Behavioral and neural activity during magnetic stimulation of *Tritonia tetraquetra* imply conditional magnetotactic response

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Blinks-NSF REU-BEACON 2014

Summer 2014

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Keywords: Tritonia tetraquetra, magnetic orientation, neuroethology, geomagnetic sense

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Abstract

A variety of species are known to sense and respond to the geomagnetic field for navigation. The sea slug *Tritonia tetraquetra* (a.k.a. *Tritonia diomedea*) has been shown to respond to the magnetic field through both behavior and electrophysiological experiments. However, it remains unclear by which mechanism this sensory information is integrated into motor commands. Additionally, the purpose for a response to magnetic stimuli has yet to be determined. While it is hypothesized that the sea slug sometimes uses a cue-switching mechanism to navigate, ultimately relying on the magnetic field for orientation, laboratory experiments thus far have failed to demonstrate this behavior. The experiments here sought to test this cue-switching hypothesis by demonstrating a response to a local distortion in the geomagnetic field. However, the behavioral response observed was an increased turning frequency upon loss of an initial attractive odor, independent of magnetic distortion, which is inconsistent with the hypothesized straight, geomagnetic-guided crawling. Additionally, in electrophysiological experiments ciliary motor neurons Pd5 and Pd6, as well as sensory nerve CeN1, failed to demonstrate a response to rotations of the magnetic field, despite previous evidence that these units are involved in *T. tetraquetra*'s magnetic response. These results, when compared with previously demonstrated responses, may imply conditional parameters under which detection and use of the magnetic field are employed, or may be due to population differences.

Introduction

A diverse range of species has been shown to respond to the geomagnetic field, including sea turtles (Lohmann, 1991), birds (Wiltschko & Wiltschko 1972), and bacteria

(Blakemore 1975). As human nervous systems are not known to be influenced by earth-strength magnetic fields, many questions arise as to how these organisms use the information obtained from this sensory input. These species have been studied to determine both the purpose and mechanism for detection of the magnetic field. There is evidence for use of this sensory information for navigation and migration, for example as demonstrated by experiments with European robins and homing pigeons (Wiltschko & Wiltschko 1972; Keeton 1971), although the purpose for its use in others remains unclear. Similarly, the identification of neural processes to detect and transduce this information has proven difficult.

The marine nudibranch mollusk *Tritonia tetraquetra* has been recognized as an animal that senses and responds to the geomagnetic field (Lohmann & Willows 1987). *T. tetraquetra* is an ideal model system within which to study neuronal responses to the magnetic field because its nervous system is made up of only about 7000 neurons (Boyle *et al.* 1983) many of which are large and colorful, making electrophysiological recording feasible. Additionally, the extensive mapping and characterization of individual neurons within *T. tetraquetra* has provided evidence for identification of the "same" neuron in different individuals. The presence of these re-identifiable neurons facilitates repetitive readings from different animals. Recordings from certain neurons have exhibited activity in response to manipulations of the ambient earth-strength magnetic field, such as that shown by experiments with the right pedal 5 (RPd5) and left pedal 5 (LPd5) neurons (Lohmann *et al.*1991; Wang *et al.* 2003; Wang *et al.* 2004). The experiments depicted enhanced electrical activity in response to changes in the ambient magnetic field, indicating the role of LPd5 and RPd5 in the animal's detection of the magnetic field.

These responses appeared several minutes after the rotation of the magnetic field, but the magnetoreceptors have yet to be located (Pavlova *et al.* 2011). Nerve recordings demonstrated the involvement of multiple nerves in sensory reception of this information, so pursuit of these nerve pathways further elucidates the mechanics of the magnetosensory system. In these experiments, nerve sensory activity was analyzed for any changes in response to rotations of the horizontal component of the ambient magnetic field. Neurons (Pd 5, 6, and 7) were chosen because they are shown to respond to magnetic field rotations, and because they affect crawling by activating cilia on the foot (Popescu 1999; Wang *et al.* 2003; Wang *et al.* 2004). The activities of these neurons were examined to determine the location of the magnetoreceptors in the body of the slug. The authors concluded that the receptors are widely distributed in the body and send input via many nerves.

At any given point on the surface of the Earth, the magnetic field creates a vector made up of both a horizontal and a vertical component. Orientation experiments, with *T. tetraquetra* in normal and canceled magnetic fields have shown it to respond to manipulations in the horizontal component of the field, i.e. it has a polarity compass, not an inclination compass (Lohmann & Willows 1987).

The ability to sense and utilize information from the geomagnetic field serves a variety of purposes for navigation and orientation. For species where other external cues are limited, such as in an underwater habitat, the magnetic field can provide a valuable source of information. In the case of *T. tetraquetra*, which do not migrate significant distances over the courses of their lives, it is unlikely that this sense is used for long-term navigation, as it is in far-ranging species like the sea turtle.

Instead, Wyeth has proposed a hypothesis that these nudibranchs integrate the information from the magnetic field with other sensory input, to navigate in a rapidly changing environment (Wyeth 2010). With *T. tetraquetra,* the direction toward which the current moves can often change much more quickly than the animal can complete its response to a stimulus. For an animal that moves 2 mm/s (Audesirk 1978), this can make maintaining orientation toward an attractive odor quite difficult. The hypothesis proposes that *T. tetraquetra'*s solution to this problem is a series of temporal shifts in priority between preferred stimuli, enabling it to remain on course. The initial stimulus could be that of an attractive odor, from either potential prey or a potential mate. The animal would first orient to this odor, but quickly switch to use of the current as the odor diffuses throughout the water. Rheotaxis, the organism's response of orienting itself in the direction of an oncoming current, induces upstream movement toward the attractant. However, the turbulent current again is a variable source of information (Wyeth *et al*. 2006). At this point the nudibranch transitions to focus on its position in relation to the magnetic field, with which it can maintain its direction of movement despite its inconstant external environment. In short, the hypothesis describes a shift in response from odor to current, and finally to geomagnetic field.

Wyeth's hypothesis has failed to be supported by laboratory experiments in 2013 and in the present study (Beron, Linney, Murray unpublished); but experiments in natural habitat are now being analyzed (Wyeth and Murray, unpublished). In the behavior experiments described below we sought to demonstrate the transition between external sensory cues through *T. tetraquetra*'s directional response, comparing that in a normal environment and that in which the magnetic field has been distorted.

Methods

Behavioral experiments

To test Wyeth's hypothesis, the behavioral response to a manipulated magnetic field was examined. Using a 2.15 m long and 0.97 m wide and 16 cm deep flow-through flume with a continuous, even (consistent across the width of the flume, and fairly consistent at all depths) current, the sea slugs were exposed to a variety of external stimuli (see Wyeth & Willows 2006 for details about this flume).

At the beginning of each trial period, nine live sea pen (*Ptilosarcus gurneyi*) were soaked in 3.5 to 4 gallons of seawater for approximately 90 minutes. As these are a natural prey species of *T. tetraquetra*, this scented seawater served as the attractant for the animals. The attractant was then poured into a plastic 6.5-gallon carbuoy, where it was dyed with fluorescein to enable visualization of the path of the odor through the flume. The dyed scent was then delivered by latex tubing to a central position at the beginning of the flume, at a height of 6.5 cm. This end of the flume contained a constructed waterfall to distribute incoming seawater uniformly across the width of the flume, creating a relatively even and constant, yet turbulent flow (current) down the length of the apparatus (Wyeth & Willows 2006) (Table 1). The odor was released at a slow enough speed that it got carried along with current, creating a plume consistent in velocity with the main current, and thus eliminating a possible confounding variable from differential water velocity. There was sometimes a back-eddy of flow on the ~1-cm deep surface of the water, which made it difficult to visualize the downstream-headed plume that was below the surface. The water ran through at an average velocity of 1.25 cm/s, an average initial range at the beginning of the flume of 1.31 cm/s to 2.17 cm/s, and an average final range at the end of the flume of 0.68 cm/s to 1.51 cm/s, calculated from three series of measurements at five equal intervals across the width of the flume (16.2cm, 32.4cm, 48.6cm, 64.8cm, 81cm) (Table 1). The current appeared to decline in speed as it moved down the flume.

The *T. tetraquetra* for these experiments were obtained from Ilahee town dock near Bremerton, and Langley off Whidbey Island (Washington) by SCUBA diving. For the behavior assays, each sea slug was run through four trials, the order of which was randomly assigned (using a random number generator): beginning on the left/right side with a magnet present, and beginning on the left/right side in the absence of a magnet. The coin-shaped magnet (1" x 1/8" Thick, Grade N50, Ni-Cu-Ni Coated Rare Earth Neodymium Disc Magnet (ND054 from MagnetForLess.com)) was positioned flat in a Petri dish hanging flat 1.6 cm above the surface of the water at the far end of the flume from the sea slug, near the release of the odor. The distance of the magnet from the slug ranged from 9 cm to the tips of its extended rhinophores to 17.5 cm from its foot. For each trial with the magnet present, the magnet was rotated such that the distortion of the magnetic field would be identical across trials (the number 1 written on top of the Petri dish pointed upstream) with the north pole down, so as to add to the intensity of the natural geomagnetic field (Figure 1). The azimuth and the intensity of the magnetic field around the magnet were measured at a height of 1.5 cm above the surface of the flume, which is the approximate height of the oral veil and the mid-height frontal plane of the animal.

The sea slugs were released from the starting position oriented perpendicular to the current and facing away from the wall, ~180 cm from the odor source at the upstream end of the flume. Each trial began with the release of *T. tetraquetra* and ended when the animal made contact with any wall of the flume (Figure 2). A trial was considered a success if the animal made it to or beyond the halfway point along the length of the flume and the odor was shut off. If the slug made it past halfway and then remained stationary for 5 minutes or more, the trial was also considered complete. As the slug oriented to the sea pen odor and traveled up the current, its movement and position were tracked with a video camera (JVC model GR-DVL9800U); time-lapsed at 1 frame per 10 seconds using SecuritySpy software v.3. When the animal reached the halfway mark, the odor was turned off and the latex tubing cleared with fresh, undyed seawater to remove any trace of the source of odor. The behavior of the sea slug was then monitored in response to the disappearance of this odor cue. The current remained constant throughout each trial.

The video recordings of each successful trial were analyzed using ImageJ software to create tracking coordinates for slug position, as well as the heading angle of the slug over time. Changes in heading angle were considered a turn if they reached a difference of at least 45° from the starting position within two minutes. A turn was considered complete when the slug proceeded forward in the same direction for at least two frames (20 seconds). The heading angle of each slug at the time the flow was removed was subtracted from the heading angle at each of these time periods. The average of each value for all four of each slug's trials were calculated, and then the data run in a Friedman test for a repeated measures one-way ANOVA. Additionally, the changes in heading angle over 10-second intervals in the 80 seconds preceding odor removal were compared with those in the 80 seconds following odor removal. The absolute value of each change in angle (over 10 seconds) was averaged for each point in time in the 80 seconds before and after the odor was cut off, first within the four trials of each sea slug to avoid pseudoreplication, and then between the ten slugs. The time scale of the trial was centered to time 0, which was set to the time at which the odor plume was cut off.

Electrophysiological experiments

T. tetraquetra were also used for electrophysiological experiments, where their brains were exposed via a semi-intact preparation that allowed recording during normal behavior (Figure 3). An incision was made on the central dorsal region of the animal, and proceeded in the anterior direction until the incision reached between the rhinophores. The brain was immobilized with stainless-steel pins on a wax-coated stainless steel platform to make it accessible to the microelectrodes. The animal was placed in a rectangular chamber filled with seawater, surrounded by a narrow gap continuously channeling through icy freshwater. This maintained an environment conducive to keeping the specimen alive while electrical recordings were obtained. All instruments involved in this procedure were constructed of non-magnetic materials to avoid any unintentional altering of the ambient magnetic field. Average rates of spikes were calculated using LabChart 7 software.

Intracellular recordings

Electrodes filled with 4M KAc and surrounding a chlorided silver wire penetrated the designated neuron and were used to obtain electrical readings from inside the cells. The electrical reading recorded upon penetration was delivered from the microelectrode to a preamplifier and amplifier (A-M Systems Model 1600) through which its intensity was amplified by a magnitude of 10x. This amplifier was sometimes operated with a notch filter to reduce 60 Hz noise from surrounding electrical appliances, emphasizing the desired measurements from neuronal output (and was otherwise processed by a Humbug noise reducing circuit). This amplified recording was then transmitted to a digitizer (Powerlab model or 4SP; at 4000 Hz) to record the output on a laptop for subsequent off-line analysis. A baseline voltage level ("resting potential") of 30-60 mV confirmed successful penetration and a healthy cell interior. Rotations of the ambient field were effected either 60° clockwise or counterclockwise relative to the geomagnetic field, achieved using two cubical Merritt coils (Merritt *et al.* 1983) 2m by 2m by 2m. Activation of this coil through running DC currents changed the direction of the horizontal component of the magnetic field while maintaining relatively stable intensity. The ambient earth-strength intensity at the beginning of the trials was recorded using a single axis fluxgate magnetometer (model DM2220 from Schonstedt Instruments, Kearneysville, WV) at 469 mG, that of the 60° clockwise-rotated field 447 mG and that of the 60° counterclockwise-rotated field 466 mG. These rotations were also recorded on the PowerLab and spike analysis was done relative to the timing of the rotations. The activity of the neuron was recorded in the time before and after these rotations, to monitor for a response to this manipulation of the magnetic field.

Each trial spanned 15 minutes, broken into three segments. The first five minutes recorded the intracellular activity in a normal magnetic field, followed by five minutes of field rotation (60° clockwise or 60° counterclockwise). Finally, the five minutes after the field rotation recorded the neuronal activity after the field was returned to its normal geomagnetic bearing. Each neuron was attempted to undergo 20 of these 15-minute trials, with the order of the 10 clockwise and 10 counterclockwise rotations randomly assigned.

Extracellular recordings

Extracellular recordings were taken using suction electrodes to draw in the cut peripheral end of nerve, from which electrical signals were recorded using an A-M Systems Model 1700 differential amplifier. This device amplifies the difference in voltage between a silver-chloride electrode inside the suction pipette and a similar electrode on the outside of the pipette. The settings on the amplifier with 1000X amplification had the high-pass filter set to 5000 Hz, and the low-pass filter set to 10 Hz. We sometimes used the notch filter to reduce 60 Hz noise, but if possible relied instead on active noise-cancellation by a Quest Scientific Humbug device, which can remove periodic signals without filtering, revealing sporadic and irregular signals such as action potentials. These signals were digitized as above, but at 10,000 Hz. Baseline noise levels for these recordings ranged between 1μV and 5 μV peak-to-peak, low enough that extracellular activity could be detected. Extracellular recordings were smoothed over 21 samples with a triangular (Bartlet) window. Stimulus timing for extracellular recordings involved one minute of recording in a normal field, one minute of a 60° field rotation, and one minute of return to normal field recording. As with the intracellular experiment, each nerve was attempted at 10 clockwise trials and 10 counterclockwise trials.

For both intracellular and extracellular recordings, the first period of each trial with baseline activity level was subtracted from the combined second (rotated field) and third (normal field) periods of the trial to normalize the data. The averages of these values from all trials were calculated for each slug, then the average across all slugs calculated.

Results

Behavioral experiments

Slugs' tracks were analyzed to determine behavioral responses to removal of the sea pen odor flow (Figure 4). Comparison of number of turns after odor stopped between trials with, and those without, a magnet present showed no significant difference (paired t-test, p=0.82; non-parametric version of t-test, p=0.46). Analysis included both total number of turns after sea pen odor removed before slug contacted the wall, as well as number of turns within the first 8 minutes after removal of the odor (Figure 5A).

In the majority of trials (53.2%), slugs made a single turn and continued straight at the new angle until making contact with a side wall of the flume to complete the trial. Slugs turned right first 71% of the times they were positioned on the left edge of the odor plume at the time it was stopped. Slugs turned left first 67% of the times they were positioned on the right edge of the odor plume at the time it was stopped. However, the evidence shows no statistical relationship was found between these variables, both with a chi-square test and a Fisher exact test (p=0.112 and p=0.118, respectively).

Heading angle was also tracked and provides evidence that slugs did not continue to crawl straight and toward the source of the odor after the odor flow was turned off. Heading angle measurements from periods before and after the odor flow is removed show that the slugs change their heading angle more (in degrees per time) after loss of the scent (Friedman test for repeated measures one-way ANOVA, Friedman statistic =10.40, p=0.0034, n=10) (Figure 5B). The change in heading angle showed the average degree by which the slugs changed absolute value of heading angle over each 10-second interval (Figure 5C). This graph shows that, on average, the slugs changed heading angle more in the 10-second intervals following the removal of the odor plume, increasing from an average rate of 49°/min (5-10° /10-sec interval) before the odor was removed to 68°/min (10-15° /10-sec interval) after the odor was removed.

Several *T. tetraquetra* obtained from each location failed to orient at all to the sea pen odor, but instead repeatedly crawled across the flume straight from their starting position, perpendicular to current and odor flow. About 75% of trials demonstrated a failure of the animal to orient to the odor flow, and these trials were excluded from analysis. About 38% of animals failed at chemotactic behavior to the degree that no successful trials were completed, whereas the other slugs seemed to encounter periods of failed orientation interspersed with periods of successful orientation upstream. In some trials a small layer of dye remained on the surface near the source of the odor, but this did not appear to influence slug movement and the rhinophores did not physically reach that upper layer.

Electrophysiological experiments

Recordings showed intracellular and extracellular activity levels before, during, and after the rotation of the magnetic field (Figures 6 and 7).

From the intracellular recordings, RPd5 and LPd5 showed no change in activity upon a 60° rotation clockwise (p=0.23 and p=0.41, respectively) or counterclockwise (p=0.72 and p=0.87, respectively) of the magnetic field (Figure 8). There was also no difference in activity found between clockwise and counterclockwise rotations in either neuron. Some trials showed an increase (25 trials) or decrease (12 trials) in activity upon rotation of the field, but these happened infrequently enough to likely be random occurrences. Recordings from RPd6 and LPd6 were compared together with RPd5 and LPd5 due to similar activity. Results from the peripheral ends of RCeN1 and LCeN1 also showed no significant difference in activity upon a 60° rotation clockwise (p=0.34 and p=0.42, respectively) or counterclockwise (p=0.21 and p=0.37, respectively), nor a difference between the direction of magnetic field rotations (These data were also not found to be significant with a non-parametric version of a t-test, based on the Wilcoxon test) (Figure 9).

Discussion

The results from these experiments showed no response from *T. tetraquetra* to a magnetic field stimulus. The behavior experiments failed to demonstrate a response of the animals to a distorted magnetic field after the removal of the odor flow. After odor flow was cut off, the behavior of the animals showed a divergence from the straight path toward the source of the odor, which resulted in a search-like behavior characterized by more frequent turning. This result is inconsistent with the hypothesis that the slugs are navigating by switching from an odor cue to information from the magnetic field (Wyeth 2010). The inability to maintain a straight course, even without distortion from the magnet, provides evidence that the slugs are not relying on a cue from the magnetic field but instead have not yet completed a switch in stimulus from the odor flow. It is possible that switching to magnetic orientation occurs only when scent is lost when water flow changes direction, and our flow did not change direction but simply stopped or went below the threshold of detection; so the cue-switching hypothesis may be valid under different circumstances. The increased turning behavior implies an attempt to recover the lost scent, although not necessarily with the use of the magnetic field. It is possible that the animals use the magnetic field to transform seemingly random searching behavior into a more systematic pattern derived from awareness of body position in relation to the geomagnetic field.

This behavior incites the question regarding the context within which sensory information about the magnetic field may be used. Perhaps there are certain conditions in which *T. tetraquetra* relies on the field for navigation, and others when it does not. In these trials the water flow was kept constant throughout the duration of the trial, and this additional sensory input may have distracted or prevented the animals from orienting to the input from the magnetic field. The environment of the slug in its natural habitat likely allows for wider parameters, such as other cues that may offer a longer period of crawling to facilitate calibration to the magnetic field.

The neurons RPd5 and LPd5 showed little to no change upon rotation of the magnetic field, contrasting previous experiments that demonstrated a response to the stimulus. These studies included a series of one-minute rotations back and forth of the field as well as few single occurrence rotations, where the field was rotated for an extended (30 min.) period of time (Lohmann *et al.* 1991). In both cases, the neurons took about 10 minutes to respond to the rotation of the field. These stimuli are exaggerated and may provide a supernormal stimulus to the animals, as it is unlikely that the slugs will be turning in alternating directions so frequently in nature; in contrast, a single very extended turn is also not necessarily natural. The more naturalistic single rotation of the field may have resulted in a less easily-detectable response to the stimulus. Further experimentation may demonstrate a conditional response of these neural systems to magnetic field rotations, such as minimum angle or period of rotation.

The location of the magnetoreceptors in the body remains to be determined, and so further investigation into sensory nerve input may lead to the discovery of this information. Identification of magnetic detection sites and mechanisms of integration into motor programs may provide insight into the context in which the animals make use of this information in their environments. CeN1 also showed no apparent response to the rotation of the magnetic field, but further analysis may elucidate differences in activity levels between individual units of the nerve. Previous experiments with CeN1 included similar single shorter-duration rotations of the field, and demonstrated an increased activity level in the nerve as a response (Pavlova *et al.* 2011).

The results of these experiments open up many questions that remain unanswered about the navigational mechanisms employed by *T. tetraquetra*. These animals live in an environment that changes more rapidly than they can respond at times, and it remains to be determined how they respond to this variable stimuli. Further investigation is needed to understand the method by which these slugs integrate sensory information from their environment to produce a motor response.

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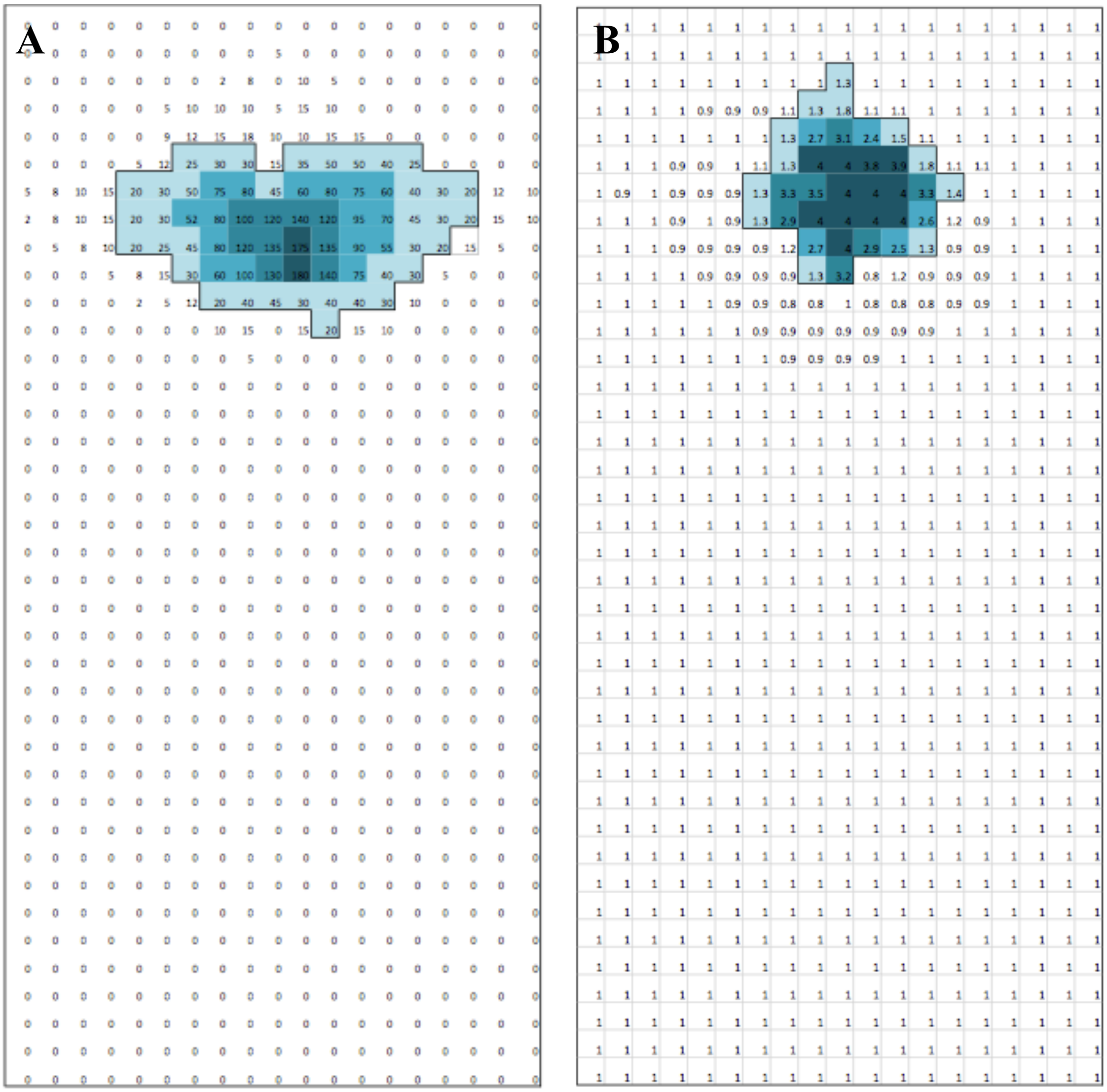
Figures



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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

B

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1	1	1	1	0.9	0.9	0.9	1.1	1.3	1.8	1.1	1.1	1	1	1	1	1	1	1
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1	1	1	0.9	0.9	1	1.1	1.3	4	4	3.8	3.9	1.8	1.1	1.1	1	1	1	1
1	0.9	1	0.9	0.9	0.9	1.3	3.3	3.5	4	4	4	3.3	1.4	1	1	1	1	1
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1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Figure 1. Map of magnetic field distortion. Both magnetic field direction and magnetic field intensity are affected by the presence of the magnet in the flume. Absolute deviation from the normal field direction is represented in **(A)** and proportion of maximum normal magnetic field intensity (measured at 510 mG during the mapping of the field) in **(B)** for each 5cm by 5cm square on the grid.

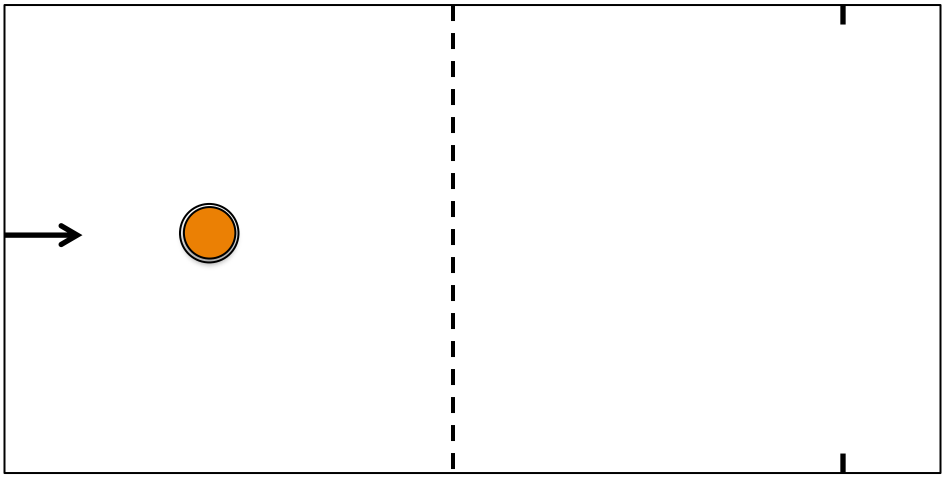


Figure 2. Diagram of behavior trial. Slugs were positioned at the tick marks at the downstream end of the flume, perpendicular to odor flow. Sea pen odor was released upstream and dyed with fluorescein for visualization of flow from the position and direction of the odor. Slug orientation and movement upstream toward the odor source was monitored and odor flow stopped when the slug reached the marker at the middle of the flume (dashed line). For distorted field trials, the magnet hung 1.6 cm above the surface of the water, represented by the orange circle.

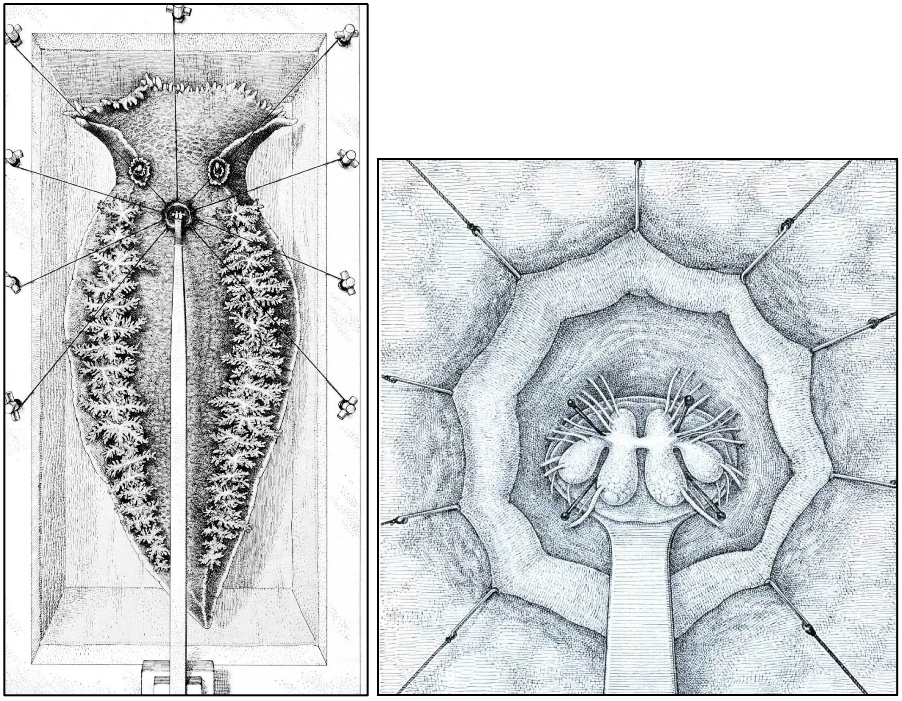


Figure 3. Diagram of semi-intact preparation (from Willows 1971). An incision was created in the anterior dorsal region of the slug's body, and the brain lofted on a wax-coated platform. Neurons and nerves were made accessible to electrophysiological recording. The slug was kept cool in a chamber of filtered seawater surrounded by a continuous flow of icy freshwater.

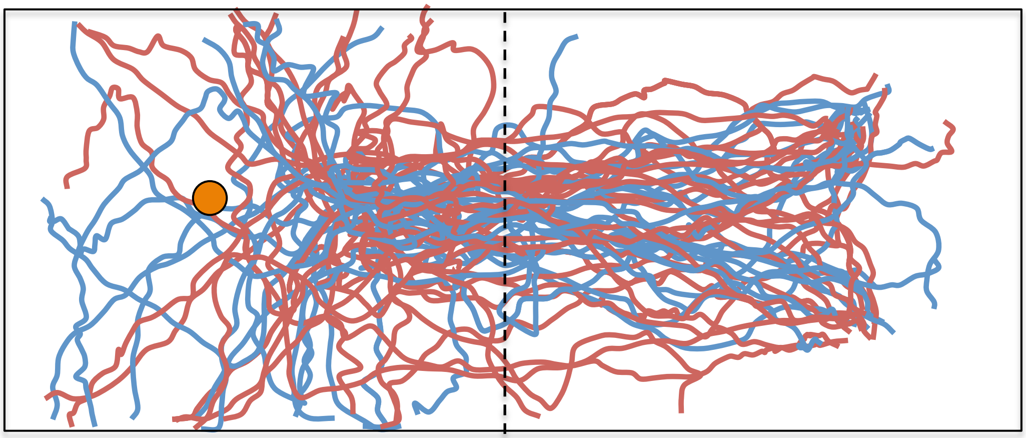
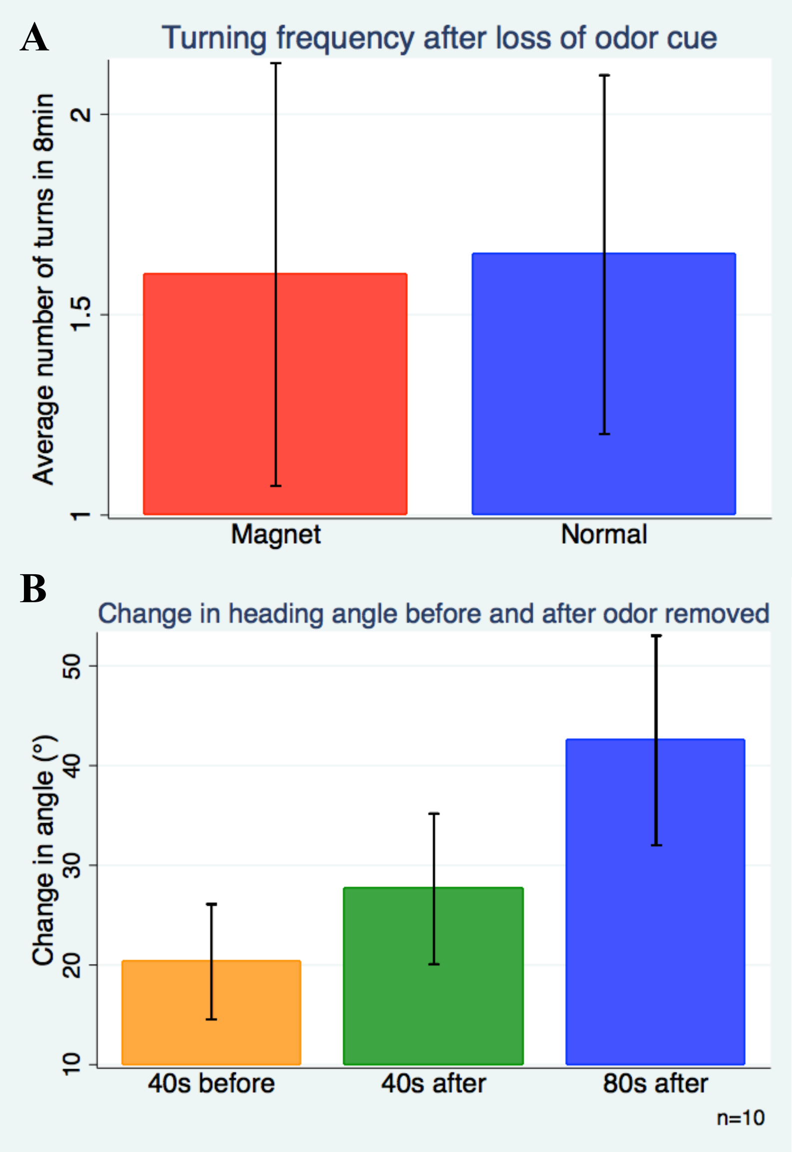


Figure 4. Compiled tracks of behavior trials. Tracks of slugs from starting position (on right) to completion of trial after odor plume stopped. Slugs oriented to flow and traveled upstream (to the left). Odor was cut off when the slug reached the dashed line. The position of the magnet (present in one-half of trials) is represented by the orange circle. Tracks in blue represent trials with a ambient magnetic field, and tracks in red represent trials with a distorted magnetic field.


A
Turning frequency after loss of odor cue
Average number of turns in 8min
1
1.5
2
Magnet
Normal
B
Change in heading angle before and after odor removed
Change in angle (°)
10
20
30
40
50
40s before
40s after
80s after
n=10

C

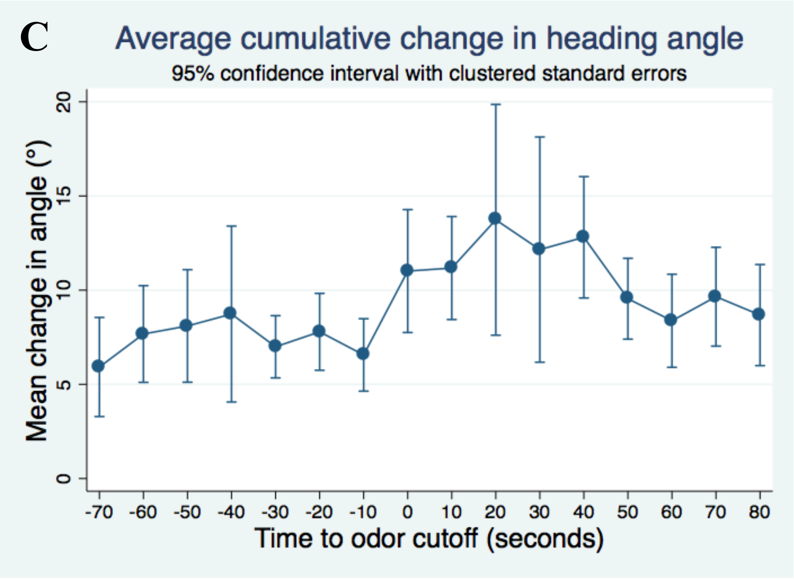


Figure 5. Behavioral responses to loss of odor. Comparison of turning rate with and without magnet present and change in directional heading at point odor flow is removed. **(A)** The first 8 min after removal of the odor flow with and without the distorted magnetic field showed no difference in number of turns. **(B)** Change in directional heading of slug (measured perpendicular to the angle between the rhinophores) 40 s before odor flow stopped (orange) vs. directional heading 40 s and 80 s after odor flow stopped (green and blue, respectively) (Friedman statistic=10.40, p=0.0034, n=10). **(C)** Change in heading angle over 10-second intervals increases after the odor is cut off, as shown at time=0 in the graph. Absolute value of change in heading angle changes more in the intervals 80 seconds following removal of odor than in those in the preceding 80 seconds. The error bars shown represent a 95% confidence interval. In this case, the standard errors that create the confidence interval are formed accounting for the clustering within slugs that occur due to the repeated trials with the same slug (Williams, 2000).

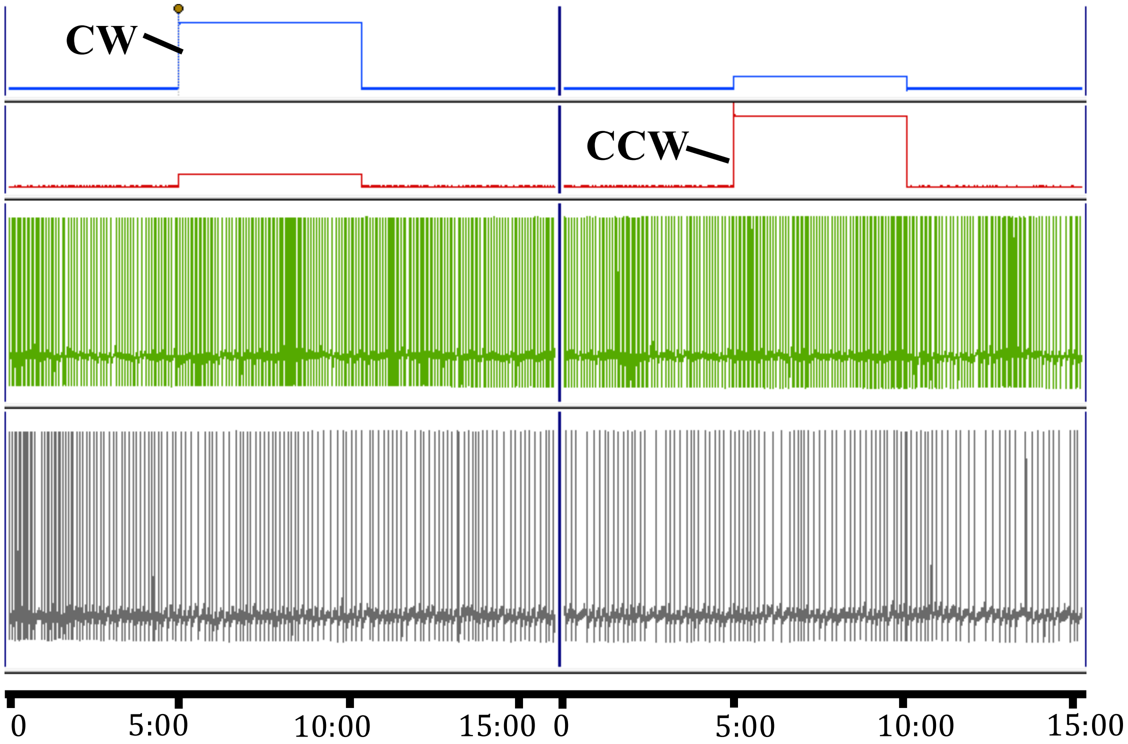


Figure 6. Sample of intracellular recording data. Intracellular recordings in RPd5 and LPd5 show voltage change in the neurons over time. The first segment records activity during 5 minutes of normal magnetic field conditions. The second segment records activity during a 5-minute 60° clockwise (CW) rotation of the field. The third segment records activity during 5 minutes of a return to normal magnetic field conditions. The first segment records 5 minutes of normal magnetic field conditions, the second segment records activity during a 5-minute 60° counterclockwise (CCW) rotation of the field, and the third segment records activity during 5 minutes of a return to normal magnetic field conditions.

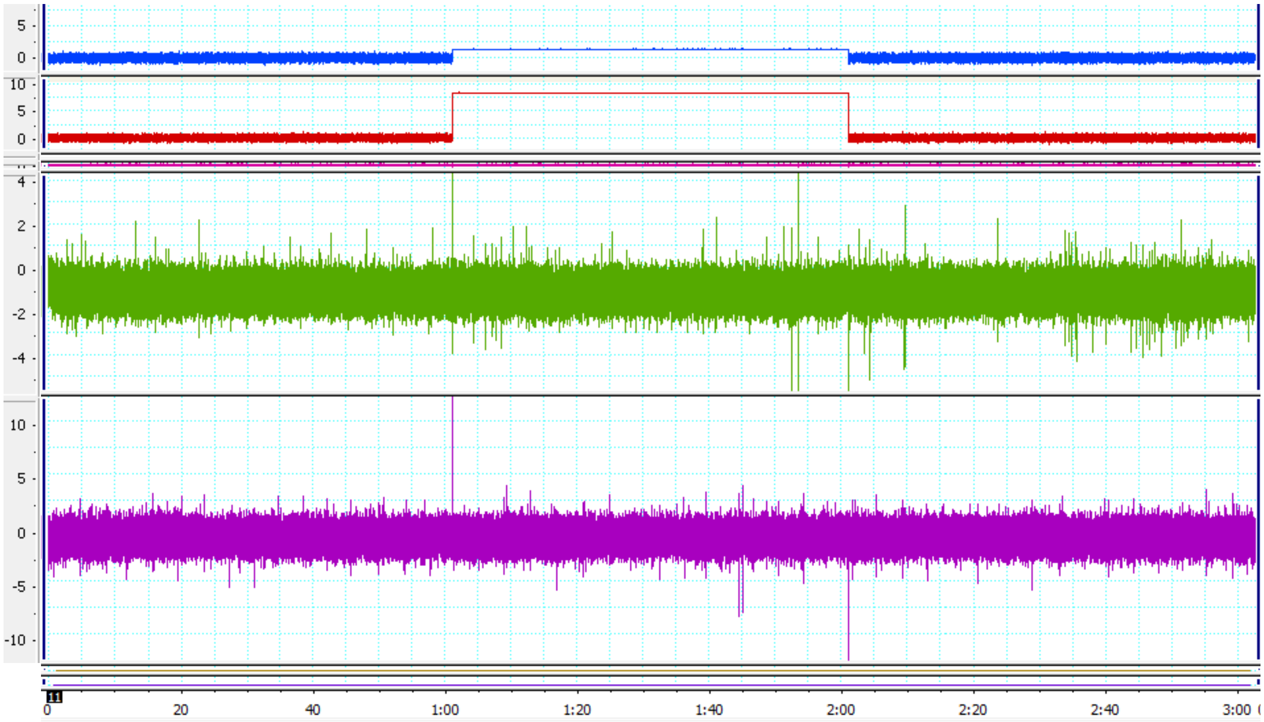


Figure 7. Sample of extracellular recording data. Extracellular recordings in RCeN1 (green) and LCeN1 (purple) show voltage change (µV) in the nerves over time (min). The first minute records smoothed activity during normal magnetic field conditions. The second minute records activity during a 60° counterclockwise rotation of the field. The third minute records activity during a return to normal magnetic field conditions.

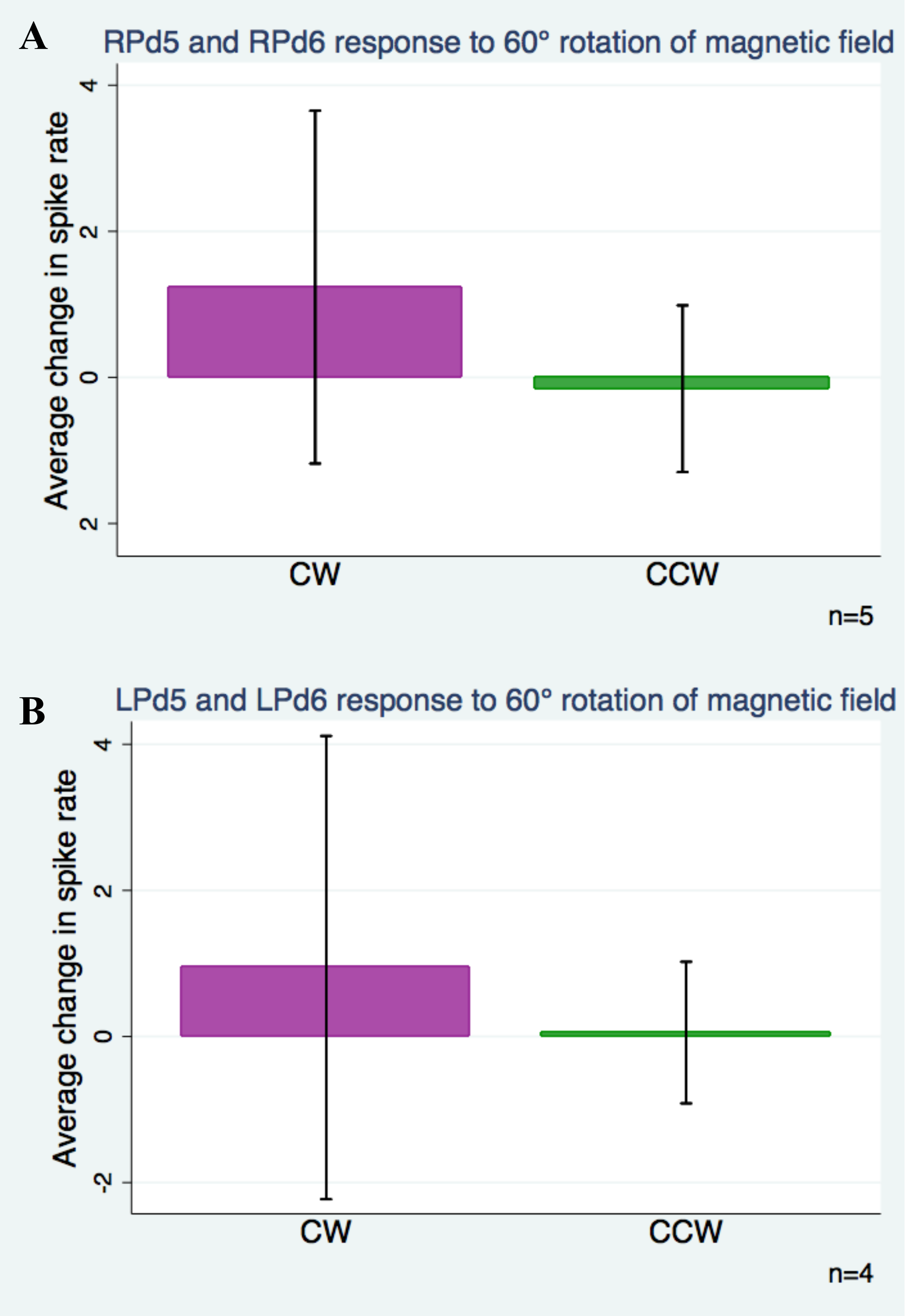


Figure 8. Pedal 5 neurons showed no response to the magnetic field. Intracellular recordings in RPd5 and LPd5 show average change in activity after a 60° rotation of the magnetic field. Each column represents the difference in average spike rate within Pd5 between the final two periods of each trial and the initial period (averaged between all 10 trials for each slug). The columns have been normalized by subtracting the average initial spike rate from each. **(A)** RPd5 shows no change in activity after a clockwise (two-tailed t-test, df=3, p=0.23, n=5) or counterclockwise (two-tailed t-test, df=3, p=0.72, n=4) rotation of the field. **(B)** LPd5 shows no change in activity after a clockwise (two-tailed t-test, df=3, p=0.41, n=4) or counterclockwise (two-tailed t-test, df=3, p=0.87, n=4) rotation of the field.

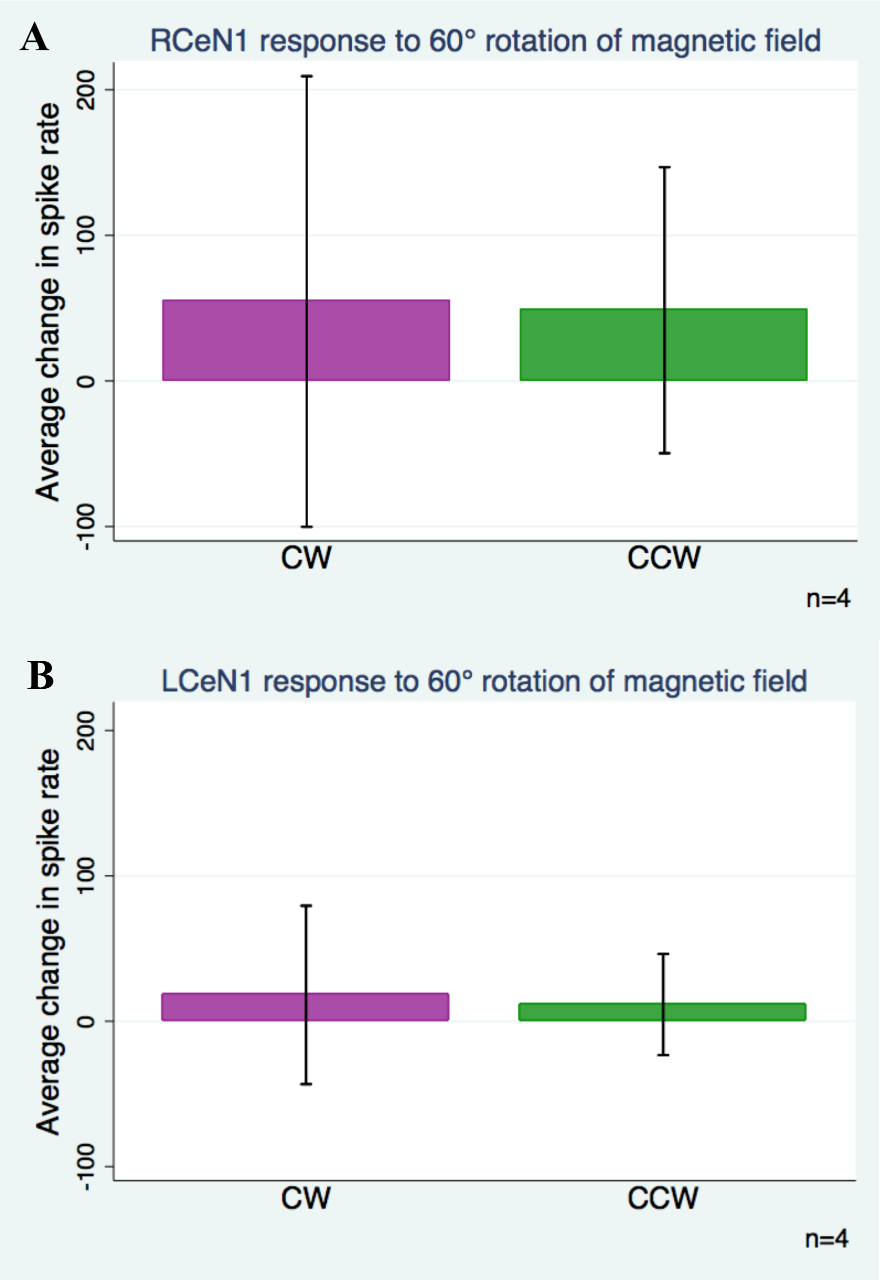


Figure 9. Cerebral nerve 1 showed no evidence of sensory detection of the magnetic field. Extracellular recordings from the peripheral ends of left and right cerebral nerve 1 show change in activity levels after a 60° rotation of the magnetic field. Each column represents the average spike rate within CeN1 during its respective time (averaged between all trials for each slug). The columns have been normalized by subtracting the average initial spike rate from each average final spike rate **(A)** RCeN1 showed no change in activity in correlation with the clockwise (two-tailed t-test, df=3, p=0.34, n=4) or counterclockwise rotations (two-tailed t-test, df=3, p=0.21, n=4) of the field. **(B)** LCeN1 showed no change in activity in correlation with a clockwise (two-tailed t-test, df=3, p=0.42, n=4) or counterclockwise (two-tailed t-test, df=3, p=0.37, n=4) rotation of the field.

	Distance drop released from flume edge (cm)				
	16.2	**32.4**	**48.6**	**64.8**	**81**
Average Velocity (cm/s)	1.2869 1.8406 1.5894	1.1593 1.4976 1.6113	0.9610 1.1733 1.6005	1.1483 0.8327 1.2566	0.9458 0.9952 0.8863
Initial Velocity (cm/s)	1.6733 2.5366 2.3033	0.9209 1.1166 1.8880	1.5381 1.1110 1.9948	1.7299 1.5032 1.6517	1.0863 1.7189 1.8766
Final Velocity (cm/s)	1.0833 1.3215 0.8039	1.6985 1.3338 1.5100	0.6666 1.0254 0.6145	1.0296 0.2173 0.8827	0.7027 0.7815 0.5456

Table 1. Speed of current measured at five points across the flume. Velocity of current in cm/s measured at five points across the width of the flume as well as at the beginning and end of the flume are recorded in the table. Each point along the flume had three trials to generate an average value.