any conclusion about the magnetic perceptions of this animal. It is also possible that *C. talarum* may have a well-developed magnetic sense but not a preferred orientation of their tunnels with respect to the earth's magnetic field.

Results of the maze experiments also suggest that *C. talarum* do not use magnetic cues for orientation. However, again other possible explanations could exist for the observed results. Contrary to what happens when obtaining positive behavioural evidence for a magnetic sense, negative evidence must be taken more carefully, as other factors, beside the failure to sense the magnetic field, can explain the lack of response of individuals to variations in the magnetic field (Wiltschko & Wiltschko 1995; Deutschlander et al. 2003). These factors, such as motivation, presence of other sensory cues or individual preferences or constraints, usually interfere with the interpretation of the results. When analysing the results obtained in the complex labyrinth, lack of motivation and presence of other sensory cues could be excluded as possible explanations for the lack of evidence for magnetic orientation in *C. talarum*, as animal motivation was increased via food deprivation and much care was taken in order to eliminate chemical, sensory or visual cues that could be used by *C. talarum* to obtain spatial information. However, it is also possible that *C. talarum* did not rely on magnetic cues to obtain spatial information for food localization or that it might not be adapted to respond to rapid changes in the magnetic field, two situations that could also account for the lack of magnetic sensitivity observed in *C. talarum*. Another possibilty is that, as recently shown by Deutschlander et al. (2003), the expression of a directional preference for nest position along a particular axis relative to the magnetic field depends on the holding conditions prior to experiments, giving another explanation for the failure to demonstrate a magnetic orientation in this rodent.

If the partial evidence obtained in this work truly reflects the absence of a magnetic sense in *C. talarum*, other mechanisms should be used by this subterranean rodent to obtain spatial information in a habitat with restricted number and variety of sensory cues. One possible mechanism to orient in the burrows could be based on the apprenticeship of a path by memorizing a fix sequence of body rotations within its space, as was previously suggested by Kimchi & Terkel (2001b) for *S. ehrenbergi*. Another possibility is that *C. talarum* may rely on internal cues for accurate spatial orientation in the subterranean environment. A mechanism of orientation based on internal cues or path integration to construct a cognitive map of the surrounding environment was shown for rats (Benhamou 1997) and hamsters (Etiene et al. 1986) and was recently suggested for a subterranean rodent, *S. ehrenbergi* (Kimchi & Terkel 2001b).

This works shows that, although magnetic compass orientation seems to have evolved in various species of subterranean rodents, generalizations of this trend should be taken carefully, as under the specific experimental conditions used in this work we did not find evidence that *C. talarum* rely on the geomagnetic field to orient underground. Additional studies are needed to clearly determine the absence of a magnetic sense in *C. talarum*, and if so, which mechanisms are used by this rodent to orient efficiently within its burrow system and to what extent is the use of the geomagnetic field to orientate spread among subterranean rodents.

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