

**Determining Magnetic Sensitivity of *Armina californica*; and of  
Neurons Homologous to those in *Tritonia tetraquetra*  
(*a.k.a.* *T. diomedea*)**

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## **Abstract**

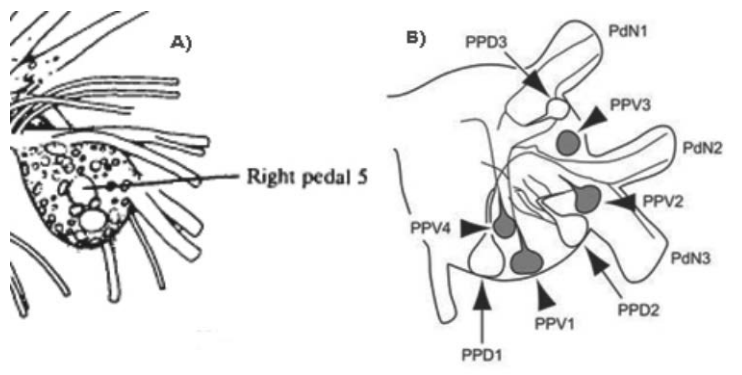
The gastropod nudibranch *Tritonia tetraquetra* is a convenient model organism for neurobiologists. It appears unique among nudibranchs in its ability to detect and orient to the magnetic field of Earth, which makes it an interesting basis for comparison. In our study, we assess the potential of magnetoreception in another nudibranch that shares a similar geography and lifestyle, *Armina californica*. We examine the neurons in *Armina* that are reported to be homologous to the known neural correlates of magnetoreception in *Tritonia*, and attempt to observe changes in activity while providing magnetic stimuli. Furthermore, we look for potential behavioral clues in the reorientation of displaced animals in natural and unnatural magnetic fields. Our results demonstrated that although *A. californica* and *T. tetraquetra* lead similar lives, *Armina* exhibited no significant responses to magnetic stimuli, either in neurophysiological or behavioral assays. These results suggest that *Tritonia* may be more unique than previously thought, and that magnetoreception may be limited to a very specific subset of gastropods.

## **Introduction**

The ability to detect the magnetic field of earth is present among diverse animal lineages. In both vertebrates and invertebrates this sense is involved with navigation and spatial positioning, such as the optimized burrowing vectors of the nematode *C. elegans* (Vidal-Gadea et al. 2015) or the pan-oceanic migration of sea turtles (Luschi et. al 2007). The nudibranch species *Tritonia tetraquetra* (formerly *Tritonia diomedea*) has been shown to be magnetically sensitive as well. Neural correlates of magnetic receptivity have been identified in *Tritonia* (Lohmann and Willows 1991; Wang et. al 2002), as well as a geomagnetic orientation behavior (Lohmann and Willows 1987), making it a

convenient model for studying the physiological and behavioral aspects of magnetic sensitivity. However, as of now, *T. tetraquetra* appears unique among nudibranchs in its ability to sense and orient to earth's magnetic field, and the mechanisms involved are still largely unknown (Pavlova et al. 2011).

*Armina californica* is another nudibranch species that inhabits the same soft sediment beds as *T. tetraquetra*, feeding upon the same colonial *cnidarian Ptilosarcus gurneyi*. Furthermore, two pairs of neurons in *T. tetraquetra* that are excited by alterations in earth strength magnetic fields (known as Pedal 5 and Pedal 6 cells) are known to be homologous both in location and function to the PPD1 and PPV1 neurons located in the pedal ganglia of *Armina californica* (Baltzley et. al, 2011). These cells are



**Figure 1.) Right pedal ganglia of *T. tetraquetra* brain (A) showing Pd5 and Pd6 below it, compared to the same ganglia of *A. californica*. (B)**

part of a motor network that utilizes a pedal peptide transmitter (Cain et al. 2005) that is conserved among many nudibranch, and non-nudibranch gastropod species (Baltzley et. al, 2011).

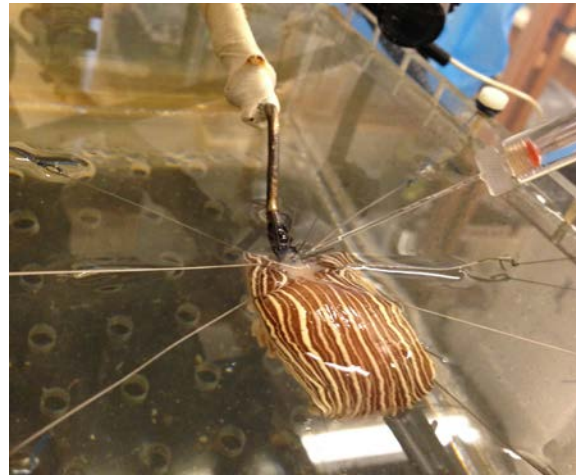
In this study, we take a two-part approach to determine if *Armina californica*, a relative of *T. tetraquetra* that shares a similar lifestyle, is sensitive to earth-strength magnetic fields. We seek to determine whether its purported homologous neurons can be excited by changes in magnetic field azimuth and, in addition, we look for behavioral clues to determine if and how magnetoreception can influence navigation in these animals.

## Methods

### Physiology

Specimens of *A. californica* were hand-collected via SCUBA from Lopez Sound, off the east coast of Whidbey Island, WA and from Squamish Harbor, WA and housed in flow-through seawater tanks (12-14° C) at Friday Harbor Labs. The animals were immobilized with hooks in a cooled salt-water dissection tank and the brain was exposed with an incision. The brain was then carefully secured with non-magnetic tungsten dissection pins onto a wax-covered non-magnetic platform and cleared of obscuring connective tissue, allowing individual neurons to be surveyed through the translucent epineurium. Positive identification of the purported homologue cells was possible due to the size of the neurons, their color, and their position on the dorsal surface of the ganglia.

Special care was taken not to damage any nerves, as that could destroy any potential responses (Lohmann and Willows, 1991). The electrical activity of these cells was measured intracellularly by impaling them with glass micro-electrodes (resistance between 6-15 M $\Omega$ ) filled with 4M potassium acetate



**Figure 2.) *Armina californica* brain exposed and immobilized. Intracellular electrode can be seen on the right.**

over a chlorided silver wire. The electrodes were made using a Sutter Instrument Micropipette puller (Model P97). Signals were detected by an A-M systems high-impedance amplifier (Neuroprobe model number 1600). If the cell membranes were

disrupted by the penetration, the cell was allowed to repair itself while applying gentle negative current to alleviate injury spiking and slowly weaning the cell off of it. After the cells are weaned from current, they were allowed to normalize themselves for at least half an hour. Once it was clear that the cells were behaving normally, magnetic trials of 30 minutes each could begin ( $n = 8$  neurons, 22 trials). Action potentials were measured for 10 minutes in the ambient magnetic field of earth, followed by 10 minutes in an earth-strength magnetic field (469 milligauss, azimuth rotated 63 degrees counter-clockwise relative to north, inclination at  $-67.5^\circ$ ) generated by a Merritt 4-coil system (Helmholtz and Kirschvink, 1992) that surrounds the experiment, and finally another 10 minutes in Earth's ambient field (469 milligauss, natural north, inclination at  $-69.5^\circ$ ). Controls were performed by not powering the coil to prevent rotating the field. Electrical activity was recorded and processed in LabChart v. 7.2.5 and Microsoft Excel. Spike histograms were compiled in order to determine average spiking rates before, during and after each rotation or control, and those values were used to perform a Kruskal-Wallis one-way ANOVA statistical analysis.

### Behavior

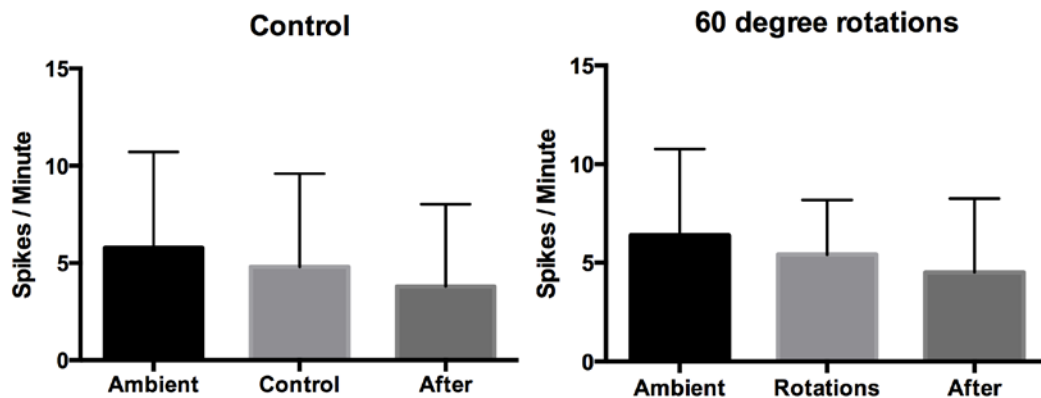
Behavioral studies were performed in a flow-through seawater table surrounded by a Lee-Whiting 4-coil system (Helmholtz and Kirschvink, 1992) that rotates the horizontal component of the field by  $185^\circ$  but at the same magnitude (469 milligauss) and similar inclination ( $-70^\circ$  while off and  $-67.5^\circ$  while activated). Inside the seawater table is a circular, plastic enclosure roughly one-half meter in diameter. The enclosure is designed to prevent the *Armina* from moving beyond the area where the artificial

magnetic field is uniform. Above the apparatus is a camera (JVC Mini DV model GR-DVL9800) set to capture time-lapse (one 640x480 pixel frame every 5 seconds) through the software SecuritySpy (v.2). In order to eliminate chemical and mechanical orientation cues, the floor of the seawater table was siphoned before each trial and water input to the table was reduced as much as possible while still maintaining temperature and dissolved oxygen content (flow inside the enclosure verified by dye flow as  $< 0.5$  cm/s). Light levels were not controlled and the opening of the enclosing structure was always brighter and located to the north northwest. Ten animals at a time were placed inside the enclosure and swirled around in a haphazard manner. After four hours, the same ten animals were once again disoriented, and the magnetic field rotated 180 degrees. The animals were allowed another 4 hours to settle and orient before the trial ended. In two trials (8 hours each), the angles of the slugs were recorded at 10 minutes, 120 minutes and 240 minutes, although the identities of each animal were not recorded before they were disoriented, making pairwise comparisons between a rotated field and the control impossible. The orientation of animals for these two trials were added together and analyzed for clustering using a Rayleigh test. A third trial was performed, in which each of the animals was tracked through both parts of the experiment. Again, the position of each animal was recorded at 10 minutes, 120 minutes and 240 minutes. In addition to allowing pairwise analysis, the greater detail of the third trial meant we were able to observe the behavior of individual animals. Each time an individual came to a rest for more than five minutes, the angle of the slug was recorded, as well as the duration of its rest. Video data were analyzed using ImageJ. Angular orientation data, being circular in nature, were analyzed in the circular statistics package Oriana (Kovach Computing Services).

## Results

### Physiology

In both the control and the active rotation trials, we typically observed a slow decline in action potentials over the course of one to two hours. Although the cells were all still healthy and undamaged, they eventually went silent and their membrane potentials dropped to between  $-50$  and  $-70$  mV. Rotating magnetic field azimuth did not appear to stimulate an increase in spike rate (comparing rotations to controls did not yield a significant result (Figure 3)).



**Figure 3.)** Average spike rates during control and active rotation trials. (n = 9 control trials, 13 rotation trials). Control vs Rotation not significant ( $p = 0.74$ )

Behavior

<u><b>Trial</b></u>	<u><b>P value</b></u>
Control, 10 minutes	0.072
Control, 2 hrs	0.56
Control, 4 hrs	0.80
Rotation, 10 minutes	0.27
Rotation, 2 hrs	0.13
Rotation, 4 hrs	0.87

**Figure 4.)** Results of predicted grouped orientations in Behavioral Trials 1 and 2.

In the first two behavioral trials the animals appeared to orient themselves randomly. There was no significant clustering of orientations during the control or the active rotation trials (Figure 4). The third trial did not demonstrate any significant grouping or orientations, either during the rotation or the control, at any of the three time intervals (Figure 5). The resting times and orientations of the slugs during third trial can be seen in (Figure 6). Analysis of the resting animals revealed no significant clustering of orientations in either the control or the rotated field (control  $p = 0.13$ , rotation  $p = 0.19$ ). Pairwise analysis of the slug resting positions before and after magnetic rotations was also not significant ( $0.2 > p > 0.1$ ).

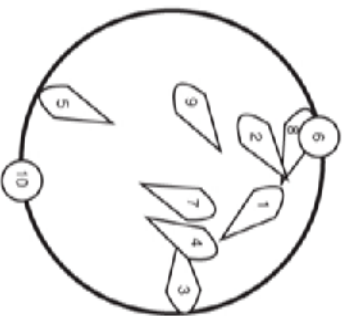
**Control**






10 minutes  
 $p = 0.09$



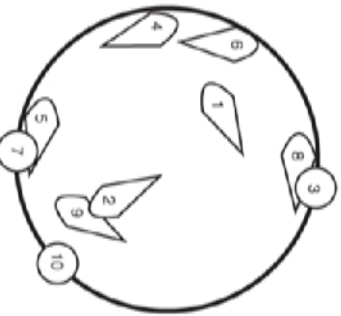
120 minutes  
 $p = 0.54$



240 minutes  
 $p = 0.61$

-  = position of slug
-  = slug is on its back
-  = slug is on the wall

**180 Degree Rotation**



10 minutes  
 $p = 0.24$

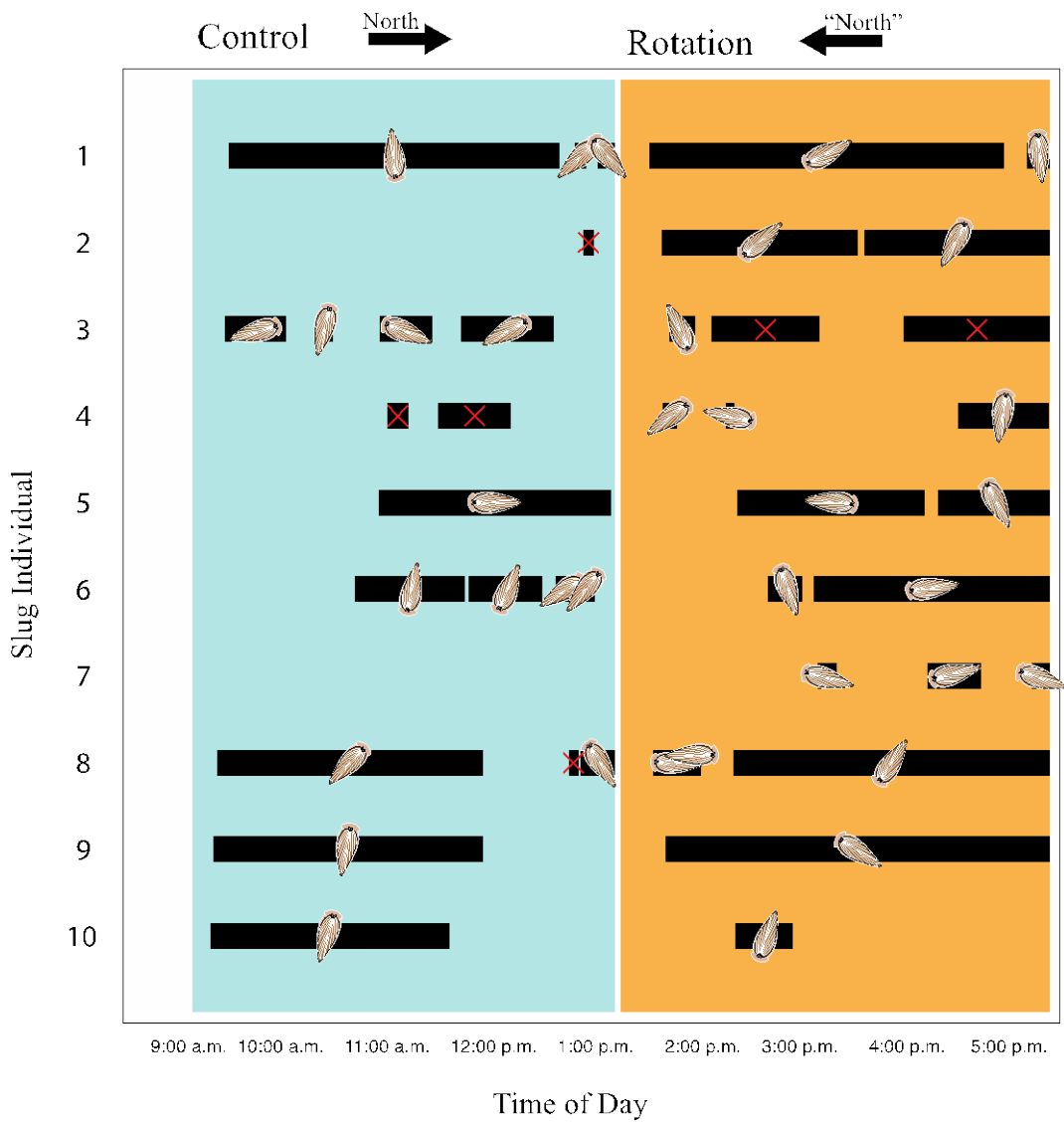


120 minutes  
 $p = 0.24$



240 minutes  
 $p = 0.31$

**Figure 5.** Positions and orientations of all 10 slugs during behavioral trial number 3.



**Figure 6.)** Times and orientations of each slug’s resting periods. Black bars represent time spent resting. Red X’s mean the animal was on its back.

## **Discussion**

While neither of the assays yielded significant results regarding the magnetoreceptive abilities of *A. californica*, this may have been a result of the scope and size of the experiments performed. It could still be possible that these animals can detect the magnetic field, but were observed at an unfavorable period. Lohmann and Willows showed that the geomagnetic responsive behavior of *Tritonia* was modulated by the lunar cycle by performing replicate experiments over a greater period of time than the trials we performed here. It may be possible that we were observing the animals during a period of the lunar cycle that was not conducive to behavioral responses. That being said, if the animals are truly detecting magnetic stimuli, we would have observed the response during the neurophysiological assay unless the neurons we were observing were not truly homologous with those of *Tritonia*. Future studies could include isolating biogenic magnetic materials (perhaps magnetite) from the tissues of both *Armina* and *Tritonia* to determine whether these materials are present in both animals. Additionally, future work elucidating the mechanisms of magnetoreception in *Tritonia* may shed light on similar mechanisms in different species. If *Tritonia* is unique in its ability to sense and orient to Earth's magnetic field, it would be an interesting example of either the conservation or derivation of magnetoreception in gastropods.

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