

# **The Mouse Magnetic Compass**

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## **ABSTRACT**

All five classes of vertebrates use the geomagnetic field for spatial orientation. The geomagnetic field can be used to derive both ‘map’ and ‘compass’ information. There is evidence for two different mechanisms used to sense the magnetic field, the radical pair mechanism (RPM) and the magnetite based mechanism (MBM). C57BL/6 laboratory mice can rely on directional information from the magnetic field to position their nests and to solve a water maze task.

The primary objective of this research was to characterize the magnetic compass of C57BL/6 laboratory mice in the plus water maze task. These experiments explored sources of variation in magnetic responses and investigated the underlying magnetic compass orientation mechanism in C57BL/6 mice. The results provide evidence that the mouse magnetic compass is sensitive to low-level radiofrequency fields, consistent with the use of the RPM for magnetic orientation. Surprisingly, the results also suggest that C57BL/6 mice have a polarity sensitive compass, consistent with the use of a MBM for magnetic orientation.

These experiments confirm that mice have a specialized magnetic compass sense. Furthermore, despite the controlled environment in which these laboratory experiments were conducted, a variety of factors can increase the variability in the response. Future experiments are needed to further characterize the mouse magnetic compass, as there is a possibility of a hybrid magnetic response where both magnetoreception mechanisms could be used for spatial orientation.

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# Introduction

## Magnetic Compass Orientation

Many vertebrates that migrate over long distances, well beyond their range of familiarity (e.g., salmon, song birds, newts and sea turtles), make use of a wide range of directional and spatial cues for navigation and homing. These include sources of directional ('compass') information such as magnetic, polarized light, star, and sun compasses (Ferguson 1971; Diego-Rasilla et al. 2005; Wiltschko & Wiltschko 2009), and sources of geographic position ('map') information such as magnetic, olfactory, and possibly gravitational and infrasound maps (Hagstrum 2000; Freake et al. 2006; Lohmann & Lohmann 2006). The high degree of redundancy evident in the multiple systems used for the map and compass components of long-distance orientation/navigation suggests that there is strong selection for accurate spatial navigation. In contrast to other classes of vertebrates, until very recently, there has been evidence for use of a time-compensated sun compass (Fluharty et al. 1976; Haigh 1979), but not a magnetic compass, in the spatial behavior of epigeic rodents (Deutschlander et al. 2003; Muheim et al. 2006; Phillips et al. 2013).

With the additional evidence for a magnetic sense in rodents, all five classes of vertebrates are now known to use the geomagnetic field for spatial orientation (Wiltschko & Wiltschko 1995; Wiltschko & Wiltschko 2005). The geomagnetic field can be used to derive both 'map' and 'compass' information. Map (i.e., geographical position) information is derived from spatial variation in the inclination and/or intensity of the geomagnetic field. For example, Eastern red-spotted newts *Notophthalmus viridescens* use the vertical alignment of the magnetic field (i.e. magnetic inclination, which varies from horizontal at the magnetic equator to vertical at the north and south magnetic poles) to derive north-south position, while they use the horizontal alignment of the magnetic field lines (i.e., magnetic polarity) to derive compass heading (Wiltschko & Wiltschko 1995; Fischer et al. 2001; Phillips et al. 2002; Freake et al. 2006).

## **Mechanisms of Magnetic Compass Orientation**

There are two mechanisms that involve fundamentally different biophysical processes used for sensing the geomagnetic field, or magnetoreception.

The Magnetite Based Mechanism (MBM) involves particles of biogenic magnetite that activate coupled membrane channels. Responses using the MBM can persist in total darkness and are sensitive to the polarity of the magnetic field. MBM responses are not affected by radio frequency (RF) fields at high intensities up to 4800 nT. However, brief, high intensity magnetic pulses that are strong enough to remagnetize magnetite particles disrupt responses mediated by this mechanism (“pulse remagnetization”; Burda et al. (1990); Marhold et al. (1997); Burger et al. (2010); Winklhofer and Kirschvink (2010)).

The Radical Pair Mechanism (RPM) is based on a light dependent biochemical reaction thought to involve cryptochromes (specialized photopigments best known for their involvement in entrainment of circadian rhythms) that form spin- correlated radical pair intermediates (Ritz et al. 2000; Rodgers & Hore 2009). Responses using the RPM are light dependent and are not sensitive to the polarity of the magnetic field (i.e., are not able to distinguish north from south). However, responses mediated by the RPM are sensitive to the axis of the magnetic field, and use the inclination or slope of the magnetic field to distinguish between the two ends of the magnetic field lines. A unique feature of the RPM is that responses are disrupted by RF fields at frequencies in the range of 1-10 MHz at intensities as low as 15nT and possibly lower (Henbest et al. 2004; Ritz et al. 2004; Thalau et al. 2005; Ritz et al. 2009). Unlike responses mediated by the MBM, responses mediated by the RPM are not affected by “pulse remagnetization” (Winklhofer & Kirschvink 2010).

In animals in which specialized photoreceptors responsible for sensing the geomagnetic field are located in the retina or compound eye (Olcese et al. 1988; Phillips et al. 2010a; Nießner et al. 2013), the RPM-based magnetic compass may cause the magnetic field to be perceived as a pattern of light intensity and color superimposed on an animal’s environment (Ritz et al. 2000; Cintolesi et al. 2003; Solov'yov et al. 2007; Rodgers & Hore 2009; Phillips et al. 2010b).

## **Magnetoreception Mechanisms Used Across Organisms**

The mechanism(s) of magnetoreception differ among taxa. For example, two families of subterranean mole rats that live in dark, underground habitats exhibit spontaneous nest building responses relative to the magnetic field. The properties of this response are consistent with a MBM, i.e. magnetic responses are independent of light and are sensitive to polarity of the field (Marhold et al. 1997). In contrast, the magnetic compass orientation of amphibians and birds exhibits properties consistent with the RPM, i.e. magnetic responses are light dependent and are sensitive to the axis, but not polarity, of the field (Wiltschko & Wiltschko 1972; Phillips 1986b; Phillips & Borland 1992; Muheim et al. 2002; Phillips et al. 2010a; Wiltschko et al. 2009).

Birds and amphibians use both types of magnetoreception mechanisms. Interestingly, in both groups, the RPM appears to provide compass information, while the MBM is used to derive map information (Phillips 1986b; Phillips & Borland 1994; Beason & Semm 1996; Munro et al. 1997; Phillips et al. 2010a).

In contrast to mole rats, amphibians, and birds, relatively little is known about the properties of the newly discovered magnetic compass sense of epigeic rodents (i.e., species that are active above ground and typically have well-developed visual systems (Deutschlander et al. 2003; Muheim et al. 2006)). The available evidence suggests that the magnetoreception mechanism involved in directional ('compass') responses may not be the same in different groups of rodents (Marhold et al. 1997). The only evidence concerning the magnetoreception mechanism underlying a magnetic compass response in a mammal is for mole rats (*Cryptomys* spp.), which exhibit properties consistent with a MBM. If mice also have a MBM, this would suggest that this mechanism was inherited from a common ancestor. However, because mole rats have evolved to live in subterranean habitats that are devoid of light, reliance on a non-light dependent MBM for compass orientation could be a recent adaptation. In contrast to mole rats, mice are epigeic rodents. Because the visual ecology of mice is more similar to that of the species of amphibians and birds than that of mole rats, mice and other epigeic rodents may have a light-dependent RPM for compass orientation.

Evidence that rodents and other mammals possess a magnetic compass like other vertebrates has been obtained only recently. For example, Siberian hamsters *Phodopus sungorus* and C57BL/6 laboratory mice (whose genome is largely contributed by *Mus musculus domesticus*, which has a worldwide distribution) can rely on directional information from



magnetic cues to position their nests (Deutschlander et al. 2003; Muheim et al. 2006). Common spatial assays can be adapted to further evaluate magnetic compass orientation in rodents.

### **Maze Assays for Spatial Cognition**

There have been a large variety of maze tasks used to study rodent spatial behavior. One of the testing paradigms used to test spatial learning and memory in rats is the radial arm maze (Olton & Samuelson 1976). The testing apparatus includes eight radial arms elevated above the floor with a food reward in a well at the end of each arm. The design ensures that, after checking for food at the end of each arm, the rat is always forced to return to the central platform before making another choice (Olton & Samuelson 1976). Two types of memory are evaluated during the performance in this task—working and reference memory. Working memory is used within a trial, while reference memory is used across trials (Carew 2000).

The Morris water maze was later developed to test the spatial learning capability of the rat in a large circular pool with milky water (Morris et al. 1982). In this assay, rats were required to locate a submerged platform in a fixed position in the water maze that can be approached from any direction. This test provided evidence that rats use distal cues (e.g., visual landmarks located around the water maze) to navigate from different start locations around the perimeter of the pool to the location of the submerged platform. Reference memory is determined by preference for the former position of the platform in probe trials with the platform removed. The spatial component of learning is assessed by quantifying the amount of time rats spend searching in the quadrant in which the submerged platform had been located. The water maze assay has since been adapted for use with other organisms such as laboratory mice (D’Hooge & De Deyn 2001; Vorhees & Williams 2006).

Experiments have also been carried out in a modified 4-arm (plus-shaped) water maze (Wahlsten et al. 2006). Phillips et al. (2013) conducted water maze experiments with mice that were tested under conditions in which an earth-strength magnetic field provided the only consistent source of directional information (using a plus-shaped water maze with a submerged platform located at the end of one of the four arms). The water maze is housed in a radially symmetrical room without directional visual, auditory, vibratory or olfactory cues, and centered in two orthogonally aligned horizontal coils wrapped on the same wooden frame (Merritt et al. 1983). The coils are positioned so that the horizontal component of the magnetic field can be

aligned along the four cardinal compass directions coinciding with any one of the four arms of the maze. The coils are double- wrapped and controlled by reversing the current flow direction in one of the two wraps (Phillips 1986a; Kirschvink 1992). These experiments and earlier nest-building experiments showed for the first time that mice have a well-developed magnetic compass sense (Phillips et al. 2013), but to fully understand the characteristics of their magnetic compass, more experiments are needed.

The primary objective of this research was to characterize the magnetic compass of C57BL/6 laboratory mice in the 4-arm (plus-shaped) water maze task. These experiments explored factors that can contribute to variation in magnetic orientation. This research was also aimed to determine whether mice have an inclination sensitive or a polarity sensitive compass and to investigate the underlying magnetic compass orientation mechanism.

**Chapter One: Variability in Magnetic Compass Response of  
C57BL/6 Mice**

## **Introduction**

Various factors can disrupt the ability for organisms to use the geomagnetic field for spatial orientation, which is required for migration, navigation, and spontaneous alignment responses. When alternative sources of directional information are available (e.g. olfactory, auditory, or visual cues) organisms are more likely to use these cues in preference to the geomagnetic field (Phillips unpublished data).

A variety of biological and biophysical factors can modify or influence an organism's use of magnetic compass orientation. Prior research on C57BL/6 mice has shown that there are a variety of influences on magnetic compass orientation despite attempts to carry out experiments in a controlled laboratory environment. To effectively study trained magnetic compass orientation in the laboratory environment, alternative cues and influences must be minimized. However, magnetoreception is a very specific system that still faces sources of variability in a controlled laboratory environment.

Surprisingly, prior experience in a mouse colony can influence magnetic compass orientation (Phillips unpublished data). For example, when a male caretaker was responsible for the colony, mice tested in a nest building experiment oriented opposite to their trained direction ( $\mu = 157^\circ$ ,  $r = 0.41$ ,  $p < 0.01$ ; Phillips unpublished data). This is consistent with a recent finding of higher stress hormone (corticosterone) levels in mice that are exposed to male experimenters (Sorge et al. 2014). In addition to variability in upbringing, environmental influences may have similar effects.

There are a variety of factors that can originate both outside and inside of behavioral testing facilities that can influence mouse behavior. Low frequency noises from outside can alter the magnetic orientation response of mice. Although experimental buildings are generally insulated from sound, low frequency sounds such as thunder, farm equipment, and fireworks cause a reversal of magnetic orientation relative to the trained direction. High winds (>12 mph) can cause the buildings to make creaking noises that alter magnetic response as well (Phillips unpublished data).

Variation in mouse magnetic responses can also be caused by factors within a testing facility. For example, asymmetrical sounds within a water maze building can cause an altered magnetic response. Asymmetrical sources present during probe trials, can cause mice to orient

topographically and ignore the magnetic field alignment. Additionally, if noises are present during training but not testing in nest building experiments, mice may orient in a magnetic direction away from the sound source, ignoring the individual trained direction (Phillips unpublished data).

Non-directional cues inside a water maze building can also alter magnetic compass orientation response. Even if precautions are taken to eliminate directional olfactory cues, testing more than two mice per litter can result in an increase in scatter of magnetic compass orientation, presumably because of the higher level of littermate odors (Phillips et al. 2013). Furthermore, an asymmetrical light source may enable mice to use visual asymmetries to orient in a water maze (Phillips unpublished data). Non-directional factors such as water temperature can also alter performance in a water maze due to susceptibility of mice to hypothermia (Iivonen et al. 2003).

Subject age and breeding pair age can also influence the use of the magnetic compass in a water maze assay. In previous experiments by Phillips (unpublished data) mice were tested with a narrow age range (60-85 days old) to minimize potential effects of age. For water maze experiments discussed in this chapter, it was necessary to pair mice for breeding at an older age due to a shortage of mice in the colony. Similar to the responses to alternative cues above, a reversal in orientation response was observed in some experiments during this time. This led to investigate whether the age of breeder mice affected the magnetic compass response of their offspring. While these alternative cues and biological influences have been considered, biophysical influences must also be considered as potential sources of variability.

Two known mechanisms used by terrestrial animals for magnetic compass orientation may involve fundamentally different biophysical processes. A unique feature of the radical pair mechanism (RPM) is that responses are disrupted by RF fields at frequencies in the range of 1-10 MHz at intensities as low as 15nT and maybe lower (Henbest et al. 2004; Ritz et al. 2004; Thalau et al. 2005; Ritz et al. 2009). When RF is introduced into a laboratory environment, magnetic compass orientation is either shifted, or lost (Ritz et al. 2004; Malkemper et al. 2015) (Phillips unpublished data). Furthermore, “electromagnetic smog” is found in every urban environment due to RF production from anthropogenic devices. For example, migratory birds could not orient using their magnetic compass due to very low levels of background electromagnetic noise present in the ambient environment on a university campus (Engels et al. 2014). Unintentional “electromagnetic smog” can also be produced within a laboratory by various sources such as

thermostats, motors, unfiltered florescent light sources, and computers (Phillips unpublished data). Shielding a laboratory environment from environmental RF fields is necessary to elicit a trained magnetic compass response in C57BL/6 mice (Muheim et al. 2006; Phillips et al. 2013); however, electromagnetic noise generated by equipment used in experiments (e.g. coils, power supplies) can also contribute to variability in magnetic responses (Phillips unpublished data).

Recently, mice tested in an East or West field (magnetic North set at geographic East or West) in water maze experiments have shown greater scatter in magnetic compass response. This led to investigate whether electromagnetic noise was generated in these two magnetic field alignments and if so, whether it caused an alteration of the response to of magnetic cues.

The purpose of this study is to examine the influences of breeding pair age and electromagnetic noise on mouse magnetic compass orientation response in water maze experiments.

## **Methods**

### *Study Site*

These experiments were performed at the Virginia Tech Behavioral Testing Facility (BTF) in Blacksburg, Virginia. The facility consists of four testing buildings constructed of non-magnetic materials, with a central ‘hub’ building supplying air for heating and cooling, as well as filtered AC and DC power through underground conduits. The water maze experiments take place in one of the testing buildings located 20 meters from the hub building.

### *Water maze*

A water maze experiment was carried out in a modified 4-arm plus shaped maze with C57BL/6 mice and is fundamentally different than the traditional Morris water maze (D’Hooge & De Deyn 2001; Vorhees & Williams 2006). During training in the plus water maze, the submerged platform was located at the end of one of four arms (coinciding with the cardinal compass directions) that could be approached from only one direction. Consequently, the plus water maze requires a greater reliance on directional, rather than spatial, information to solve the task. The water in the plus water maze was made opaque with white tempura paint so that the location of the submerged platform was not visible to the test subjects. Water temperature was maintained between 27.5-29.5 °C. The plus water maze was centered in two orthogonally aligned

horizontal coils wrapped on the same wooden frame (Merritt et al. 1983). The coils are positioned so that the horizontal component of the magnetic field could be aligned along any one of the four cardinal compass directions coinciding with one of the four arms of the maze. The coils were double-wrapped and controlled by reversing the current flow direction in one of the two wraps (Phillips 1986a; Kirschvink 1992).

### *Experimental subjects*

Subjects used were female C57BL/6 laboratory mice between 60-85 days old. C57BL/6 laboratory mice were used because they performed best in the water maze assay of all strains previously tested (Wahlsten et al. 2006). Mice were originally purchased from Jackson Labs and then bred in our laboratory colony, which is maintained on a L: D (15:9) light cycle. Groups of 6-8 mice were transported by car in grouped cages from the breeding colony to the BTF in the morning (8:00-10:00) and placed in individual cages on a holding shelf outside the testing room. The light cycle in the holding room was the same as the breeding colony L: D (15:9). During all tests, the holding shelf was shielded to minimize radio frequency interference (RFI) with a maximum signal strength of  $\sim 0.05$  nT (0.1-100 MHz).

### *Training*

In the afternoon (14:00-16:00) of the same day the mice were transported to the BTF, they were given two brief ( $< 60$  sec) training trials. A Plexiglas partition was used to restrict the movements of the mouse to one arm of the maze with the submerged platform at the outer end. For each training trial, a mouse was removed from the holding shelf in its cage. The lid of the cage was removed, and the mouse was carried into the testing room in its holding cage. The mouse was grasped gently by the base of the tail, and placed into the training arm facing the center of the maze. The observer remained in the room until the training trial was complete. The position of the observer changed between both training trials to reduce the likelihood of the observer being a potential cue. The mouse would swim until it encountered the submerged platform and would then climb out of the water and from there, up onto the side of the plus maze. The mouse was then given a delay of 10 seconds before it was returned to its holding cage, carried once around the water maze and then returned to the outer room. The cage was placed on a drying shelf beneath a 125 W infrared light to dry the mouse's fur. The back half of the drying shelf was shaded so the mouse could thermoregulate by moving between the front and back of

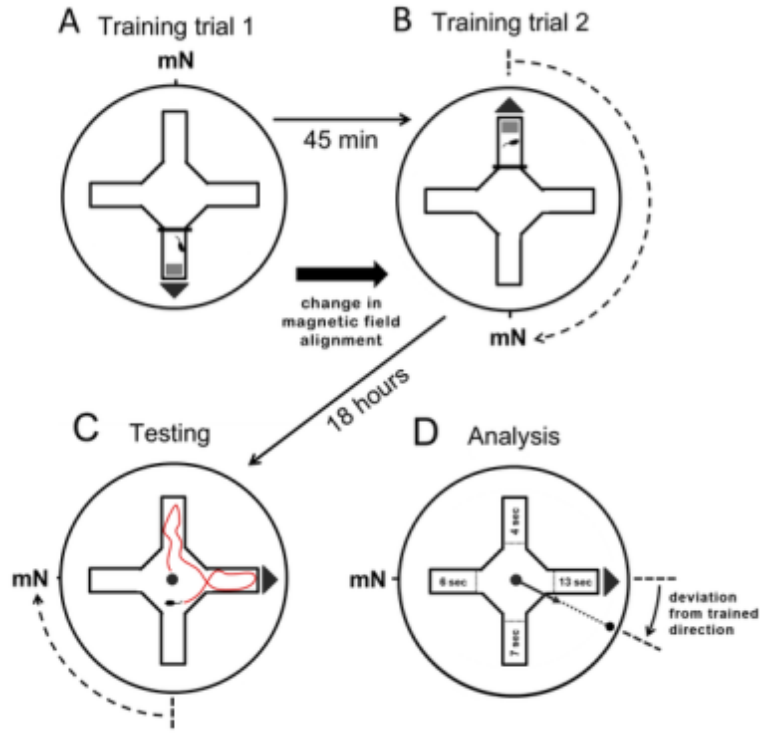
the cage. After the fur of each mouse was dry (approximately 10 min), its cage was moved backed to the holding shelf, where it had access to water and food available *ad libitum*.

After an interval of at least 45 minutes, each mouse was given a second training trial in the opposite arm of the maze. Prior to the second training trial, the alignment of the magnetic field was rotated by 180 degrees so that magnetic north was in the same alignment relative to the training arm and to the submerged platform at the end of the arm as in the initial training trial. As in the first training trial, the mouse was allowed to crawl up from the submerged platform onto the wall of the maze and remain there for 10-second delay before being placed back in its cage. It was then transported in the cage by an indirect route to the drying shelf in the outer room (Figure 1.1). In both training and testing, the holding room was maintained at a temperature of 25.5-30°C and the room containing the water maze was maintained at 26.5-31°C and relative humidity of 55-85%.

### *Testing*

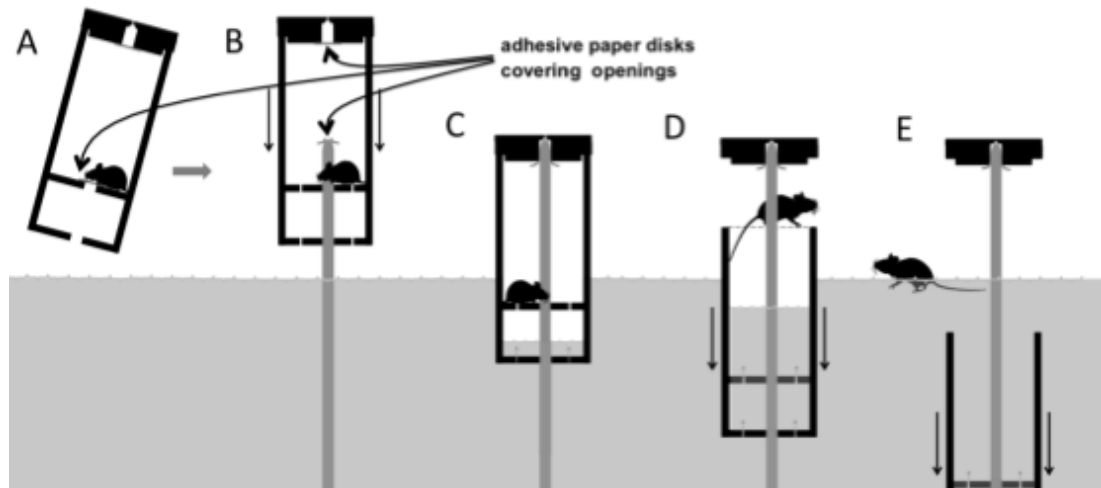
Each mouse was given a single testing “probe” trial the following morning. For testing, the submerged platform and the partition used to isolate one arm of the maze for training were removed. The mouse was transferred from its holding cage to an opaque cylindrical plexiglass container. The cylinder is rotated slowly clockwise around its long axis as the mouse is carried into the testing room. The cylinder is then placed in the center of the arena from a constant direction onto a round-topped acrylic rod in the center of the plus maze to serve as the release device (Figure 1.1). Once the release device is in place, the observer quietly leaves the room and closes the intervening doors. The release device is designed to gradually fill with water and, after ~50 seconds, sink below the surface of the water forcing the mouse to swim (Figure 1.2).





**Figure 1.1: Plus water maze training and testing protocol.**

Figure used with permission from Phillips et al. (2013). Mice with no prior experience with the water maze were given two training trials in different arms of the water maze with the submerged platform in the same relative alignment to the magnetic field (A & B). In this example, the mouse is being trained to orient to magnetic south. For testing the following morning, the submerged platform was removed (C). The mice were released individually from a central release device (see Figure 1.2) and had free access to all four arms of the maze. Magnetic field alignment was changed between trials, and data pooled across testing groups, so an equal number of mice were tested in each of the four magnetic field alignments, i.e., magnetic North (mN) at geographic North, East, South or West; each mouse tested only once in one of the four fields. The tracking software calculated orientation direction as the vector sum of the times spent in the four arms during the 60 sec testing trial (D).



**Figure 1.2: Plus water maze release device.**

Figure used with permission from Phillips et al. (2013). A) Mouse transported from the holding shelf to the testing arena inside the opaque release device. Disks of paper towel attached with a ring cut from a self-adhesive label covered openings in both the top and bottom of the chamber in which the mouse was confined to prevent its tail from being pinched. B) The release device was then slid slowly down over a vertical plexiglass rod extending up above the water in the center of the plus maze. The top of the rod inserted firmly into a socket on the inside of the lid. C) The buoyancy of the lower section of the release device and the rate of inflow of water through four small openings were adjusted so when the release device was in place, the lower section remained snugly against the lid long enough for the observer to quietly exit the testing room and close the intervening door without being observed. D) Water slowly entered the lower chamber, and eventually the upper chamber, through 4 small holes, causing the lower section of the release device to gradually separate from the lid and sink lower in the water. As the lower section filled with water and separated from the lid, the mouse crawled up onto the top rim of the cylinder and spent 20-35 seconds walking around the rim looking in all directions. E) The lower section of the release device gradually submerged, taking ~50 sec for the top rim to sink below the surface, forcing the mouse to swim.

### *Statistical Analysis*

Standard circular statistics were used to analyze directional responses (Batschelet 1981). Custom Watermaze 2.6 Software, written by Rachel Muheim, computed the mean vector bearing ( $\mu$ ) and mean vector length ( $r$ ) from individual mice by vector addition of the times spent in each of the four arms. The mean vectors from individual mice were grouped relative to the water maze arena (topographic bearings), relative to the alignment of magnetic north (magnetic

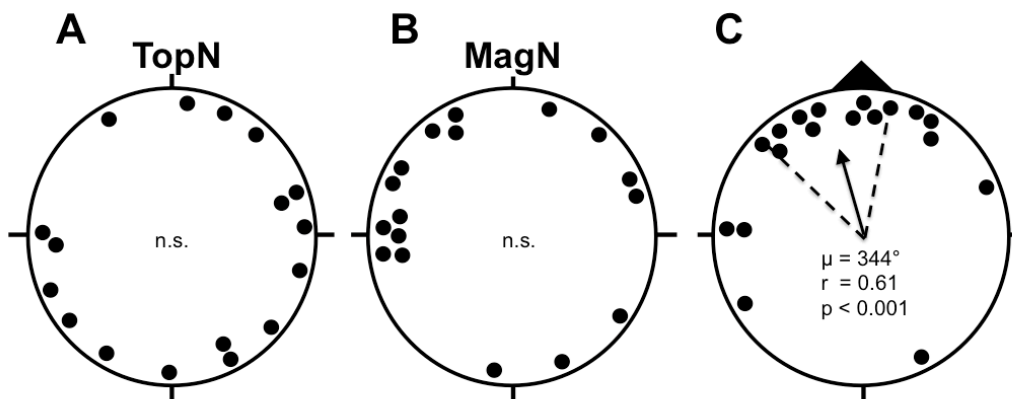
bearings), and relative to the trained magnetic direction (relative to trained bearings, or RTT). A Rayleigh test was used to test for a non-random distribution of bearings. The 95% confidence intervals were used to determine whether the mean vector bearing from the distribution of individual bearings included the trained magnetic direction.

To investigate the effect of breeding pair age on water maze performance, a Watson's  $U^2$  test was used to compare the distribution of RTT bearings obtained from mice born from breeding pairs that were older than 118 days with that of mice born from breeding pairs that were younger than 118 days. The mean age of the breeding pairs that produced mice used in these experiments was 118 days.

To investigate whether a problem with the magnetic field producing stimulus was affecting the responses of the mice, the distribution of RTT bearings obtained from mice tested in North and South fields was compared to the distribution of RTT bearings obtained from mice tested in East and West fields (Watson  $U^2$  test). To confirm that a problem with the coil stimulus was interfering with the magnetic compass orientation in the East and West fields, I compared responses of mice that had been trained in the East and West fields versus North and South fields (Watson  $U^2$  test). All statistics were performed with Oriana: Version 4.

## **Results**

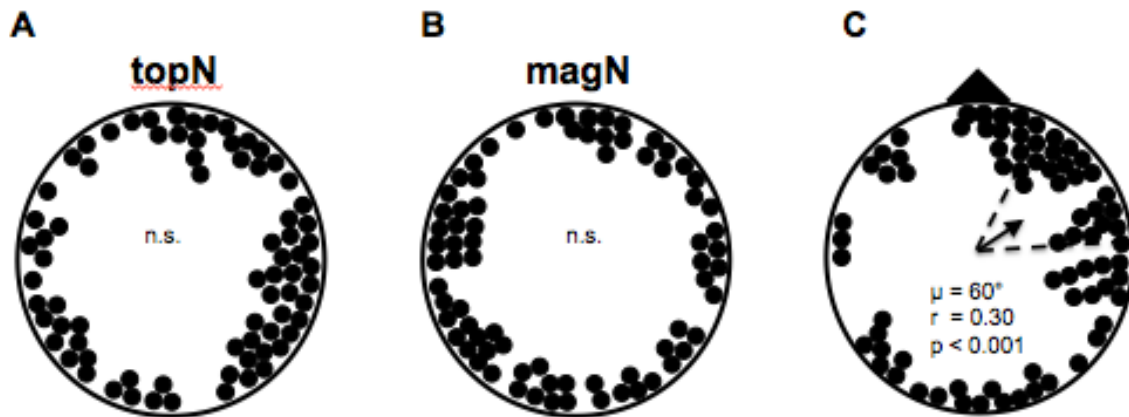
Laboratory mice can be trained to orient in a specific direction relative to the magnetic field (Phillips et al. 2013)(Arnold & Snyder unpublished data; Figure 1.3). We tested mice for magnetic compass orientation in the 4-armed "plus" water maze assay.



**Figure 1.3: Fall 2013 replication of 'plus' water maze response.**

(Arnold and Snyder unpublished data) (A) The distribution of topographic bearings and (B) the distribution of magnetic bearings were indistinguishable from random ( $p > 0.10$ , Rayleigh test). In contrast, (C) The distribution of bearings relative to the trained magnetic direction (black triangle) was non-randomly distributed ( $p < 0.001$ ; Rayleigh test), and the 95% confidence interval for the mean vector bearing contained the trained direction. Each data point is the directional response of a single mouse tested in one of the four magnetic field alignments (see Methods & Methods). The arrow in the center of is the mean vector for distributions of bearings that are non-randomly distributed. The length of the arrow is proportional to the mean vector length ( $r$ ), a measure of the clustering of bearings ranging from 0 to 1; radius of the circle corresponds to  $r = 1$ . Dashed lines show the 95% confidence interval for the mean vector bearing (Batschelet 1981). 'n.s.'- not significant.

The distributions of bearings obtained from the 75 mice tested for magnetic compass orientation were indistinguishable from random when the bearings were pooled with respect to topographic north (i.e., absolute bearings ignoring magnetic field alignment;  $p > 0.10$ , Rayleigh test, Figure 1.4a) and with respect to magnetic north (ignoring trained magnetic direction; Figure 1.4b,  $p > 0.10$ , Rayleigh test). In contrast, the distribution of RTT bearings was significantly oriented  $60^\circ$  clockwise of the trained direction, and the 95% confidence intervals did not contain the trained direction ( $\mu = 60^\circ \pm 29.37^\circ$ ,  $r = 0.30$ ,  $p < 0.001$ ; Rayleigh test; Figure 1.4c).



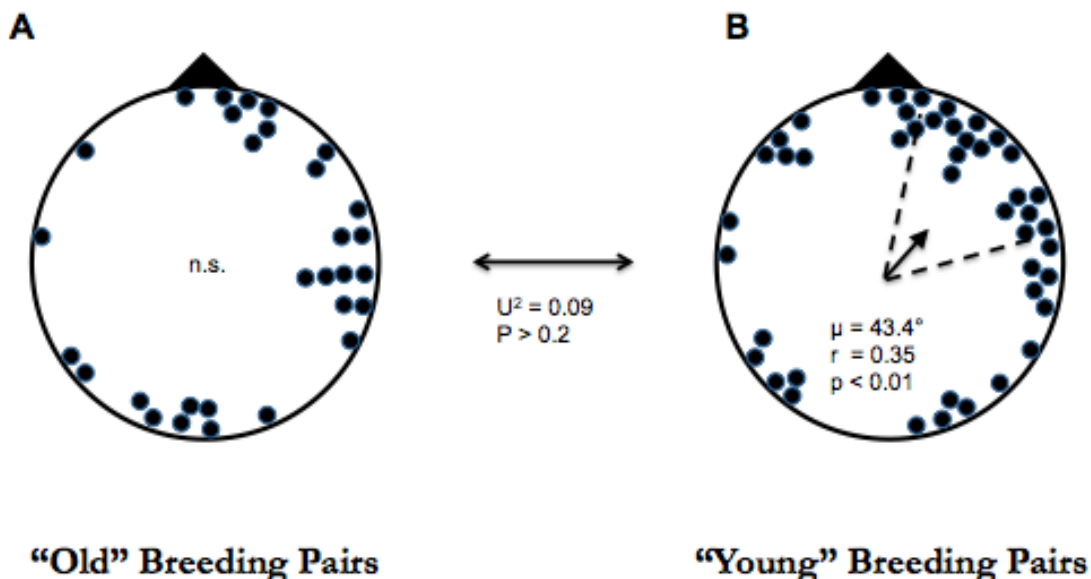
**Figure 1.4. Bearings of orientation in test series.**

( $n=75$ ) Each data point is the directional response of a single mouse tested in one of the four magnetic field alignments. A) The distribution of topographic bearings was indistinguishable from random ( $p > 0.10$ ; Rayleigh test). B) The same was true of the distribution of magnetic bearings; i.e. relative to the alignment of magnetic north (magN). C) In contrast, the distribution of bearings relative to trained (black triangle) was non-randomly distributed, and the 95% confidence interval (CI) for the mean vector bearing did not contain the trained direction ( $89.37^\circ \geq \mu \geq 30.63^\circ$ ; Rayleigh test). The length of the arrow is proportional to the mean vector length ( $r$ ), a measure of the clustering of

bearings ranging from 0 to 1; the radius of the circle corresponds to  $r = 1$ . The dashed lines show the 95% CI for the mean vector bearing ( $\mu$ ). ‘n.s.’- not significant ( $p > 0.05$ ; Rayleigh test).

Two factors differed from earlier experiments in which mice tested in the water maze assay oriented in the trained direction (Phillips et al. 2013; Figure 1.3); older breeding pairs were used to produce some of the mice that were tested and later, a short circuit was discovered in one of the coils used to produce the stimulus fields. Because of this, supplemental analyses were conducted to determine whether these factors contributed to the greater variation and clockwise shift relative to the trained direction in magnetic compass response.

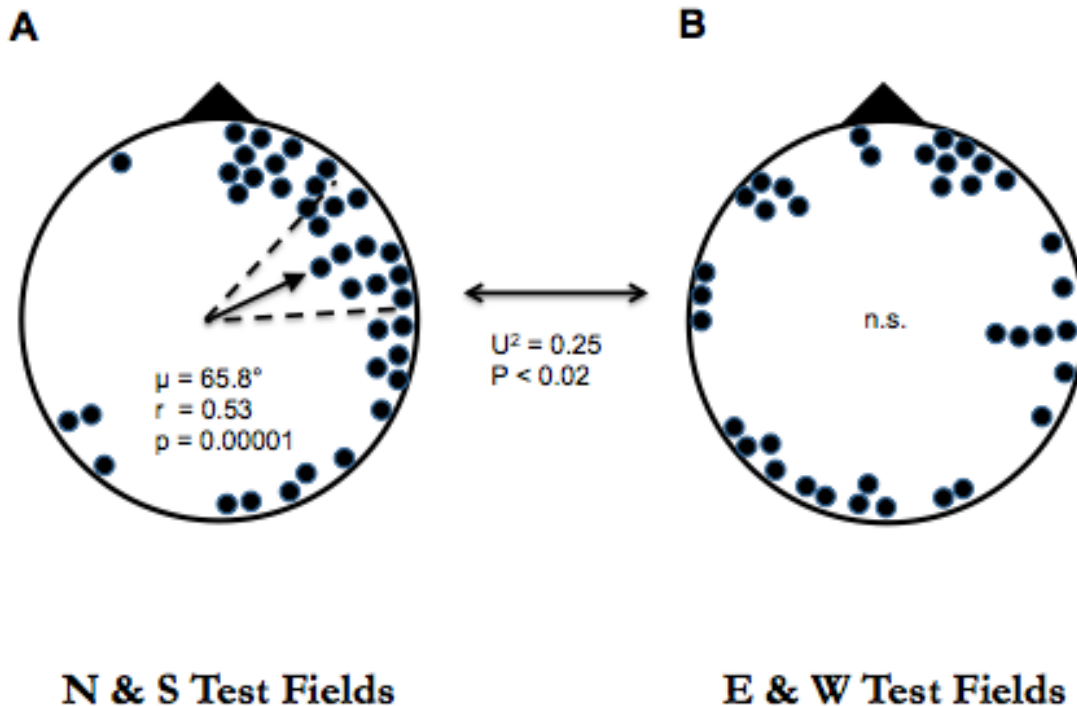
The average age of breeding pairs, on the date of birth of mice used in these experiments, was 118 days. Therefore, breeding pairs were considered “old” if they were older than 118 days and “young” if they were younger than 118 days. The distribution of RTT bearings obtained from mice born from old breeding pairs was not significantly different from the distribution of RTT bearings obtained from mice born from young breeding pairs ( $p > 0.20$ ; Watson’s  $U^2$  test; Figure 1.5). However, the distribution of RTT bearings obtained from mice born from older breeders were indistinguishable from random ( $\mu = 89.9^\circ$ ,  $r = 0.3$ ,  $p = 0.066$ ; Rayleigh test; Figure 1.5a), while that of mice from younger breeders was non-randomly distributed ( $\mu = 43.4^\circ$ ,  $r = 0.35$ ,  $p < 0.01$ ; Rayleigh test; Figure 1.5b), although the 95% confidence intervals did not include the trained direction ( $76^\circ \geq \mu \geq 10^\circ$ ), indicating that the mice were orienting in a consistent direction clockwise of the trained direction.



**Figure 1.5: Comparison between mice born of older breeding pairs to younger breeding pairs.**

Each data point is the directional response of a single mouse tested in one of the four magnetic field alignments. A) The distribution of RTT bearings obtained from mice born from “old breeding pairs” (> 118 days old; n = 30) was indistinguishable from random ( $p > 0.10$ ; Rayleigh test). B) The distribution of RTT bearings obtained from mice born from “young breeding pairs” (< 118 days old; n = 45) was non-randomly distributed, although, the 95% confidence interval (CI) for the mean vector bearing did not contain the trained direction ( $76^\circ \geq \mu \geq 10^\circ$ ; Rayleigh test). The length of the arrow is proportional to the mean vector length ( $r$ ), a measure of the clustering of bearings ranging from 0 to 1; the radius of the circle corresponds to  $r = 1$ . The dashed lines show the 95% CI for the mean vector bearing ( $\mu$ ). ‘n.s.’ - not significant ( $p > 0.05$ ; Rayleigh test). The two distributions were not significantly different ( $U^2 = 0.09$ ,  $p > 0.20$ ; Watson  $U^2$  test). The black triangle at the top of each circular diagram is the trained magnetic direction.

Based on initial observations suggesting that magnetic compass response varied with the alignment of the testing field (Figure 1.4), the distribution of bearings RTT for mice tested in North and South fields were combined and compared to the comparable distribution for mice tested in East and West fields in a post hoc analysis. Mice tested in the North and South fields exhibited significant orientation relative to, but clockwise of, the trained direction ( $\mu = 65.8^\circ \pm 22.52^\circ$ ,  $r = 0.53$ ,  $p = 0.00001$ ; Rayleigh test; Figure 1.6a). In contrast, the distribution of relative to trained bearings from mice tested in East and West test fields was indistinguishable from random ( $\mu = 24.5^\circ$ ,  $r = 0.09$ ,  $p = 0.73$ ; Rayleigh test; Figure 1.6b). The two distributions were significantly different ( $U^2 = 0.25$ ;  $P < 0.02$ ; Watson  $U^2$  test).



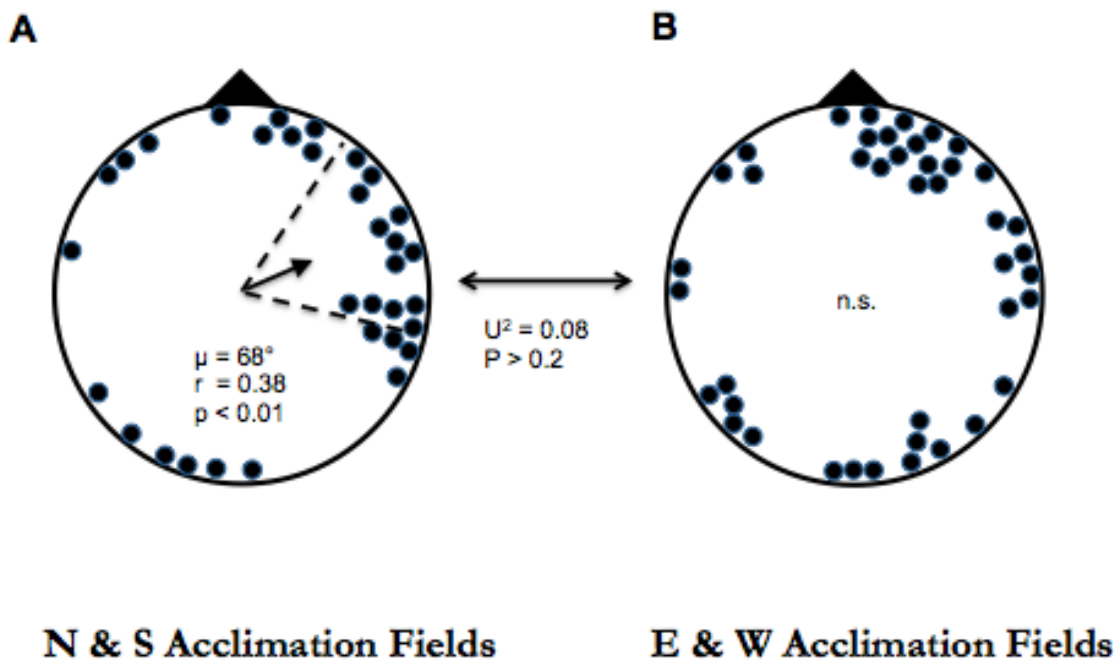
**Figure 1.6: Comparison of North and South to East and West test fields.**

Each data point is the directional response of a single mouse tested in one of the four magnetic field alignments. A) The distribution of bearings RTT (black triangle) obtained from mice tested in North and South fields was non-randomly distributed ( $r = 0.53$ ;  $p < 0.0001$ ; Rayleigh test), and the 95% confidence interval (CI) for the mean vector bearing did not contain the trained direction ( $88.32^\circ \geq \mu \geq 43.28^\circ$ ; Rayleigh test). B) The distribution of RTT bearings obtained from mice tested in East and West fields (magnetic North set to geographic East or West) was indistinguishable from random ( $p > 0.10$ ; Rayleigh test). The length of the arrow is proportional to the mean vector length ( $r$ ), a measure of the clustering of bearings ranging from 0 to 1; the radius of the circle corresponds to  $r = 1$ . The dashed lines show the 95% CI for the mean vector bearing ( $\mu$ ). ‘n.s.’- not significant ( $p > 0.05$ ; Rayleigh test). The two distributions were significantly different ( $U^2 = 0.25$ ;  $P < 0.02$ ; Watson  $U^2$  test). The black triangle at the top of each circular diagram is the trained magnetic direction.

Because the comparison of the responses of mice tested in the North and South versus East and West fields was carried out after we noticed an apparent difference in the strength of the response in the different test fields and subsequently found a short circuit in the west stimulus coil, a second independent analysis was carried to verify that the stimulus fields were differentially affecting the response of the mice. Because the magnetic field alignment in which a mouse was tested was independent of the magnetic field alignments (either North and South, or

East and West) in which the mouse was trained (see Methods), we predicted that mice trained in the East and West fields would exhibit more scatter in the distribution of RTT bearings (regardless of the alignments of the magnetic field in testing) than would mice trained in the North and South field.

As predicted, mice trained in North and South fields exhibited significant orientation relative to, but clockwise of, the trained direction, and the 95% confidence intervals did not include the trained direction ( $\mu = 68^\circ \pm 34.84^\circ$ ,  $r = 0.38$ ,  $p < 0.01$ ; Rayleigh test; Figure 1.7a). In contrast, the distribution of bearings from mice trained in East and West test fields was indistinguishable from random ( $\mu = 50.4^\circ$ ,  $r = 0.25$ ,  $p = 0.073$ ; Rayleigh test; Figure 1.7b). However, the two distributions were not significantly different ( $U^2 = 0.08$ ;  $P > 0.2$ ; Watson  $U^2$  test).



**Figure 1.7: Comparison of North and South to East and West acclimation fields.**

A) The distribution of relative to trained (black triangle) bearings obtained from mice trained in North and South fields (magnetic North set to geographic North or South) was non-randomly distributed, and the 95% confidence interval (CI) for the mean vector bearing did not contain the trained direction ( $102.84^\circ \geq \mu \geq 33.16^\circ$ ; Rayleigh test). B) The distribution of relative to trained (black triangle) bearings obtained from mice trained in East and West fields (magnetic North set to geographic East or West) was



indistinguishable from random ( $p > 0.10$ ; Rayleigh test). However, the two distributions were not significantly different ( $U^2 = 0.08$ ;  $P > 0.2$ ; Watson  $U^2$  test). Each data point is the directional response of a single mouse tested in one of the four magnetic field alignments. The length of the arrow in the center of the circular diagram (B) is proportional to the mean vector length ( $r$ ), a measure of the clustering of bearings ranging from 0 to 1; the radius of the circle corresponds to  $r = 1$ . The dashed lines show the 95% CI for the mean vector bearing ( $\mu$ ). ‘n.s.’ - not significant ( $p > 0.05$ ; Rayleigh test). The black triangle at the top of each distribution is the trained magnetic direction.

## **Discussion**

The results of this experiment provide further evidence that C57BL/6J mice have a magnetic compass that they can use to orient in a consistent direction relative to the magnetic field in a 4-arm “plus” water maze. The mice were significantly oriented when the magnetic bearings are pooled relative to the trained direction. However, unlike preliminary data (Figure 1.3), the 95% confidence interval did not include the trained direction (Figure 1.4c). The absence of a consistent clustering of bearings relative to topographic or magnetic north, ignoring the training directions (Figure 1.4a & b) indicates that the mice were not orienting in a fixed direction relative to topographic or magnetic cues. Although the mice relied on the magnetic compass to orient, and their directional responses depended on the direction of training (compare Figure 1.4b & c), their ability to correctly determine the trained direction appears to have been compromised.

Previous studies have shown that animals can be trained to orient in a particular magnetic direction, but in some cases will exhibit consistent deviations clockwise or counter-clockwise of the trained direction due to differences in the pattern of input from the magnetic field that they experience in training and testing. For example, in experiments that provided the first evidence for a light-dependent magnetic compass, newts trained to orient in a shoreward compass direction under full spectrum (white) light, but then tested under long-wavelength ( $\lambda > 500$  nm) light, exhibited magnetic orientation that was shifted 90° counterclockwise of the trained direction (Phillips & Borland 1992). In other studies, turtles and mice exhibiting spontaneous magnetic alignment responses have been shown to rotate the direction of magnetic alignment when exposed to low-level RF stimuli, which also suggests a change in pattern of magnetic input (Landler et al. 2015; Malkemper et al. 2015).

In an attempt to understand the unusually high degree of variability in the most recent water maze experiments (Figure 1.4), we examined two factors: one that may have affected the

physiological state of the mice or their ability to detect and orient with respect to the magnetic field (breeding pair age), and the second a possible source of low-level RF noise (generated by a defect in one of the stimulus coils) that could affect a radical pair-based magnetoreception mechanism.

A comparison of the effects of breeding pair age on offspring performance in the water maze showed no difference between the distributions of RTT bearings from breeders of different ages (Figure 1.5). Although the distribution of RTT bearings exhibited by mice from younger breeding pairs were non-randomly distributed (Figure 1.5b), while that of older breeding pairs was indistinguishable from random (Figure 1.5a), the similarity in the mean vector bearings from the two groups suggests that the larger sample size of mice from younger breeders is primarily responsible for this difference in significance level. Consequently, these data provide no basis for limiting the age of breeders used to produce mice for future studies of magnetic compass orientation. This conclusion should be treated with caution, however, due to the possibility that an effect of breeder age may have been masked by a problem with our coil system that was later found. Future tests with a fully-functional coil system should be done to further characterize if age of breeding pairs truly has an effect on offspring magnetic compass orientation in the plus water maze.

The second analysis showed that a major source of variability in these experiments was a short circuit in one of the coils used to change the alignment of the magnetic field. The first clue that there was a problem with the coil system came from the absence of consistent orientation exhibited by mice tested in East and West fields (Figure 1.6). Following this analysis, a short circuit was found in the coils that produce the West field. A second, independent analysis comparing the responses of mice that had been exposed to different magnetic field alignments in training confirmed that the coil system was a source of scatter in the data (Figure 1.7).

Interestingly, there was no detectable effect of the short circuit on the intensities of the static magnetic fields used in training and testing. A more likely explanation is that the coil defect was a source of electromagnetic noise in the radio frequency range. Low-level RF fields have shown to affect the response of a RPM-based compass (Henbest et al. 2004; Ritz et al. 2004; Thalau et al. 2005; Ritz et al. 2009). Recent studies of the European wood mouse, *Apodemus sylvaticus*, have shown that a spontaneous magnetic response can be altered by exposure to RF fields, although at relatively high intensities (Malkemper et al. 2015).

Preliminary evidence from subsequent water maze experiments indicate that mice are able to detect the polarity of the magnetic field (See Chapter Two), which is consistent with the response of a MBM-based, rather than a RPM-based, compass. Combined, these results suggest that mice have a hybrid magnetoreception mechanism. Future experiments are needed to directly test if a hybrid magnetic system that receives input from both MBM and RPM like that of birds and amphibians (Phillips 1986b; Phillips & Borland 1994; Beason & Semm 1996; Munro et al. 1997; Phillips et al. 2010a) is involved in mediating the responses in C57BL/6 mice and, if so, how and why these mechanisms interact.

In summary, magnetic compass orientation has proven to be extremely difficult to study in mice, even under highly controlled laboratory conditions. Findings of the present experiments, as well as those of other recent experiments, have identified a wide variety of factors that can alter the response of C57BL/6 mice. Nevertheless, it is clear that these mice have a magnetic compass sense, because of their consistent orientation to a particular angle relative to the trained magnetic direction. A better characterization of the mechanism(s) of magnetoreception in mice is clearly necessary in order to gain a more complete understanding of the role of the geomagnetic field in spatial orientation.

## **Chapter Two: Functional Properties of the Magnetic Compass in C57BL/6 Mice**

## **Introduction**

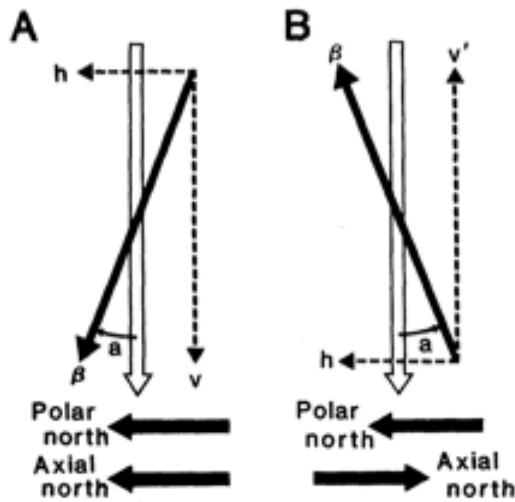
In terrestrial animals, there are two known mechanisms that involve fundamentally different biophysical processes used for sensing the geomagnetic field, or magnetoreception.

The Magnetite Based Mechanism (MBM) involves particles of biogenic magnetite that are believed to activate coupled membrane channels. Responses using the MBM can persist in total darkness and are sensitive to the polarity of the magnetic field. Additionally, MBM responses are not affected by radio frequency (RF) fields at intensities as high as 4800 nT. However, brief, high intensity magnetic pulses that are strong enough to remagnetize magnetite particles have been shown to disrupt responses mediated by this mechanism (Burda et al. 1990; Marhold et al. 1997; Burger et al. 2010; Winklhofer & Kirschvink 2010).

The Radical Pair Mechanism (RPM) is based on a light dependent biochemical reaction thought to involve cryptochromes (specialized photopigments best known for their involvement in entrainment of circadian rhythms) that form long-lived spin-correlated radical pair intermediates (Ritz et al. 2000; Rodgers & Hore 2009). Responses using the RPM are light dependent and are insensitive to the polarity of the magnetic field (i.e., are not able to distinguish north from south). However, responses mediated by the RPM are sensitive to the axis of the magnetic field, and use the inclination or slope of the magnetic field to distinguish between the two ends of the magnetic field lines. A unique feature of the RPM is that responses are disrupted by radiofrequency fields in the range of 1-10 MHz at intensities as low as 15nT and possibly lower (Henbest et al. 2004; Ritz et al. 2004; Thalau et al. 2005; Ritz et al. 2009). Unlike responses mediated by the MBM, responses mediated by the RPM are not affected by “pulse remagnetization” (Winklhofer & Kirschvink 2010).

The mechanism(s) of magnetoreception differ among taxa. The goal of this experiment is to determine whether C57BL/6 mice have an inclination magnetic compass like that shown in birds (Wiltschko & Wiltschko 1972) and amphibians (Phillips 1986b) or polarity-sensitive magnetic compass like that shown in mole rats (Marhold et al. 1997). A diagnostic test, in which the vertical component of the magnetic field is inverted, can be used to differentiate between sensitivity to ‘polarity’, that is a characteristic of a magnetite based mechanism, and sensitivity to ‘inclination’, that is a characteristic of a radical pair mechanism, i.e. where the slope, rather than the polarity of the magnetic field is used to distinguish between two ends of the magnetic axis.

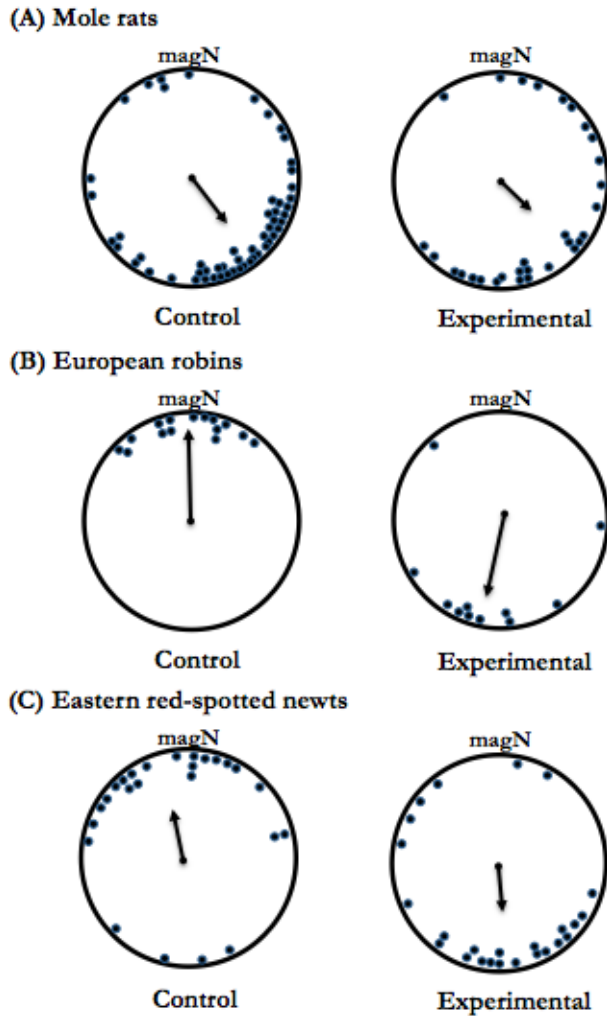
Inverting the vertical component of the magnetic field does not affect the horizontal polarity, but reverses inclination of the magnetic field (Figure 2.1; Phillips (1986b)).



**Figure 2.1: Axial and polar magnetic responses**

Figure used with permission from John Phillips (A) For any horizontal component ( $h$ ), when the vertical component of the magnetic field ( $v$ ) points downward (in the Northern hemisphere of the earth), polar and axial norths coincide. (B) Inversion of the vertical component ( $v'$ ) reverses the inclination but does not change the horizontal polarity of the magnetic field (in the Southern hemisphere of the earth). When the vertical component is inverted, axial and polar norths are in opposite direction (Phillips 1986b).

Magnetic compass orientation of organisms using a polarity sensitive MBM will be unaffected when the vertical component of the magnetic vector is inverted (i.e. reversing inclination, while leaving the horizontal component unchanged), which is consistent with the response exhibited by functionally blind, subterranean mole rats (*Cryptomys* spp.) (Figure 2.2a, right hand distribution). In contrast, inversion of the vertical component causes animals using an inclination-sensitive RPM to change their direction of orientation by approximately 180 degrees due to the reversal of the inclination of the magnetic field, as shown in European Robins (*Erithacus rubecula*) (Figure 2.2b, right hand distribution) and Eastern red-spotted newts (*Notophthalmus viridescens*) (Figure 2.2c, right hand distribution).



**Figure 2.2: Polarity and inclination sensitive magnetic compasses.**

Control distributions signify a vertical “down” component. Experimental distributions signify a vertical “up” component. (A) Orientation of mole rats *Cryptomys* spp. providing evidence for the use of a polarity compass (data from Marhold et al. (1997)). (B) Orientation of migratory European robins providing evidence for an inclination sensitive compass. The robins were tested in the spring when their seasonally appropriate migratory direction was to the North (data from Wiltschko and Wiltschko (2005)). (C) Orientation of shoreward orienting Eastern red-spotted newts providing evidence for an inclination sensitive compass. The newts were trained to orient toward magnetic North in the local geomagnetic field (data from Phillips (1986b)).

If C57BL/6 mice use a RPM-based, inclination sensitive magnetic compass, like that found in amphibians and birds, the direction of orientation should depend on the alignment of the vertical component of the magnetic field. Inversion of the vertical component, reversing the

inclination, should result in an approximately 180° reversal in the direction of magnetic compass orientation. In contrast, if these mice use a MBM-based polarity compass, like that found in mole rats, inversion of the vertical component, reversing the inclination, magnetic compass orientation should not be affected.

## **Methods**

### *Study Site*

These experiments were performed at the Virginia Tech Behavioral Testing Facility (BTF) in Blacksburg, Virginia. The facility consists of four testing buildings constructed of non-magnetic materials, with a central ‘hub’ building supplying air for heating and cooling, as well as filtered AC and DC power through underground conduits. The water maze experiments take place in one of the testing buildings located 20 meters from the hub building.

### *Water maze*

A water maze experiment was carried out in a modified 4-arm “plus” maze with C57BL/6 mice and is fundamentally different than the traditional Morris water maze (D’Hooge & De Deyn 2001; Vorhees & Williams 2006). During training in the plus water maze, the submerged platform was located at the end of one of four arms (coinciding with the cardinal compass directions) that could be approached from only one direction. Consequently, the plus water maze requires a greater reliance on directional, rather than spatial, information to solve the task. The water in the plus water maze was made opaque with white tempura paint so that the location of the submerged platform was not visible to the test subjects. Water temperature was maintained between 27.5-29.5 °C. The plus water maze was centered in two orthogonally aligned horizontal coils wrapped on the same wooden frame (Merritt et al. 1983). The coils are positioned so that the horizontal component of the magnetic field could be aligned along any one of the four cardinal compass directions coinciding with one of the four arms of the maze. The coils were double-wrapped and controlled by reversing the current flow direction in one of the two wraps (Phillips 1986a; Kirschvink 1992).



### *Experimental subjects*

Subjects used were female C57BL/6 laboratory mice between 60-85 days old. C57BL/6 laboratory mice were used because they performed best in the water maze assay of all strains previously tested (Wahlsten et al. 2006). Mice were originally purchased from Jackson Labs and then bred in our laboratory colony, which is maintained on a L: D (15:9) light cycle. Groups of 6-8 mice were transported by car in grouped cages from the breeding colony to the BTF in the morning (8:00-10:00) and placed in individual cages on a holding shelf outside the testing room. The light cycle in the holding room was the same as the breeding colony L: D (15:9). During all tests, the holding shelf was shielded to minimize radio frequency interference (RFI) with a maximum signal strength of  $\sim 0.05$  nT (0.1-100 MHz).

### *Training*

In the afternoon (14:00-16:00) of the same day the mice were transported to the BTF, they were given two brief (< 60 sec) training trials. A Plexiglas partition was used to restrict the movements of the mouse to one arm of the maze with the submerged platform at the outer end. For each training trial, a mouse was removed from the holding shelf in its cage. The lid of the cage was removed, and the mouse was carried into the testing room in its holding cage. The mouse was grasped gently by the base of the tail, and placed into the training arm facing the center of the maze. The observer remained in the room until the training trial was complete. The position of the observer changed between both training trials to reduce the likelihood of the observer being a potential cue. The mouse would swim until it encountered the submerged platform and would then climb out of the water and from there, up onto the side of the plus maze. The mouse was then given a delay of 10 seconds before it was returned to its holding cage, carried once around the water maze and then returned to the outer room. The cage was placed on a drying shelf beneath a 125 W infrared light to dry the mouse's fur. The back half of the drying shelf was shaded so the mouse could thermoregulate by moving between the front and back of the cage. After the fur of each mouse was dry (approximately 10 min), its cage was moved backed to the holding shelf, where it had access to water and food available *ad libitum*.

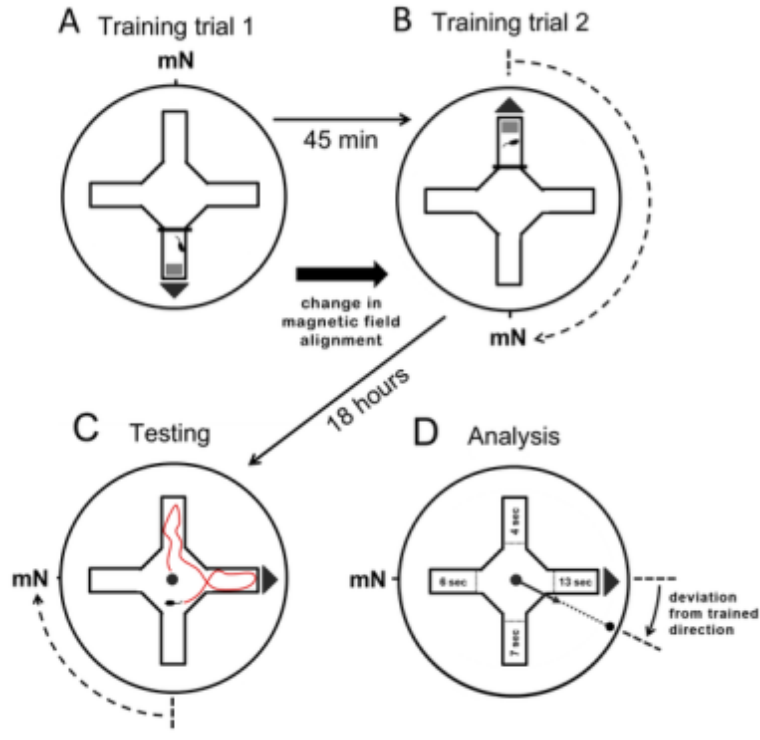
After an interval of at least 45 minutes, each mouse was given a second training trial in the opposite arm of the maze. Prior to the second training trial, the alignment of the magnetic field was rotated by 180 degrees so that magnetic north was in the same alignment relative to the

training arm and to the submerged platform at the end of the arm as in the initial training trial. As in the first training trial, the mouse was allowed to crawl up from the submerged platform onto the wall of the maze and remain there for 10-second delay before being placed back in its cage. It was then transported in the cage by an indirect route to the drying shelf in the outer room (Figure 2.3).

In both training and testing, the holding room was maintained at a temperature of 25.5-30°C and the room containing the water maze was maintained at 26.5-31°C and relative humidity of 55-85%. Mice were only trained to the North and South directions but were exposed to all four magnetic field alignments (magnetic north set at geographic north, east, south, or west). Mice were trained consistently with the vertical component of the field set to “down” (consistent with the ambient environment).

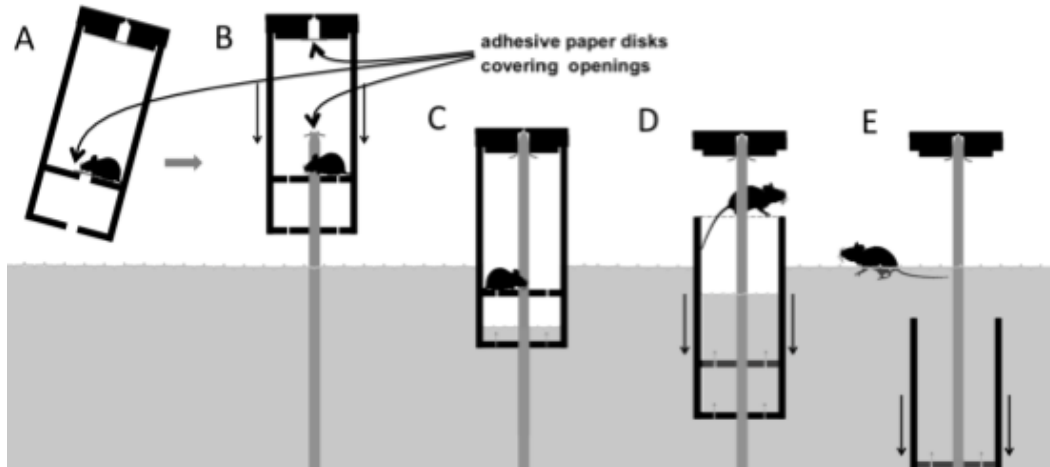
### *Testing*

Each mouse was given a single testing “probe” trial the following morning. For testing, the submerged platform and the partition used to isolate one arm of the maze for training were removed. The mouse was transferred from its holding cage to an opaque cylindrical plexiglass container. The cylinder is rotated slowly clockwise around its long axis as the mouse is carried into the testing room. The cylinder is then placed in the center of the arena from a constant direction onto a round-topped acrylic rod in the center of the plus maze to serve as the release device (Figure 2.3). Once the release device is in place, the observer quietly leaves the room and closes the intervening doors. The release device is designed to gradually fill with water and, after ~50 seconds, sink below the surface of the water forcing the mouse to swim (Figure 2.4). Overall, each mouse was tested in one of the four horizontal field alignments (magnetic north aligned to geographic north, east, south, or west) and one of the two vertical field alignments (“down” for control mice and “up” for experimental mice).



**Figure 2.3: Training and testing protocol.**

Figure used with permission from Phillips et al. (2013). Mice with no prior experience with the water maze were given two training trials in different arms of the water maze with the submerged platform in the same relative alignment to the magnetic field (A & B). In this example, the mouse is being trained to orient to magnetic south. For testing the following morning, the submerged platform was removed (C). The mice were released individually from a central release device (Figure 2.2) and had free access to all four arms of the maze. Magnetic field alignment was changed between trials, and data pooled across testing groups, so an equal number of mice were tested in each of the four magnetic field alignments, i.e., magnetic North (mN) at geographic North, East, South or West; each mouse tested only once in one of the four fields. The tracking software calculated orientation direction as the vector sum of the times spent in the four arms during the 60 sec testing trial (D).



**Figure 2.4: Water maze release device.**

Figure used with permission from Phillips et al. (2013). A) Mouse transported from the holding shelf to the testing arena inside the opaque release device. Disks of paper towel attached with a ring cut from a self-adhesive label covered openings in both the top and bottom of the chamber in which the mouse was confined to prevent its tail from being pinched. B) The release device was then slid slowly down over a vertical plexiglass rod extending up above the water in the center of the plus maze. The top of the rod inserted firmly into a socket on the inside of the lid. C) The buoyancy of the lower section of the release device and the rate of inflow of water through four small openings were adjusted so when the release device was in place, the lower section remained snugly against the lid long enough for the observer to quietly exit the testing room and close the intervening door without being observed. D) Water slowly entered the lower chamber, and eventually the upper chamber, through 4 small holes, causing the lower section of the release device to gradually separate from the lid and sink lower in the water. As the lower section filled with water and separated from the lid, the mouse crawled up onto the top rim of the cylinder and spent 20-35 seconds walking around the rim looking in all directions. E) The lower section of the release device gradually submerged, taking ~50 sec for the top rim to sink below the surface, forcing the mouse to swim.

### *Statistical Analysis*

Standard circular statistics were used to analyze directional responses (Batschelet 1981). Custom Watermaze 2.6 Software, written by Rachel Muheim, calculated the mean vector bearing ( $\mu$ ) and mean vector length ( $r$ ) by vector addition. The mean vector bearings from individual mice were grouped relative to the water maze arena (topographic bearings), relative to the alignment of magnetic north (magnetic bearings), and relative to the trained magnetic direction (relative to trained bearings, or RTT). By combining approximately equal numbers of bearings

from each of the eight treatment conditions (2 trained directions x 4 alignments of the magnetic field in testing), the variance in the directional responses was partitioned into topographic, magnetic, and trained magnetic components. Rayleigh tests were used to test for non-random distributions. All statistics were performed with Oriana: Version 4.

The distributions of bearings in the control and experimental conditions were compared. For each individual test series, the mean vector ( $\mu$ ) of topographic, magnetic, and relative to trained bearings for mice tested in a “down” vertical field alignment and “up” vertical field alignment were calculated separately. We then calculated the deviation of the mean vector of the bearings for mice tested in the “up” vertical field alignment from the mean vector of the bearings for mice tested in a “down” vertical field alignment for each test for each of the three distributions. A Rayleigh test was used to test for unimodal orientation of the “up” mean vectors relative to the controls.

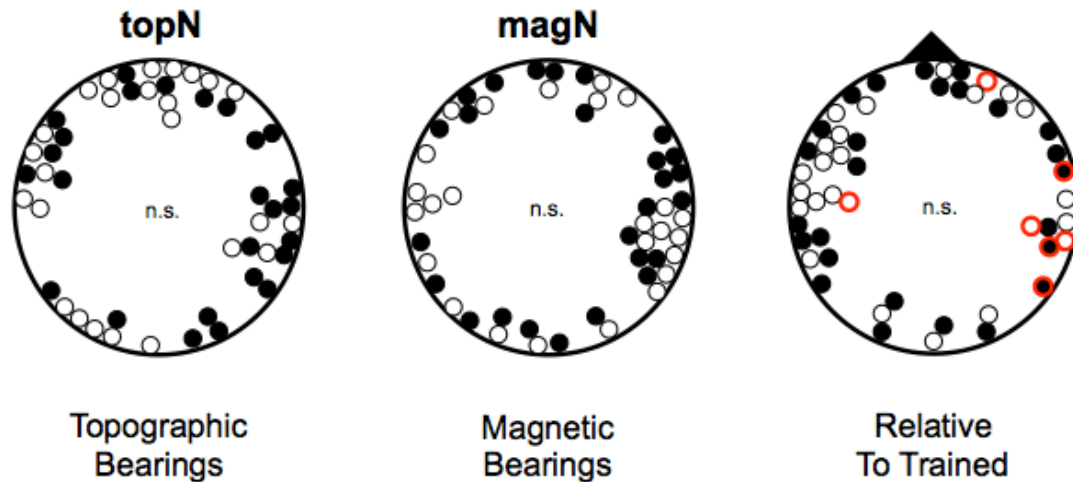
## **Results**

Mice were tested in the 4-armed “plus” water maze assay to further characterize the mouse magnetic compass. In order to determine which of the two magnetic compass mechanisms mice use to orient with respect to the magnetic field, the vertical component of the magnetic field was inverted (vertical component up) during probe trials for experimental mice and compared to control mice (vertical component down; local geomagnetic field).

The distribution of bearings obtained from the experimental and control mice tested for magnetic compass orientation were indistinguishable from random when the bearings were pooled together with respect to topographic north (i.e., absolute bearings ignoring magnetic field alignment;  $p > 0.06$ , Rayleigh test, Figure 2.5a) and with respect to magnetic north (ignoring trained magnetic direction; Figure 2.5b,  $p > 0.10$ , Rayleigh test,). Additionally, when the bearings were pooled together relative to the trained magnetic direction for both experimental and control mice, the distribution was also indistinguishable from random ( $p > 0.07$ ; Rayleigh test; Figure 2.5c).

Furthermore, when experimental and control mice were analyzed separately, the distribution of bearings were indistinguishable from random when the bearings were pooled together with respect to topographic north (control:  $p > 0.05$ ; experimental:  $p > 0.1$ ; Rayleigh test) and magnetic north (control:  $p > 0.2$ ; experimental:  $p > 0.8$ ; Rayleigh test). Likewise, the

distribution of bearings relative to the trained magnetic direction was also indistinguishable from random for both control and experimental mice when analyzed separately (control:  $p > 0.5$ ; experimental:  $p > 0.1$ ; Rayleigh test). These topographic, magnetic, and relative to trained responses were not statistically different between control and experimental mice (topographic:  $U^2 = 0.11$ ,  $p > 0.2$ ; magnetic:  $U^2 = 0.042$ ,  $p > 0.5$ ; relative to trained:  $U^2 = 0.069$ ,  $p > 0.2$ ; Watson  $U^2$  tests).



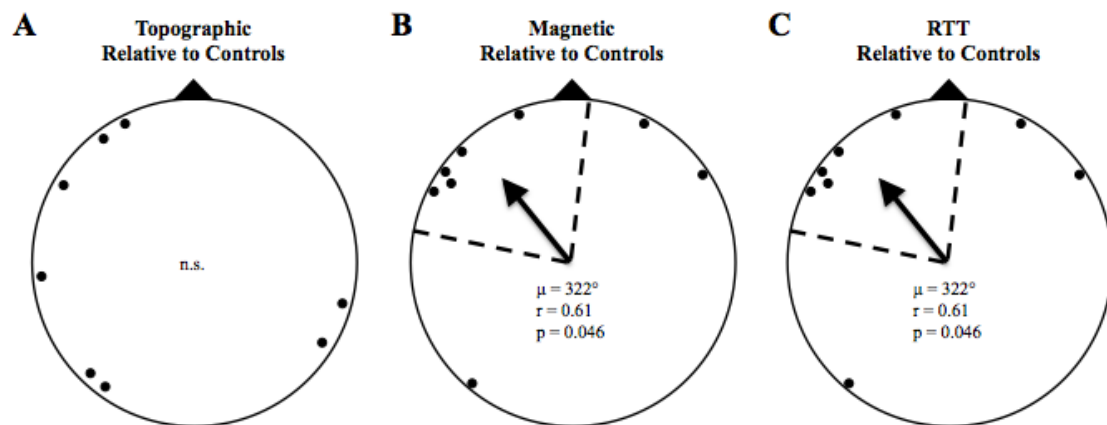
**Figure 2.5: Bearings of orientation in inclination experiment series.**

( $n = 51$ ) Each data point is the directional response of an individual mouse tested in one of the four horizontal magnetic field alignments. Open circles signify experimental mice; filled signify controls. Data points lined in red in (C) represent an individual test with  $>25$  mph wind speeds. A) The distribution of topographic bearings was indistinguishable from random ( $p > 0.06$ ; Rayleigh test). B) The distribution of magnetic bearings; i.e. relative to the alignment of magnetic north (magN) was also indistinguishable from random ( $p > 0.10$ ; Rayleigh test). C) The same was true of the distribution of bearings relative to trained ( $p > 0.07$ ; Rayleigh test). ‘n.s.’ - not significant

Due to variation in the direction of orientation relative to the trained magnetic direction of orientation between test groups, further analysis was conducted to determine if there was a consistent relationship between orientation of the controls (“vertical down”) and experimental (“vertical up”) mice within testing groups. Since the deviations of experimental mean vector bearings were calculated relative to the control mean vector bearings, the analysis is the same for the magnetic and RTT distributions. A consistent response of experimental subjects that is indistinguishable from controls for magnetic and RTT distributions would suggest a mechanism

of magnetoreception that is sensitive to polarity, while a consistent response that is opposite that of controls for magnetic and RTT distributions would suggest a mechanism that is sensitive to inclination, rather than polarity.

When we examined the deviations of the mean vector bearings calculated from the topographic distributions, the distribution of experimental mean vector bearings from the “vertical up” mice was indistinguishable from random ( $\mu = 258^\circ$ ;  $r = 0.23$ ;  $p > 0.6$ ; Rayleigh test; Figure 2.6a). In contrast, when we examined the magnetic and RTT distribution of deviations of the mean vector bearings of experimental mice from control mice, the distribution of experimental mean vector bearings from the “vertical up” mice was significantly oriented ( $\mu = 322^\circ$ ;  $r = 0.61$ ;  $p < 0.05$ ; Rayleigh test; Figure 2.6b & c). Additionally, the 95% confidence interval included the mean direction of controls ( $279.1^\circ \geq \mu \geq 5.7^\circ$ ; Rayleigh test; Figure 2.6b & c). Overall, the magnetic orientation of mice tested in the inverted vertical field is indistinguishable from controls.



**Figure 2.6: Comparison of experimental mice to control mice.**

(A) The topographic distribution of experimental mice relative to controls was indistinguishable from random ( $p > 0.6$ ; Rayleigh test). Both the (B) magnetic and (C) RTT distributions of experimental mice relative to controls were non-randomly distributed ( $p < 0.05$ ; Rayleigh test), and the 95% confidence interval (CI) for the mean vector bearing contained the mean direction of controls ( $279.1^\circ \geq \mu \geq 5.7^\circ$ ). Each data point is the mean vector of mice tested in an “up” vertical field alignment for a single test group (8 tests total; number of experimental mice = 25), plotted as a deviation from the mean vector of controls (“down” vertical field alignment). Control direction of orientation is signified by the black triangle. The length of the arrow is proportional to the mean vector length ( $r$ ), a measure of the clustering of bearings ranging from 0 to 1; the radius of the circle corresponds to  $r = 1$ . The dashed lines show the 95% CI for the overall mean vector bearing ( $\mu$ ).

## **Discussion**

Although the overall response of both experimental and control mice tested relative to the trained direction was indistinguishable from random (Figure 2.5c), the consistency in orientation of the two treatment groups within individual tests suggests that the mice were exhibiting consistent responses relative to the trained magnetic direction but that this direction varied between tests (see red symbols; Figure 2.5c). Mice appear to be unaffected when the vertical component of the magnetic vector was inverted (i.e. reversing inclination, while leaving the horizontal component unchanged). The similarity in the magnetic responses of mice exposed to the vertical up and vertical down conditions indicate that C57BL/6 mice use a polarity sensitive magnetic compass (Figure 2.6b & c). These results are consistent with previous responses exhibited by functionally blind, subterranean mole rats, suggesting that C57BL/6 mice have a MBM like that found in the magnetic compass response of mole rats (Marhold et al. 1997).

Birds and amphibians have both MBM and RPM magnetoreception systems. Interestingly in both cases, the RPM appears to provide compass information, while the MBM is used to derive map information (Phillips 1986b; Phillips & Borland 1994; Beason & Semm 1996; Munro et al. 1997; Phillips et al. 2010a). During seasonal migrations, when newts are showing map-based homing, (i.e. “true navigation” that requires both map and compass information) their response to the magnetic field shows properties of both mechanisms; i.e. sensitivity to magnetic field polarity, a characteristic of a MBM, and to the wavelength of light, a characteristic of a RPM (Phillips 1986b; Phillips & Borland 1994).

A unique feature of the RPM is that responses mediated by this mechanism are disrupted by RF fields at frequencies in the range of 1-10 MHz at intensities as low as 15nT and maybe lower (Henbest et al. 2004; Ritz et al. 2004; Thalau et al. 2005; Ritz et al. 2009). Recent experiments have provided evidence that the magnetic compass of mice is sensitive to RF (Malkemper et al. 2015; see Chapter One). Furthermore, there is a possibility of a hybrid magnetic mechanism (implicating both MBM and RPM) is involved in mediating the responses in C57BL/6 mice.

In order to fully understand how mice use the geomagnetic field for spatial orientation, future experimentation is needed. Future experiments should test whether both sensitivity to the polarity of the magnetic field and to low-level RF are present in the same strain of mice. This



information, along with experiments exploring whether the magnetic response is dependent on light, are needed to determine if mice exhibit a hybrid response (involving input from both a MBM and a RPM) like that implicated in migratory birds and homing responses of newts.

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