

Small differences in weak electromagnetic fields disrupt magnetic compass orientation of C57 BL/6 mice (Rodentia: Muridae)

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Abstract. Studies of learned magnetic compass orientation by C57 BL/6 mice were carried out to determine if responses to magnetic cues were disrupted by exposure to a very low-level (1–2 nT) 1.46 MHz radio frequency field. Findings show that exposure to the same intensity of the 1.46 MHz RF in training and testing (1.0 to 1.2 nT) had no effect on magnetic compass orientation. However, exposure to a slightly higher intensity of RF in training (1.3 to 2.1 nT) eliminated magnetic compass orientation either because the stronger intensity was above a critical threshold for disruption of the underlying magnetoreception mechanism, or because the difference in the RF intensities in training and testing resulted in qualitatively different patterns of magnetic input ('magnetic modulation patterns'). Importantly, although similar effects of RF on magnetic compass orientation have been reported in other organisms, sensitivity to such low intensities of RF fields cannot be explained by current models of the mechanisms of magnetoreception in terrestrial organisms. Consequently, future research to determine if the findings from C57 BL/6 reported here are replicable is of the first importance.

Key words. Magnetic compass, murine rodents, low-level RF, radical pair mechanism.

INTRODUCTION

Murine rodents ("murids") have a robust magnetic compass sense (DEUTSCHLANDER et al. 2003, MUHEIM et al. 2006, PAINTER et al. 2018). As shown in other vertebrates with well-developed visual systems (PHILLIPS & BORLAND 1992, HORE & MOURITSEN 2016, PHILLIPS et al. 2010, LANDLER et al. 2015, PHILLIPS & DIEGO-RASILLA 2022), the available evidence suggests that the magnetic compass in murids is mediated by a light-dependent quantum process (the so-called radical pair mechanism or RPM; RITZ et al. 2000). Evidence for the RPM in murids includes: (i) the presence of light-dependent magnetoreceptors in the retina (reviewed by OLCESE 1990), (ii) a complex, axially-symmetrical pattern of magnetic input (PAINTER et al. 2013), and (iii) disruption of magnetic orientation by extremely weak electromagnetic fields at frequencies in the low MHz range (<10 MHz; MALKEMPER et al. 2015) that are thought to alter the quantum spin state of the radical pair (RITZ et al. 2004, MAEDA et al. 2012, WILTSCHKO et al. 2015, HORE & MOURITSEN 2016). Studies of a variety of non-mammalian vertebrates, and invertebrates, have also provided evidence that a light-dependent RPM, or similar quantum process, underlies the magnetic compass (PHILLIPS & BORLAND 1992, PHILLIPS & SAYEED 1993, RITZ et al. 2004,

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VÁCHA et al. 2009, WILTSCHKO et al. 2015, MUHEIM & LIEDVOGEL 2015, TOMANOVÁ & VÁCHA 2016, HORE & MOURITSEN 2016, PINZON-RODRIGUEZ & MUHEIM 2017).

Despite the growing evidence that murids, and perhaps mammals generally, rely on the magnetic field for a variety of orientation behaviors (BEGALL et al. 2013, BURDA et al. 2020), a majority of laboratory studies of mammalian spatial behavior and cognition have failed to find evidence for sensitivity to magnetic cues (e.g., SHARPE et al. 2001). A review of behavioral studies of murine rodents suggests that the presence of low-level radio frequency (RF) fields in laboratory settings may be an important source of variability in magnetic orientation experiments (PHILLIPS et al. 2022). In earlier laboratory studies of C57 BL/6 mice carried out by our laboratory, sources of RF fields, including those generated by laboratory equipment, as well as intermittent bursts of RF noise produced by a nearby electric fence, were found to disrupt responses to the magnetic field. Reliable magnetic compass orientation was elicited from C57 BL/6 mice only after installing electromagnetic shielding in the training and testing facilities, as well as RF filters in the electrical circuits powering stimulus coils to minimize exposure to low-level RF fields from laboratory equipment and the surrounding environment (MUHEIM et al. 2006, PAINTER et al. 2018, PHILLIPS et al. 2022).

The present experiments were carried out using a nest building assay shown to elicit robust learned magnetic compass orientation in C57 BL/6 mice when training and testing facilities were electromagnetically shielded to eliminate exposure to laboratory and ambient RF fields (MUHEIM et al. 2006, PAINTER et al. 2018). In contrast to MUHEIM et al. (2006), however, the intent of this study was to probe the sensitivity of learned magnetic compass orientation using RF fields close to the Lamour frequency, which has been shown to disrupt magnetic responses in other vertebrates (RITZ et al. 2004, LANDLER et al. 2015). Specifically, we investigated the effects of exposure to a low-intensity (~1–2 nT), near Lamour frequency radio frequency (LFRF) field (1.46 MHz)* in both the training and testing environment on magnetic compass orientation in C57 BL/6 mice. Due to the variation in the intensity of the static magnetic field in training and testing ($\pm 2\%$), the 1.46 MHz signal used in these experiments falls within the range of the LFRFs for the magnetic intensities in both training and testing environments, but did not precisely match the LFRF for all positions as a mouse moved about the training cage or testing arena (see below). Therefore, we refer to the RF field as ‘near-LFRF’.

METHODS

The behavioral testing facility (BTF) at Virginia Tech, Blacksburg, VA USA was designed for studies of magnetoreception and magnetic orientation behavior in a variety of animals (newts, mice, flies). The buildings housing these experiments were constructed of non-magnetic materials and designed to minimize exposure to low-level radio frequency fields produced by equipment required to carry out behavioral experiments, i.e., thermostats, heaters, air conditioners, electric motors, computers, power supplies, etc. Temperature and humidity in the buildings was regulated by means of underground air ducts coming from a central utility building, supplemented during the warmer months with air conditioning units in the training and testing buildings that were turned off when experiments were underway. Electrical power was provided to the training and testing buildings by underground cables from the utility building equipped

* The frequency of the RF field to which mice were exposed in training and testing was 1.456 MHz. However, due to the ~2% variation in magnetic field intensities (see above), this frequency did not provide a precise ‘match’ to the LF in *all locations* within the training cages and testing arena. Therefore, the ‘near-LFRF’ frequency used in these experiments was rounded to 1.46 MHz, matching the true LF in some, but not all, areas mice were exposed to during training and testing.

with RF filters. All C57 BL/6 mice used in these experiments were bred from a laboratory colony and then transferred to the BTF for training and testing.

Training procedure

Mice were trained individually in separate non-magnetic Plexiglas cages (48×25.5×16 cm) with nestlets (pulped, sterilized, cotton fiber pressed into flat, 5×5 cm pieces; Ancare) provided for nest construction, and with food and water *ad lib*. A black plastic nest box was placed at the back of the cage, shaded by an overhead shelf, with the entrance facing toward the center of the cage (Fig. 1A & B). Four shelving units, each holding three mouse cages on separate shelves, were centered in the middle of the training room (3.5×3.5 m), so that the shelving units faced in magnetic directions that differed by 90° (i.e., 70°, 160°, 250°, and 340°; Fig. 1B). The cages were aligned on the shelves so that the nest box was positioned

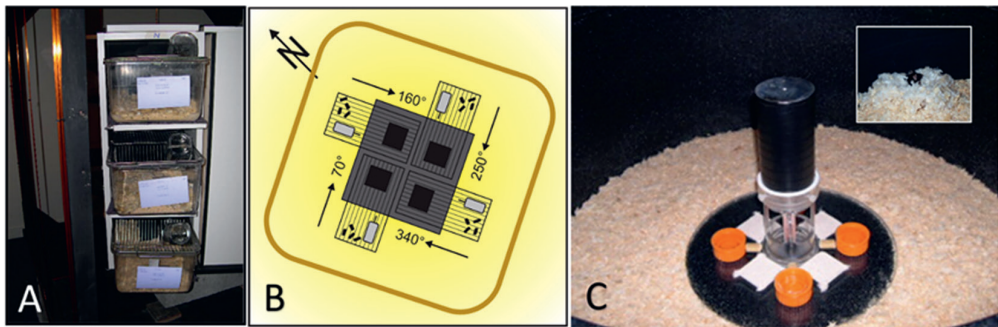


Fig. 1. Training and Testing Apparatus. (A) Three training cages aligned along one of the four trained directions. Stainless steel lids had relatively little effect on the static magnetic field. The interior of the shelves was painted flat black and the overlying shelves shaded the ‘dark’ end of each cage. The ‘light’ end of each cage was unshaded and exposed to overhead light. (B) Schematic of the top cage on each set of shelves aligned along one of the trained directions. Black rectangles show nest boxes that were closed on three sides, with the open side (i.e., entrance) toward the light end of the training cage. Light grey rectangle and small black rectangles located towards the light end of the cage represent water bottles and food pellets, respectively. Large brown square (with rounded corners) surrounding the four cages shows one element of a two element, Helmholtz-style antenna used to expose mice in training to a RF field. The training shelves and antenna were centered in a double-wrapped, orthogonal pair of cube surface coils (not shown) identical to those surrounding the testing arena (see below). However, the coils surrounding the training shelves were connected to a second channel of the power supply used to produce the test fields, but with the current turned to zero. The trained magnetic direction for the four set of shelves are listed. (C) Interior of the testing arena showing equally spaced ‘nestlets’ (i.e., nest-building material), food pellets, and orange plastic water dishes. The arena was centered in a double Helmholtz-style antenna identical to that surrounding the training shelves (not shown). The arena and antenna were centered in an orthogonal pair of cube surface coils that were used to produce the four symmetrically aligned testing fields, with $\text{magN} = \text{north, east, south, or west}$ (MUHEIM et al. 2006) and a small subset of bearings were collected with $\text{magN}=345^\circ$ (see text for details). Each coil was double-wrapped, and turned on and off by reversing the direction of current flow in one of the two wraps (KIRSCHVINK 1992). The release device in the center was attached to a plexiglass disk on which the nest material, food, and water were positioned. Picture inset shows example of nest constructed overnight.

towards the back wall of the training shelf that was painted flat black. A 75-W, 12-VDC tungsten/halogen light source (ECY; EIKO Ltd.) projected through a 1.2×1.2 m white Plexiglas sheet centered above the training shelves provided diffuse illumination during the light phase of the photoperiod (15:9-h light:dark photoperiod (light, 0500–2000 h, EST). In each training cage, the shadow of the overhanging shelf created a light gradient with the nest box positioned against the back wall at the dark end of cage (Fig. 1A).

Individual mice were assigned to one of the four training directions and were placed in the training cages for a minimum of 5 days (average, 11 days; range, 5–24 days) before being tested for magnetic compass orientation.

Testing procedures

The mice, on average 74 days old (range, 60–85 days), were tested individually, one per night in a large (88 cm diameter), radially symmetric, circular arena centered below an overhead light source, identical to that used in training and synchronized to the same photoperiod the mice were exposed to in training. Tests began in the late afternoon, 2–3 h before the beginning of the dark phase of their light cycle, and ended the next morning. Each mouse was tested only once in one of the magnetic field alignments (see earlier discussion). Before a mouse was placed into the arena, the arena floor was covered with a thin layer of pine shavings. Four pieces of food, four nestlets, and four small cups with water were placed symmetrically around the center of the arena (Fig. 1C).

Once the arena was prepared, a mouse was removed from its cage in the training building, placed in a light-tight transfer cylinder, and carried ~50 m by the investigator to the testing building while slowly rotating the cylinder. Once inside the testing building, the transfer cylinder served as a release chamber that was placed on top of a cylindrical scaffolding at the center of the arena, confining the mouse in an elevated chamber above the center of the arena while the experimenter exited the testing room (Fig. 1C). After a 60 sec acclimation period, the experimenter lowered the bottom of the release chamber by means of a plexiglass rod extending up through the floor of the arena and inserted into the bottom of the release chamber. The rod was lowered using a hydraulic mechanism located in an adjacent room, releasing the mouse into the arena. Between trials, the wood shavings, nestlets, and food pieces were removed from the arena and discarded, and the arena was thoroughly cleaned first with with BioKleen (BioSentry Inc.), and then with 70% ethanol.

Testing fields

A majority of the mice were tested in one of four different alignments of the magnetic field: the ambient magnetic field (magnetic north [mN]=360°) and three shifted fields with magnetic north at geographic east (mN=90°), south (mN=180°), or west (mN=270°; Fig. S1). In addition, a smaller number of mice (n=12) were tested in a magnetic field shifted 45° counterclockwise (mN=315°; Fig. S1; see further discussion below). Each mouse was tested only once in one of the magnetic field alignments.

All test fields closely resembled the ambient geomagnetic field in inclination ($\pm 1.5^\circ$) and total intensity ($\pm 2\%$), measured with a Fluxgate magnetometer (Applied Physics Systems, model 520A). Magnetic fields were produced by a pair of horizontal, perpendicularly aligned, cube-surface coils wrapped on a wooden frame with a linear dimension of ~1.2 m surrounding the testing arena (see PHILLIPS 1986a). The coils were powered by a custom, current-regulated power supply (Design Solutions Inc.) located in a central hub building and connected to the coils by shielded electrical wire run through an underground conduit. The outputs of the power supply were equipped with EMI filters (Dearborn 1JX2459) to minimize low-level radio frequency fields. Each coil was double-wrapped in a four-element configuration (MERRITT et al. 1983) and was controlled by reversing the direction of current flow in one of the two strands of wire (PHILLIPS 1986b, KIRSCHVINK 1992).

The training shelves were centered in a second set of double-wrapped coils identical to those used in testing. The coils surrounding the training shelves were connected to a second channel of the power supply

used to produce the test fields, but with the current turned to zero. These ‘dummy’ coils were used to make sure that any background levels of RF generated by the power supply was the same in training and testing.

RF Exposure

A 1.46 MHz (near LFRF) field was produced using a pair of horizontally aligned square (1×1 m), loop antennas (Helmholtz design) surrounding the training shelves. Due to the differences in vertical shelf position relative to the RF antennas, there were slight differences in the intensity of the LFRF signal within and between the training cages. The LFRF field intensities that mice were exposed to on the four

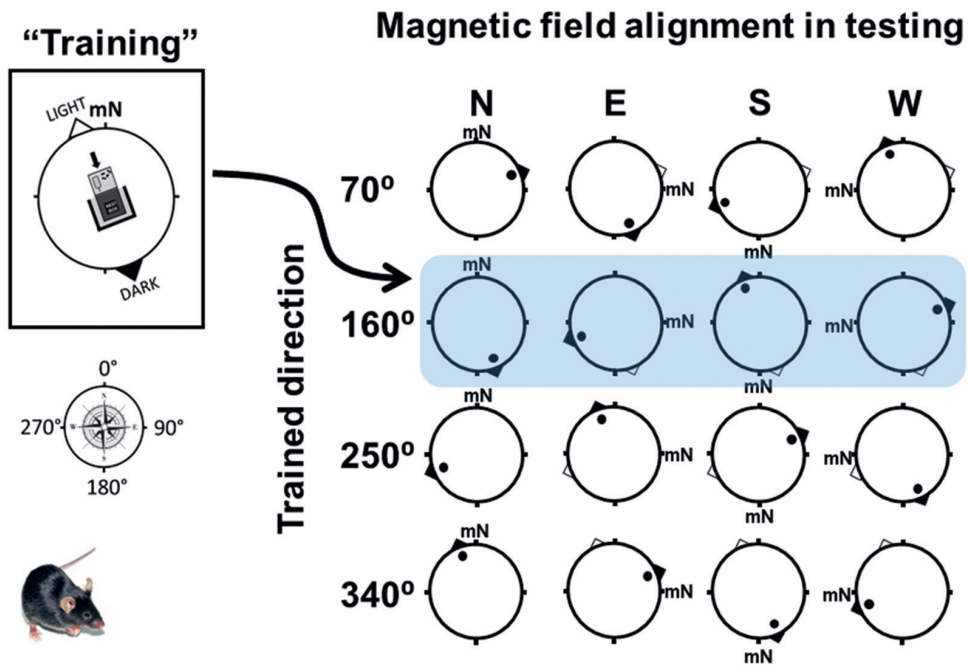


Fig. 2. Testing Format. Individual mice in the study of MUHEIM et al. (2006) were tested only once in one alignment of the magnetic field. A majority of mice were exposed to one of 16 training/testing conditions – 4 trained directions × 4 magnetic field alignments. Left side of figure shows outline of testing arena; inset shows how alignment of a cage on one of the training shelves translates into ‘trained direction’ (i.e., dark end of trained axis). Compass rose shows geographic directions. Photograph is a C57 BL/6 mouse. Right side of figure shows trained directions rotated 20° counter-clockwise from the cardinal directions to allow cages to bypass the frame of the dummy coil when being removed from the training shelves (Fig. 1A). Separate groups of mice were trained in four different directions (blue shaded rectangle shows one of the four trained directions). Mice trained in each of the four directions then tested in 4 symmetrical magnetic field alignments (i.e., magN = north, east, south, or west). Each mouse was tested only once in one of the four symmetrical magnetic field alignments, or in a magnetic field alignment with magN = northwest (315°; not shown).

middle shelves ranged from 1.0 to 1.3 nT and closely matched the range of intensities mice were exposed to in the test arena (see below). In contrast, the LFRF intensities that mice were exposed to on the upper and lower sets of training shelves ranged from 1.3 to 2.1 nT, with minimal overlap with the intensities measured in the testing arena (see Fig. 4).

As in training, a pair of horizontal loop antennas (square Helmholtz configuration) were used to produce the 1.46 MHz near-LFRF that was broadcast in the test arena. In contrast to the variation of LFRF intensity in training (see previous discussion), the LFRF intensity was less variable in the testing arena because the movement of the mice was limited to the plane of the arena floor, centered between the two coil elements. As a consequence, the LFRF intensities mice were exposed to in testing ranged from 1.1 nT near the edges of the arena to 1.2 nT at the center of the arena.

The RF fields that the mice were exposed to in training and testing (Fig. S2) were produced by Agilent 33250A Function Generators, and measured with a 6 cm loop antenna (calibrated by Fred Dietrich, Electric Power Associates) connected to an Agilent E4402B ESA-E Series Spectrum Analyzer.

Data collection and testing criteria

The following morning, after a mouse had an opportunity to build a nest overnight in the arena, the investigator recorded the position and quality of the nest. The directional choice of the mouse was determined by recording the bearing of the nest from the center of the arena to the nearest 5°. The topographic bearing (location within the arena independent of the magnetic field alignment), magnetic bearing (bearing relative to magnetic north in the testing field), and trained bearing (bearing relative to the trained magnetic direction) were recorded for each nest. The following criteria, established previously (MUHEIM et al. 2006), were used in these experiments. (1) Mice were between 60 and 85 days old then they were put in testing. (2) The temperature of the testing room was between 18 and 20.5 °C. (3) Mice were not tested on nights when thunderstorms were in the area, and tests were excluded when a thunderstorm unexpectedly occurred while a mouse was in the test arena. (4) A nest had to incorporate at least 50% of the nesting material, be cup shaped, and be positioned in the outer two thirds of the arena (nests were excluded if they were in contact with the base of the release device, which consisted of a 34-cm-diameter, 10-mm-thick Plexiglass disk in the center of the arena floor). Nests that did not meet these *prior* criterion were excluded from the analysis. For more detailed Methods see MUHEIM et al. (2006) and PAINTER et al. (2018).

Summary of test series

Three series of experiments were carried out to probe the effects of near-LFRF fields on mouse learned magnetic compass orientation, with the same training and testing procedures that were used in the earlier experiments without RF exposure by MUHEIM et al. (2006); findings from MUHEIM et al. (2006) shown here in Fig. 3.

Series I: Mice were trained on the three shelves aligned in each of the four trained directions while being exposed to the near-LFRF using a pair of square, horizontally aligned loop antennas in a Helmholtz configuration (Fig. 1B; and see KIRSCHVINK 1992). In addition to mice tested in four alignments of the magnetic field with magnetic north at one of the cardinal compass directions (magN = north, east, south, or west), this series of experiments included mice tested in a magnetic field shifted by 45° CCW (magN=315°)*. Series I was carried out from April to July 2010 (Fig. 4).

Series II: Training shelves were repositioned so the intensity and range of variation of the LFRF in training more closely resembled the LFRF in testing. To do this, the middle shelves were removed. The upper and

* The experiments in which mice were tested with magN=315° were carried out as a prelude to future research to determine if the quadrimodal tendency in the responses of mice trained on the lower shelves (Fig. 4; lower distribution) is replicable and, if so, whether the quadrimodal pattern is a response to magnetic or topographic cues.

lower shelves were moved closer together, and centered between the two coil elements of the RF antenna, so the RF field intensities the mice were exposed to on the upper and lower shelves in training closely resembled the intensities they were exposed to in the testing arena. These experiments were carried out from May to August 2011 (Fig. 6).

Series III: A new group of mice was used in a replication of the Series II without the presence of loud noises that may have caused a reversal in the direction of magnetic compass orientation of some of the mice tested in Series II (see below). These experiments were carried out from January to February 2012 (Fig. 7).

RESULTS

In the earlier study by MUHEIM et al. (2006), mice were trained and tested in shielded enclosures that minimized exposure to low-level RF fields. On each of the three shelf positions (upper #1, middle #2, lower #3), the magnetic bearings pooled from mice trained in the four directions (see Figs. 1A, B) exhibited significant magnetic compass orientation in all three shelf positions that coincided with the trained direction (Fig. 3; distributions on the right).

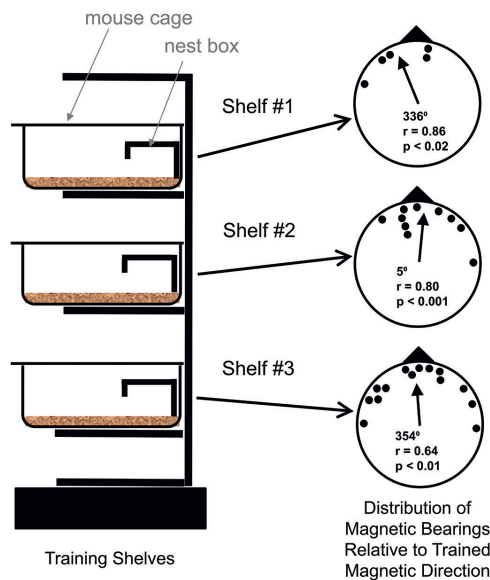


Fig. 3. Magnetic compass orientation of C57 BL/6 mice from original experiments in which training and testing was carried out in electromagnetically shielded enclosures (MUHEIM et al. 2006). Left diagram shows cross section through training cages on the lower, middle, and upper shelves for one of the four trained directions; for top view showing alignments of cages see Fig. 1B. Circular diagrams on the right show magnetic compass orientation of mice trained in cages on the corresponding shelves; responses pooled from the four trained directions and from mice tested in four different magnetic field alignments; each mouse tested only once in one trained direction and one of the four magnetic field alignments. Bearings are plotted relative to the trained magnetic direction (black triangle at the top of each diagram; see Fig. 2A). Note: the training shelf was not reported for one data point in MUHEIM et al. (2006) and was excluded from this figure.

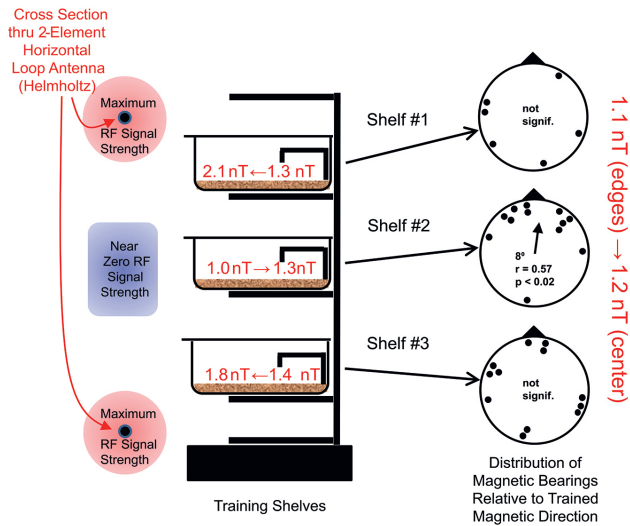


Fig. 4. Magnetic compass orientation of mice exposed to low-level RF field in training and testing (1.46 MHz). Left side of the figure shows a cross-section through the Helmholtz antenna elements used to expose the mice to the RF field in training. Red shading shows regions of maximum intensity of the RF field near the coil elements. Blue shading shows the regions of minimum intensity of the RF field centered between the two antenna elements where the RF fields from the two antenna elements effectively cancel out. Center of the figure shows a cross section through the training shelves aligned along one of the four trained directions. Red type shows the range of RF field values from the outside ('light') end of each training cage to the inside ('dark') end of each training cage. Circular diagrams on the right show magnetic compass orientation of mice trained in cages on the corresponding shelves (see Fig. 3). Red type at the right side of the figure shows the range of RF intensities from the minimum near the outside walls of the testing arena to the maximum at the center of the testing arena. The RF field generated by the Helmholtz antenna was aligned vertically, and the 6 cm dia loop antenna used to measure the RF field intensities was aligned horizontally to measure the vertical RF fields. Mice trained in cages on the middle shelf in each trained direction, where the RF field intensities were nearly identical to those in the testing arena, were well oriented with respect to the trained magnetic direction, exhibiting magnetic compass orientation comparable to that shown by mice on all three shelves (lower, middle, upper) in the original experiments (Fig. 3). However, mice trained in cages on the upper or lower shelves in each trained direction, where the variation in RF field intensities is larger and does not overlap the intensities in testing, failed to exhibit consistent directions of orientation relative to the magnetic field. Black triangle at the top of the distribution shows the trained magnetic direction. See text for more detailed explanation.

In contrast to MUHEIM et al. (2006; Fig. 3), the distribution of nest bearings obtained from mice that were exposed to the 1.46 MHz LRRF field in training and testing appeared to depend on the position of the shelf on which they were trained (i.e., lower, middle, upper), each exposed to a different range of intensities of the 1.46 MHz field (Fig. 4). Mice trained in all four directions on the middle shelves, exposed to the same range of intensities of LFRF that they experienced in the testing arena, exhibited significant magnetic compass orientation in the trained direction (Fig. 4; middle distribution; $p < 0.05$, Rayleigh Test). In contrast, mice trained on the

upper and lower shelves, exposed to a wider range and higher absolute intensities of the LFRF field (Fig. 4; top and bottom distributions), failed to exhibit consistent directions of orientation relative to the trained magnetic direction (in both cases, $p > 0.10$, Rayleigh Test). The combined distribution of bearings from mice exposed to different LFRF intensities in training and testing (top and bottom distributions in Fig 4) was also indistinguishable from random (distribution on the right in Fig. 5, $p > 0.50$, Rayleigh test). The difference in the distribution of bearings from mice trained on the middle shelf (left distribution) versus the combined distribution of bearings from mice trained on the upper and lower shelves (right distribution) approached significance (Fig. 5, $p < 0.10$; Watson U^2 test).

The data in Fig. 4 are consistent with variation in the learned magnetic compass response being affected either by the position of the shelves (upper and lower) or the higher intensity or the greater variation of the RF field mice were exposed to on the upper and lower shelves. In the earlier experiments by MUHEIM et al. (2006), there appeared to be no effect of shelf position (Fig. 3), suggesting that the differences in orientation reported here may have been a response to the differences in the intensity of the RF fields the mouse were exposed to in training. In order to confirm these findings, the middle shelf in each trained direction was removed and the upper and lower shelves in all four trained directions were moved closer together so that the intensity of the RF field the mice experienced in training more closely resembled the intensity of the RF field in the testing arena. In the first of these experiments, the mice exposed to RF field intensities on the newly positioned upper and lower shelves, similar to the intensities they were exposed to in testing, exhibited bimodal magnetic compass orientation along the trained magnetic axis (Fig. 6). These findings suggest that the absence of learned magnetic compass orientation by mice trained on the upper and lower shelves in the experiments shown in Fig. 4 was likely a consequence of the larger variation in intensities, and/or the higher absolute intensities, of LFRF, rather than an effect caused by shelf position alone.

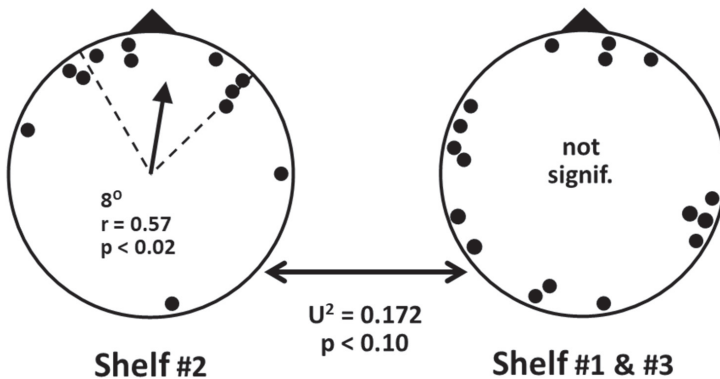


Fig. 5. Data from Fig. 4 comparing the responses of mice trained on the middle shelves where they were exposed to intensities of near-LFRF that were the same as in testing (Shelf #2) to the responses of mice trained on the upper and lower shelves where they were exposed to greater variation and overall higher absolute intensities of the LFRF field in training compared to testing (Shelf #1 and #3).

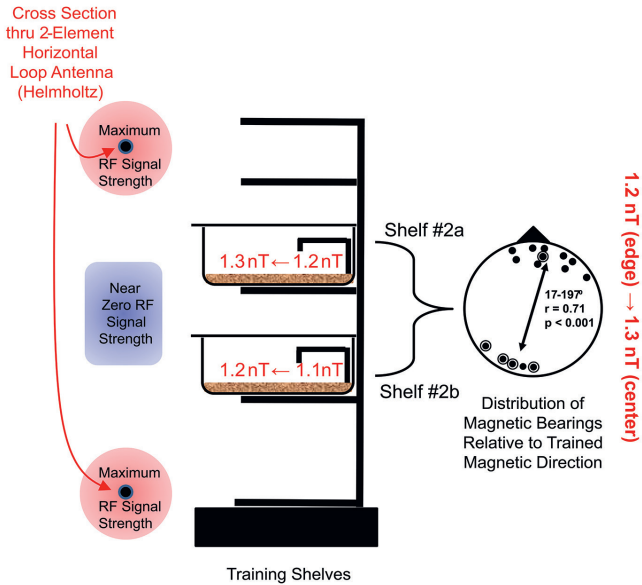


Fig. 6. Control experiments carried out with the middle shelf in each trained direction removed, and the upper and lower shelves moved closer together, so that the intensity of the RF field that the mice were exposed to in training more closely resembled that in testing. Circled symbols were from mice tested on the days leading up to 4 July (USA Independence Day Holiday) when fireworks were heard throughout the day and evening (see text).

However, an unexpected feature of the responses in Fig. 6 is the clustering of a subset of bearings opposite the trained direction. Most of these bearings were from mice tested on the days leading up to 4 July (USA Independence Day) Holiday when the sound of fireworks was heard throughout the day and evening (circled symbols). Mice exhibited similar responses in pilot experiments carried out before the experiments shown in Fig. 3 when thunder was heard overnight while a mouse was in the testing arena, and so these responses were excluded from consideration in the experiments carried out by MUHEIM et al. (2006) based on a *priori* criterion (see Methods). Interestingly, reversals in the direction of magnetic compass orientation have been shown previously in nest building experiments in response to other factors (e.g., odors of predators in the vicinity the testing building, a change from a female to a male mouse caretaker in the mouse colony, retesting of individual mice), highlighting the plasticity underlying rodent magnetic compass orientation (PAINTER et al. 2018, and unpubl. data).

Due to the possibility that the sound of fireworks leading up to the July 4 holiday affected the direction of orientation along the trained magnetic axis of some mice (Fig. 6), this series of experiments was repeated after 4 July holiday when the loud noises were no longer present. Like the mice trained on the middle shelf in the experiments shown in Fig. 4, mice exposed to similar intensities of near LFRF fields in training and testing exhibited unimodal orientation in the trained magnetic direction (Fig. 7).

DISCUSSION

Evidence that input from the magnetic compass can be altered or eliminated by low-level RF fields has been obtained in a variety of vertebrates (RITZ et al. 2004, ENGELS et al. 2014, MAL-KEMPER et al. 2015, PHILLIPS et al. 2022, MCKENNA et al. in prep.). Experiments with LFRF fields that were higher in intensity (35–50 nT) than those used in the current experiments have shown that yearling snapping turtles *Chelydra serpentina* will spontaneously associate an RF-dependent pattern of input from the magnetic field (‘magnetic modulation pattern’) with novel surroundings (LANDLER et al. 2015). LANDLER et al. (2015) found that if turtles were initially introduced to the testing arena *without* exposure to LFRF fields, they would align themselves with respect to the magnetic field in subsequent testing trials only in the absence of LFRF fields, and were disoriented when tested in the presence of the LFRF stimulus. Conversely, if the turtles were initially exposed to the testing arena *with* LFRF fields, in subsequent sessions they would align themselves with respect to the magnetic field only with the LFRF fields and were disoriented when tested *without* LFRF fields. These data suggest that in some cases exposure to RF fields may qualitatively alter the pattern of input (‘magnetic modulation pattern’), rather than eliminate magnetic input altogether (see also PHILLIPS et al. 2022).

Effects of ambient anthropogenic RF fields of only ~1 nT have been shown to disrupt magnetic compass orientation of migratory European robins *Erithacus rubecola* (ENGELS et al. 2014), although the nature of the effect (i.e., a response to a change in the magnetic modulation pattern vs elimination of the pattern altogether) was not determined. Further evidence suggests that

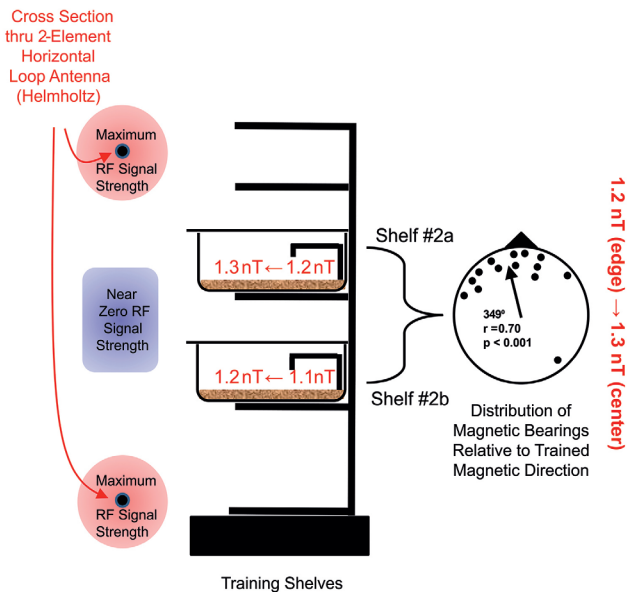


Fig. 7. Magnetic compass orientation of mice exposed to similar intensities of a low-level LFRF field in training and testing (1.46 MHz); replication of Fig. 6.

RF fields of this intensity can be a source of uncontrolled variability in laboratory experiments in a variety of other organisms, including murine rodents (MALEWSKI et al. 2018, PHILLIPS et al. 2022).

The findings reported here show that the presence of a ~ 1 nT LFRF field *per se* does not disrupt learned magnetic compass orientation by C57 BL/6 mice; mice exposed to a low-level (~ 1 nT) LFRF field spanning a narrow intensity range that overlapped the intensity range in testing (Figs. 4–6) showed comparable orientation to that observed in the earlier experiments without RF exposure (Fig. 3; MUHEIM et al. 2006). In contrast, both distributions of magnetic bearings from mice exposed to a larger range, and higher intensities, of the LFRF field in training, that did not overlap the narrow intensity range in testing, failed to exhibit magnetic compass orientation in the trained directions (Fig. 4, upper and lower distributions; Fig. 5). Further research is needed to determine if these findings are replicable and, if so, to better characterize the threshold (both the absolute threshold and the relative threshold for intensity differences between training and testing) for such effects.

The findings in Fig. 4 are consistent with at least two possible explanations. (1) The intensities used in these experiments spanned the threshold for effects of the RF fields on magnetic compass orientation, i.e., the intensities to which the mice were exposed on the upper and lower shelves were above a critical threshold altering or eliminating the magnetic modulation pattern, preventing the mice from learning the magnetic compass direction of the nest box in the training cages. Alternatively, (2) the difference in the RF field intensities that mice were exposed to on the upper and lower shelves from the intensities mice were exposed to in the testing arena were sufficient to produce different patterns of magnetic input (magnetic modulation patterns) so that the mice could not reconcile the pattern they learned in training with the pattern produced by the weaker LFRF stimulus exposed to in testing. If so, the disorientation of mice trained on the upper and lower shelves (Fig. 4) was due to an effect similar to that observed by LANDLER et al. (2015) when yearling snapping turtles were exposed to a near-LFRF field an order of magnitude higher in intensity than those used in the present experiments (see earlier discussion).

It is important to emphasize, however, that both explanations for the current findings suggest a level of sensitivity to low-level near-LFRF fields that cannot be explained by current models of the RPM (KAVOKIN et al. 2014, KATTNIG et al. 2016, HORE & MOURITSEN 2016). Nevertheless, dismissing these findings without an attempt at replication would be difficult to justify given the emerging evidence that RF fields at intensities of ~ 1 nT can alter the magnetic compass orientation in a variety of other animals (ENGELS et al. 2014, TOMANOVÁ & VÁCHA 2016, MALEWSKI et al. 2018, PHILLIPS et al. 2022). In future experiments using three training shelves in each direction (Figs. 3 and 4), mice trained on the middle shelves could be exposed to a slight increase in the intensity of the near-LFRF field (i.e., to 1.3 to 2.1 nT) to provide a further test of whether slight differences in the intensity of the RF field in training and testing are sufficient to disrupt the learned magnetic compass response. Confirmation that such weak RF fields alter the response of the mouse magnetic compass would raise a number of interesting questions about the underlying biophysical process, contribute to a better understanding of potential sources of variability in the response of murine rodents to magnetic cues in laboratory experiments, and raise the possibility of a widespread impact of electromagnetic fields generated by human activity on natural populations of wild animals (BELMORI 2015). Moreover, if these findings prove replicable, they would establish the nest-building assay with C57 BL/6 mice as an excellent model system with which to characterize the effects of exposure to low-level RF fields on the mammalian magnetic compass.

Acknowledgments

Hynek BURDA's research on the ecology, sensory biology, reproductive physiology, neuroanatomy, and social behavior of mole-rats is a *tour de force* showing the remarkable insights that can be gained from comparative studies of a unique group of animals adapted to an extreme environment. The work provides a telling illustration of how adaptation involves the whole animal, documenting the complex interplay among multiple sensory, physiological, morphological, and behavioral systems. As an offshoot of this work, Hynek's lab pioneered studies on the involvement of magnetic field cues in the spatial behavior of mammals; our lab's early attempts to show magnetic field sensitivity in small mammals were unsuccessful, and it was only after we began using the nest-building assay developed by Hynek's group that we were able to embark on a multi-year study of magnetoreception in murine rodents. In addition, Hynek and his colleagues have provided the first systematic studies of magnetic field sensitivity in large mammals, revealing both the wide taxonomic distribution of this sensory ability and a novel class of responses (magnetic alignment behavior) that has since been demonstrated in other major classes of vertebrates. We look forward to future contributions that Hynek and the members of his lab will continue to make across many fields of biological research.

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MagN at Cardinal Compass Directions

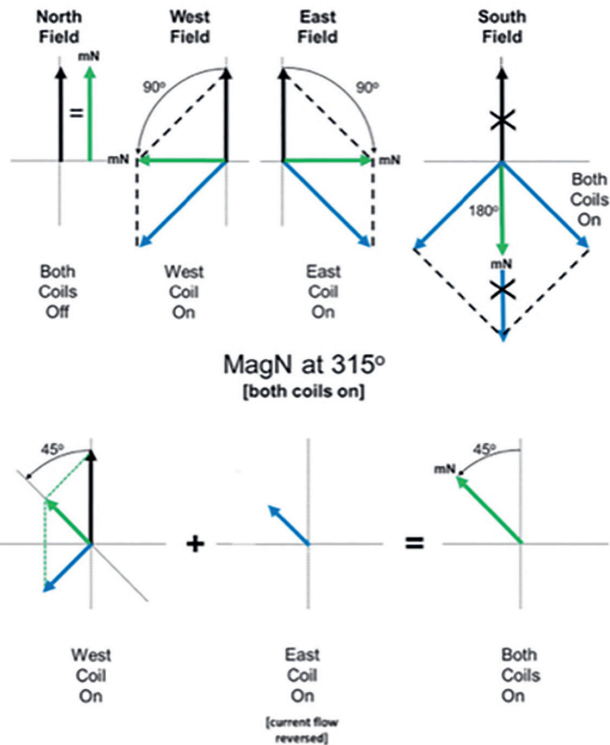


Fig. S1. Magnetic field alignments used in testing (top view). The coil system made it possible to produce three alignments of the magnetic field with magN at east (90°), south (180°) or west (270°) that matched the intensity and inclination of the ambient magnetic with magN at north (0°) using only a magnetic compass (MUHEIM et al. 2006). However, a magnetometer was used to adjust the intensity of the magnetic field with magN at northwest (315°). Black arrows show horizontal component of ambient magnetic field. Blue arrows indicate artificial field produced by orthogonal cube-surface-coils aligned to produce horizontal fields along the northeast-southwest ($45\text{--}225^\circ$) axis – West Coil, or the northwest-southeast ($135\text{--}315^\circ$) axis – East Coil. “X” indicates components of the ambient and/or artificial magnetic fields that canceled out. To produce testing fields with magN at east (90°) or west (270°), the current in the respective coil was increased until a dipole compass placed at the center of the coil was rotated by 90° clockwise or counterclockwise. The same current settings were used with both the east and west coils on to produce the field with magN at south (180°). Green arrows indicate the horizontal component of the ambient field (magN=north) or horizontal component of the resultant field (magN = west, east, or south) used in testing. To generate the magnetic field with magN at northwest (315° ; lower diagram), the intensity of the west coil was adjusted until the alignment of the resultant horizontal component was rotated 45° counterclockwise. The polarity of the east coil was then reversed and the current increased until the intensity of the horizontal component matched that of the ambient magnetic field. As in previous experiments (e.g., MUHEIM et al. 2006) only the horizontal component of the magnetic field was manipulated. The vertical component was unchanged so the resulting total field intensity ($\pm 2\%$) and inclination ($\pm 1.5^\circ$) of the altered field closely matched the ambient field.

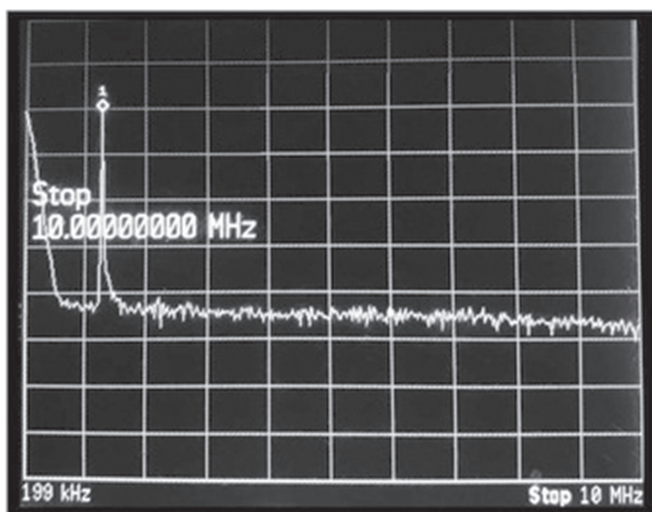


Fig. S2. RF stimulus used in training and testing plotted on a scale from ~ 0 to 10 MHz. The intensity of the RF field varied from 1.0 to 2.1 nT (see text).