Mechanisms of Compass Orientation in C57BL/6 Laboratory Mice

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ABSTRACT

Compass orientation or menotaxis is defined as the ability to orient at a specific angle relative to a directional cue. Cues used for compass orientation include the sun, stars, moon, geomagnetic field and polarized light. While there is evidence in a variety of organisms for compass orientation, the ability of mammals to use cues for compass orientation has been relatively unexplored.

The goal of this research was to explore whether laboratory mice could use either magnetic or auditory cues for compass orientation. The results indicate that mice are able to learn to position their nest using a magnetic compass. The development of a magnetic compass assay in laboratory mice will allow the investigation of the mechanism of magnetic compass orientation in mammals, a goal that has been unattainable to this point.

In addition, this research has provided preliminary evidence that mice are able to learn to position their nests using an auditory compass. While there is evidence in several organisms for place navigation using auditory cues (i.e. the ability to locate a specific spatial position using auditory cues), this is the first evidence in any organism for an auditory compass (i.e. the ability to calculate a directional heading relative to an auditory cue).

In conclusion, both experiments provide evidence for specialized compass systems in mice and suggest that further research is necessary to fully understand the role of these systems in the behavioral ecology of mice.

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INTRODUCTION

Methods of Compass Orientation

Spatial orientation plays an important role in many aspects of behavior including dispersal, migration, foraging, searching for mates, and escaping predators. Since these activities are crucial to individual fitness, almost all living organisms have developed some sort of orientation system. However, because the needs of organisms differ and because the availability of orientation cues can be unpredictable, a variety of orientation systems have evolved. The simplest types of orientation are those involving taxes, which are essentially movements towards (positive taxes) or away (negative taxes) from certain stimuli. For example, many insects exhibit positive or negative phototaxis (movement towards or away from light). There are many additional types of taxes including phonotaxis (movement relative to sound), chemotaxis (movement relative to a chemical gradient), and geotaxis (movement relative to gravity), and an individual organism may employ several different types of taxes.

More complex forms of orientation include piloting, compass orientation, and true navigation. Piloting refers to the use of familiar reference points, such as landmarks, for orientation. There is evidence that rodents primarily use landmark cues for orienting about their home territories (Alyan and Jander, 1994). Compass orientation (also referred to as menotaxis), on the other hand, refers to orientation in which the animal is able to derive and maintain a directional heading or bearing relative to (but not necessarily towards or away from) an external cue, and is referred to as menotaxis. Path integration, a specialized form of compass orientation, involves the use of compass information to keep track of the path of an outward trip. This information is then integrated into a direct

path back to the initiation point. Path integration has been documented in insects and

mammals (Wehner and Wehner, 1986; Etienne et al., 1988). True navigation, on the

other hand, requires an ability to determine geographic position relative to a specific goal

or destination in unfamiliar surroundings without reference to landmarks or information

obtained during the displacement. True navigation, which enables an animal to home

from an unfamiliar territory, requires both a compass (sense of direction) and a map

(sense of geographic position).

Compass orientation is widespread in the animal kingdom. While the variety and

redundancy of compass systems (see below) is surprising, it illustrates the important role

that spatial orientation plays in the survival and/or reproduction of many species of

animals. Numerous types of compass systems have been found in both invertebrates and

vertebrates including polarized light compasses, sun compasses, star compasses, and

magnetic compasses (Ferguson, 1971; Able, 1980; Bovet, 1992; Quinn and Dittman,

1992; Wiltschko and Wiltschko, 1995a; Lohmann and Lohmann, 1998; Phillips, 1998;).

Many organisms are able to use the sun as a source of compass information,

which requires a circadian clock to derive angular information from the changing position

of the sun in the sky (see Able, 1980 for review). Experimentation with insects has

revealed that honeybees communicate information about the location of food sources by

performing waggle dances that encode foraging directions as an angle relative to the sun

(von Frisch, 1967). Pond-dwelling fish (large-mouth bass) have been shown to utilize a

sun compass in order to orient perpendicular to shoreline (y-axis orientation) (Loyacano

et al., 1977; Goodyear and Bennett, 1979). Furthermore, bluegill can be trained to seek a

shelter relative to the sun's position (Hasler and Wisby, 1958) and parrot fish orientation

was disrupted under overcast skies (Winn et al., 1964). There are also numerous studies indicating the presence of sun compass orientation in amphibians (Ferguson, 1971; Adler, 1976), reptiles (Newcomer et al., 1974; DeRosa and Taylor, 1980; Adler and Phillips, 1985), and birds (Wiltschko, 1983). The evidence for sun compass orientation is more limited in mammals. Sun compass orientation has only been convincingly demonstrated in the meadow vole, the thirteen-lined ground squirrel, and common dolphins (Pilleri and Knuckey, 1969; Fluharty et al., 1976; Haigh, 1979). However, there have also been instances recorded where bats have used the glow of the setting sun as a compass cue (Buchler and Childs, 1982).

The sun compass appears to involve only the azimuth of the sun and not its altitude. Since the sun moves at an average rate of 15 degrees per hour across the sky, an organism must have an internal clock to compensate for the movement of the sun. Clock shift experiments have indicated that indeed, an internal clock is used to compensate for sun movement (Papi, 1955).

Polarized light patterns [i.e. the plane in which the electrical vector (e-vector) of light oscillates] have been shown to be another cue for compass orientation used by a variety of animals. Interestingly, because polarized light patterns are centered about the axis of the sun, a polarized light compass can theoretically be used to determine the position of the sun when it is not actually visable (requiring only a patch of blue sky), and therefore can aid in sun compass orientation. Polarized light compasses have been extensively studied in bees and ants (Rossel and Wehner, 1986; Wehner, 1989). A number of insects have been shown to have a neural template that approximates the natural celestial polarization patterns, a mechanism termed a "matched sensory filter"

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(Wehner, 1987). There is more limited evidence that fish (Hawryshyn et al., 1990), amphibians (Adler and Taylor, 1973), and birds (Munro and Wiltschko, 1995) are capable of using polarized light patterns. However, birds are also thought to use polarization patterns as a calibration reference for other compass systems including the sun compass (Helbig, 1990; Phillips and Moore, 1992; Able, 1993). To date, however, there is no evidence for the use of a polarized light compass in any mammal.

Another form of compass orientation, used by nocturnal species, is orientation with respect to star patterns. Star compass orientation has only been convincingly described in birds (Emlen, 1967, 1975), and unlike the sun compass, a star compass does not require a circadian clock to compensate for the movement of the stars throughout the night (Emlen, 1967). While the exact mechanism of star compass orientation is unknown, there is evidence that exposure to celestial rotation is necessary for birds to learn the directional properties of individual star patterns (Emlen, 1970a; Wiltschko and Wiltschko, 1991).

There has also been some evidence suggesting the use of the moon as a possible compass cue. For instance, a lunar compass has been suggested for orientation in salmon (Brannon et al., 1981). However, this experiment is controversial and could possibly be explained by other cues. Furthermore, since moon cues are not very reliable (i.e. the visibility of the moon varies throughout the month and the moon moves more slowly than the sun and would therefore require a separate internal clock), it seems unlikely that a moon compass will be of widespread occurrence.

Magnetic Compass Orientation

Finally, the most recently discovered compass, found to be increasingly widespread in the animal kingdom, is the magnetic compass. Since the 18th century, it has been thought that magnetic fields have effects on organisms, however, until recently there has been much difficulty in providing evidence that animals are indeed sensitive to the geomagnetic field. Many of the early attempts to demonstrate a magnetic sense in birds ended with failure (Emlen, 1970b; Kreithen and Keeton, 1974), causing doubt that animals were able to sense the magnetic field. However, the development of a reliable magnetic compass assay in migratory birds provided solid evidence for this sensory system (Wiltschko and Wiltschko, 1972), and since this time, an enormous variety of animals have been shown to be sensitive to the geomagnetic field (e.g. Quinn, 1980; Dejong, 1982; Lohmann, 1985; Phillips, 1986a; Lohmann, 1991; Phillips and Sayeed, 1993; Wiltschko and Wiltschko, 1995a; Kimchi and Terkel, 2001).

Blakemore (1975) reported the discovery of magnetotactic bacteria that contain small particles of magnetite (Fe_3O_4) causing the organism to align itself with its anterior end towards either north (northern hemisphere) or south (southern hemisphere). This ability appears to have arisen from a need to remain buried in sediment, as the bacteria are anaerobic. Since the inclination in the northern hemisphere is positive (i.e. pointing into the ground) and the inclination in the southern hemisphere is negative (i.e. pointing upward), magnetotactic bacteria can use the magnetic field to align themselves to either north or south, ensuring they are positioned to swim in a direction that leads them back into the sediment and, therefore, to an anaerobic environment. Additionally, the tendency

to position the body relative to a certain axis of the magnetic field has been described in insects (Altman, 1981) and fish (Tesch and Lelek, 1973).

While magnetotaxis and magnetic alignment behaviors are certainly present in the animal kingdom, magnetic compass orientation appears to be a much more widespread phenomenon. Magnetic compass orientation has been demonstrated in many taxonomic classes of both invertebrates and vertebrates. Invertebrates shown to have magnetic compasses include molluscs (Lohmann and Willows, 1987), crustaceans (Arendse, 1978; Lohmann, 1985; Ugolini and Pezzani, 1992), and insects (Baker and Mather, 1982; Dejong, 1982; Rickli and Leuthold, 1988; Phillips and Sayeed, 1993). While many invertebrates use their magnetic compass for either homing (Rickli and Leuthold, 1988; Lohmann et al., 1995) or migration (Baker and Mather, 1982), it was found that several species could be trained to use their magnetic compasses to orient relative to an ecologically significant cue. For example, drosophila and mealworm beetles were trained to orient magnetically, in a direction corresponding to a light (Arendse and Vrins, 1975; Phillips and Sayeed, 1993), and beachhoppers and isopods were trained to use a magnetic compass to orient perpendicular to a shoreline (Arendse, 1978; Ugolini and Pezzani, 1992). Such "trained" responses have proved useful in developing behavioral assays that can be used to characterize the magnetic compass in organisms that do not exhibit migration or homing responses in the laboratory.

Magnetic compass orientation in vertebrates has also been studied quite extensively. In fish, studies of magnetic orientation have focused primarily on eels (elasmobranchs) and salmon since they both undertake long migrations (eels develop in fresh water habitats and return to the ocean to spawn, whereas salmon spend most of their

lives in the open ocean and then return to their natal stream to spawn). Europeans eels were first shown to orient along a consistent magnetic axis when tested in a circular arena with six exit tubes (Branover et al., 1971; Gleizer and Khodorkovsky, 1971). Similarly, sockeye salmon were shown to use a magnetic compass when tested in a circular tank with four openings. In this study, salmon fry oriented in their appropriate migratory direction, and maintained this direction relative to magnetic north when the magnetic field was rotated (Quinn, 1980; Taylor, 1986).

Convincing evidence for magnetic compass orientation in reptiles has only been obtained in turtles. Box turtles were trained to swim to a directional shoreline and, when tested in a circular arena, they oriented towards the trained shoreward direction. This response was abolished when strong bar magnets were attached to the carapace, suggesting the use of a magnetic compass (Mathis and Moore, 1988). Additionally, young hatchling Loggerhead and Leatherback sea turtles use a magnetic compass for open ocean migratory orientation (Lohmann, 1991; Lohmann and Lohmann, 1993).

The first evidence for use of a magnetic compass in amphibians was found in the cave salamander. Salamanders were trained to orient along either a north-south magnetic axis or an east-west magnetic axis, and when tested, the salamanders exhibited a weak preference for their trained direction (Phillips, 1977). Since this time, experiments in which Eastern Red-spotted newts were trained to orient towards a directional shoreline have shown that they have a reliable magnetic compass sense (Phillips, 1986a). Furthermore, several studies on toads have indicated that magnetic cues play a role in migration to breeding sites (Sinsch, 1987, 1992). More recently, it was found that larval

bullfrogs are able to use a magnetic compass to orient along a shore/deep water axis (Freake et al., 2002).

The largest body of evidence for magnetic compass orientation has been obtained in birds. It was noted that caged migratory birds exhibited migratory restlessness (Zugunruhe) by attempting to escape from their cage in the appropriate migratory direction. This characteristic was exploited by placing the bird in the center of a paper funnel on top of an inkpad. Consequently, when the bird jumped on the side of the funnel while attempting to escape, it would leave footprints on the funnel in the direction it was attempting to fly (Emlen, 1967). Emlen funnels have proven to be an extremely important tool for investigating the directional cues used for migratory orientation, including the magnetic compass. To date, there are approximately twenty species of birds for which magnetic compass orientation has been documented (see Wiltschko and Wiltschko, 1995a).

In contrast to the expansive literature on magnetic compass orientation in birds, there are relatively few studies on magnetic compass orientation in mammals. Mather and Baker (1981) were the first to provide evidence for a magnetic compass in a mammal. In their experiment, European woodmice (*Apodemus sylvaticus*) altered their homing behavior after being displaced in a reversed magnetic field. However, the methods used in this experiment (mice were observed through a hole in a cardboard box) made the validity of these findings uncertain. A similar experiment by August et al. (1989) provided evidence for magnetic compass orientation in *Peromyscus leucopus*. However, because the methods were derived from those of Mather and Baker (1981), the results were met with criticism. Evidence for a magnetic compass has also been cited in

humans, however attempts to replicate these experiments reported negative results (see

Kirschvink et al., 1985; Baker, 1987). More rigorous experiments have demonstrated the

presence of a magnetic compass in mole rats from two different families, Bathyergidae

and Muridae (Burda et al., 1990; Marhold et al., 1997b; Kimchi and Terkel, 2001). In the

most recent experiment, Kimchi and Terkel (2001) showed that blind mole rats (*Spalax

ehrenbergi*) consistently built food and nest chambers in the southeast sector of an eight

armed radial maze, relative to the magnetic field. Finally, recent experiments from our

laboratory have provided evidence that Siberian hamsters (*Phodopus sungorus*) show

magnetic compass orientation along a trained axis when tested in a circular arena

(Deutschlander et al., 2003).

Mechanisms of Magnetic Compass Orientation

Despite numerous attempts to characterize the properties of the magnetic sense in

terrestrial organisms, the physiological mechanism responsible for this extraordinary

ability has proved to be exceedingly complex. Currently, there are two plausible theories

for the mechanisms of magnetoreception in animals, a light-dependent, photoreceptor-

based magnetic compass and a non-light-dependent, magnetite-based magnetic compass.

These two mechanisms have primarily been investigated in migratory birds, newts, and

mole rats, which have well-defined, reliable assays for testing magnetic compass

orientation.

Light-Dependent Magnetic Compass

Models of light-dependent magnetoreception were initially proposed in response to the discovery that the magnetic compass of birds is sensitive to inclination, rather than polarity, of the magnetic field (referred to as an inclination compass) (Wiltschko and Wiltschko, 1972). It was found that birds reversed their direction of compass orientation when the vertical component of the magnetic field was inverted, thereby reversing inclination. Additionally, birds were disoriented in a horizontal magnetic field (inclination = 0°) (Wiltschko and Wiltschko, 1972). Furthermore, Eastern red-spotted newts were found to use an inclination compass to orient relative to a shoreline (Phillips, 1986b). The only other organism found to have an inclination compass are loggerhead sea turtles (Lohmann and Lohmann, 1994). Consistent with a light-dependent mechanism, magnetic compass orientation in birds and newts has been shown to be sensitive to changes in the wavelength and intensity of light (see below). Contrary to the findings in birds and newts, however, young leatherback sea turtles were able to use their magnetic compass in complete darkness suggesting that the magnetic compass of sea turtles is not light-dependent (Lohmann and Lohmann, 1993).

The possibility that the mechanism of magnetoreception involved a photoreceptor was first suggested by Hong (1977), who explored effects of magnetic fields on the photopigment rhodopsin. However, Hong's model required field strengths many times that of the geomagnetic field and was quickly replaced by the more plausible optical pumping model (Leask, 1977, 1978). In this model, a molecule is elevated to an excited state via photon absorption. The molecule can then reach a triplet state by excitation transfer, where the molecule has a magnetic moment that can interact with the

geomagnetic field via a double resonance process. Detection of the magnetic field would result from monitoring the rate of decay of molecules from the triplet state to ground state and would then depend upon the alignment of the molecule with the ambient magnetic field. The plausibility of Leask's model has been questioned, however, because it requires an internal source of radio-frequency energy which is not known to exist in biological systems.

Schulten (1982) proposed a variation on Leask's optical pumping model, that involves molecules elevated to the singlet excited state that dissociate into a radical pair. An external magnetic field can then interact with the radical pairs causing a change in the ratio of singlet and triplet excited molecules. Moreover, the product of recombination of radical pairs in a singlet state has different chemical properties than the product of recombination of radical pairs in a triplet state. Therefore, depending on the alignment of the animal with the geomagnetic field, levels of chemical products (recombined radical pairs) can vary and alter transduction pathways involved (e.g.) in photoreception (Schulten and Windemuth, 1986).

More recently, Ritz et al. (2000) proposed a slightly different version of Schulten's radical pair model. This model also proposes that the ambient magnetic field influences anisotropic hyperfine coupling interactions between unpaired electrons, and thus produces different yields of molecules in the triplet state depending upon the alignment of the molecule with the magnetic field. Ritz et al. (2000) was able to show, by modeling a simple radical pair system, that earth strength magnetic fields can theoretically produce different ratios of chemical products which could serve as the basis for a magnetoreceptor (Ritz et al., 2000).

Additionally, Ritz et al. (2000) implicated the involvement of cryptochromes, a recently discovered class of photopigments involved in the entrainment of circadian rhythms, in the process of magnetoreception. Cryptochromes are believed to have evolved from photolyases, a group of proteins responsible for light-dependent DNA repair in plants and animals, which generate a radical pair upon excitation by light (Cashmore et al., 1999). Thus, it is probable that cryptochromes can also generate a radical pair upon excitation by light, an essential characteristic for the model of a photoreceptor-based magnetoreceptor proposed by Ritz et al. (2000).

Moreover, in the inner nuclear layer (INL) of the retina of mice, cryptochromes were found in the vicinity of large displaced ganglion cells which have neural connections with the nucleus of the basal optic root (nBOR), a site which has been shown to respond to magnetic stimuli (Semm and Demaine, 1986; Miyamoto and Sancar, 1998). Furthermore, cryptochromes were found to be distributed rather evenly in the INL of the retina of mice (Miyamoto and Sancar, 1998), which would be an essential characteristic for a magnetoreceptor given that there must be molecules in a variety of different alignments in order to accurately measure magnetic field direction.

Evidence for a photoreceptor-based magnetic compass mechanism was found in experimental studies showing wavelength dependent effects of light on the direction of magnetic orientation and a lack of magnetic orientation in complete darkness. The first experimental evidence for a light-dependent magnetic compass was found in young pigeons. Wiltschko and Wiltschko (1981) found that young inexperienced pigeons, shown in earlier experiments to use magnetic compass cues to determine the direction of

displacement, were unable to correctly home after being displaced in total darkness. This provided evidence that there was a possible link between light and magnetoreception.

The role of photopigments in the light-dependent magnetic compass of birds was further explored by exposing birds to monochromatic (40 nm bandwidth) light of different wavelengths and recording changes in magnetic orientation. It was found that European robins, Australian Silvereyes, and Garden Warblers all were oriented in their appropriate migratory direction under wavelengths of light below about 565nm (Wiltschko and Wiltschko, 1995b, 1999, 2001). However, under wavelengths of light above 570nm, the birds were randomly oriented suggesting that birds were only able to obtain directional information from the magnetic field under monochromatic blue and green light (Wiltschko et al., 1993; Rappl et al., 2000). Experiments conducted on Australian Silvereyes and European robins under 565nm green light (7 x 10^{15} quanta s^{-1} m^{-2} light intensity), near where the transition between orientation and disorientation occurred, yielded normal magnetic compass orientation unlike the disorientation seen under higher wavelengths of light (Wiltschko et al., 2000a, b). However, both Australian Silvereyes and European Robins significantly shifted their orientation when tested under 565nm green light at an intensity of 43 x 10^{15} quanta s^{-1} m^{-2}, seven times that of the standard intensity (Wiltschko et al., 2000a, b; Wiltschko and Wiltschko, 2001). Furthermore, when tested under high intensity blue (424nm) and turquoise (510nm) light, European Robins showed similar shifts in magnetic compass orientation. In contrast, under high intensity red (635nm) and yellow (590nm) light, the birds were disoriented. Interestingly however, when exposed to 635nm light for one hour before the test at an intensity that had been shown to produce disorientation in earlier experiments (7 x 10^{15}

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quanta s^{-1} m^{-2}), robins were then oriented in their appropriate migratory direction (Moller et al., 2001), suggesting that they were able to acclimate to long wavelength light and then orient in the appropriate trained direction.

In more recent experiments using narrow band (10 nm bandwidth) monochromatic light, it was found that at a low light intensity (3.9 x 10^{15} quanta s^{-1} m^{-2}) European robins oriented in the appropriate migratory direction under 560.5nm green light, were disoriented under a range of intensities of 567.5nm green-yellow light, and shifted from their normal migratory direction under 617nm red light (Muheim et al., 2002). From these experiments it is clear that restricting the exposure of birds to a narrow range of wavelengths at various intensities disrupts magnetic compass orientation.

Eastern red-spotted newts (*Notophthalamus viridescens*) have also been shown to have a light-dependent magnetic compass. It was shown that newts can be trained to use their magnetic compass to orient towards an artificial shoreline (Phillips, 1986a) and further exploration revealed that newts were able to orient towards the appropriate trained direction when tested under either white light or wavelengths $\leq$ 450 nm (19.9 x 10^{15} quanta s^{-1} m^{-2} to 39.8 x 10^{15} quanta s^{-1} m^{-2} light intensity) (Phillips and Borland, 1992c). However, newts were unable to use their magnetic compass when tested under near infra-red light (Phillips and Borland, 1992b), again suggesting that magnetoreception has light-dependent properties. In addition, newts were shown to exhibit a 90° counterclockwise shift in magnetic orientation when tested under wavelengths of light $\geq$ 500nm (19.9 x 10^{15} quanta s^{-1} m^{-2} to 39.8 x 10^{15} quanta s^{-1} m^{-2} light intensity) (Phillips and Borland, 1992c), suggesting that newts were either perceiving a shifted magnetic field or were motivated to orient parallel to shore under certain wavelengths of light. To differentiate

between these two hypotheses, newts were trained to a shoreline under long wavelength light ($\geq$ 500nm) and then tested under white light. The newts were shown to display a 90° clockwise shift in orientation, which would be expected if the newts perceived a shifted magnetic field under long wavelengths of light. Thus, this experiment demonstrated that light is directly affecting the magnetoreception mechanism rather than having motivational effects (Phillips and Borland, 1992a).

A hypothesis for the affects of different wavelengths of light on magnetic orientation in newts was presented by Phillips and Borland (1992a) and reviewed by Deutschlander et al., 1999b. This hypothesis suggests that magnetoreception is elicited via an antagonistic interaction between two light-sensitive mechanisms, one mechanism sensitive to short wavelengths and one mechanism sensitive to long wavelengths. However, to explain the 90° counterclockwise shift in orientation under long wavelength light ($\geq$ 500nm) and the continued shoreward orientation under both full spectrum and short wavelength light (450nm), the short wavelength mechanism must be more sensitive than the long wavelength mechanism. In addition, Phillips and Borland (1992a) hypothesized that under an intermediate wavelength (i.e. 475 nm) of light, the sensitivity of both short and long wavelength mechanisms should balance and therefore, newts should be unable to perceive the magnetic field. As predicted, newts were randomly oriented when tested under 475nm light (Phillips and Borland, 1992a), thus providing support for the antagonistic photoreceptor theory.

The only other species that has been shown to exhibit wavelength dependent shifts in magnetic compass orientation is the fruit fly (*Drosophila melanogaster*). *D. melanogaster* were trained to orient towards a 365nm ultraviolet light gradient and when

tested under 365nm light, *D. melanogaster* correctly oriented towards their trained direction. However, when tested under 500nm light, *D. melanogaster* oriented 90° clockwise to the trained direction, indicating that fruit flies also have a light-dependent magnetic compass (Phillips and Sayeed, 1993). The reason that newts shift their orientation 90° counterclockwise under long wavelength light, while flies shift 90° clockwise remains unknown.

Additional evidence for a light-dependent magnetic compass has been obtained from electrophysiological recordings. In pigeons, it was found that units in the vestibular system are responsive to changes in the magnetic field, and these units were not responsive when the animal was held in complete darkness (Semm et al., 1984). Additionally, the nucleus of the basal optic root (nBOR), which contains cells that are highly responsive to both movement and direction in the visual field, was shown to respond to slow changes in the horizontal and vertical components of the magnetic field (Semm et al., 1984). The greatest response of the nBOR was seen when the eyes were exposed to either 503nm or 582nm light, again suggesting differential effects of specific wavelengths of light on magnetoreception (Semm and Demaine, 1986). Additional evidence for the involvement of the visual system in magnetoreception was recently reported in European robins. Wiltschko et al. (2002b) reported that magnetoreception is strongly lateralized, with robins correctly orienting in their migratory direction when using only their right eye, but failing to correctly orient when using only their left eye. This experiment suggests that magnetorecption is linked to vision and that the right eye, or left brain hemisphere, dominates this sense.

In newts, the magnetoreceptor was localized to extraocular photoreceptors in or near the pineal. In a series of experiments, newts were fitted with either clear, long wavelength or short wavelength "pineal caps" that allowed either white light, long wavelength light, or short wavelength light, respectively, to reach photoreceptors in the pineal. This enabled the experimenters to differentiate between retinal and pineal photoreceptors, thus allowing the investigation of the anatomical location of the light-dependent magnetic compass. The results of these experiments indicate that the pineal or a nearby structure, rather than the retina, is the location of the magnetoreceptor in newts (Deutschlander et al., 1999a; Phillips et al., 2001).

While there have also been several studies in birds and rodents that have found electrophysiological responses of the pineal to magnetic stimuli, as well as effects of electromagnetic stimuli on the pineal hormone melatonin, there is no definitive evidence that the magnetoreceptor of birds and rodents is located in the pineal (Semm et al., 1980; Semm, 1983; Demaine and Semm, 1985; Olcese and Ruess, 1986; Stehle et al., 1988; Schneider et al., 1994).

Non-Light-Dependent Magnetic Compass

The second theoretical mechanism for magnetoreception implicates either single-domain or superparamagnetic particles of biogenic magnetite (magnetite-based mechanism). While there is no explicit evidence that magnetite is the main physiological component involved in magnetoreception, high concentrations of magnetic material have consistently been found in tissues in the heads of mammals, birds, fish, and newts (Walcott et al., 1979; Mather and Baker, 1981; Walker et al., 1984; Brassart et al., 1999).

Superparamagnetic (SPM) particles of magnetite are small (< 0.04μm) and are not able to

hold a stable magnetic moment. Therefore, the magnetic moments track the external

magnetic field, without the particle rotating (see Kirschvink et al., 1985 for review). In

contrast, single-domain (SD) particles of magnetite are fairly large (0.04-0.12 μm) and

have a stable magnetic moment. As a consequence, the entire particle can be passively

rotated by an external magnetic field. In theory, a SD magnetite-based receptor would be

able to detect the polarity (referred to as a polarity compass) of the magnetic field only if

it is fixed (not freely rotating) in the surrounding tissue, thereby constraining its magnetic

moment. On the other hand, a SPM particle or a freely rotating SD particle would be

unable to differentiate between the magnetic poles, since either the magnetic moment

(SPM) or the entire particle (freely rotating SD) can rotate freely. However, these freely

rotating particles could mediate an inclination magnetic compass, whereas a magnetic

compass involving fixed single domain particles could mediate a polarity magnetic

compass (see Kirschvink and Walker, 1985 for review).

Superparamagnetic magnetite

Because SPM particles of magnetite are much smaller and have weaker magnetic

moments that will track an external magnetic field without physical rotation of the

particle, the only plausible design that could serve as a magnetoreceptor would be an

array of interacting particles. For instance, a row of membrane-bound SPM particles

spaced at an appropriate distance side by side would have slight interactions with each

neighboring particle (i.e. attractive or repulsive forces), that could cause either expansion

(repulsion) or contraction (attraction) of the cell membrane because the magnetic

moments would track the alignment of an external magnetic field. The particle

configuration and, thus, the force on the membrane (expansion or contraction) would

depend on the external magnetic field alignment (Kirschvink et al., 1985).

Recent experiments have revealed the presence of SPM magnetite particles in a

layer of skin on the underside of the upper beak of homing pigeons (Hanzlik et al., 2000;

Winklhofer et al., 2001) and further exploration indicated that the arrangement of the

particles was such that detection of the magnetic field would be possible by converting

magnetic stimuli into mechanical force (Shcherbakov and Winklhofer, 1999).

Furthermore, in the same region of the beak of homing pigeons, the trigeminal nerve was

found to terminate at clusters of superparamagnetic particles, indicating that the

trigeminal nerve may innervate a SPM magnetoreceptor (Fleissner et al., 2003). This

theory is further supported by electrophysiological recordings indicating that the

trigeminal nerve is sensitive to changes in earth-strength magnetic fields (Beason and

Semm, 1987; Semm and Beason, 1990). This is by far the most compelling evidence

indicating the location of a magnetite-based magnetoreceptor in any organism.

Single-domain magnetite

There are also several theories of the mechanism of magnetoreception involving

SD magnetite, including the possibility that such particles are connected to stretch

receptors or hair cells that can then lead to a transduction pathway (Kirschvink et al.,

1993). One interesting theory, involving a three dimensional array of SD magnetite

particles located in carotenoid-containing oil droplets of a specialized photoreceptor, is

based on the idea that magnetite particles may track an external magnetic field and thus

alter the alignment of surrounding carotenoid particles. Therefore, exposure to an

external magnetic field could alter the alignment of these molecules, which in turn, would

change the intensity of light reaching the photopigment contained in the outer segment of

the receptor (Edmonds, 1996). Another example of a possible magnetoreception

mechanism involving SD magnetite is a torque detector, where several SD magnetite

particles are aligned in a chain so that, when magnetized, they produce a greater torque

which could be perceived by a receptor cell (i.e. hair cell or pressure receptor)

(Kirschvink and Gould, 1981; Kobayashi and Kirschvink, 1995). Another plausible

model for magnetoreception using SD magnetite is that of a magnetically sensitive ion

channel. In this model, a particle of magnetite is connected to an ion channel gate via a

filament. The filament would transfer torque from the particle of magnetite (produced by

an external magnetic field) to the filament, causing the ion channel gate to either open or

close depending upon the alignment of the magnetic field (Kobayashi and Kirschvink,

1995).

To investigate the involvement of SD magnetite in magnetic compass orientation,

animals have been tested using a procedure referred to as demagnetization, where the

organism is treated with slowly decreasing alternating magnetic fields starting with an

initial intensity that is greater than the coercivity of the magnetite particles. This

procedure is thought to randomize the magnetization of SD magnetite particles, because

as the strength of the alternating current (AC) field decreases, this treatment leaves some

particles magnetized in one direction (larger particles with higher coercivity drop out

first) and others magnetized in the opposite direction, and therefore will randomize

magnetic compass orientation if fixed SD particles are involved. Another similar

procedure called pulse remagnetization exposes the subject to a brief high intensity magnetic pulse intended to reverse the polarity of SD magnetite particles. Several studies have reported deflections in orientation behavior in birds and mammals exposed to pulse remagnetization (Beason et al., 1994, 1995; Marhold et al., 1997a). However, only recent experiments which exposed birds to a "biasing field" (a magnetic field approximately 20x the geomagnetic field intended to align all magnetite particles in a known direction) before pulse remagnetization, have provided solid evidence that pulse remagnetization is effective for detecting the presence of SD magnetite (Wiltschko et al., 2002a). In contrast, attempts to 'demagnetize' animals have had no convincing effects on magnetic orientation behavior [Gould et al., 1980 (bees); Walcott et al., 1988 (pigeons)], presumably because the intensity of the experimental magnetic field was not greater than the coercivity of the magnetite particles, and therefore would only temporarily rotate particles of magnetite.

Evidence that SD magnetite particles are involved in magnetoreception has been revealed in trout. Recently Walker et al. (1997) found SD magnetite particles in the olfactory epithelium, which were linked to the brain via a magnetically responsive segment [superficial ophthalmic ramus (ros V)] of the trigeminal nerve. Furthermore, Diebel et al. (2000), recently confirmed that the single-domain magnetite particles found in the trout are magnetic, and an earlier three-dimensional reconstruction revealed that the particles are arranged in a chain, a structure consistent with models of a SD magnetite-based magnetoreceptor (Kobayashi and Kirschvink, 1995). While these studies are intriguing, they provide no conclusive evidence that a SD magnetite-based mechanism is actually being used for magnetoreception.

Magnetic Map

There is evidence for both photoreceptor-based and magnetite-based magnetoreception mechanisms. Even more unusual is the fact that in birds and newts, both a magnetite-based mechanism and a photoreceptor-based mechanism may be present in the same organism. Many species of migrating birds are born with an innate magnetic compass sense that designates the species or population appropriate migratory direction (Berthold, 1988), and the birds then eventually learn a map as a byproduct of their normal movements (Perdeck, 1958; Wiltschko and Wiltschko, 1995b). Thus, it appears that inexperienced juvenile migrants have a magnetic compass that allows them to migrate in the appropriate direction, but have not yet developed a magnetic map (Perdeck, 1958; Berthold, 1988). When inexperienced juvenile Tasmanian silvereyes were tested under red light, they were disoriented exactly like adults suggesting the juveniles and adults possessed a light-dependent magnetic compass. In contrast, pulse remagnetization has been shown to affect migratory orientation in adult birds, but not juveniles, suggesting that a magnetite-based map system is used only by adult migrants (Munro et al., 1997a,b).

Similar evidence exists for newts (Phillips, 1986b; Phillips and Borland, 1994; Phillips et al., 2002). It was found that newts trained to use a magnetic compass for shoreward orientation were responsive to a reversal of the inclination of the magnetic field, while newts exhibiting homeward orientation were not responsive to such changes in inclination, but rather to changes in the polarity of the magnetic field (Phillips, 1986b).

Additionally, shoreward orienting newts were found to display shifts in magnetic orientation under long wavelengths of light (see above), while homeward orienting newts were randomly oriented under long wavelengths of light (Phillips and Borland, 1994). Phillips and Borland concluded that there are two different magnetoreception mechanisms in newts, a photoreceptor-based mechanism for magnetic compass orientation and a magnetite-based mechanism for the map component of homing.

Comparable to results with birds, these experiments suggest the existence of a magnetite-based magnetic map and a photoreceptor-based magnetic compass. However, because certain wavelengths of light have effects on the homing orientation of newts, it has been suggested that a "hybrid" magnetic mechanism exists. The theory suggests the magnetic compass is used to position the so-called "map detector" in a fixed alignment with respect to the magnetic field in order to obtain the precise measurements necessary to derive map information from spatial variation in the magnetic field (Phillips and Borland, 1994; see also Phillips et al., 2002). Nonetheless, more research is needed to either prove or disprove this hypothesis.

Magnetic Compass Orientation in Mammals

While there is an abundance of information about the magnetic compasses of birds, newts, and even insects, there is an obvious need to understand more about the magnetic compass mechanism of mammals. Evidence for magnetic compass orientation has only been obtained in mice, mole rats, and hamsters (see above) and the mechanism(s) behind magnetic orientation in mammals have only been explored in mole rats.

Evidence suggests that mole rats use a non-light-dependent magnetic compass, because they are able to orient using magnetic cues in total darkness (Marhold et al., 1997a, b; Kimchi and Terkel, 2001). Furthermore, pulse remagnetization experiments have altered magnetic compass orientation in mole rats providing evidence for the presence of permanent magnetic material (Marhold et al., 1997a). The properties of this compass (i.e. disorientation after pulse remagnetization, ability to orient in total darkness, and sensitivity to the polarity and not inclination of the magnetic field) are consistent with a magnetoreception mechanism involving fixed single-domain particles of biogenic magnetite (Kirschvink and Walker, 1985).

In contrast to the response seen in mole rats, studies of the magnetoreception mechanism in some species of birds and amphibians have provided evidence for a light-dependent magnetic compass (Phillips and Borland, 1992a; Wiltschko et al., 1993) consistent with theoretical models implicating specialized photoreceptors in magnetoreception (Ritz, et al., 2000) (see above). Since rodents such as mice and hamsters are phylogenetically more closely related to mole rats than to birds and amphibians, it might be expected that they would employ a non-light-dependent magnetic compass. However, mole rats live virtually their entire lives underground in total darkness and they are functionally blind. Yet, in comparison to mole rats, most rodents, including nocturnally active species, are exposed to much higher light levels in their natural environment and have an intact, fully functional visual system. Thus, in terms of their visual ecology, rodents that are active above ground are more similar to nocturnally migrating birds and newts, suggesting that they might employ a light-dependent magnetic compass. Therefore, studies of magnetic compass orientation in rodents such as mice and

hamsters will help to determine if the mechanism of magnetoreception is primarily

dependent on phylogeny or ecology.

The primary objective of this research was to develop an assay for magnetic

compass orientation in C57BL/6 laboratory mice. We used laboratory mice because they

make it possible to use genetic techniques to investigate the molecular mechanisms that

underlie sensitivity to the magnetic field. Future experiments using this assay would be

aimed at determining whether mice have a light-dependent or a non-light-dependent

magnetic compass and, also, at exploring the genetic components of this system.

LITERATURE CITED

Able, K. P. (1980). Mechanisms of orientation, navigation, and homing. In: *Animal Migration, Orientation, and Navigation* (ed. S.A. Gauthreaux Jr.), pp. 283-373. New York: Academic Press.

Able, K. P. (1993). Orientation cues used by migratory birds: a review of cue-conflict experiments. *Trends Ecol. Evol*. **8**, 367-371.

Adler, K. (1976). Extraocular photoreception in amphibians. *Photochem. Photobiol*. **23**, 275-298.

Adler, K. and Phillips, J. B. (1985). Orientation in a desert lizard (Uma notata): Time-compensated compass movement and polarotaxis. *J. Comp. Physiol. A* **156**, 547-552.

Adler, K. and Taylor, D. H. (1973). Extraocular perception of polarized light by orienting salamanders. *J. Comp. Physiol.* **87**, 203-212.

Altmann, G. (1981). Untersuchung zur Magnetotaxis der Honigbiene, Apis mellifica L. *Anz Schadlingskd Pflanzenschutz Umweltschutz* **54**, 177-179.

Alyan, S. and Jander, R. (1994). Short-range homing in the house mouse, *Mus musculus*: Stages in the learning of directions. *Anim. Behav.* **48**, 285-298.

Arendse, M. C. (1978). Magnetic field detection is distinct from light detection in the invertebrates *Tenebrio* and *Talitrus*. *Nature* **274**, 358-362.

Arendse, M. C. and Vrins, J. C. M. (1975). Magnetic orientation and its relation to photic orientation in *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). *Neth. J. Zool*. **25**, 407-437.

August, P. V., Ayvazian, S. G., and Anderson, J. G. T. (1989). Magnetic orientation in a small mammal, *Peromyscus leucopus*. *J. Mammal.* **70**, 1-9.

Baker, R. R. (1987). Human navigation and magnetoreception: the Manchester experiments do replicate. *Anim. Behav*. **35**, 691-704.

Baker, R. R. and Mather, J. G. (1982). Magnetic compass sense in the large yellow underwing moth, *Noctua pronuba* L. *Anim. Behav.* **30**, 543-548.

Beason, R. C., Dussourd, N., Deutschlander, M. (1995). Behavioral evidence for the use of magnetic material in magnetoreception by a migratory bird. *J. Exp. Biol*. **198**, 141-146.

Beason, R. C., Harper, J., McNulty, S., Dussourd, N., and Freas, J. (1994). Magnetic effects on homing bank swallows. *J. Ornithol*. **135** (special issue), 88.

Beason, R. C. and Semm, P. (1987). Magnetic responses of the trigeminal nerve system of the bobolink (*Dolichonyx oryzivorus*). *Neurosci. Lett*. **80**, 229-234.

Berthold, P. (1988). The control of migration in European warblers. In: *Acta XIX Congressus Internationalis Orithologicus*. (ed. H. Ouellet.), pp. 215-249. Ottawa: University of Ottawa Press.

Blakemore, R. P. (1975). Magnetotactic bacteria. *Science* **19**, 377-379.

Bovet, J. (1992). Mammals. In: *Animal Homing*. (ed. F. Papi), New York: Chapman and Hall.

Brannon, E. L., Quinn, T. P., Lucchetti, G. L., and Ross, B. D. (1981). Compass orientation of sockeye salmon fry from a complex river system. *Can. J. Zool*. **59**, 1548-1553.

Branover, G. G., Vasil'yev, A. S., Gleizer, S. I., Tsinober, A. B. (1971). A study of the behavior of the eel in natural and artificial magnetic fields and an analysis of its reception mechanism. *J. Ichthyol.* **11**, 608-614.

Brassart, J., Kirschvink, J. L., Phillips, J. B. and Borland, S. C. (1999). Ferromagnetic material in the eastern red-spotted newt, *Notophthalmus viridescens*. *J. Exp. Biol*. **202**, 3155-3160.

Buchler, E. R. and Childs, S. B. (1982). Use of post-sunset glow as an orientation cue by the big brown bat (*Eptesicus fuscus*). *J. Mammal.* **63**, 243-247.

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

Cashmore, A., Jarillo, J., Wu, Y-J. and Liu, D. (1999). Cryptochromes: blue light receptors for plants and animals. *Science* **284**, 760-765.

DeJong, D. (1982). Orientation of comb building by honeybees. *J. Comp. Physiol.* **147**, 495-501.

Demaine, C. and Semm, P. (1985). The avian pineal as an independent magnetic sensor. *Neurosci. Lett*. **62**, 119-122.

DeRosa, C. T. and Taylor, D. H. (1980). Homeward orientation mechanisms in three species of turtles (*Trionyx spinifer, Chrysemys picta,* and *Terrapene Carolina*). *Behav. Ecol. Sociobiol.* **7***,* 15-23.

Deutschlander, M. E., Borland, S. C., and Phillips, J. B. (1999a). Extraocular magnetic compass in newts. *Nature* **400**, 324-325.

Deutschlander, M. E., Freake, M. J., Borland, S. C., Phillips, J. B., Anderson, L. E. and Wilson, B. W. (2003). Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus*. *Anim. Behav.* **65**, 779-786.

Deutschlander, M. E., Phillips, J. B., and Borland, S. C. (1999b). The case for light-dependent magnetic orientation in animals. *J. Exp. Biol*. **202**, 891-908.

Diebel, C. E., Proksch, R., Green, C. R., Neilson, P., and Walker, M. M. (2000). Magnetite defines a vertebrate magnetoreceptor. *Nature* **406**, 299-302.

Edmonds, D. T. (1996). A sensitive optically detected magnetic compass for animals. *Proc. Roy. Soc. Lond*. B **263**, 295-298.

Emlen, S. T. (1967). Migratory orientation in the indigo bunting. *Passerina cyanea*. *Auk* **84,** 309-342.

Emlen, S. T. (1970a). Celestial rotation: its importance in the development of migratory orientation. *Science* **170**, 1198-1201.

Emlen, S. T. (1970b). The influence of magnetic information on the orientation of the indigo bunting, *Passerina cyanea*. *Anim. Behav*. **18**, 215-224.

Emlen, S. T. (1975). The stellar-orientation system of a migratory bird. *Sci. Amer*. **233**, 102-111.

Etienne, A. S., Maurer, R., and Saucy, F. (1988). Limitations in the assessment of path dependent information. *Behav*. **106**, 81-111.

Ferguson, D. E. (1971). The sensory basis of orientation in amphibians. *Ann. N.Y. Acad. Sci.* **188**, 30-36.

Fleissner, G., Holtkamp-Rotzler, E., Hanzlik, M., Winklhofer, M., Fleissner, G., Petersen, N., and Wiltschko, W. (2003). Ultrastructural analysis of a putative magnetoreceptor in the beak of homing pigeons. *J. Comp. Neurol*. **458**, 350-360.

Fluharty, S. L., Taylor, D. H., and Barrett, G. W. (1976). Sun-compass orientation in the meadow vole *Microtus pennsylvanicus*. *J. Mammal.* **57**, 1-9.

Freake, M. J., Borland, S. C. and Phillips, J. B. (2002). Use of a magnetic compass for Y-axis orientation in larval bullfrogs, *Rana catesbeiana*. *Copeia* **2002**, 466-471.

Gleizer, S. I. and Khodorkovsky, V. A. (1971). Experimental determination of geomagnetic reception in the European eel. *Dokl. Akad. Nauk. SSSR Ser. Biol*. **201**, 964-967.

Goodyear, C. P., and Bennett, D. H. (1979). Sun compass orientation in immature bluegill. *Trans Am. Fish. Soc*. **108**, 555-559.

Gould, J. L., Kirschvink, J. L., Deffeyes, K. S., Brines, M. L. (1980). Orientation of demagnetized bees. *J. Exp. Biol*. **86**, 1-8.

Haigh, G. R. (1979). Sun-compass orientation in the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *J. Mammal.* **60**, 629-632.

Hanzlik, M., Heunemann, C., Holtkamp-Rotzler, E., Winklhofer, M., Petersen, N., and Fleissner, G. (2000). Superparamagnetic magnetite in the upper beak tissue of homing pigeons. *Biometals* **13,** 325-331.

Hasler, A. D. and Wisby, W. J. (1958). The return of displaced largemouth bass and green sunfish to a 'home' area. *Ecology* **39**, 289-293.

Hawryshyn, C. W., Arnold, M. G., Bowering, E., and Cole, R. L. (1990). Spatial
orientation of rainbow trout to plane-polarized light: the ontogeny of E-vector
discrimination and spectral sensitivity characteristics. *J. Comp. Physiol.* **166**, 565-
574.

Helbig, A. J. (1990). Depolarization of natural skylight disrupts orientation of an avian
nocturnal migrant. *Experientia* **46**, 755-758.

Hong, F. T. (1977). Photoelectric and magneto-orientation effects in pigmented
biological membranes. *J. Colloid Interface Sci*. **58**, 471-497.

Kimchi, T. and Terkel, J. (2001). Magnetic orientation in the blind mole rat *Spalax
ehrenbergi. J.Exp. Biol.* **204**, 751-758.

Kirschvink, J. L. and Gould, J. L. (1981). Biogenic magnetite as a basis for magnetic
field detection in animals. *Biosystems* **13**, 181-201.

Kirschvink, J. L., Jones, D. S., MacFadden, B. J. (1985). *Magnetite
biomineralization and magnetoreception in organisms: A new biomagnetism.*
New York: Plenum Press.

Kirschvink, J. L., Ricci, R. D., Nesson, M. H., and Kirschvink, S. J. (1993).
*Magnetite-based magnetoreceptors in animals: structural, behavioral and
biophysical studies*. Palo Alto, CA: Electric Power Research Institution (EPRI).

Kirschvink, J. L. and Walker, M. M. (1985). Particle-size considerations for magnetite-
based magnetoreceptors. In: *Magnetite biomineralization and magnetoreception
in organisms*. (ed. J.L. Kirschvink, D.S. Jones and B.J. MacFadden), pp.243-256.
New York: Plenum Press.

Kobayashi, A. and Kirschvink, J. L. (1995). Magnetoreception and electromagnetic field effects: sensory perception of the geomagnetic field in animals and humans. In: *Electromagnetic fields: Biological interactions and mechanisms*. (Ed. M. Blank), pp. 367-394. Danvers, Massachusetts: American Chemical Society.

Kreithen, M. L. and Keeton, W. T. (1974). Attempts to condition homing pigeons to magnetic stimuli. *J. Comp. Physiol.* **91**, 355-362.

Leask, M. J. M. (1977). A physico-chemical mechanism for magnetic field detection by migratory birds and homing pigeons. *Nature* **267**, 144-145.

Leask, M. J. M. (1978). Primitive models of magnetoreception. In: *Animal migration, navigation, and homing*. (ed. K. Schmidt-Koenig and W.T. Keeton), pp. 318-322. New York: Springer.

Lohmann, K. J. (1985). Geomagnetic field detection by the Western Atlantic spiny lobster, *Panulirus argus*. *Mar. Behav. Physiol.* **12**, 1-17.

Lohmann, K. J. (1991). Magnetic orienation by hatchling loggerhead sea turtles (*Caretta caretta*). *J. Exp. Biol.* **171**, 1-13.

Lohmann, K. J. and Lohmann, C. M. F. (1993). A light-independent magnetic compass in the leatherback sea turtles. *Biol. Bull.* **185**, 149-151.

Lohmann, K. J. and Lohmann, C. M. (1994). Detection of magnetic inclination angle by sea turtles: a possible mechanism for determining latitude. *J. Exp. Biol.* **194**, 23-32.

Lohmann, K. J. and Lohmann, C. M. (1998). Migratory guidance mechanisms in marine turtles. *J. Avian Biol.* **29**, 585-596.

Lohmann, K. J., Pentcheff, N. D., Nevitt, G. A., Stetten, G. D., Zimmer-Faust, R. K., Jarrard, H. E., and Boles, L. C. (1995). Magnetic orientation of spiny lobsters in the ocean: experiments with undersea coil systems. *J. Exp. Biol.* **198**, 2041-2048.

Lohmann, K. J. and Willows, A. O. D. (1987). Lunar-modulated geomagnetic orientation by a marine mollusk. *Science* **235**, 331-334.

Loyacano Jr., H. A., Chappell, J. A., and Gauthreaux, S. A. (1977). Sun compass orientation in juvenile largemouth bass *Micropterus salmoides*. *Trans Am. Fish. Soc*. **106**, 77-79.

Marhold, S., Burda, H., Kreilos, I., & Wiltschko, W. (1997a). Magnetic orientation in common mole rats from Zambia. In: *Orientation and Navigation - Birds, Humans and other Animals* (ed. by the RIN), pp.5-1 - 5-9. Oxford: Royal Institute of Navigation.

Marhold, S., Wiltschko, W., and Burda, H. (1997b). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* **84**, 421-423.

Mather, J. G., and Baker, R. R. (1981). Magnetic sense of direction in woodmice for route-based navigation. *Nature* **291**, 152-155.

Mathis, A. and Moore, F. R. (1988). Geomagnetism and the homeward orientation of the box turtle, *Terrapene carolina*. *Ethology* **78**, 265-274.

Miyamoto, Y. and Sancar, A. (1998). Vitamin B_2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proc. Natl. Acad. Sci. USA*. **95**, 6097-6102.

Moller, A., Gesson, M., Noll, C., Phillips, J. B., Wiltschko, R., and Wiltschko, W. (2001). Light-dependent magnetoreception in migratory birds: previous exposure to red light alters the response to red light. In: *Orientation and Navigation – Birds, Humans, and Other Animals.* Proceedings of the RIN 01 Conference. pp. 6-1 - 6-6. Oxford: Royal Institute of Navigation.

Muheim, R., Backman, J., and Akesson, S. (2002). Magnetic compass orientation in European robins is dependent on both wavelength and intensity of light. *J. Exp. Biol*. **205**, 3845-3856.

Munro, U., Munro, J. A., Phillips, J. B., Wiltschko, R. and Wiltschko, W. (1997a). Evidence for magnetite-based navigational 'map' in birds. *Naturwissenschaften* **84**, 26-28.

Munro, U., Munro, J. A., Phillips, J. B. and Wiltschko, W. (1997b). Effect of wavelength of light and pulse magnetization on different magnetoreception systems in a migratory bird. *Aust. J. Zool*. **45**, 189-198.

Munro, U. and Wiltschko, R. (1995). The role of skylight polarization in the orientation of a day-migrating bird species. J. *Comp Physiol. A* **177**, 357-362.

Newcomer, R. T., Taylor, D. H., and Guttman, S. I. (1974). Celestial orientation in two species of water snakes (*Natrix sipedon and Regina septemvittata*). *Herpetologica* **30**, 194-200.

Olcese, J. and Ruess, S. (1986). Magnetic effects on pineal gland melatonin synthesis: Comparative studies of albino and pigmented rodents. *Brain Res*. **369**, 365-368.

Papi, F. (1955). Experiments on the sense of time in Talitrus saltator (Crustacea: Amphipoda). *Experientia* **11**, 201-202.

Perdeck, A. C. (1958). Two types of orientation in migrating starlings, *Sturnus vulgaris* L., and chaffinches, *Fringilla coelebs* L., as revealed by displacement experiments. *Ardea* **46**, 1-37.

Phillips, J. B. (1977). Use of the earth's magnetic field by orienting cave salamanders (*Eurycea lucifuga*). *J. Comp. Physiol*. **121**, 273-288.

Phillips, J. B. (1986a). Magnetic compass orientation in the Eastern red-spotted newt (Notophthalamus viridescens). J. Comp. Physiol. **158**,103-109.

Phillips, J. B. (1986b). Two magnetoreception pathways in a migratory salamander. *Science* **233**, 765-767.

Phillips, J. B. (1998). Magnetoreception. In: *Amphibian Biology, Vol. 3, Sensory Perception* (ed. H. Heatwole and M. Dawley), Chipping Norton: Surrey Beatty & Sons.

Phillips, J. B. and Borland, S. C. (1992a). Behavioral evidence for use of a light-dependent magnetocreception mechanism by a vertebrate. *Nature* **359**, 142-144.

Phillips, J. B. and Borland, S. C. (1992b). Magnetic compass orientation is eliminated under near-infrared light in the eastern red-spotted newt *Notophthalamus viridescens*. *Anim. Behav.* **44**, 796-797.

Phillips, J. B. and Borland, S. C. (1992c).Wavelength-specific effects of light on magnetic compass orientation of the eastern red-spotted newt *Notophthalmus viridescens*. *Ethol. Ecol. Evol*. **4,** 33-42.

Phillips, J. B. and Borland, S. C. (1994). Use of a specialized magnetoreception system for homing by the eastern red-spotted newt *Notophthalamus viridescens. J.Exp. Biol*. **188**, 275-291.

Phillips, J. B., Borland, S. C., Freake, M. J., Brassart, J., and Kirschvink, J. L. (2002). 'Fixed-axis' magnetic orientation by an amphibian: non-shoreward-directed compass orientation, misdirected homing or positioning a magnetite-based map detector in a consistent alignment relative to the magnetic field? *J. Exp. Biol*. **205**, 3903-3914.

Phillips, J. B., Deutschlander, M. E., Freake, M. J., Borland, S. C. (2001). The role of extraocular photoreceptors in newt magnetic compass orientation: parallels between light-dependent magnetreception and polarized light detection in vertebrates. *J. Exp. Biol*. **204**, 2543-2552.

Phillips, J. B. and Moore, F. R. (1992). Calibration of the sun compass by sunset polarized light patterns in a migratory bird. *Behav. Ecol. Sociobiol*. **31**, 189-193.

Phillips, J. B. and Sayeed, O. (1993). Wavelength-dependent effects of light on magnetic compass orientation in *Drosophila melanogaster*. *J. Comp. Physiol*. **172**, 303-308.

Pilleri, G. and Knuckey, J. (1969). Behaviour patterns of some Delphinidae observed in the Western Mediterranean. *Z. Tierpsychol*. **26**, 48-72.

Quinn, T.P. (1980). Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *J. Comp. Physiol.* **137**, 243-248.

Quinn, T.P. and Dittman, A.H. (1992). Fishes. In: *Animal Homing*. (ed. F. Papi), pp. 145-211. New York: Chapman and Hall.

Rappl, R., Wiltschko, R., Weindler, P., Berthold, P. and Wiltschko, W. (2000). Orientation behavior of garden warblers (*Sylvia borin*) under monochromatic light of various wavelengths. *Auk* **1**, 256-260.

Rickli, M. and Leuthold, R. H. (1988). Homing in harvester termites: evidence of magnetic orientation. *Ethology* **77**, 209-216.

Ritz, T., Adem, S., and Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. *Biophys. J.* **78**, 707-718.

Rossel, S. and Wehner, R. (1986). Polarization vision in bees. *Nature* **323**, 128-131.

Schneider, T., Thalau, H., Semm, P. (1994). Effects of light or different Earth-strength magnetic fields on the nocturnal melatonin concentration in a migratory bird. *Neurosci. Lett*. **168**, 73-75.

Schulten, K. (1982). Magnetic field effects in chemistry and biology. *Festkorperprobleme* **22**, 60-83.

Schulten, K. and Windemuth, A. (1986). Model for a physiological magnetic compass. In: *Biophysical effects of steady magnetic fields*. (ed. G. Maret, N. Boccara and J. Kiepenheuer), pp. 99-106. New York: Springer.

Semm, P. (1983). Neurobiological investigations on the magnetic sensitivity of the pineal gland in rodents and pigeons. *J. Comp. Biochem. Physiol*. **76**, 683-689.

Semm, P. and Beason, C. (1990). Responses to small magnetic variations by the trigeminal system of the bobolink. *Brain Res. Bull*. **25**, 735-740.

Semm, P. and Demaine, C. (1986). Neurophysiological properties of magnetic cells in the pigeon's visual system. *J. Comp. Physiol. A* **159**, 619-625.

Semm, P., Nohr, D., Demaine, C. and Wiltschko, W. (1984). Neural basis of the magnetic compass: Interactions of visual, magnetic and vestibular inputs in the pigeon's brain. *J. Comp. Physiol*. **155**, 283-288.

Semm, P., Schneider, T., and Vollrath, L. (1980). Effects of an earth-strength magnetic field on electrical activity of pineal cells. *Nature* **288**, 607-608.

Shcherbakov, V. P. and Winklhofer, M. (1999). The osmotic magnetometer: a new model for magnetite-based magnetoreception in animals. *Euro. Biophys. J. Biophys. Lett*. **28,** 380-392.

Sinsch, U. (1987). Orientation behavior of toads (*Bufo bufo*) displaced from the breeding site. *J. Comp. Physiol. A* **161**, 715-727.

Sinsch, U. (1992). Sex-biased site fidelity and orientation behaviour in reproductive natterjack toads (*Bufo calamita*). *Ethol. Ecol. Evol*. **4**, 15-32.

Stehle, J., Ruess, S., Schroeder, H., Henschel, M., and Vollrath, L. (1988). Magnetic field effects on pineal N-acetyltransferase activity and melatonin content in the gerbil – Role of pigmentation and sex. *Physiol. Behav*. **44**, 91-94.

Taylor, P. B. (1986). Experimental evidence geomagnetic orientation in juvenile salmon, *Onchorhynchus tschawytscha*. *J. Fish. Biol*. **28**, 607-624.

Tesch, F. W. and Lelek, A. (1973). Directional behavior of transplanted stationary and migratory forms of the eel, *Anguilla anguilla*, in a circular tank. *Neth. J. Sea Res*. **7**, 46-52.

Ugolini, A. and Pezzani, A. (1992). Learned solar orientation in Idotea baltica. *Mesogee (Bull Mus Hist Nat, Marseille)* **52**, 77.

von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Cambridge: Harvard University Press.

Walcott, C., Gould, J. L, and Kirschvink, J. L. (1979). Pigeons have magnets. *Science*. **205**, 1027-1029.

Walcott, C., Gould, J. L., Lednor, A. J. (1988). Homing of magnetized and demagnetized pigeons. *J. Exp. Biol*. **134**, 27-41.

Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montogomery, J. C. and Green, C. R. (1997). Structure and function of the vertebrate magnetic sense. *Nature* **390**, 371-376.

Walker, M. M., Kirschvink, J. L., Chang, S-B. R., Dizon, A. E. (1984). A candidate magnetic sense organ in the yellowfin tuna, *Thunnus albacares*. *Science* **224**, 751-753.

Wehner, R. (1987). 'Matched filters' – neural models of the external world. *J.Comp. Physiol. A* **161**, 511-531.

Wehner, R. (1989). The hymenopteran skylight compass: matched filtering and parallel coding. *J. Exp. Biol.* **146**, 63-85.

Wehner, R. and Wehner, S. (1986). Path integration in desert ants. Approaching a long-standing puzzle in insect navigation. *Monitore Zool. Ital*. **20**, 309-331.

Wiltschko, R. and Wiltschko, W. (1995a). *Magnetic orientation in animals. Zoophysiology vol 33*. Berlin: Springer-Verlag.

Wiltschko, W. (1983). Compasses used by birds. *Comp. Biochem. Physiol. A* **76A**, 709-717.

Wiltschko, W., Munro, U., Ford, H., and Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature* **364**, 525-527.

Wiltschko, W., Munro, U., Wiltschko, R., and Kirschvink, J. L. (2002a). Magnetite-based magnetoreception in birds: the effect of a biasing field and a pulse on migratory behavior. *J. Exp. Biol*. **205**, 3031-3037.

Wiltschko, W., Traudt, J., Gunturkun, O., Prior, H., Wiltschko, R. (2002b). Lateralization of magnetic compass orientation in a migratory bird. *Nature* **419**, 467-470.

Wiltschko, W. and Wiltschko, R. (1972). Magnetic compass of European robins. *Science* **176**, 62-64.

Wiltschko, W. and Wiltschko, R. (1981). Disorientation of inexperienced young pigeons after transportation in total darkness. *Nature* **291**, 433-434.

Wiltschko, W. and Wiltschko, R. (1991). Magnetic orientation and celestial cues in migratory orientation. In: *Orientation in Birds*. (ed. P. Berthold), pp. 16-37. Basel: Birkhauser Verlag.

Wiltschko, W. and Wiltschko, R. (1995b). Migratory orientation of European robins is effected by the wavelength of light as well as by a magnetic pulse. *J. Comp. Physiol. A* **177**, 363-369.

Wiltschko, W. and Wiltschko, R. (1999). The effect of yellow and blue light on magnetic compass orientation in European robins, *Erithacus rubecula*. *J. Comp. Physiol*. *A* **184**, 295-299.

Wiltschko, W. and Wiltschko, R. (2001). Light-dependent magnetoreception in birds: the behavior of European robins, *Erithacus rubecula*, under monochromatic light of various wavelengths and intensities. *J. Exp. Biol*. **204**, 3295-3302.

Wiltschko, W., Wiltschko, R., and Munro, U. (2000a). Light-dependent magnetoreception in birds: does directional information change with light intensity? *Naturwissenschaften* **87**, 366-369.

Wiltschko, W., Wiltschko, R., and Munro, U. (2000b). Light-dependent magnetoreception in birds: the effect of intensity of 565nm green light. Naturwissenschaften **88**, 387-390.

Winklhofer, M., Holtkamp-Rotzler, E., Hanzlik, M., Fleissner, G., and Petersen, N. (2001). Clusters of superparamagnetic magnetite particles in the upper-beak skin of homing pigeons: evidence of a magnetoreceptor? *Euro. J. Mineral*. **13,** 659-669.

Winn, H. E., Salmon, M., and Roberts, N. (1964). Sun-compass orientation by parrot fishes. *Zeits. Tierpsychol*. **21**, 798-812.

CHAPTER 1

Magnetic Compass Orientation in C57BL/6 Laboratory Mice

ABSTRACT

Use of the geomagnetic field as a source of directional information has been demonstrated in a wide variety of organisms including birds, reptiles, and mammals. The mechanism of this magnetic compass is unknown, but theoretical models have implicated the involvement of specialized photoreceptors in birds and newts. In contrast, subterranean mole rats that are functionally blind appear to use a non-light dependent magnetic compass that likely involves single-domain particles of the mineral magnetite. Here, we present results indicating C57BL/6J laboratory mice can be trained to use a magnetic compass to position their nests in a specific magnetic direction. This assay can be used to characterize the magnetic compass of a rodent with normal vision (i.e. to determine whether mice have a light-dependent or non-light-dependent magnetic compass) and will also allow the use of behavioral genetic techniques to explore the underlying mechanism of magnetoreception.

Introduction

Evidence that animals are able to derive compass information from the geomagnetic field has been found in a variety of species including fish, amphibians, reptiles, birds, and mammals, as well as a variety of invertebrates (Quinn, 1980; Dejong, 1982; Lohmann, 1985; Phillips, 1986a; Lohmann, 1991; Phillips and Sayeed, 1993; Wiltschko and Wiltschko, 1995; Kimchi and Terkel, 2001). Early studies on birds indicated that magnetoreception was primarily used for migration and homing, and experimenters have focused magnetic orientation experiments on species known to migrate or accurately home. Unfortunately, few investigations have focused on non-migratory species because of the difficulty of eliciting a consistent response from a group of experimental subjects. Thus, the magnetic compass of mammals has remained relatively unexplored.

The first evidence for magnetic sensitivity in a mammal was found in woodmice (*Apodemus sylvaticus*). In this experiment, woodmice were transported approximately 40m from their capture site in either the geomagnetic field or in a magnetic field shifted by 180°. The results suggested that woodmice use the magnetic field to keep track of the direction of displacement in order to home back to their capture site (Mather and Baker, 1981). However, these results were widely discounted since the mice were tested inside a cardboard box and were observed by the experimenter through peep-holes visible to the mice. A demonstration of magnetic orientation in the white-footed mouse (*Peromyscus leucopus*) produced variable results in different groups of *P. leucopus* and was subject to criticism, because the experimental design was derived from Mather and Baker (1981) (August et al., 1989). In addition, an attempt to train the Siberian hamster (*Phodopus*

sungorus) and the bush opossum (*Monodelphis domesticus*) to search for food in a specific magnetic direction failed, increasing skepticism that mammals have a magnetic sense (Madden and Phillips, 1987).

In contrast to the examples mentioned above, reliable evidence for a magnetic compass response in a mammal has been obtained in several species of mole rats. Zambian Gray's common mole rat (*Cryptomys* spp.) were shown to have a spontaneous preference for building nests to magnetic southeast, and when the magnetic field was rotated, the mole rats rotated the position of their nests accordingly (Burda et al., 1990). The blind mole rat (*Spalax ehrenbergi*) was also found to have an innate preference for building nests to magnetic southeast (Burda et al., 1991; Marhold et al., 2000; Kimchi and Terkel, 2001).

More recent experiments demonstrated that mole rats are able to position nests relative to the magnetic field in total darkness, indicating that they have a non-light-dependent magnetic compass (Marhold et al., 1997a,b; Kimchi and Terkel, 2001). The properties of the non-light-dependent magnetic compass are consistent with a magnetoreception mechanism involving single domain particles of biogenic magnetite (Marhold et al., 1997a,b; Munro et al., 1997a,b; Kimchi and Terkel, 2001). For example, Zambian Gray's common mole rats were tested for magnetic orientation after being subjected to pulse remagnetization, using a magnetic pulse of 0.5 tesla (5ms), which is strong enough to remagnetize single-domain magnetite. Following pulse remagnetization, mole rats shifted their direction of orientation by approximately 73°, indicating that ferromagnetic particles may be involved in the mechanism of magnetoreception in mole rats (Marhold et al., 1997a). Furthermore, several experiments

have shown that the non-light-dependent magnetic compass of mole rats is sensitive to magnetic polarity (north vs. south) ("polarity compass") (Marhold et al., 1997b, Kimchi and Terkel, 2001), which is incompatible with photoreceptor-based models of magnetoreception (e.g. Edmonds, 1996; Ritz et al., 2000: see below).

In contrast to the response seen in mole rats, studies of the magnetoreception mechanism in some species of birds, amphibians, and insects have provided evidence for a light-dependent magnetic compass consistent with theoretical models implicating specialized photoreceptors in magnetoreception (Ritz et al., 2000). For example, magnetic compass orientation by the eastern red-spotted newt (*Notophthalmus viridescens*) undergoes a shift of approximately 90° under specific wavelengths of light. The wavelength dependent shift results from a direct effect of light on the underlying mechanism, and is mediated by photoreceptors located in or near the light sensitive pineal organ (Phillips and Borland, 1992; Deutschlander et al., 1999).

Similarly, wavelength dependent effects of light on magnetic orientation have been found in several species of birds, and electrophysiological experiments have revealed magnetic sensitivity in visual centers in the brain of bobolinks (Beason and Semm, 1987; Semm and Beason, 1990; Wiltschko et al., 1993; Wiltschko and Wiltschko, 1995). In contrast to the magnetic compass of mole rats, organisms with a light-dependent magnetic compass are reported to show sensitivity to the axis, rather than the polarity of the magnetic field (Wiltschko and Wiltschko, 1972; Phillips, 1986b), and use the inclination or dip angle to distinguish "poleward vs. equatorward" directions along the magnetic axis ("inclination compass").

Since mice and other rodents are closely related to mole rats taxonomically, it would be expected that they would also have a non-light-dependent magnetic compass mechanism. However, unlike *S. ehrenbergi* and mole rats in genus *Cryptomys,* most rodents have acute vision and are typically active at light levels similar to nocturnally migrating birds and newts. Thus based on visual ecology, typical rodents would be expected to have a magnetic compass like that of birds or newts.

To distinguish between functional and phylogenetic explanations for the type of magnetic compass found in different groups of mammals, the properties of magnetoreception of a more typical rodent must be explored. Recently, our laboratory has developed a magnetic compass assay in Siberian hamsters, in which the hamsters are trained to build their nest along a specific axis of the magnetic field (Deutschlander et al., 2003). While the development of this assay was important in confirming that a magnetic compass is not restricted to subterranean rodents, the orientation of the hamsters was bimodal (nests were positioned at both ends of the trained axis), and therefore would not be suitable for experiments designed to characterize the type of compass mechanism. For instance, in order to test whether rodents have a polarity or inclination compass, it must be determined whether mice reverse their direction of orientation in response to a change in the magnetic field, and this can not be detected if the orientation is bimodal.

Using an assay similar to the one used in the experiments on Siberian hamsters, we attempted to develop a unimodal magnetic compass assay in strain C57BL/6J laboratory mice. Laboratory mice were selected for the study of magnetic compass orientation because they would allow the use of genetic techniques (knockouts) to investigate the underlying molecular mechanisms.

Methods

Animals

Eighteen 2-4 month old male C57BL/6J mice were used in the experiment. Male mice were held in sibling groups in clear polycarbonate cages lined with wood shavings under a 15:9 h light:dark cycle (light 0600-2100 hours) until transported to the training facility. Food (rodent pellets 2018, Harlan Teklad), water, and nest material (compact cotton squares: Nestlets, Ancare) were provided ad libitum.

Training

Mice were transported from the breeding colony approximately 4 miles by automobile to the Behavioral Testing Facility (BTF). The BTF consists of a "hub" building which supplies filtered electricity and air for heating and cooling via underground ducts to four satellite testing buildings, which helps to minimize potential sound cues and electromagnetic disturbance in each satellite building. Experimental mice were housed in a single building with training and testing located in two separate but adjacent rooms of the same building. After transport to the BTF, mice were immediately placed in training. The experimental methods for both training and testing were derived from earlier experiments in our lab investigating the magnetic compass in Siberian hamsters (Deutschlander et al., 2003).

The mice were trained individually in large polycarbonate cages (48.3cm x 25.4cm x 15.2cm) with a layer of pine shavings as bedding, and a shelter (nest box) at one end. The cages were aligned along either the 45° - 225°(dark end - light end)

magnetic axis or the 135° - 315° (dark end - light end) magnetic axis, and a light gradient

was used to encourage mice to build their nest to one end (dark end) of the cage. To

produce the light gradient along the axis of the training cages, a 75 watt tungsten halogen

light (Eiko, MR16 EYC) located above each set of shelves was directed at a white panel

aligned vertically at a distance of 1.4 m from the open end of the cages (light intensity:

17-28 lux). Food and water were provided at the light end of the cage, and a shelter with

nest material (nestlets) was provided at the dark end of the cage (Figure 1). The mice

built their nest in the shelter at the dark end of the training axis, to the northeast (45°) or

southeast (135°) relative to the magnetic field. The training cages were located on a

narrow vertical shelving unit (each shelf: 28cm x 28cm x 30.5cm), so that the dark end of

the cage was enclosed by the shelf and the light end extended beyond the edge of the

shelf (Figure 1). Each training shelf unit contained 5 vertically stacked shelves, with

each shelf housing a single cage. Individual mice were randomly assigned one of the two

trained directions and were trained a minimum of 5 days before being tested for magnetic

compass orientation.

During both training and testing periods, mice were held on a 15:9 h light:dark

cycle (light 0600-2100 hours), maintaining the same light cycle of the main colony where

the mice were bred.

Testing

Mice were tested individually, one per night. Tests began in the late afternoon

(1700-1830 hrs) and ended the next morning (0800-0900 hrs). Individual mice were

tested in a large (89 cm diameter), radially symmetric circular arena. The arena was

surrounded by a system of coils, which allowed the horizontal component of the magnetic field to be manipulated (see "coil system" below). Each mouse was tested in one of four magnetic field alignments, with magnetic north at either geographic north, south, east, or west (inclination = $-67.0° \pm 0.5°$; total intensity = 53300 nT ± 400 nT). A total of 18 mice were tested, with four individuals in north and south fields and 5 individuals in east and west fields (Table 1). Individual mice were tested only once.

A 33 cm plexiglass disk, centered on the floor of the testing arena, provided a base for the release device. The arena floor surrounding the disk was covered with a thin layer of pine shavings, and food and nesting material (4 nestlets) were placed symmetrically on the center plexiglass disk. The mouse to be tested was transferred from its training cage to the testing arena in the adjacent room inside the "holding tube" component of the release device (Figure 2a). A plexiglass "plug" fit inside the holding tube to contain the mouse, and the entire holding tube was covered with a black cloth bag so the mouse was in total darkness during the transport. The holding tube containing the mouse was then placed on the release device "base", with the plexiglass "rod" fitting inside the plexiglass "plug" (see Figure 2b), and the experimenter quietly left the room. The mouse was left in the release device for a period of approximately 1 minute before being released into the arena. The mouse was then released in the center of the arena using a system of hydraulic syringes, controlled from the outer room, which lowered the plexiglass plug, and therefore the mouse, from the holding tube to the floor of the arena (Figure 2b). This design allowed the experimenter to operate the release device from another room, thus eliminating potential disturbances caused by the experimenter leaving the test room after a mouse was released into the arena.

Experiments typically began in the evening between 18:00 and 19:00 hours (approximately 2-3 hours before the beginning of the dark phase of the light cycle) and ran overnight. Results were recorded the following morning.

The mouse's directional response was determined the following morning by recording the bearing of the nest in the arena. The outside rim of the arena was marked in 10° intervals, and the bearing of the nest was recorded by placing a vertically aligned rod in the center of the nest and measuring the location of the nest to the nearest 5°. The topographic bearing (location inside the arena), magnetic bearing (bearing relative to magnetic north), and trained bearing (bearing relative to the trained magnetic direction) were recorded for each nest, along with a detailed description of the nest quality.

After testing, all mice were either returned to the main colony or euthanized with CO_2 gas. All experimental procedures were approved by the Virginia Tech Animal Care Committee (protocol # 1-140-Biol).

Coil System

The coil system surrounding the testing arena consisted of two 1.2 m, double-wrapped, four-square coil systems (Merritt et al., 1983) arranged perpendicularly (see Phillips, 1986a), and was powered by a Lambda power supply (model LQD-421) located in the hub building. Both coils were wrapped with two strands of insulated copper wire (see Kirschvink et al., 1993). This design allowed us to run current through the two strands in either a parallel or antiparallel fashion. When current through the two strands was running in parallel, an artificial field was produced that duplicated the intensity and inclination of the geomagnetic field, but was aligned with magnetic north at either

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geographic east, west, or south (depending on which of the two coils was activated; see Phillips, 1986a). However, when the current running through the two strands was antiparallel, the resultant fields canceled each other leaving the ambient magnetic field (i.e. magnetic north at geographic north), although the same current was running through the two strands of each coil. Thus, the output of the power supply (including any sound, vibration, etc.) was the same in all four alignments of the magnetic field. The advantage of testing equal numbers of animals in four symmetrical alignments of the magnetic field was that the pooled distribution of magnetic bearings factored out any consistent non-magnetic component of the mice's orientation.

Testing Criteria

Prior to beginning the final series of tests reported here, we generated a set of preliminary data to establish our testing criterion. Both rain and strong winds ($\geq$ 15 mph) were found to effect the magnetic compass orientation of mice, presumably because the mice responded to the noise created by these events. Unexpectedly, however, in the preliminary data set it appeared that mice tested during nights with heavy rain oriented opposite the trained direction (Figure 3c,d). If this response is real, it could represent either an adaptive response (e.g. to leave the burrow to avoid flooding or to forage during heavy rain when food items might be dislodged from foliage) or a non-specific response to the noise produced by the rain contacting the building. To avoid this source of variability in the data, we avoided testing on nights when heavy rains or high winds were expected.

Furthermore, there were several occasions where the experimenter accidentally made noise while leaving the testing room, which in preliminary tests had been found to bias the mice's orientation, and these experiments were immediately discarded. However, because the tests ran overnight, we were unable to ascertain whether any unusual noises occurred later during the testing period. Therefore, once the experimenter left the building, the mouse's response was counted, except in the case of an unexpected rain shower occurring after the start of the experiment.

Another objective for collecting a preliminary data set was to establish criteria for what we considered to be an acceptable nest. In our earlier study of hamsters, individuals that constructed poor nests also failed to position the nests in a consistent relationship to the magnetic field, and such nests were excluded (Deutschlander et al., 2003). In the present experiments, in order to be counted, a nest had to contain shredded nest material and also have a "cup shape" impression in the center where the mouse had obviously slept. Nests containing only pine shavings and no nest material, or nests that were not "cup shaped" (i.e. nest material was scattered and not in a compact nest), were not included in the data set. Furthermore, mice typically built their nests along the edge of the arena wall. Therefore, nests that were built with over half of their volume on the center plexiglass disk, thereby making the measurement of a directional bearing difficult, were also excluded from the data set (see also Deutschlander et al., 2003).

Statistical Analysis

Directional responses were analyzed using standard circular statistics (Batschelet, 1981). Because we had four different alignments of the magnetic field, data were pooled

relative to the test arena (absolute or "topographic" bearings), relative to the alignment of magnetic north (magnetic bearings), or relative to the trained magnetic direction (trained bearings). A mean vector bearing (MVB) and mean vector length (r) was calculated by vector addition. A Rayleigh test was then performed on the pooled distributions of data to test for a non-random distribution. 95% confidence intervals were used to determine whether the mean vector bearing included the trained magnetic direction. Furthermore, Watson's U^2 test was used to compare the distribution of magnetic bearings obtained from mice trained to 45° with the distribution of magnetic bearings obtained from mice trained to 135°, to test whether the two distributions were significantly different from one another.

Results

The preliminary data set suggested that laboratory mice could be trained to build their nest in a specific direction relative to the magnetic field (Figure 3). Using this information and the criteria described above, we tested 18 mice for magnetic compass orientation in a second test series.

The 18 mice tested for magnetic compass orientation were significantly oriented towards their trained direction [4° ± 27° (MVB ± 95% C.I.), r = 0.651, p < 0.001; Figure 4d). In Figure 4d, data from both trained directions were pooled so that the expected direction of orientation was 0°. In contrast, the topographic distribution of nests was random indicating that mice were orienting with respect to the magnetic field, rather than to some other, non-magnetic cue present in the test arena (87°, r = 0.188, p = n.s.; Figure 4a). When the orientation of each trained group was analyzed separately, we found that

mice trained to 135° were significantly oriented in their trained direction (126° ± 30°, r = 0.770, n=10; p< 0.001; Figure 4c), while mice trained to 45° were not significantly oriented towards their trained direction (72°, r = 0.574, n = 8; p = n.s.; Figure 4b). The distribution of mice trained to 45° was also not significantly different from the distribution of mice trained to 135° (p > 0.20, Watson's U^2 test), and when the data from both trained directions were pooled relative to magnetic north, the mice were significantly clustered towards magnetic east (106°, r = 0.615, p< 0.001). The individual bearings of all mice are listed in Table 1.

Discussion

The results of this experiment indicate that C57BL/6J mice have a magnetic compass that they use to position their nests in a consistent direction relative to the magnetic field. However, from the data obtained so far, it is unclear whether the mice are exhibiting a learned directional preference or if they have an innate preference for building their nests to the east/southeast, similar to the spontaneous southeasterly orientation found in mole rats. While the mice are significantly oriented when the magnetic bearings are pooled with respect to the trained direction (Figure 4d), and the mean vectors of the 45° group and 135° group only differ from the expected directions by 27° and 9° respectively, the difference between the two distributions was not significant. Furthermore, the distribution of magnetic bearings of mice trained to 45° was indistinguishable from random, although the response approached significance (72°, r = 0.574, n = 8, p = 0.1; Figure 4b). Therefore, additional data are clearly needed to

determine whether or not mice trained to 45° will orient in the trained magnetic direction, and to determine whether mice can be trained to other (e.g. 225° and 315°) directions.

While mice are relying on their magnetic compass to position their nests, it seems that their ability to correctly position their nests relative to the magnetic field is disrupted when auditory (i.e. wind, rain), visual (i.e. asymmetries in the arena), or olfactory (i.e. odors associated with the nests of previously tested animals) cues are present during the experiment. These observations indicate that, at least under some conditions, nest placement is influenced by a number of different types of cues, and that auditory, visual and olfactory cues may take precedence over magnetic cues.

Alternatively, the mice may not have been significantly motivated to build their nests in the appropriate magnetic direction, causing them to become easily distracted when extraneous cues are present. This possibility is further supported by the observation that in training some mice did not build their nest toward the dark end of the training cage, but rather towards the light end or middle of the cage. This suggests that the conditions used in training may not have sufficiently motivated the mice to learn the appropriate magnetic direction. Using a more elongated cage and more distinct light and dark areas could provide more motivation for the animals by emphasizing the difference between the nest (dark) area and the food and water (light) area. Alternatively, a cage consisting of two enclosed boxes (one for a nest and the other for food and water) connected to each other by a mouse "play tunnel" would provide only one location suitable for nest construction and therefore would sufficiently emphasize the magnetic direction of the nest. Yet another possibility would be to train and test mice in more similar environments (i.e. training and testing in a circular arena) or perhaps even in the

same environment, which might increase their motivation to build a nest in a learned relationship to the magnetic field, since the mice could better associate the testing environment with the training environment. However, training and testing in the same apparatus might also increase the likelihood that the mice would become familiar with and learn to orient with respect to other, non-magnetic cues present in the apparatus.

In contrast to experiments on Siberian hamsters, which show bimodal magnetic orientation, this is the first assay to show that a visually intact rodent, that is regularly active aboveground, exhibits unimodal magnetic compass orientation. While more experiments are necessary to determine whether the response is learned or innate, a unimodal response will allow the investigation of mechanism of magnetic compass orientation in mice. For example, if mice reverse their direction of orientation when the vertical component of the magnetic field is inverted, then they have an inclination compass like birds and newts, rather than a polarity compass like mole rats. An answer to this question will help to determine whether the mechanism of magnetic compass orientation is linked to the ecology or the phylogeny of the animal, thereby, providing insight into the evolution of the magnetic sense.

In addition, laboratory mice allow the use of behavioral genetic techniques that can be used to investigate the molecular mechanisms underlying the magnetic compass. Recently, Ritz et al. (2000) implicated cryptochromes, a newly discovered class of photopigments involved in circadian rhythm regulation (Cashmore et al., 1999), in the process of magnetoreception. Cryptochromes have several characteristics required by a radical-pair based magnetoreceptor (Ritz et al., 2000), including 1) the likely generation of radical pair intermediates, similar to the photolyases from which cryptochromes

evolved (Sancar, 1994), 2) an even distribution within the retina (Miyamoto and Sancar, 1998), and 3) localization near displaced ganglion cells that project from the retina to the magnetically sensitive nucleus of the basal optic root (nBOR) (Semm and Demaine, 1986). Therefore, cryptochromes are a likely candidate for involvement in magnetic field detection. In C57 BL/6 mice, there are 'knockouts' available of both crytochrome genes. Consequently, C57 BL/6 mice provide an opportunity to explore the role of cryptochromes in the magnetic compass.

Figure 1: Diagram of the training apparatus. Mice were held in rectangular plexiglass cages (48.3cm x 25.4cm x 15.2cm) with stainless steel lids. Each cage was placed on a shelf (28cm x 28cm x 30.5cm), so that one end was sheltered, while the other end of the cage extended beyond the shelf edge into the light. This provided a light gradient that encouraged the mouse to build its nest in the provided nest box at the dark end of the cage. To reinforce this axis, food and water were placed at the light end of the cage, so the mouse had to periodically come into the light. The light source was located above the training shelves and was directed towards a white reflective surface. Additionally, a painted black square of plexiglass was placed over the dark end of the cage to block any incoming light. Mice were trained individually for a minimum of 5 days.
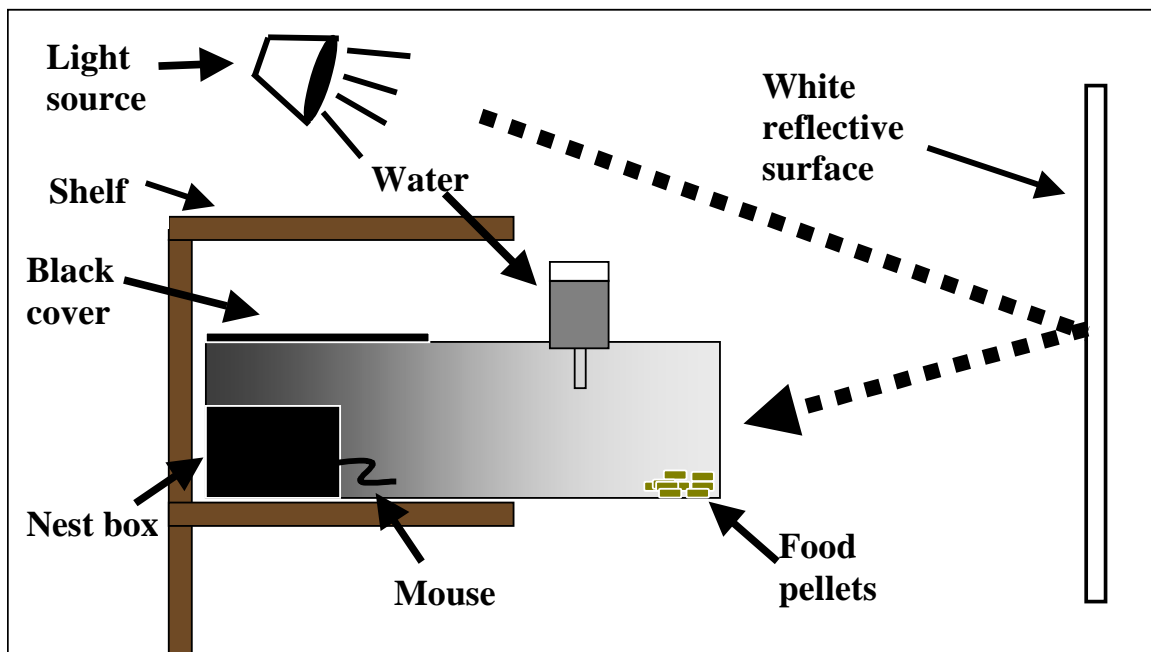
Table 1: Bearings of nests of individual mice tested in each horizontal alignment of the magnetic field. Animals are listed in the order they were tested and are given arbitrary numbers in the table. The column heading labeled "direction of magnetic north" refers to the geographic direction that magnetic north was aligned during the test. "Topographic bearing" refers to the absolute position in the arena, "magnetic bearing" refers to the position of the nest relative to magnetic north, and "trained bearing" refers to the position of the nest relative to the trained direction.

Animal	Trained Direction	Direction of Magnetic North	Topographic Bearing	Magnetic Bearing	Trained Bearing
1	135°	270°	80°	170°	35°
2	135°	90°	280°	190°	55°
3	135°	0°	100°	100°	325°
4	45°	180°	215°	35°	350°
5	135°	270°	65°	155°	20°
6	135°	180°	360°	180°	45°
7	45°	180°	15°	285°	240°
8	45°	270°	305°	35°	350°
9	135°	0°	95°	95°	320°
10	45°	270°	60°	150°	105°
11	45°	0°	90°	90°	45°
12	45°	180°	260°	80°	35°
13	135°	90°	200°	110°	335°
14	45°	0°	115°	115°	70°
15	45°	90°	145°	55°	10°
16	135°	180°	260°	80°	305°
17	135°	270°	25°	115°	340°
18	135°	90°	170°	80°	305°

Figure 2: Plexiglass release device. (a) External view of the release device. The base of the release device was fixed in the center of the arena for each test and was surrounded by a circular plexiglass disk (33 cm dia.). There were four openings in the sides of the base for the mouse to exit from once released. The holding tube was used to transport the mouse from training to testing and to hold the mouse prior to release into the testing arena. Once the mouse was placed in the holding tube to be transported to the testing arena, a plexiglass "plug" was placed into the holding tube. (b) Cut-away view of the release device. The holding tube was placed in a bag consisting of 2 layers of black cloth before it was carried into the testing room. Once in the testing room, the holding tube was inverted and placed on top of the base with the plexiglass rod inserting into a hole in the bottom of the plexiglass plug. When the finger grips used to hold the plug in place during transport were released, the plug was free to move down flush with the arena floor when the rod was lowered. The mouse was released by lowering the rod and therefore the plug, using a system of hydraulic syringes controlled from an adjacent room. The tapered design of the plug prevented the mouse from building its nest inside the base of the release device.
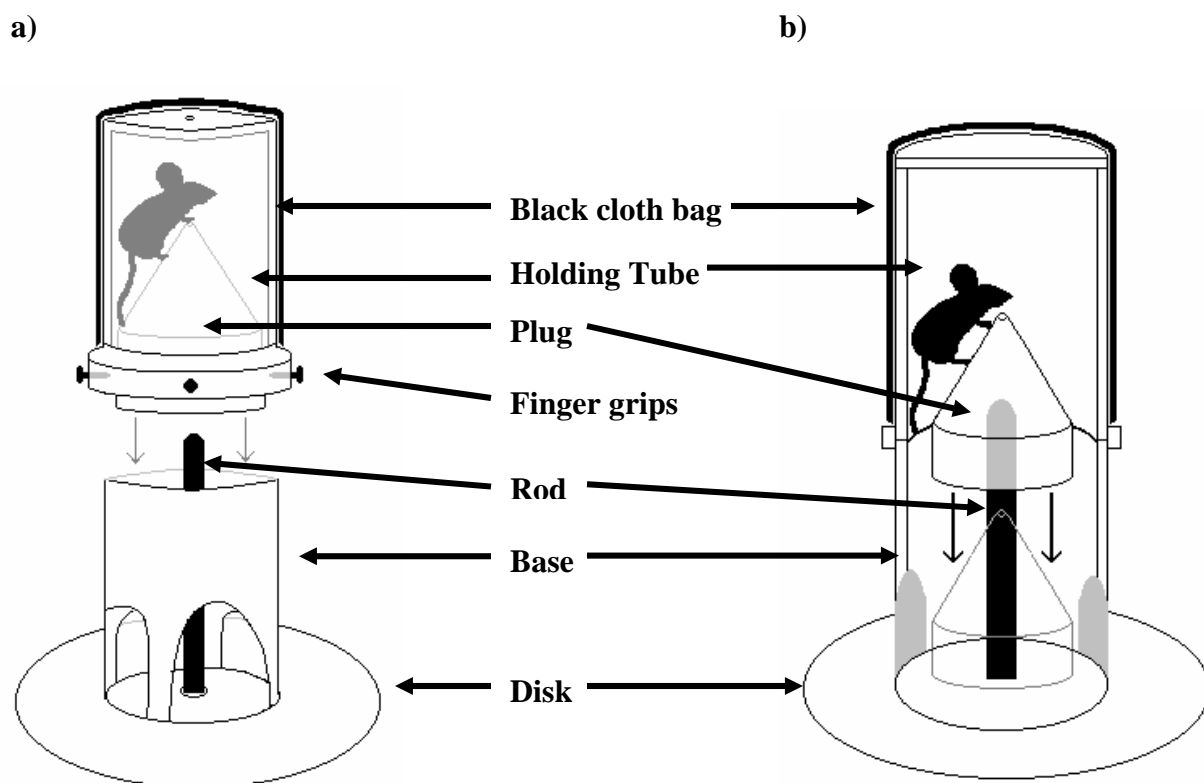
a)   b)

Black cloth bag

Holding Tube

Plug

Finger grips

Rod

Base

Disk

Figure 3: Preliminary data set (n =25) used to define testing criteria. This data set excludes all experiments performed on nights with high winds or noise caused by the experimenter, which were both found to elicit poor orientation in the mice. Solid circles represent bearings of mice trained to 135° and tested on nights without rain, open circles represent bearings of mice trained to 45° and tested on nights without rain, and X's represent test nights with rain. a) Bearings plotted topographically relative to geographic north (indicated by the arrow outside the circle). The bearings, including data from both trained directions, were not significantly oriented (51°, r = 0.059, p = n.s.). b) Bearings of mice trained to 45° plotted relative to magnetic north. The data excluding rainy nights are significantly oriented in the trained direction (38° ± 48° (MVB ± 95% C.I.), r = 0.572, n =10, p<0.05; see solid arrow inside circle). The trained direction is indicated by the open arrow outside the circle. c) Bearings of mice trained to 135° plotted relative to magnetic north. The data excluding rainy nights are significantly oriented in the trained direction (156° ± 26°, r = 0.864, n = 9, p<0.001; see solid arrow inside circle). The trained direction is indicated by the solid arrow outside the circle. The bearings obtained on non-rainy nights exhibited highly significant orientation in the each of the trained directions (b and c) and the two distributions were significantly different (U^2 = 0.984, p< 0.001, Watson U^2 test). d) Bearings plotted relative to the expected or "trained" magnetic direction (indicated by the solid arrow outside the circle). While the mean direction of all data was significantly clustered in the trained direction (5°, r = 0.37, n = 25, p <0.05; see solid arrow), the data obtained on rainy nights was roughly opposite the trained direction (200°, r = 0.634, n = 6, p = n.s.). In contrast, the bearings obtained on only non-rainy nights exhibited highly significant orientation in the trained direction (10°, r = 0.681, n = 19, p < 0.001; see dashed arrow). In all figures, the length of the arrow inside the circle is proportional to the length of the mean vector "r", with the radius of the circle equal to 1.
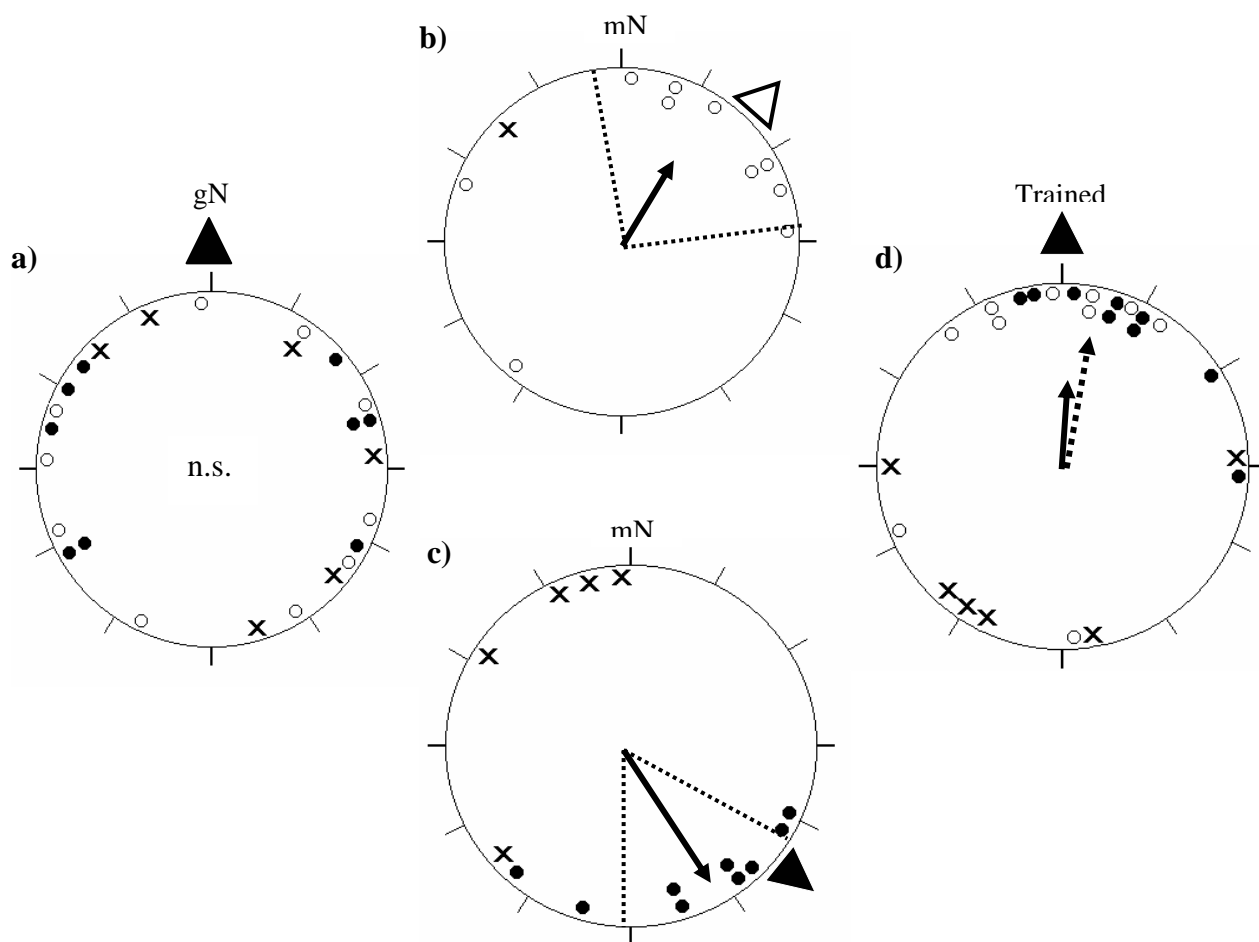
Figure 4: Bearings of nests in test series (n = 18). Solid circles represent bearings of mice trained to 135° and open circles represent bearings of mice trained to 45°. a) Bearings plotted topographically relative to geographic north (indicated by the arrow outside the circle). The bearings, including data from both trained directions, were not significantly oriented (mean vector = 87°, r = 0.188, n = 18, p = n.s.). b) Bearings of mice trained to 45° plotted relative to magnetic north were not significantly oriented in the trained direction (72°, r = 0.574, n = 8, p = n.s.). The trained direction is indicated by the open arrow outside the circle. c) Bearings of mice trained to 135° plotted relative to magnetic north, were significantly oriented in the trained direction (126° ± 29° (MVB ± 95% C.I.), r = 0.770, n = 10, p < 0.001; see solid arrow inside circle). The trained direction is indicated by the solid arrow outside the circle. d) Bearings plotted relative to the expected or "trained" magnetic direction (indicated by the solid arrow outside the circle). The bearings, including data from both trained directions, were significantly oriented in the trained (4° ± 27°, r = 0.651, n = 18, p<0.001; see solid arrow inside circle). In all figures, the length of the arrow inside the circle is proportional to the length of the mean vector "r", with the radius of the circle equal to 1.

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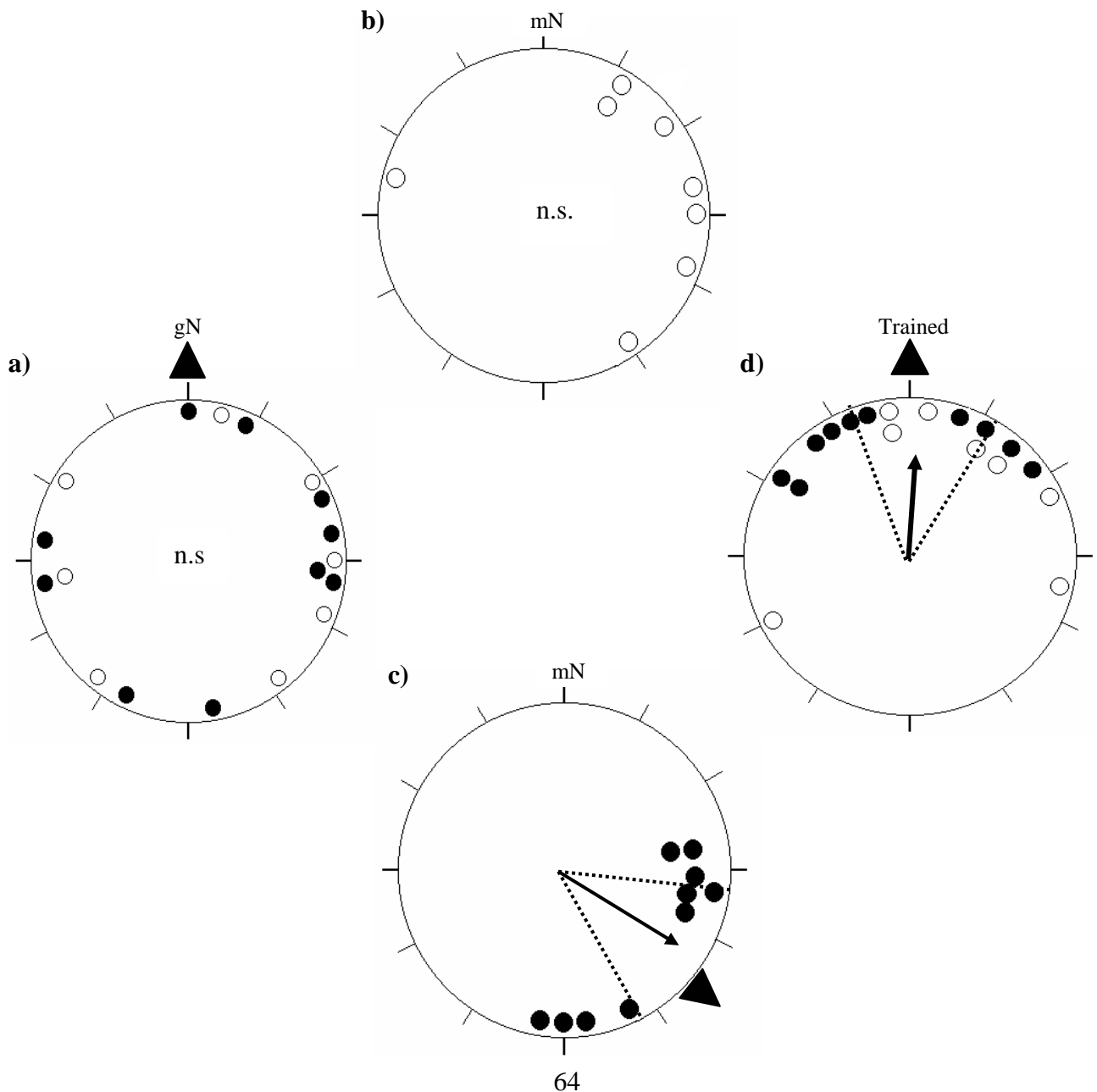
LITERATURE CITED

August, P. V., Ayvazian, S. G., and Anderson, J. G. T. (1989). Magnetic orientation in a small mammal, *Peromyscus leucopus*. *J. Mammal.* **70**, 1-9.

Batschelet, E. (1981). Circular Statistics in Biology. Academic Press.

Beason, R. C. and Semm, P. (1987). Magnetic responses of the trigeminal nerve system of the bobolink (*Dolichonyx oryzivorus*). *Neurosci. Lett*. **80**, 229-234.

Burda, H., Beiles, A., Marhold, A., Simson, S., Nevo, E., and Wiltschko, W. (1991). Magnetic orientation in subterranean mole rats of the superspecies *Spalax ehrenbergi*: experiments, patterns and memory. *Isr. J. Zool*. **37**, 182-183.

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

Cashmore, A., Jarillo, J., Wu, Y-J. and Liu, D. (1999). Cryptochromes: blue light receptors for plants and animals. *Science* **284**, 760-765.

DeJong, D. (1982). Orientation of comb building by honeybees. *J. Comp. Physiol.* **147**, 495-501.

Deutschlander, M. E., Borland, S. C., and Phillips, J. B. (1999a). Extraocular magnetic compass in newts. *Nature* **400**, 324-325.

Deutschlander, M. E., Freake, M. J., Borland, S. C., Phillips, J. B., Anderson, L. E. and Wilson, B. W. (2003). Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus. Anim. Behav*. **65**, 779-786.

Edmonds, D. T. (1996). A sensitive optically detected magnetic compass for animals. Proc. Roy. Soc. Lond. B **263**, 295-298.

Kimchi, T. and Terkel, J. (2001). Magnetic orientation in the blind mole rat *Spalax ehrenbergi. J.Exp. Biol.* **204**, 751-758.

Kirschvink, J. L., Ricci, R. D., Nesson, M. H., and Kirschvink, S. J. (1993). *Magnetite-based magnetoreceptors in animals: structural, behavioral and biophysical studies*. Palo Alto, CA: Electric Power Research Institution (EPRI).

Lohmann, K. J. (1985). Geomagnetic field detection by the Western Atlantic spiny lobster, *Panulirus argus. Mar. Behav. Physiol.* **12**, 1-17.

Lohmann, K. J. (1991). Magnetic orienation by hatchling loggerhead sea turtles (*Caretta caretta*). *J. Exp. Biol*. **171**, 1-13.

Madden, R. C. and Phillips, J. B. (1987). An attempt to demonstrate magnetic compass orientation in two species of mammals. *Anim. Learn. Behav*. **15**, 130-134.

Marhold, S., Beiles, A., Burda, H., and Nevo, E. (2000). Spontaneous directional preference in a subterranean rodent, the blind mole-rat, *Spalax ehrenbergi. Folia Zool*. **49**, 7-18.

Marhold, S., Burda, H., Kreilos, I., & Wiltschko, W. (1997a). Magnetic orientation in common mole rats from Zambia. In: *Orientation and Navigation - Birds, Humans and other Animals* (ed. by the RIN), pp.5-1 - 5-9. Oxford: Royal Institute of Navigation.

Marhold, S., Wiltschko, W., and Burda, H. (1997b). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* **84**, 421-423.

Mather, J. G., and Baker, R. R. (1981). Magnetic sense of direction in woodmice for route-based navigation. *Nature* **291**, 152-155.

Merritt, R., Purcell, C., and Stroink, G. (1983). Uniform magnetic field produced by three, four, and five square coils. *Rev. Sci. Instrum*. **54**, 879-882.

Miyamoto, Y. and Sancar, A. (1998). Vitamin B_2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proc. Natl. Acad. Sci. USA*. **95**, 6097-6102.

Munro, U., Munro, J. A., Phillips, J. B., Wiltschko, R. and Wiltschko, W. (1997a). Evidence for magnetite-based navigational 'map' in birds. *Naturwissenschaften* **84**, 26-28.

Munro, U., Munro, J. A., Phillips, J. B. and Wiltschko, W. (1997b). Effect of wavelength of light and pulse magnetization on different magnetoreception systems in a migratory bird. *Aust. J. Zool*. **45**, 189-198.

Phillips, J. B. (1986a). Magnetic compass orientation in the Eastern red-spotted newt (*Notophthalamus viridescens*). *J. Comp. Physiol*. **158**,103-109.

Phillips, J. B. (1986b). Two magnetoreception pathways in a migratory salamander. *Science* **233**, 765-767.

Phillips, J. B. and Borland, S. C. (1992). Behavioral evidence for use of a light-dependent magnetocreception mechanism by a vertebrate. *Nature* **359**, 142-144.

Phillips, J. B. and Sayeed, O. (1993). Wavelength-dependent effects of light on magnetic compass orientation in *Drosophila melanogaster*. *J. Comp. Physiol*. **172**, 303-308.

Quinn, T.P. (1980). Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *J. Comp. Physiol*. **137**, 243-248.

Ritz, T., Adem, S., and Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. *Biophys. J.* **78**, 707-718.

Sancar, A. (1994). Structure and function of DNA photolyase. *Biochem.* **33**, 2-9.

Semm, P. and Beason, C. (1990). Responses to small magnetic variations by the trigeminal system of the bobolink. *Brain Res. Bull*. **25**, 735-740.

Semm, P. and Demaine, C. (1986). Neurophysiological properties of magnetic cells in the pigeon's visual system. *J. Comp. Physiol. A* **159**, 619-625.

Wiltschko, W., Munro, U., Ford, H., and Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature* **364**, 525-527.

Wiltschko, W. and Wiltschko, R. (1972). Magnetic compass of European robins. *Science* **176**, 62-64.

Wiltschko, W. and Wiltschko, R. (1995). Migratory orientation of European robins is effected by the wavelength of light as well as by a magnetic pulse. *J. Comp. Physiol. A* **177**, 363-369.

CHAPTER 2

Auditory Compass Orientation in C57BL/6 Laboratory Mice

ABSTRACT

Animals use a number of redundant sources of directional information for menotactic (compass), such as a sun compass, polarized light compass, magnetic compass, and star compass. Interestingly, all of these sources of compass information are based on photoreception, suggesting that the processing of compass information may be uniquely associated with brain regions that receive photic input or that compass information may be restricted to regions of the brain that process light information. Contrary to this conclusion, however, here we present preliminary evidence that C57BL/6J laboratory mice are able to use directional auditory cues as a source of compass information. While there is evidence indicating that rodents may use auditory cues for place navigation (a spatial task), this is the first evidence that demonstrates rodents are able to use auditory cues for compass orientation (a directional task). Our results suggest that compass orientation is distinctly different from place navigation, and that spatial and directional information are processed through different pathways in the nervous system.

Introduction

Compass orientation or menotaxis is defined as the ability to orient at a specific angle relative to a directional cue (Kuhn, 1919). Cues used for compass orientation include the sun, moon, stars, polarized light, and the geomagnetic field (Wiltschko and Wiltschko, 1972; Emlen, 1975; Wehner, 1989). Interestingly, these types of compasses, including the light-dependent magnetic compasses of at least some species (e.g., Phillips and Borland, 1992a,b; Phillips and Sayeed, 1993, Wiltschko et al., 1993, 2000a,b, 2002b), share a common characteristic in that they depend on photoreception to obtain compass information. This raises the possibility that the processing of sensory information necessary to derive a menotactic response may have originated from, or be uniquely associated with, photoreception and/or vision. Here we investigate whether directional cues from an entirely different sensory modality (sound) can be used by laboratory mice for menotactic orientation.

Auditory cues have been shown to play a role in many orientation tasks. Until now, however, there has been no compelling evidence that any organism uses directional auditory cues as a source of compass information. Auditory cues are used for echolocation in groups such as bats, dolphins, shrews, and even rats (Rosenzweig et al., 1955; Griffin, 1958; Buchler, 1976; Forsman and Malmquist, 1988; Harley et al., 2003). These species emit sound (e.g. high frequency ultrasound for most terrestrial echolocating species) that reflects back to the animal from nearby objects (Griffin, 1958), providing information about the direction and distance to objects in space. Thus, they can use this information to orient through their habitat and even to detect prey.

Numerous species also use acoustic cues for locating and orienting either towards or away from conspecifics (phonotaxis; see Bright, 1984 for review). For instance, females of many species of frogs and crickets are attracted to the calls of males of the same species and use phonotaxis to approach calling males (Alexander, 1961; Gerhardt, 1974). While it is clear that auditory cues play an important role for survival, to date, there is no evidence that any animal is able to use auditory cues as a source of directional compass information.

While the role of visual cues in spatial orientation has been studied extensively in rodents, the role of auditory cues in spatial orientation has been investigated far less. Sutherland and Dyck (1984) explored the possibility that rats were able to use auditory "beacons" to locate the spatial position of a hidden platform in a variation of the Morris water maze. In this study the hidden platform was located in the southeast quadrant of the pool, while two auditory beacons were placed outside the pool at the northwest and southwest quadrants. In addition, a white noise source was placed directly east of the pool about 1.5 meters away. The results indicated that rats trained with the auditory beacons present were not able to better locate the platform than rats trained without auditory beacons. However, when the white noise source was removed, rats trained with the auditory beacons were better able to find the hidden platform than rats trained without the auditory beacons.

These results suggest that the white noise was masking the other two auditory cues and that rats are able to use auditory information for spatial orientation. However, the results do not take into account the possibility that the rats might be instinctively swimming away from the auditory beacons (located just outside the NW and SW

quadrants) when they were not masked by the white noise and, thus, finding the platform more quickly because they are swimming east, away from the noise. Alternatively, they may have been repelled by the white noise source, causing them to avoid the east side of the pool, which could also explain why they found the hidden platform on this side of the pool when the white noise source was turned off. Moreover, it is possible that the rats in the study by Sutherland and Dyck (1984) actually used a cue other than sound to orient (e.g., magnetic field, light gradient, substrate vibrations). To distinguish between these possibilities it would be necessary to shift the auditory beacons to other locations around the pool to see if the rats orient in the same direction relative to the auditory cue.

Additional evidence that auditory cues are important for spatial orientation in rats was reported by Rossier et al. (2000). In this study, it was found that rats are able to successfully locate a hidden platform in a variation of the Morris water maze task when they are trained to associate several directional sources of auditory and visual cues with a spatial location. Interestingly, rats were unable to correctly orient to the platform when only auditory or only visual information was available, suggesting that auditory cues may aid in spatial orientation, but rats do not use them independently of other types of (e.g., visual) cues .

It is important to emphasize that the tasks used by Sutherland and Dyck (1984) and by Rossier et al. (2000) required the subjects to locate the spatial position of the underwater platform (place navigation), rather than to maintain a directional heading relative to a distance sound source (compass orientation). Place navigation requires the animal to reach a specific goal or location with respect to a two-dimensional configuration of two or more directional cues (e.g. auditory beacons). In effect, the

animal must triangulate its position relative to the configuration of cues to localize a specific spatial position. Furthermore, these beacons are located at a finite distance relative to the range of movement of the organism so that the direction to each of the beacons changes as the animal moves with respect to the configuration of cues. In contrast to place navigation, compass orientation requires the animal to calculate a fixed directional heading as an angle relative to a single directional cue. Additionally, compass orientation requires that the source of directional information remains constant as an animal moves, so that it will provide a consistent directional reference.

More recently, evidence consistent with the use of auditory compass cues has been obtained in a study of magnetic compass orientation by Siberian hamsters (Deutschlander et al., 2003). In this experiment, Siberian hamsters were trained in the ambient magnetic field to position their nest along a particular magnetic axis. The hamsters were then tested in an adjacent room in a circular arena in which magnetic north could be aligned to geographic north, south, east, or west. In initial experiments, Siberian hamsters only positioned their nests along the trained magnetic axis when magnetic north was aligned to geographic north in testing, i.e., the same alignment of magnetic north that the hamsters were exposed to in training. The nests of hamsters tested with magnetic north rotated to the east, south or west, were randomly distributed.

In the hamster experiments, pneumatic pumps located on either side of the training/testing building provided a constant source of low frequency sound pulses that could be easily heard by human observers in both the training room and adjacent testing room. Thus, one possible explanation for the findings of these experiments was that the hamsters were integrating both auditory and magnetic directional cues, and experienced a

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'cue conflict' when the relationship between the two sources of directional cues was altered (i.e., when the magnetic field was rotated to east, south or west). In a later series of experiments, in which training was moved to a location where the auditory cues were not audible, the hamsters showed consistent orientation with respect to both the normal (magnetic north at north) and rotated fields (Deutschlander et al., 2003). While use of auditory compass cues is not the only possible explanation for the hamster results, these findings suggested that a more rigorous investigation of the role of auditory cues in the compass orientation of rodents was warranted.

The experiments reported here demonstrate auditory compass orientation in mice. Mice are well-suited to investigate the possibility of auditory compass orientation, because they have well developed hearing and their territories are relatively small (e.g., compared to birds). Consequently, there are likely to be able to detect auditory cues [e.g. streams, bird calls, chorusing frogs and crickets, etc.] that originate well beyond their territory boundaries and, therefore, may provide a relatively constant directional reference for spatial orientation. Unlike the methods used by Rossier et al. (2000) and Sutherland and Dyck (1984), we trained mice in a manner that has been shown to elicit directional (compass) orientation in rodents (see Burda et al., 1990; Marhold et al., 1997a,b; Deutschlander et al., 2003). Furthermore, we used only a single sound source to train and test the mice, and trained mice in two different directions with respect to the sound source. We also eliminated visual, olfactory, and magnetic cues that could be sources of bias in the test arena.

Our results indicate that mice are capable of accurate auditory compass orientation. The evidence presented here not only reveals that menotactic processing of

directional information is not limited to photoreception, but sheds new light on the mechanisms of spatial and compass orientation in mammals.

<u>Methods</u>

Animals

Two to four month old male C57BL/6J mice were used in the experiment. Prior to training, male mice were held in the breeding colony in sibling groups in clear polycarbonate cages lined with wood shavings under a 15:9 h light:dark cycle (light 0600-2100 hours) until transported to the training facility. Food (rodent pellets 2018, Harlan Teklad), water, and nest material (Nestlets, Ancare) were provided ad libitum. During both training and testing, mice were held on a 15:9 h light:dark cycle (light 0600-2100 hours), maintaining the same light cycle of the main colony. After the experiments, all mice were either returned to the main colony or euthanized with CO_2 gas. All experimental procedures were approved by the Virginia Tech Animal Care Committee (protocol # 1-140-Biol).

Training

Male mice were transported from the breeding colony and were placed directly in training. Individual mice were randomly assigned to one of two trained directions and were trained an average of 10 days before being tested (range 6-18 days). Mice were trained individually in large cages (49.5cm x 29.2cm x 33.0cm; Fig 1a). The base of the cage was made of wood and the inside was lined with pine shavings. The side walls and

the top of the cage were made of hardware cloth to ensure the cages were transparent to sound.

Mice were trained to build their nests in one of two directions relative to an auditory cue aligned perpendicular to the long axis of the cage. A small (12.7cm x 8.9cm x 7.6cm) black shelter (nest box) was located at one end of each cage, either 90° clockwise (90° CW) or 90° counterclockwise (90° CCW) from the sound source (Figure 1b). Cages for each trained direction were placed on vertical shelving units (one shelving unit for each trained direction), which could hold up to four cages, and were surrounded on three sides with acoustic foam to help attenuate sounds other than the sound source used for training (Figures 1b and 2). The fourth side was left open so that light entered the cage from the end opposite the nest box (see below) and the experimenters had access to the cages.

Light entering from the open end of the shelving unit formed a light gradient in the training cage. Food and water were provided at the light end of the cage and a shelter and nest material (nestlets) at the dark end of the cage. This method of training encouraged mice to build their nest at only one end of the training cage (dark end) and, therefore, to learn a specific position of their nest relative to the sound cue. The light source used to produce the light gradient in the training cages consisted of a 12 Volt 75 Watt projector bulb located above each training shelf and directed towards the wall opposite the open side of the shelf.

The sound used for both training and testing was produced by a Sharper Image Sound Soother 20 set to mimic the sound of running water in a "brook", which is a sound cue that mice could potentially encounter in nature. The sound was sent to an amplifier

and two 8.4 cm diameter speakers that were fixed to the bottom and the top of a 2.1m

vertically aligned 7.6 cm diameter PVC pipe. At each of the four training shelves on a

single shelving unit, a T-junction was connected to a 10.2 cm length of pipe that

conducted the sound through a hole in the acoustic foam covering the sides of the shelves

(Figure 2). The end of the pipe was covered with gray cloth that closely matched the

gray color of the acoustic foam. The PVC T-junctions were used to produce a directional

sound source in each cage perpendicular to the long-axis of the cage and was either 90°

CW or 90° CCW of the sheltered end (Figure 1b and 2). The sound level for each

training cage was adjusted to approximately 65 decibels (db)[1] at the position of the nest

box using a digital sound level meter (Radioshack, model # 33-2055), which

corresponded to a moderately high conversational level. The sound level was sufficient

to mask most extraneous sound originating in the rooms surrounding the training and

testing room.

Testing

Individual mice were tested overnight in a large (89 cm diameter), radially

symmetric circular arena. There were four circular openings spaced at 90° intervals

around the sides of the arena approximately 14.0 cm above the floor, through which PVC

T-junctions, attached to a 33.5 cm height of PVC pipe, were placed. Similar to training,

8.4 cm diameter speakers were placed in the top of the PVC pipe, and sound was

conducted through the T-junction into the arena. The end of the T-junction that entered

the arena was covered, first with screening to prevent the mice from climbing into the

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[1] Reference: 0 db = 0.0002 Micro Bar

PVC pipe, and then with thick black plastic so the pipes would blend with the black walls of the arena as much as possible. One of the speakers was given an arbitrary bearing of 0° and the remaining three were assigned angles of 90°, 180°, and 270° in a clockwise direction around the arena. Each of the four speakers was adjusted so that the level of emitted sound was approximately 65 db, when measured along the wall of the arena at an angle of 90° (either CW or CCW) from the speaker.

During a test each mouse was semi-randomly tested with the sound originating from only one of the four possible positions. To date, a total of 7 mice have been tested, with 4 mice tested in the 90° speaker alignment, 2 mice at the 180° speaker alignment, and 1 mouse at the 0° speaker alignment. Individuals were tested only once. Two of the 7 mice were trained to 90° CCW and these mice were tested with sound coming from the 90° and 180° speakers. The remainder of the mice were trained to 90° CW (see Table 1).

Before each test, the arena floor was lined with a thin layer of pine shavings. Four food pellets (Rodent chow 2018, Harlan Teklad) and four nestlets (Ancare) were arranged symmetrically around the center of the arena. Mice were transported from the training room to the adjacent testing room in an opaque plastic cup, which doubled as a release device. The cup was covered with a 10 cm square of plastic to prevent mice from escaping during the transport, and the cup containing the mouse was placed in the center of the testing arena with the open side down on top of the plastic cover. The closed end of the plastic cup was fitted with a hook, which connected to a length of monofilament fishing line. The monofilament line went straight up through a small hole in the center of a light diffuser (translucent plexiglass) above the arena, and was then connected to a series of pulleys that enabled the experimenter to raise the cup from an adjacent room,

releasing the mouse into the arena. Once the experimenter left the room, the mouse was held in the release device for approximately one minute before the cup was raised and the mouse was released. A typical experiment began in the evening approximately 2-3 hours before "lights out" and ran overnight.

The mouse's directional response was obtained the following morning by recording the bearing of the nest in the arena. Tests where mice did not build a nest using at least half of the nest material (2 of 4 nestlets) were eliminated (see Deutschlander et al., 2003). The outside rim of the arena was marked in 10° intervals, and the bearing of the nest was recorded by placing a vertical PVC rod in the center of the nest and measuring the location where this rod intersected the rim to the nearest 5°. The topographic bearing (location with respect to the arena), the bearing relative to the sound source (clockwise deviation from the active speaker), and the bearing relative to the trained direction (clockwise deviation from the trained direction) were recorded for each nest along with a detailed description of the nest quality.

Care was taken to eliminate other potential sources of directional information in the test arena besides the auditory cue. A cube-surface coil (Merritt et al., 1983) was positioned around the arena to cancel out the horizontal component of the geomagnetic field, thus eliminating the potential use of a magnetic compass for orientation. Visual cues were also eliminated. The arena was circular and was made of black plastic with lighter colored speckles that helped to mask any flaws or scratches. Furthermore, the arena was surrounded on all four sides by black curtains that hung from the top of the coil frame, eliminating visual cues from the surrounding room. A plexiglass diffuser covering the top of the coil frame insured an even distribution of light.

Statistical Analysis

All analyses were carried out using standard circular statistics (Batschelet, 1981). Because we had four different speaker positions, data were pooled either topographically, relative to the activated speaker, or relative to the trained direction. A mean vector bearing (MVB) and mean vector length (r) was calculated by vector addition. The Rayleigh test was then performed to test for significant orientation. Finally, 95% confidence intervals (95% C.I.) were used to test for orientation with respect to the trained direction.

Results

The 7 mice tested for auditory compass orientation were significantly oriented in the trained direction [23° $\pm$ 35° (MVB $\pm$ 95% C.I.), r = 0.795, n = 7, p < 0.01; Figure 3]. In Figure 3, the bearings from all seven mice are pooled so that the top of the distribution (0°) corresponds with the expected or trained direction (i.e. either 90° CW or 90° CCW of the sound source). In contrast to the strong orientation in the trained direction, when the bearings are plotted topographically (i.e. according to the absolute position in the arena), the distribution of the seven nests is indistinguishable from random (199°, r = 0.363, n = 7, p = n.s.; Figure 4). The individual bearings of all mice are listed in Table 1.

Discussion

The results of this experiment provide preliminary evidence that mice can be trained to use auditory cues as a source of compass information. Because the mice oriented at the trained angle relative to the sound source, the data suggest that mice were

exhibiting menotactic (i.e. compass) orientation, rather than positive or negative phonotaxis. However, our data are primarily from only one trained direction. Of the seven mice tested, 5 were trained to 90° CW of the sound source, while only two were trained to 90° CCW of the sound source. Thus, additional data from the 90° CCW trained direction are needed to provide a more rigorous test of whether mice have an auditory compass.

When the seven nest bearings are plotted topographically (Figure 4), the distribution is indistinguishable from random. Thus, there is no evidence to suggest that non-auditory cues were biasing the orientation of the mice. The small cluster of bearings at 200° (i.e. the direction toward the training room) suggests that some individuals may have been keeping track of inertial cues during the transport from training to testing, and then using this information to position their nests in the direction of the training room. If this pattern continues in future trials, a simple solution would be to disorient the mice (by rotation or spinning) prior to testing.

While our experiment did not control for reflections of sound waves, we used only one sound source in both training and testing thereby reducing the potential for confounding auditory cues. However, there is still the possibility that reflections of sound around the room and off the inside of the arena walls have created conflicting auditory information in the testing room and this could have caused some of the scatter we see in the data. Acoustic foam on the walls of the testing arena could be used to minimize reflectance, although any sound absorbing material would have to be designed so that mice can not chew on the material or use it to climb the sides of the arena.

The results of our experiment agree with Sutherland and Dyck's (1984) finding that the orientation of rodents is influenced by auditory cues. However, by using a single auditory cue in one of four positions in each trial, we were able to show that mice are capable of orienting at a learned angle relative to the auditory cue, rather than directly toward or away from the sound source (phonotaxis). The design used in the experiments reported here also rules out the involvement of other types of orientation cues (e.g. magnetic field, light gradients, substrate vibrations, etc.), which were possible sources of bias in the Sutherland and Dyck (1984) experiment.

Moreover, in contrast to the evidence obtained by Rossier et al. (2000), our results suggest that auditory cues can be used in isolation (i.e. without directional visual cues) for orientation. A major difference between the two experiments is that we trained mice using a consistent directional relationship to the sound source, along with a nest building assay that has been shown in previous studies to elicit compass orientation in rodents (see Burda et al., 1990; Marhold et al., 1997a,b; Deutschlander et al., 2003). On the other hand, in Rossier et al.'s (2000) experiments, the azimuth of the underwater platform varied relative to the sound source as rats moved about the water maze during training. The rats were also required to perform a task that required spatial (place navigation), rather than directional (compass), orientation.

Furthermore, Rossier et al. (2000), only investigated navigation in two conditions using strictly auditory information. Under the first condition, rats were trained and tested in the water maze using a single auditory beacon directly associated with the location of the underwater platform, but failed to perform significantly better than controls. Although this condition used a single auditory cue, it differs from a directional compass

task in that the escape platform is not located against the wall of the arena, but rather at a remote location in space. Therefore, this condition required the mouse to learn a spatial location rather than a compass bearing. Under the second condition in Rossier et al.'s (2000) experiment, rats trained and tested in the water maze using three auditory beacons unassociated with the direction of the escape platform, also failed to perform at greater than chance levels. Thus, in the second condition, Rossier et al. (2000) were essentially testing whether rats could triangulate their spatial position using a configuration of three auditory beacons. This is fundamentally different from our experiment where we demonstrate that mice are able to learn a fixed directional heading relative to a single auditory cue, and therefore, are able to use auditory cues for menotactic orientation.

When our findings are taken in conjunction with those of the two water maze studies discussed above, they suggest that auditory cues can be used to derive compass information, but that compass information may be of limited utility in spatial navigation. Evidence from studies of Scrub Jays (*Aphelocoma coerulescens*) suggests, however, that the sun compass can be used in conjunction with spatial cues for food caching and recovery (Wiltschko and Balda, 1989; Balda and Wiltschko, 1991), which indicates that compass cues may augment spatial orientation. It is plausible, therefore, that under some conditions, mice and other animals, might also use an auditory compass to aid in spatial, as well as directional, orientation.

In contrast to the experimental conditions used in our laboratory, rodents in nature are able to use several different types of cues to derive compass information (i.e. sun, magnetic cues, sound). Our earlier experiments with hamsters (Deutschlander et al., 2003), for example, suggest that magnetic and auditory compass cues may be used

together to determine nest location (see earlier discussion). Consequently, restricting

mice to only one source of directional information in the present experiments may cause

us to underestimate the accuracy with which they can position their nests when given

access to multiple sources of directional information.

As discussed previously, many organisms exhibit phonotactic responses to sound

sources (see Bright, 1984 for review), and the findings of Rossier et al. (2000) and

Sutherland and Dyck (1984) suggest that auditory cues may play a role in spatial

orientation (place navigation) in rodents. In addition, the results presented here indicate

that auditory cues may also be used for compass orientation. Phonotaxis requires only

that an animal move towards (or away from) a sound source, while both place navigation

and compass orientation require the animal to learn an arbitrary position or direction,

respectively, relative to the sound source. Thus, while all three of these tasks involve the

use of auditory cues, each may require a different type of sensory processing. To locate a

specific place relative to a configuration of landmarks/beacons (place navigation),

requires an animal to attend to changes in the directions (azimuths) of the

landmarks/beacons resulting from its own movements.

In contrast, to maintain a constant directional heading (compass orientation), an

animal must rely on a reference that remains more or less in a fixed direction (i.e.

constant azimuth) as the individual moves. This suggests that place information and

direction information are likely to be processed by different systems. Consistent with this

possibility, neurons in the hippocampus termed "place cells" were found to fire when the

animal was in a specific spatial location, and these cells collectively form a spatial map of

the animal's location (Muller et al., 1987). In contrast, cells in the hippocampus termed

"head direction cells" were found to fire only when the animal's head is pointing in a specific direction (Taube et al., 1996). These cells are sensitive only to the direction the animal is pointing, and not to spatial location like the place cells. Thus, place cells are clearly involved in the processing of spatial information, while head direction cells have properties indicating they could play a role in the processing of compass information.

Evidence that mice are able to use auditory cues for compass orientation in the laboratory, suggests that mice may also use auditory cues for spatial orientation under natural conditions. It is possible that auditory information produced, (e.g.) by territorial birds, chorusing frogs and crickets, streams or waterfalls, and even the sound of wind associated with forest edges, could provide useful auditory information for rodents and other animals in nature. While there are very few stable sources of environmental sounds (i.e. streams freeze or dry up and calls from birds, frogs, and crickets are seasonal), the same is true of other sources of compass information (e.g. celestial cues), the availability of which changes with time of day and weather conditions. Our findings suggest, therefore, that mice may be able to opportunistically make use of intermittent sources of directional auditory cues in their environment. Furthermore, the multiple sources of compass cues available in a natural setting provide redundant sources of directional information. Not only is this likely to be important when one or more of these cues are not available (i.e. the sun is not visible on a cloudy day or the magnetic field is obscured by a magnetic storm), but in addition, integration of compass information from different modalities may make it possible for animals to orient more accurately. The use of auditory compass information in a natural setting would, therefore, be likely to increase both the versatility and accuracy of compass orientation ability in mice.

If natural sounds provide orientation cues for animals, then it follows that animals may also be attuned to anthropogenic noises. With humans increasingly encroaching on natural habitats, we bring with us the noise of traffic, airplanes, trains, and construction. While some of these auditory cues may provide useful cues for orientation, they may also adversely affect the behavior of animals. For instance, it has been shown that richness and diversity of bird species significantly decreased near a heavily traveled highway (Rheindt, 2003). Additionally, the presence and breeding attempts of grassland birds were significantly reduced up to 1200m from a highway carrying a heavy volume of traffic (Forman et al., 2002). Furthermore, it has recently been reported that birds exposed to high noise levels by living in or near urban areas typically sing at higher pitches, presumably to adapt to the altered spectrum or higher volume of background noise (Slabbekoorn and Peet, 2003). Species or individuals with higher-pitched songs are better able to communicate with conspecifics and consequently less susceptible to harmful effects of anthropogenic noise (Rheindt, 2003). It remains to be determined, however, whether there is an energetic cost to singing higher pitched songs. Therefore, if birds are able to make use of auditory compass information, it is plausible that some anthropogenic noise may be a useful cue for compass orientation, but higher levels of noise can be detrimental to certain species not only by affecting acoustic communication, but perhaps also, by masking natural sources of orientation information. Moreover, it is unknown whether organisms are able to use acoustic information produced by other species (i.e. rodents orienting based on the location of frog choruses). If this is the case, loss of species diversity caused by habitat destruction and fragmentation, may have

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negative effects for many animals that depend on auditory cues produced by other species for orientation.

In conclusion, our results provide preliminary evidence that mice are able to use auditory cues for compass orientation. These results have interesting implications for the processing of auditory information by the central nervous system, and for the relative importance of different types of sensory information in mediating various forms of spatial behavior (i.e. place navigation vs. compass orientation). Future studies may explore what qualities of sound (e.g. volume, frequency, wavelength) are important for auditory compass orientation in rodents. Further study is needed to determine under what circumstances the auditory compass is used under natural conditions, and whether the use of an auditory compass is limited to a few species (e.g.) of mice, or is found in other more taxonomically and ecologically diverse groups of animals.

Figure 1: a) Diagram of the training cage. The base of the cage is made of wood, while the body of the cage consists of hardware cloth (wire mesh), which allows sound to pass into the cage. b) Diagram showing a view of the training cage from above. The nest box was located either 90° clockwise or 90° counterclockwise (90° CCW depicted) from the sound source. Acoustic foam surrounded the cage on three sides to attenuate extraneous noise, and minimize reflection of sound from nearby structures. The fourth side was left open to allow light to enter and so the experimenter had access to the cage.

a)

b)

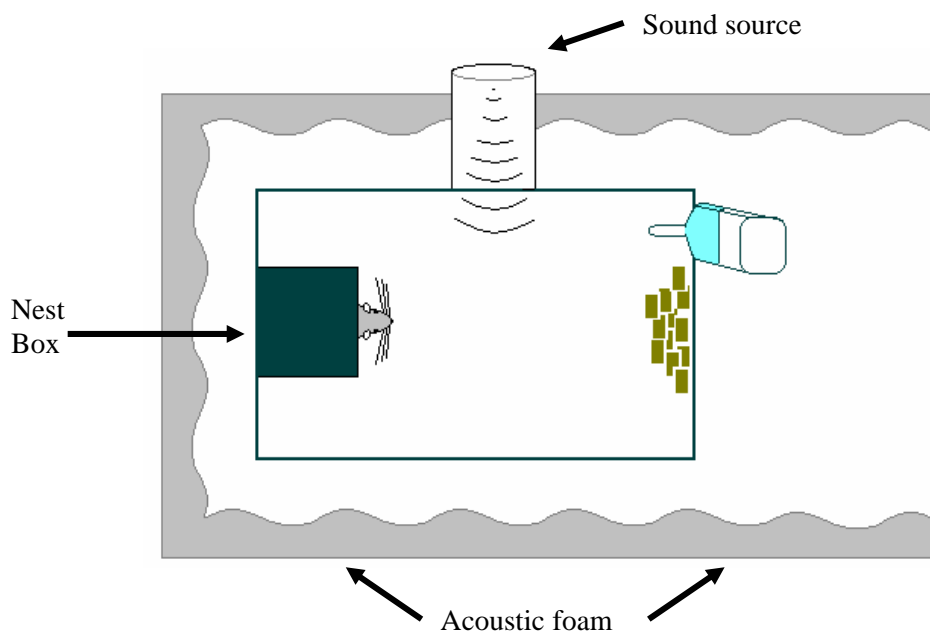Sound source

Nest Box
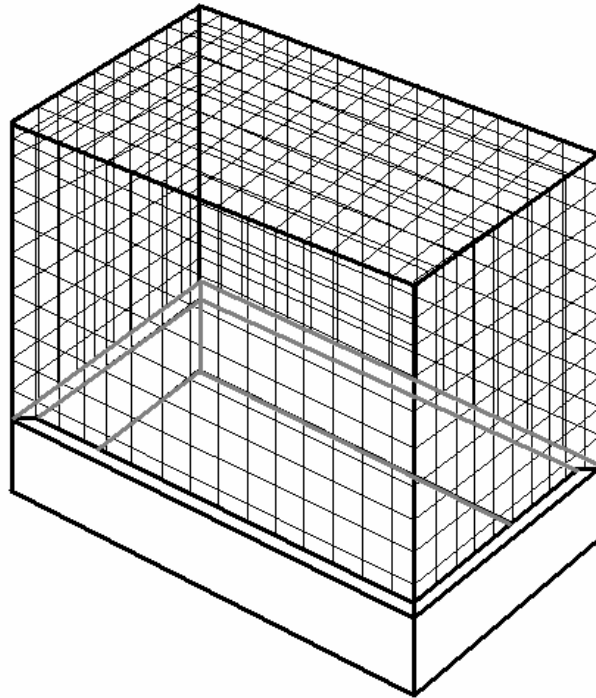
Acoustic foam

Figure 2: Diagram of one set of training shelves (90° CW). The PVC pipe was used to funnel sound from the speakers to each of the training shelves, which contained the mouse cages. The shelves were surrounded on three sides with acoustic foam, which shielded the mice from external noises, and minimized internal reflection of the sound stimulus. The overhead light source was attached to the ceiling, and was directed at the wall opposite the open side of the shelf. This created a light gradient, where the nest boxes were located towards the enclosed end of the shelf (dark end) and food and water were located towards the light end. This caused the mice to build their nests toward the dark end of the cage and thereby learn the location of their nest in a consistent direction relative to the sound source.
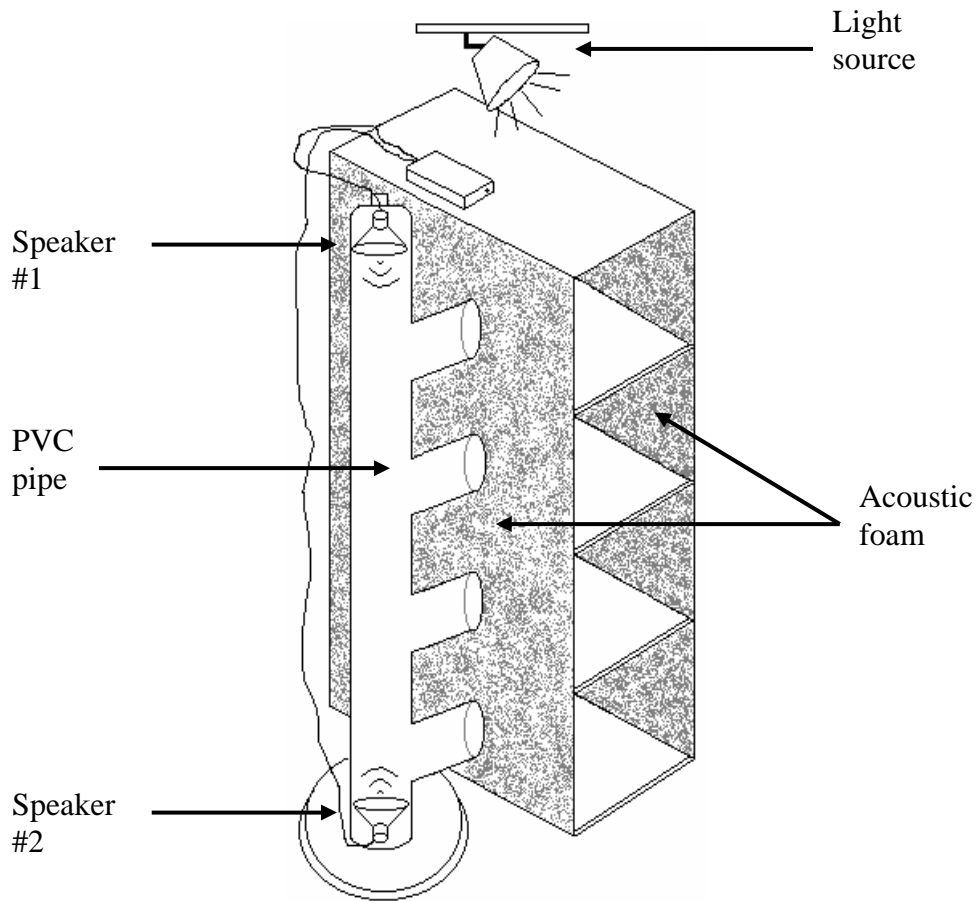
Table 1: Bearings of nests of individual mice tested with a speaker emitting sound from one of four locations (see table heading "position of speaker"). Animals are listed in the order they were tested and are given arbitrary numbers in the table. "Topographic bearing" refers to the absolute position in the arena, "bearing relative to speaker" refers to the position of the nest relative to the "on" speaker, and "bearing relative to trained" refers to the position of the nest relative to the trained direction.

Animal	Trained Direction	Position of Speaker	Topographic Bearing	Bearing Relative to Speaker	Bearing Relative to Trained
1	90° CW	0°	110°	110°	20°
2	90° CCW	90°	350°	260°	350°
3	90° CCW	180°	200°	20°	110°
4	90° CW	90°	210°	120°	30°
5	90° CW	180°	310°	130°	40°
6	90° CW	90°	160°	70°	340°
7	90° CW	90°	200°	110°	20°

Figure 3: Distribution of nest locations plotted relative to the trained or expected direction. The trained direction is at the top of the circle, indicated by the arrow outside the circle. Nest locations from mice trained clockwise (CW) relative to the sound source are indicated by the filled circles. Nest locations from mice trained counterclockwise (CCW) are indicated by the open circles. The mean vector bearing (MVB) of all seven nests was significantly oriented in the trained direction [$23° \pm 35°$ (MVB $\pm$ 95% C.I.), r = 0.795, p < 0.01; solid arrow], and the 95% confidence interval (95% C.I.) includes the trained direction (heavy dashed lines). The mean vector of the mice trained 90° CW to the sound source (solid symbols) was also significantly oriented ($18° \pm 30°$, r = 0.938, p < 0.01; dashed arrow), and the 95% confidence interval for these nests includes the trained direction. The sample size of bearings from mice trained $90°$ CCW (n = 2; open symbols) was too small for statistical analysis.
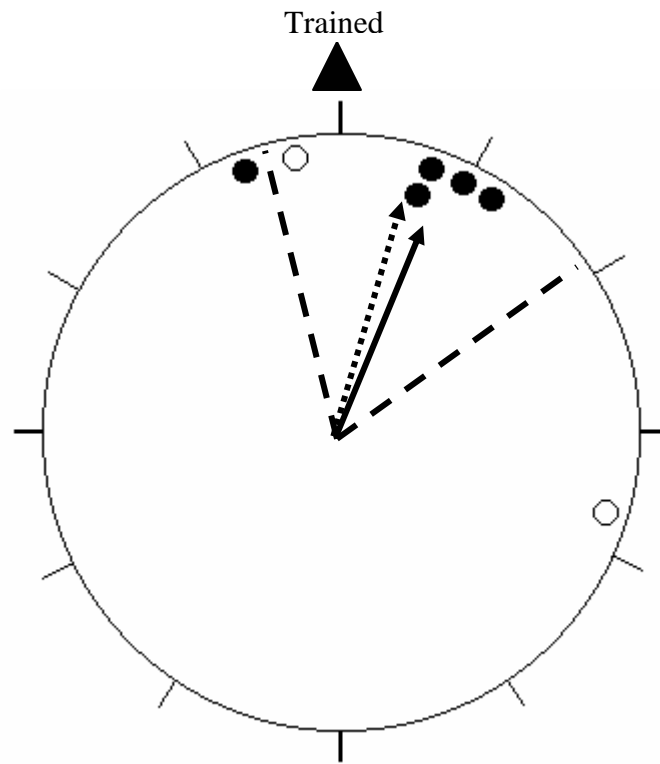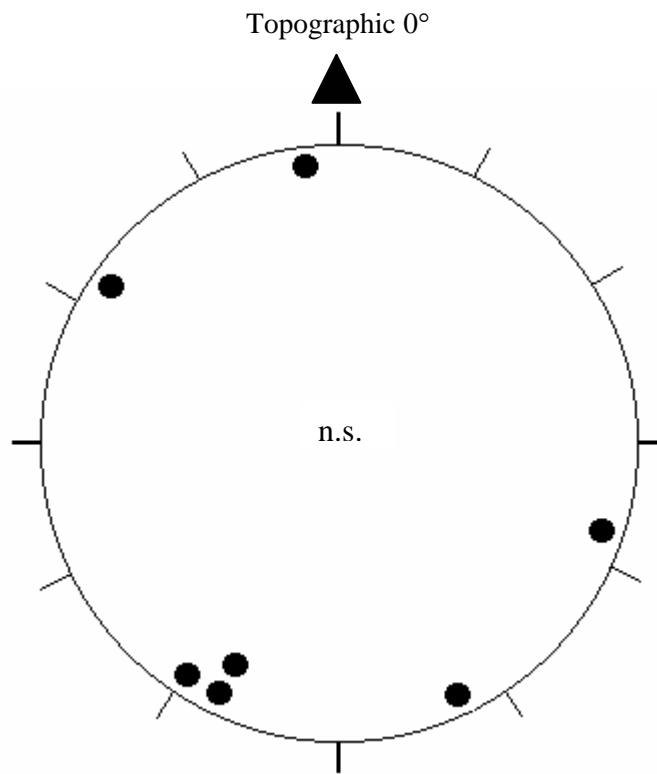

Trained

Figure 4: Distribution of nests plotted topographically, or in their absolute position in the testing arena. Each nest is represented by a solid circle. The distribution is not significant (mean vector = 199°, r = 0.363, n = 7, p = n.s.), suggesting the mice are not responding to an arbitrary (non-auditory) cue.

Topographic 0°

n.s.

LITERATURE CITED

Alexander, R. D. (1961). Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour* **17**, 130-223.

Balda, R. P. and Wiltschko, W. (1991). Caching and recovery in scrub jays: transfer of sun-compass directions from shaded to sunny areas. *Condor* **93**, 1020-1023.

Batschelet, E. (1981). Circular Statistics in Biology. Academic Press.

Bright, M. (1984). Animal Language. Cornell University Press.

Buchler, E. R. (1976). The use of echolocation by the wandering shrew (*Sorex vagra*ns). *Anim. Behav.* **24**, 858-873.

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

Deutschlander, M. E., Freake, M. J., Borland, S. C., Phillips, J. B., Anderson, L. E. and Wilson, B. W. (2003). Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus*. *Anim. Behav.* **65**, 779-786.

Emlen, S. T. (1975). The stellar-orientation system of a migratory bird. *Sci. Amer*. **233**, 102-111.

Forman, R. T. T., Reineking, B., Hersperger, A. M. (2002). Road traffic and nearby grassland bird patterns of in a suburbanizing landscape. *Environ. Manage.* **29**, 782-800.

Forsman, K. A. and Malmquist, M. G. (1988). Evidence for echolocation in the common shrew, Sorex araneus. *J. of Zool.* **216**, 655-662.

Gerhardt, H. C. (1974). Some spectral features in mating call recognition in the green treefrog (*Hyla cinerea*). *J. Exp. Biol*. **61**, 229-241.

Griffin, D. R. (1958). Listening in the Dark. New Haven: Yale University Press.

Harley, H. E., Putman, E. A., and Roitblat, H. L. (2003). Bottlenose dolphins perceive object features through echolocation. *Nature* **424**, 667-669.

Kuhn, H. (1919). Die Orientierung der Tiere im Raum. Fischer, Jena.

Marhold, S., Burda, H., Kreilos, I., & Wiltschko, W. (1997a). Magnetic orientation in common mole rats from Zambia. In: *Orientation and Navigation - Birds, Humans and other Animals* (ed. by the RIN), pp.5-1 - 5-9. Oxford: Royal Institute of Navigation.

Marhold, S., Wiltschko, W., and Burda, H. (1997b). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* **84**, 421-423.

Merritt, R., Purcell, C., and Stroink, G. (1983). Uniform magnetic field produced by three, four, and five square coils. *Rev. Sci. Instrum*. **54**, 879-882.

Muller, R. U., Kubie, J. L., and Ranck Jr., J. B. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J. *Neurosci*. **7**, 1935-1950.

Phillips, J. B. and Borland, S. C. (1992a). Behavioral evidence for use of a light-dependent magnetocreception mechanism by a vertebrate. *Nature* **359**, 142-144.

Phillips, J. B. and Borland, S. C. (1992b). Magnetic compass orientation is eliminated under near-infrared light in the eastern red-spotted newt *Notophthalamus viridescens*. *Anim. Behav*. **44**, 796-797.

Phillips, J. B. and Sayeed, O. (1993). Wavelength-dependent effects of light on magnetic compass orientation in *Drosophila melanogaster*. *J. Comp. Physiol*. **172**, 303-308.

Rheindt, F. E. (2003). The impact of roads on birds: Does song frequency play a role in determining susceptibility to noise pollution? *J. Fur Ornith*. **144**, 295-306.

Rosenzweig, M. R., Riley, D. A., Krech, D. (1955). Evidence for echolocation in the rat. *Science* **121**, 600.

Rossier, J., Haeberli, C., and Schenk, F. (2000). Auditory cues support place navigation in rats when associated with a visual cue. *Behav. Brain Res.* **117**, 209-214.

Slabbekoorn, H. and Peet, M. (2003). Birds sing at a higher pitch in urban noise. *Nature* **424**, 267.

Sutherland, R. J. and Dyck, R. H. (1984). Place navigation by rats in a swimming pool. *Can. J. Psych.* **38**, 322-347.

Taube, J. S., Goodridge, J. P., Golob, E. J., Dudchenko, P. A., and Stackman, R. W. (1996). Processing the head direction cell signal: A review and commentary. *Brain Res. Bull*. **40**, 1-8.

Wehner, R. (1989). The hymenopteran skylight compass: matched filtering and parallel coding. *J. Exp. Biol.* **146**, 63-85.

Wiltschko, W. and Balda, R.P. (1989). Sun compass orientation in seed-caching scrub jays (Aphelocoma coerulescens). *J. Comp. Physiol. A* **164**, 717-721.

Wiltschko, W., Munro, U., Ford, H., and Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature* **364**, 525-527.

Wiltschko, W., Traudt, J., Gunturkun, O., Prior, H., Wiltschko, R. (2002b).

Lateralization of magnetic compass orientation in a migratory bird. *Nature* **419**,

467-470.

Wiltschko, W. and Wiltschko, R. (1972). Magnetic compass of European robins.

Science **176**, 62-64.

Wiltschko, W., Wiltschko, R., and Munro, U. (2000a). Light-dependent

magnetoreception in birds: does directional information change with light

intensity? *Naturwissenschaften* **87**, 366-369.

Wiltschko, W., Wiltschko, R., and Munro, U. (2000b). Light-dependent

magnetoreception in birds: the effect of intensity of 565nm green light.

Naturwissenschaften **88**, 387-390.

CONCLUSTIONS AND FUTURE DIRECTIONS

Magnetic Compass Assay

The results of the magnetic compass assay in laboratory mice indicate that mice are able to use the magnetic field as a source of directional compass information. The development of this assay is important for several reasons. In contrast to a similar assay developed in Siberian hamsters (Deutschlander et al., 2003), the orientation of mice in this assay is unimodal (towards one end of the trained axis). This property will allow the investigation of the mechanism of magnetoreception because it allows the evaluation of certain behavioral responses (i.e. whether mice exhibit a reversal in orientation when the magnetic inclination is inverted) that will enable us to determine if mice have a polarity or inclination compass (see Wiltsckho and Wiltschko, 1972; Phillips, 1986b; Marhold et al., 1997a,b).

Additionally, this assay will be important to determine whether mice have light-dependent or non-light-dependent magnetic compasses. Previous experiments have shown that newts and birds alter magnetic compass orientation under monochromatic light of different wavelengths (Phillips and Borland, 1992a; Wiltschko et al., 1993; Wiltschko and Wiltschko, 2001), and were unable to use their magnetic compass in total darkness (Phillips and Borland, 1992b), suggesting that light is an essential component of magnetoreception. On the other hand, mole rats are able to use their magnetic compass in total darkness (Marhold et al., 1997a,b; Kimchi and Terkel, 2001), and they show behavioral responses (i.e. shifts in magnetic orientation after pulse remagnetization) indicating that particles of magnetite may be involved in the mechanism of magnetoreception (Marhold et al., 1997a). Therefore, this assay can be used to explore whether mice have properties of a light-dependent (i.e. wavelength-dependent shifts in magnetic compass orientation) or a non-light-dependent (i.e. magnetic compass

orientation in total darkness and shifts in magnetic compass orientation in response to pulse remagnetization) magnetic compass. These results will then help to elucidate the properties of the mechanism of magnetoreception in mammals and clarify whether magnetoreception is more closely linked to phylogeny or ecology.

Because this assay was developed in laboratory mice, the potential for behavioral genetic manipulations is available through selective use of mutants and knockouts. Future studies employing this assay will be aimed at exploring the function of certain genes suspected to be involved in magnetoreception. For example, Ritz et al. (2000) suggested cryptochromes, a newly discovered photoreceptor involved in the entrainment of circadian rhythms (Cashmore et al., 1999), as a candidate gene involved in the mechanism of magnetoreception. Mice with this gene removed [cryptochrome (*Cry*) knockout mice] can be tested using this assay to determine if this gene plays a role in magnetoreception.

Furthermore, the assay could be used to test other strains of genetic knockout mice for potential effects. If cryptochromes turn out to be involved in magnetoreception, the possibility exists that the magnetic compass may have evolved from the circadian system and, thus, other genes involved in the circadian mechanism may play a role in magnetoreception. For example, the mammalian *period* genes (*mPer1, mPer2, mPer3*) are homologs of the drosophila *Period* gene (*dPer*), which has a specific regulatory function in the circadian system (Dunlap 1999). *MPer1* and *mPer2* are important components of the circadian system involved in light-induced phase shifts (Wakamatsu et al., 2001), however, it has recently been shown that *mPer3* is not a necessary component for circadian rhythm function in mice (Shearman et al., 2000). Also, the mammalian *timeless* gene (*mTim*) is a homolog of drosophila *timeless* (dTim), which is known to

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have a specific circadian function (Dunlap, 1999). There is, however, no evidence that *mTim* has a circadian function in mammals (Gotter et al., 2000). Both *mPer3* and *mTim* knockout mice are available, and can be used in the newly developed assay to investigate whether these genes are involved in magnetoreception. Clearly, laboratory mice provide a powerful tool for investigating the physiological and genetic components controlling behavior, thus the newly developed magnetic compass assay in laboratory mice represents a significant advance in our ability to investigate molecular mechanisms underlying magnetoreception.

Auditory Compass Assay

The results of our attempt to develop an auditory compass assay in laboratory mice indicate that mice can be trained to use a single auditory cue as a source of directional compass information. This assay is the first known example of auditory compass orientation in any organism and it has several interesting implications. The discovery that auditory, as well as visual and other light-dependent (e.g., magnetic), cues can be used as sources of compass information suggests that there may be a common brain center(s) involved in processing compass information ("compass center") which receives input from multiple sensory modalities (visual, auditory, magnetic). Moreover, the evidence reported here for an auditory compass in mice contrasts with the failure to find compelling evidence for the use of auditory cues for spatial orientation (place navigation) in rodents (Sutherland and Dyck, 1984; Rossier et al. 2000). This suggests that the system(s) responsible for processing compass information may be distinct from those involved in processing spatial information. Consistent with the existence of separate processing systems for spatial and directional information, distinct classes of

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cells in the hippocampus have been shown to track an animal's spatial location (place cells), and the direction that it is facing (head direction cells) (Muller et al., 1987; Taube et al., 1996).

The evidence for an auditory compass in mice suggests that auditory cues present under natural conditions (e.g. streams, calls of neighboring animals, and even anthropogenic sounds) may provide an important source of directional compass information for at least some rodents. Further study is needed to determine whether mice will use sounds of any frequency in their range of hearing for compass orientation, or preferentially use frequencies that are audible over longer distances ("sound windows"), as has been found for the auditory communication signals of other species (Slabbekoorn, et al., 2002).

We plan to use the auditory compass assay as a control for behavioral genetic experiments aimed at dissecting the molecular basis of magnetoreception in rodents. Typically, behavioral genetic experiments using knockout mice require a battery of control experiments to determine whether any behavioral deficit is specific to the response being investigated or, instead, results from a non-specific effect of the missing gene product on the motivation or physiological condition of the mouse (Crawley and Paylor, 1997; McIlwain et al., 2001). However, multiple redundant sources of compass information have been shown to be utilized by numerous animals for orientation behaviors as diverse as foraging, migratory orientation, homing, and nest positioning (e.g., von Frisch, 1967; Ferguson, 1971; Arendse, 1978; Dejong, 1982; Wiltschko, 1983; Deutschlander et al., 2003). For example, migratory birds will use directional information from the sun compass, magnetic compass, star compass and, at least in some cases, polarized light compass to orient in a seasonally-appropriate migratory direction

(Wiltschko, 1983). Consequently, by comparing mice trained to exhibit the same behavior (e.g., positioning of nests in a learned direction), but using different sources of compass information (e.g. auditory and magnetic), the battery of tests normally required to determine whether effects of the genetic knockout are specific to (e.g.) the magnetic compass can potentially be reduced to a single experiment. Therefore, evidence that cryptochrome knockout mice ($mCry1^- / mCry1^-$ or $mCry2^- / mCry2^-$) show a deficit in magnetic compass orientation, but still are able to use an auditory compass, would strongly suggest that the missing gene is playing a role in magnetoreception, rather than having an unspecified effect on physiology, motivation, or behavior. If, as we expect, one of the cryptochromes is involved in the light-dependent magnetic compass, the mouse system provides the exciting possibility of using behavioral genetic analyses to identify the molecular alterations that have occurred during the evolution of one sensory mechanism (circadian photoreception) into an entirely different sensory system (light-dependent magnetoreception).

LITERATURE CITED

Arendse, M. C. (1978). Magnetic field detection is distinct from light detection in the invertebrates *Tenebrio* and *Talitrus*. *Nature* **274**, 358-362.

Cashmore, A., Jarillo, J., Wu, Y-J. and Liu, D. (1999). Cryptochromes: blue light receptors for plants and animals. *Science* **284**, 760-765.

Crawley, J. N. and Paylor, R. (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Horm. Behav*. **31**, 197-211.

DeJong, D. (1982). Orientation of comb building by honeybees. *J. Comp. Physiol.* **147**, 495-501.

Deutschlander, M. E., Freake, M. J., Borland, S. C., Phillips, J. B., Anderson, L. E. and Wilson, B. W. (2003). Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus*. *Anim. Behav*. **65**, 779-786.

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell* 96, 271-290.

Ferguson, D. E. (1971). The sensory basis of orientation in amphibians. *Ann. N.Y. Acad. Sci.* **188**, 30-36.

Gotter, A. L., Manganaro, T., Weaver, D. R., Kolakowski Jr., L. F., Possidente, B., Sriram, S., MacLaughlin, D. T., and Reppert, S. M. (2000). A time-less function for mouse *Timeless. Nat. Neurosci*. **3**, 755-756.

Kimchi, T. and Terkel, J. (2001). Magnetic orientation in the blind mole rat *Spalax* ehrenbergi. J.Exp. Biol. **204**, 751-758.

Marhold, S., Burda, H., Kreilos, I., & Wiltschko, W. (1997a). Magnetic orientation in common mole rats from Zambia. In: *Orientation and Navigation - Birds, Humans and other Animals* (ed. by the RIN), pp.5-1 - 5-9. Oxford: Royal Institute of Navigation.

Marhold, S., Wiltschko, W., and Burda, H. (1997b). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* **84**, 421-423.

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., and Paylor, R. (2001). The use of behavioral test batteries: Effects of training history. *Physiol. Behav*. **73**, 705-717.

Muller, R. U., Kubie, J. L., and Ranck Jr., J. B. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J. *Neurosci*. **7**, 1935-1950.

Phillips, J. B. (1986b). Two magnetoreception pathways in a migratory salamander. *Science* **233**, 765-767.

Phillips, J. B. and Borland, S. C. (1992a). Behavioral evidence for use of a light-dependent magnetocreception mechanism by a vertebrate. *Nature* **359**, 142-144.

Phillips, J. B. and Borland, S. C. (1992b). Magnetic compass orientation is eliminated under near-infrared light in the eastern red-spotted newt *Notophthalamus viridescens*. *Anim. Behav.* **44**, 796-797.

Ritz, T., Adem, S., and Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. *Biophys. J.* **78**, 707-718.

Rossier, J., Haeberli, C., and Schenk, F. (2000). Auditory cues support place navigation in rats when associated with a visual cue. *Behav. Brain Res.* **117**, 209-214.

Shearman, L. P., Jin, X., Lee, C., Reppert, S. M., and Weaver, D. R. (2000). Targeted disruption of the *mPer3* gene: subtle effects on circadian clock function. *Molec. Cell. Biol.* **20**, 6269-6275.

Slabbekoorn, H., Ellers, J., and Smith, T.B. (2002). Birdsong and sound transmission: The benefits of reverberations. *Condor* **104**, 564-573.

Sutherland, R. J. and Dyck, R. H. (1984). Place navigation by rats in a swimming pool. *Can. J. Psych*. **38**, 322-347.

Taube, J. S., Goodridge, J. P., Golob, E. J., Dudchenko, P. A., and Stackman, R. W. (1996). Processing the head direction cell signal: A review and commentary. *Brain Res. Bull*. **40**, 1-8.

von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Cambridge: Harvard University Press.

Wakamatsu, H., Takahashi, S., Moriya, T., Inouye, S. T., Okamura, H., Akiyama, M., and Shibata, S. (2001). Additive effect of mPer1 and mPer2 antisense oligonucleotides on light-induced phase shift. *NeuroReport* **12**, 127-131.

Wiltschko, W. (1983). Compasses used by birds. *Comp. Biochem. Physiol. A* **76A**, 709-717.

Wiltschko, W., Munro, U., Ford, H., and Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature* **364**, 525-527.

Wiltschko, W. and Wiltschko, R. (1972). Magnetic compass of European robins. *Science* **176**, 62-64.

Wiltschko, W. and Wiltschko, R. (2001). Light-dependent magnetoreception in birds: the behavior of European robins, *Erithacus rubecula*, under monochromatic light of various wavelengths and intensities. *J. Exp. Biol*. **204**, 3295-3302.

Appendix A **History of events during the development of the magnetic compass assay in laboratory mice.**

Dates	Problem or Event	Solution
3/17/02-4/5/02	First odor bias. Around 270° -330° topographic.	Began cleaning sides of arena along with cleaning the floor of the arena.
4/9/02-4/18/02	Nest building problem. Many "bad" nests or no nests.	Turned on A/C in fan room to cool down training and testing rooms. Colder = nests
4/30/02-5/9/02	Electric fence on at farm next to mouse building. Saw orientation along the 135°-315° axis when plotted relative to trained.	Electric fence was rewired so the section by the mouse building was permanently off.
5/22/02-5/30/02	Topographic bias (odor bias) around 210°-280°	Repainted arena on 6/5/02
6/7/02-6/12/02	Topographic bias at 240°-325° still present.	Cooling unit emitting high pitched noise from back of hub building (approximately east of the mouse building) was possible source of bias. Placed soapstone in front of cooling unit to block the noise
6/19/02-6/22/02	New power supply for testing light was placed in hub building (old one was in training room and emitting a high frequency sound). I forgot to put a timer on the new power supply and mice were exposed to constant light in testing and they built their nests in the center of the arena.	Put timer on power supply
6/27/02	Put vertical coil up, but did not ground it or connect it to the power supply.	
6/24/02-7/7/02	Bias to topographic north, possibly because more light was entering the north corner b/c the curtains were too small as a result of adding the vertical coil.	Attached Velcro to the curtains and the aluminum coil frame so the curtains would "stick" closed and not allow any light to sneak in.
7/25/02-7/28/02	Odor bias around 170°-190°	On 7/29/02 used ethanol to clean floor of arena.
7/30/02-8/15/02	Problems with doors creaking and noises on cement floor in testing.	Installed carpet in testing room and oiled doors. Also put carpet pieces on training cages to expose mice to the carpet odor.

8/19/02-8/22/02	Poor orientation in mice, possibly from carpet odor or chipped paint in arena.	Attempt to repaint arena, but make slow progress, therefore we switch to the plastic arena on 9/5/02…Also when we put connectors on the coils.
10/2/02	Still having odor bias problems	Began using only 70% ethanol to clean sides and floor of arena.
9/23/02-10/2/02	Mice not orienting well	Put up small curtains in the corners of the arena to prevent light from entering through openings in the curtains
10/7/02-10/17/02	Mice had different pine shavings in training than in testing and were poorly oriented.	Use the same bag of shavings in training and testing for each mouse.
11/4/02-11/9/02	Strong odor bias	Began cleaning floor and sides of arena first with BioKleen and then with 70% ethanol. This procedure is currently used.
9/19/02-11/11/02	Problems with multiple designs of release devices. See log book "release device history"	Designed the plexiglass release device currently being used. Release device complete on 1/4/02
1/4/03-1/8/03	Burning smell from a radiator we put in the training room to heat the building. Also a source of RF.	We removed the space heater.
1/10/03-1/17/03	Had many windy nights. Mice did not orient well on nights with $\geq$ 15 mph winds.	From this point on we did not test on nights with $\geq$ 15 mph winds.
1/17/03-1/30/03	"Shadow bias" Bias around 270° topographic. Rotated arena by 180° and the bias was still at 270°, therefore, it was not an olfactory bias.	First used sock to cover the holding tube of the release device so mice could not see light and shadows. This solved the problem and John then made a 2 layer cloth cover for the holding tube (2/2/03)
2/9/03	Beginning of test series	
2/28/03-4/9/03	Nest building problem found to be caused by male cat that was spraying around the hub building.	Hosed down the hub building and kept our female cat inside.
6/1/03	Vertical coil was connected to a power supply and grounded sometime at the beginning of June.	This provided additional shielding from RF noise to mice in testing.
6/20/03	Last point in test series	

6/22/03-6/23/03	Cage in training flooded on 6/22 and caused a strong odor.	Did not count data collected on 6/22 and 6/23 or future tests when odor from a flooded cage was a concern.
6/26/03-12/17/03	Attempting to get response back.	
8/14/03-10/1/03	Problems with voltage at BTF and specifically with the testing light power supply. 8/19/03 voltage was increased so light intensity was 28lux, but it kept decreasing. 9/5/03 voltage was increased so light intensity was 32lux, but then it continued to increase.	Tech electric replaced our transformer with a new one on 9/17/03. I put a new bulb in testing on 9/30 but the light intensity was 44lux. I decreased the voltage so the intensity was back to 30lux where we saw good orientation. No problems since.
11/20/03-11/21/03	135° trained direction was shielded with aluminum screening to prevent RF exposure	
12/2/03-12/17/03	Vertical coil, shielding in training, and wires from the wall conduit were removed because of possible RF	
1/2/04-present	Wires are back in the conduit on the wall next to the training shelves.	

Effects of radio frequencies on magnetic compass orientation in C57BL/6J laboratory mice.

The most recent model for light-dependent magnetoreception involves interactions of the magnetic field with electrons in a radical pair based photoreceptor mechanism. Recently, Ritz et al. (2000) modeled this system and predicted that electromagnetic fields in the radio frequency (RF) range (i.e., 1-40 MHz) should disrupt the hyperfine interactions that underlie the magnetic field's effect. This would therefore interfere with the ability of the organisms to derive directional information from the earth's magnetic field (Ritz et al. 2000). Recent experiments in birds (Ritz et al. 2004) and newts (J.B. Phillips, unpubl. data) support this hypothesis. In both cases, animals were randomly oriented when exposed to radio frequencies (0.1-10 MHz), compared to controls that were able to use their magnetic compass for either migratory (birds) or trained shoreward (newts) orientation. These results are consistent with the prediction that radio frequencies are interacting with the underlying radical pair mechanism and preventing the animals from obtaining directional information from the magnetic field.

Recent results from our lab suggest that radio frequencies may also affect the magnetic compass of mice. Male C57BL/6J laboratory mice were trained to build their nests in a specific magnetic direction and were then tested for magnetic compass orientation by recording the position of their nests in a circular testing arena. The testing arena was surrounded by a system of coils that allowed magnetic north to be shifted to geographic north, south, east, or west.

Results from tests occurring between April and June of 2003, as well as from preliminary tests carried out earlier, indicated that mice were orienting in the appropriate

trained direction (Figure 1a). However, this response changed abruptly at the end of June; from July to November of 2003 the mice oriented approximately 180° away from the trained direction (Figure 1b). In addition to a distinct shift in magnetic compass orientation, the mice tested from July through November 2003 showed a sharp decrease in nest-building behavior (from 56% acceptable nests in April/June to 29% acceptable nests in July/November). Thus, it appeared that mice had very suddenly shifted their direction of magnetic orientation and also exhibited a decrease in their motivation for nest building.

With the assistance of Chris Anderson, a doctoral student in the Electrical Engineering Department at Virginia Tech, we found that a wire in a conduit on the wall adjacent to the shelves used to train the mice was acting as an antenna and causing a gradient of radio frequencies (Figure 2-shading). While mice from both time periods were trained in this environment, around the end of June an additional coil that was to be used in future experiments was placed around the testing arena and connected to ground. Unbeknownst to us at the time, this coil acted as a "shield" by blocking electromagnetic waves, including those in the radio frequency range. Thus, the mice tested from April through June were trained and tested in similar environments (high RF), while mice tested from July through November 2003 were trained in an environment with relatively high RF, but were tested in a relatively clean, RF free, environment.

During the July to November 2003 time period, none of the mice exposed to high RF levels in training subsequently built nests in the test arena. The mice that did build nests in testing were those that were exposed to lower levels of RF in training (Figure 2). No such pattern was observed prior to the addition of the coil surrounding the testing

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arena. However, these mice showed a tendency to build their nests in a direction

opposite to the trained magnetic direction (Figure 1b). These results suggest that a

difference in environmental RF between training and testing may have been affecting

both nest building and magnetic compass orientation in mice. While it is unclear how RF

is causing these responses, one possibility, based on predictions made by Ritz et al.

(2000), is that RF is interfering with the mechanism of magnetoreception.

Recently, one of the coils surrounding the testing arena has been removed and an

immediate improvement in nest building has resulted, providing further evidence that the

difference in environmental RF between training and testing was preventing mice from

building nests. Additional tests need to be carried out with the coils removed to determine

whether the mice will also show improvement in magnetic compass orientation (i.e.

orientation in the trained direction rather than opposite the trained direction). If mice

once again exhibit consistent nest building behavior and magnetic compass orientation

after removal of the coil that shielded the testing arena, experiments will be initiated to

provide more rigorous tests of whether RF actually disrupts magnetic compass

orientation in mice.

Figure 1: a) Bearings of nests obtained in experiments run from April 2003 to June 2003 plotted relative to the trained magnetic direction. The data are significantly oriented in the trained direction (mean vector bearing = 357°, r = 0.75, p = 0.001). b) Bearings of nests obtained in experiments run from July 2003 to November 2003. The data are significantly oriented opposite of the trained magnetic direction (mean vector bearing = 180°, r = 0.59, p = 0.05).

a)

b)
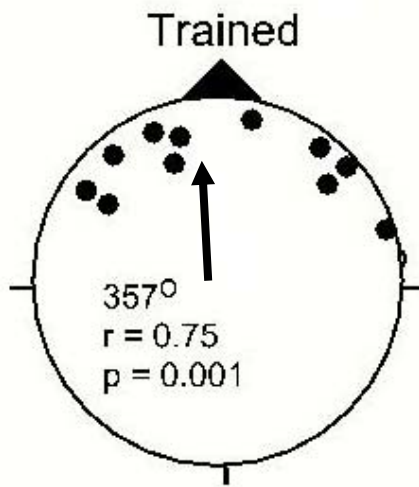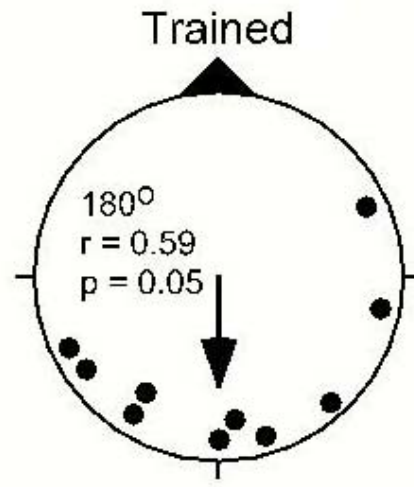
Figure 2: Representation of radio frequency levels found around the training shelves used in the experiments. The effects of RF on nest building behavior of mice are also indicated. The shading represents a gradient in the amount of RF found at each training shelf (darker shading = higher level of radio frequency). The behavior of individual mice tested from July 2003 to November 2003 is indicated next to the shelf where each mouse was trained. Bearings given are in degrees relative to the trained magnetic direction. Clearly, mice exposed to higher levels of radio frequencies in training were less likely to build a nest when tested for magnetic compass orientation. Those mice that did build nests were typically oriented opposite their trained direction.
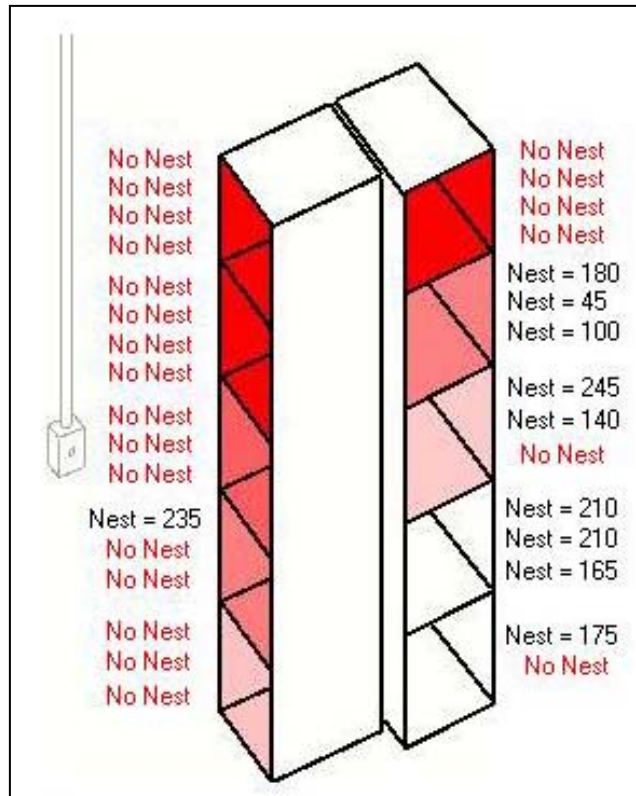
LITERATURE CITED

Ritz, T., Adem, S., and Schulten, K. (2000). A model for photoreceptor-based

magnetoreception in birds. *Biophys. J.* **78**, 707-718.

Ritz, T., Thalau, P., Phillips, J.B., Wiltschko, R. and Wiltschko, W. (2004)

Resonance effects indicate a radical-pair mechanism for avian magnetic compass.

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EDUCATION

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HONORS/AFFILLIATIONS

Phi Beta Kappa
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EXPERIENCE

Research

Master's Student, Department of Biology, Virginia Tech, Blacksburg, VA
August 2001 - present
-Design and conduct behavioral experiments on the Siberian hamster *Phodopus sungorus* and on laboratory mice (strain C57 BL/6) to characterize the mechanism of magnetoreception.

Research Assistant, Department of Biology, Virginia Tech, Blacksburg, VA
September 1998 – August 2001
-Analysis of organic matter dynamics in streams
-Water quality assessment
-Analysis of nutrient processing in streams
-Data Analysis

Research Assistant, Department of Psychology, Virginia Tech, Blacksburg, VA, September 2000 – May 2001
-Undergraduate assistant with study on autonomic responses to frustration
-Administered frustrating tasks to human subjects
-Applied electrodes to human subjects

REU Student: Anatomy and Neurobiology Department, University of Kentucky, Lexington, KY May 2000 – July 2000
-Project title: Dopamine Induced Mitochondrial Death in a Model for
  Huntington's Disease.
-Ran protein analysis assay
-Performed dissections of the striatum from the brains of laboratory rats
-Isolated synaptosomes from striatum of rat brains

Undergraduate Research: Animal and Poultry Sciences Department, Virginia Tech, Blacksburg, VA, January 2000 – May 2000
- Project title: Effects of Intracerebroventricular Injection of DL-methionine and Alimet on Food and Water Intake in chickens.

Lab Technician: Hoffman LaRoche, Belvidere, NJ, Quality Control Laboratories. May 1999 – August 1999
-Prepared assay for analysis of Chlortetracycline in animal feed.

Teaching

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-Currently teaching two sections of Mammalogy Laboratory, Aug. 2002 - present

GRANTS

GRDP (Graduate Research Development Project) Grant Program (Virginia Tech research grant program)
-Characterizing the mechanism of magnetoreception in the Siberian hamster, Phodopus sungorus. Submitted 2/11/02
-Grant funded for $300.00, 4/11/02

Sigma Xi GIAR (Grants in aid of Research) Program
-Exploring the magnetic sense in rodents. Submitted 3/15/02
-Grant denied; May 2002

Sigma Xi GIAR (Grants in aid of Research) Program
-Do laboratory mice (strain C57BL/6J) use an inclination compass or a polarity for magnetic orientation. Submitted 10/15/02
-Grant funded for $500, 1/6/03

<u>American Society of Mammalogists</u> Grants in aid of Research Program
-*Exploring the behavioral and genetic components behind magnetic orientation
 in laboratory mice, strain C57BL/6J.* Submitted 3/1/03
-Grant denied; May 2003

PRESENTATIONS

Edgar, NM 2003. Exploring the magnetic sense in laboratory mice, strain C57BL/6J. Virginia Academy of Sciences 81st annual meeting. Charlottesville, Va.

ABSTRACTS/POSTERS

Pumphrey, B.G., Friedman, B.H., Edgar, N.M., Chan, V. 2001. Autonomic responses to frustration and the Rosenzweig Picture-Frustration Study. Society for Psychophysiological Research (SPR), Montreal, Quebec, Canada.

Rockich, K.T., Edgar, N.M., Fletcher-Turner, A., Maragos, W.F. 2001. Catecholamine-induced alterations in mitochondrial membrane potential and oxidative stress. Society for Neuroscience. Abstr., vol. 27, Program No. 572.14

REVIEWED PAPERS

"Light dependent magnetic orientation in the alpine newt, *Triturus alpestris* (Caudata: Salamandridae)", by F. Javier Diego-Rasilla. Ethology, Ecology & Evolution MS #760. Reviewers: Dave Dommer, Nicole Edgar & John Phillips (Invited review solicited to John B. Phillips).