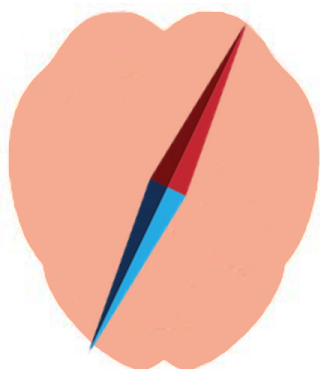

MAGNETSEARCH

A community-wide collaboration to discover the biological basis of magnetosensation

Collaboration guide



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Summary

The scientific goal of *MagnetSearch* is to identify neurons that encode the magnetic field of the Earth in the brain of any species. This could point the way to the long-sought magnetoreceptors, which would solve a long-standing biophysical puzzle, but also open opportunities in biological engineering for cellular control with magnetic fields. To pursue this goal we will conduct a large-scale search for magnetically responsive neurons across many species and brain areas. The participating laboratories – who are generally engaged in multi-neuronal recording for other purposes – each agree to contribute a few experiments using magnetic stimuli. The coordinators at Caltech will provide the required technology, hardware and software, and coordinate the team collaboration.

Background

Magnetosensation

Certain animals have the fascinating ability to sense the Earth's magnetic field, and to use this information for orientation and navigation (see the special issue introduced by Winklhofer (2010)). A fascinating body of behavioral studies – both in the field and the laboratory – has now documented magnetoreception in all major groups of vertebrates, as well as many invertebrates (Lohmann, 2010). However, the mechanisms by which the magnetic field couples to neural signals and behavior remain unknown. Indeed, magnetoreception is perhaps the last great mystery of sensory biology.

Two biophysical processes for magnetoreception have been proposed: The first postulates that the magnetic field exerts a torque on some magnetic particle, like a crystal of magnetite. This is indeed the mechanism used by magnetotactic bacteria for aligning their cells with the magnetic field lines (Bazylinski and Frankel, 2004). Multicellular animals may have found a way to couple such a “compass needle” with a mechanoreceptor cell (Kirschvink et al., 2001). A competing proposal for magnetosensation suggests that the magnetic field acts on single molecules in certain light-dependent biochemical reactions (Ritz et al., 2010). In this so-called “radical pair mechanism” the products of an electron transfer reaction depend on the equilibrium between singlet and triplet states of a reaction intermediate, and this equilibrium can be biased by an applied magnetic field. These two hypotheses and their respective predictions for magnetosensation have been reviewed extensively (Johnsen and Lohmann, 2005, Kirschvink et al., 2010).

So far, a direct search for magnetoreceptor cells has been inconclusive (Hore and Mouritsen, 2016, Shaw et al., 2015). While magnetic particles have been found in auspicious body parts of diverse species, none of them have been confirmed as associated with neural receptors (Treiber et al., 2012). On the side of the “radical pair” hypothesis, many hopes are placed in cryptochrome proteins as the light-absorbing molecule, and they are

proposed to be located in retinal photoreceptors (Hore and Mouritsen, 2016). On the other hand, the retina has been subjected to quantitative physiology experiments at least since Adrian's experiments in the 1920s, and no credible report of magnetic responses has surfaced.

Despite decades of intense research, it therefore remains unclear where exactly the magnetoreceptors are located, which molecules are involved, and how the primary magnetoreception processes work.

Coupling the biophysical mechanism of magnetosensation to overt behavior of an animal requires an extended neural circuit. This network of interneurons carries magnetic field information from the magnetoreceptor cells to the action-selection and motor-control circuits of the brain. Therefore, a plausible entry into magnetosensation would be to search for magnetically-driven neural responses anywhere in the brain. Remarkably little is known about the neurophysiology of magnetic processing. A series of early reports on responses in the avian visual system (Semm and Demaine, 1986) have since been discredited (Ramirez et al., 2014, Ritz et al., 2002). Another study on neurophysiology in the trout (Walker et al., 1997) has not been followed up in twenty years, and other attempts at replicating magnetic responses have failed (Mouritsen and Hore, 2012). More recently a tantalizing report emerged about cells in vestibular nuclei of the pigeon, whose firing rate varies in strict proportion to the magnetic field, as projected on the neuron's preferred axis (Wu and Dickman, 2012). For some reason this promising evidence has not been pursued further. The main reason for a dearth of data in this field is that relatively few laboratories are dedicated to magnetosensation, and among those only a small number have an interest in neurophysiology (Ramirez et al., 2014). Our team project aims to change this situation dramatically.

Magnetogenetics

There has been great interest in methods to manipulate cellular physiology with external control signals. Such capabilities can be used in research to perturb biological networks and observe the resulting effects. They also serve in clinical medicine to restore or replace defective cellular signals. The classic method of control is pharmacology. More recently, the advent of optogenetics introduced the ability to manipulate cells with light (Deisseroth, 2015). Because the effector mechanisms – light sensitive pumps and channels – are expressed genetically, this tool can direct the manipulation to specific cell types in the body. Optogenetics caused a revolution in circuit neuroscience: Today it is difficult to find a conference talk that doesn't incorporate one version or another of this method. It is also finding applications in other areas of biomedical research and may soon enter clinical practice (Lüscher et al., 2015).

Could one accomplish similar cellular manipulation using magnetic fields as the control signal? This would offer a powerful advantage over light, because magnetic fields penetrate the body almost undisturbed, and thus can reach every region equally well. By comparison, optogenetics is limited to a few hundred microns below the surface, and requires invasive probes to access deeper regions.

A few attempts have been made to construct a magnetoreceptor *ab initio*. They are based on coupling a biogenic iron particle (ferritin) to an ion channel, with the hope that magnetic effects on the iron particle will open or close the channel. While there are claims that this has been achieved (Stanley et al., 2015, Wheeler et al., 2016), they remain controversial, and appear to violate basic principles of biophysics (Meister, 2016). Fundamentally, ferritin is a tiny paramagnetic particle that couples too weakly with the magnetic field to transduce any appreciable effects (Anikeeva and Jasanoff, 2016).

A more promising route is to ask how Nature has solved the problem, and then develop that molecular mechanism into a bio-engineered system. This is precisely the approach that worked for optogenetics. Researchers did not set out to design a light-sensitive ion channel from scratch, but instead copied one from microorganisms (Nagel et al., 2002), then evolved it for use in animal cells. Magnetotactic bacteria could be used as a lead, but while their magnetite crystals provide a sensor, they don't offer a useful effector mechanism. For the bacterium floating in water the relevant effect is simply an alignment of the cell with the magnetic field lines. This will not be effective for a nerve cell embedded in tissue. Thus we argue that one should search directly for the mechanisms that sense magnetic fields in multicellular animals, since that will likely offer the entire signal transduction chain needed for modulating a cell's function.

A collective science approach

For both the biophysical understanding and magnetogenetics applications one needs to find magnetoreceptors and their cellular mechanisms. We suggest a solid beachhead to start a search for receptors would be the discovery of unambiguous neural signals somewhere in the brain that encode the magnetic field under laboratory conditions. With that in hand, one can continue lab-based controlled experiments to follow the functional and anatomical circuits back to the receptor neurons. It seems prudent to expand the search for magnetoreceptors beyond the particular species that have been certified with magnetosensation behavior. Behavioral experiments are challenging and may have failed to reveal the use of magnetic cues at the level of the whole animal. Under most conditions, non-magnetic sensory cues may dominate an animal's behavior, and special environmental triggers may be needed to release magnetic orienting and navigation. However, neurons connected to magnetoreceptors will still fire, even if the rest of the brain is not always "paying attention". The visual system of mice can serve as an analogy: Proving that mice can see requires rather careful behavioral experiments, and many scientists still believe they are functionally blind. But neurophysiological recording reveals visual responses as soon as one sinks an electrode into the back of the mouse brain. Taking this argument one step further, in certain species the magnetic sense may be purely vestigial, yet could still lead us to biologically active magnetoreceptors.

Similarly, one should extend the search to many brain areas that have not been the favorite targets for magnetoreception so far. A good example is the finding of magnetic responses in a vestibular nucleus (Wu and Dickman, 2012). More generally, if magnetic signals have any influence on the animal's navigation behavior they must enter those circuits at some

stage. So the hippocampus would be a plausible location, as is the cerebellum where many sensory and motor channels are combined. Therefore, a simple approach is to avoid any prior bias or expectation and look for these signals everywhere.

On that background, the mission of *MagnetSearch* is to search for magnetic responses across many brain areas in many different species. There are many laboratories worldwide that engage in large-scale neurophysiology, recording signals from neuronal populations every day. Each of these groups of course has a different research problem in mind and has trained their electrodes or microscopes on a different brain area or species. If one could borrow each of these existing experimental setups (and their operators) for just 10 minutes a day one could collect magnetic responses from a vast trove of neural populations.

Collaboration plan

MagnetSearch is coordinated by the Meister and Wagenaar research groups at Caltech (the “hub”) together with partner laboratories around the world (the “spokes”). In brief, the collaboration is planned as follows:

1. A potential spoke lab contacts the hub and proposes a series of experiments, specifying animal model, brain areas, measurement modality.
2. The hub ships to the spoke lab an electromagnetic stimulator (or several), and other instrumentation as needed.
3. The spoke lab conducts neural recordings under electromagnetic stimulation and pre-processes the data to yield neuronal responses (e.g. spike-sorting of electrical recordings, or ROI analysis of optical recordings). The spoke lab deposits these data visible to all members of the collaboration.
4. The hub and other teams proceed with data analysis to test for magnetically responsive signals. A positive result will be subject to immediate replication. Negative results will be expressed in terms of an upper bound on the magnetic response based on detection limits of the analysis.
5. After a suitable search period – determined in consultation among participants – the results will be collated for publication. All teams that contributed data by this point will share authorship. Following this first publication, the goals of the collaboration will be reassessed.

Instrumentation

Electromagnet

The spoke labs require a simple method to generate a magnetic field. What are the desired parameters? Biological magnetoreceptors need to sense the geomagnetic field with

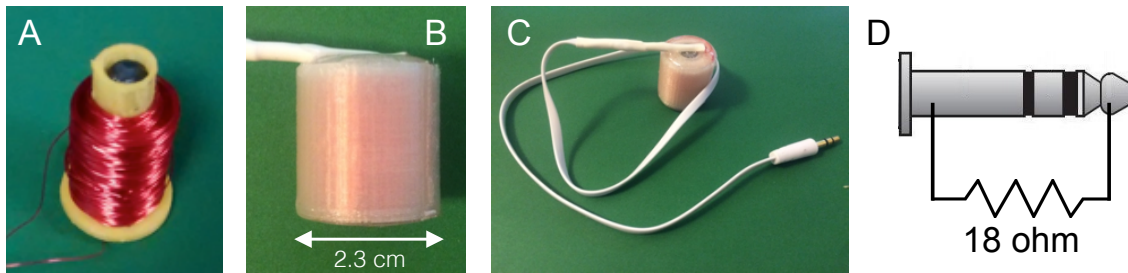


Figure 1: Electromagnetic stimulator. (A) Solenoid on bobbin with iron rod core. (B-C) Finished package. (D) Wiring of the solenoid to the audio plug.

a strength of about $50 \mu\text{T}$. They will also experience temporal variations in the strength of the field, including full reversals, for example as the animal moves its body or head. This type of stimulus can be provided with a simple iron-core solenoid, driven by a current that varies sinusoidally in time, or with any other desired timecourse.

The stimulator should fit easily into most common experimental setups without disturbing any existing components. For example, the large Helmholtz coils commonly used to produce flat magnetic fields simply aren't an option, since they would require clearing out all the other apparatus near the experimental preparation. On the other hand, because we are conducting a broad search for any kind of magnetic response, there is no need for excessively precise shaping of the magnetic field. We deem it unlikely that cellular magnetoreceptors are somehow tuned to such a special condition.

We have designed and fabricated a solenoid that measures $2.3 \times 2.3 \times 2.5 \text{ cm}$ (Figure 1). Its cable has a 3.5 mm audio plug that matches the audio output jack of mobile phones. When driven by a mobile phone app this magnet can produce a field up to 10x the geomagnetic strength at 2 cm distance. For experiments on head-fixed animals the magnet can be positioned within a few cm of the head, and moved to different positions in order to probe various magnetic field orientations. For freely moving animals, the magnet can be incorporated into the arena at a location that the animal visits frequently, for example near a water reward port. This will produce discrete episodes during the task when the animal experiences the field. If the field is modulated at a precise frequency, the magnetic responses can be easily distinguished from other task-related modulations in activity. The Caltech hub will ship this electromagnet to the spoke labs.

Stimulus generator

The electromagnet must be driven by a controllable current source, and our magnet is designed for the audio output jack of a mobile phone. Apps are available on both iOS and Android that implement very respectable signal generators. Our favorite is the Audio Function Generator by Thomas Gruber, which offers dual channel voltage signals including sine waves, square waves, frequency sweeps, and random noise (Figure 2A). This solution may already suffice for many applications.

The signal level on the audio output jack varies considerably across mobile phone models,

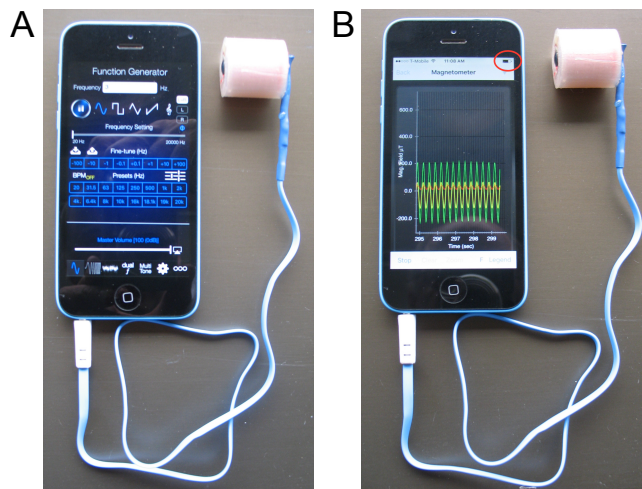


Figure 2: Magnetic stimulation and measurement using a mobile phone. (A) An iPhone 5c with the Function Generator App drives the electromagnet with a 3 Hz sine wave. **(B)** A magnetometer App (Sensor Kinetics Pro) measures and records the time-varying magnetic field. The magnetic sensor is located near the battery symbol (red circle).

so be sure to check the magnetic field amplitude produced by yours (see below). If you need a stronger voltage source, use whatever function generator you have in the lab. Don't exceed ~ 10 V: In our hands that produces about $50 \mu\text{T}$ at 10 cm distance, but it also heats the magnet noticeably. If that happens you may want to limit stimulation to a few minutes at a time, and allow for cooling in between. If you need a different or stronger source for the magnetic field, get in touch with the Caltech hub.

Magnetometer

Investigators will want to confirm that the stimulator generates the desired amplitude and modulation of the field at the relevant location of the experimental animal. The magnetometer should measure fields on the order of $50 \mu\text{T}$ with a precision of a few percent. Most mobile phones contain such a sensor and offer apps to read it out. Our preferred app on both iPhones and Android phones is Physics Toolbox Magnetometer from Vieyra Software. On the iPhone 5c the magnetic field sensor is located right under the battery symbol (Figure 2B). Find out where yours is by moving the magnet around while monitoring the magnetometer readout. If your experimental setup doesn't allow you to place a mobile phone at the location of interest, get in touch with the Caltech hub. We can provide a custom magnetometer that has a small probe on a cable.

Suggestions for experiments

Measurements

Spoke labs are free to experiment as they wish, but we suggest one common protocol that should be included by every team:

1. Start recording the neural signal.
2. After collecting baseline activity for about 10 s, apply a sinusoidal magnetic field with amplitude of $\sim 100 \mu\text{T}$ and frequency ~ 3 Hz.

3. Record the voltage applied to the magnet on the same time axis as the neural response data.
4. Collect data for about 10 min.
5. Move the magnet to produce a different field direction, and repeat.

Some comments:

1. The field should be measured at the suspected location of magnetoreceptor cells. Obviously that location remains uncertain. For a small brain (mouse, finch, zebrafish) one can get that field strength throughout the brain volume. For larger brains it may help to scan the brain volume by exploring a few magnet positions. The AC modulation of the field will make detection of a stimulus-locked signal easier than with a constant field. Also modulations of a few Hz should be expected in Nature, resulting from movements of the animal. Finally this frequency is low enough to minimize interference with electrical measurements via magnetic induction. Choose the exact frequency so it is different from any other modulations present in your environment.
2. Without belaboring the obvious, keeping a record of the stimulus is essential for reliable interpretation of the responses.
3. At 3 Hz this will give about 2000 stimulus periods, which should yield a respectable signal-to-noise ratio (SNR) in detecting stimulus-locked modulations. Of course, longer recordings are better, but the improvement of SNR goes only as the square-root of time, so better to use the time for a different experiment (see point 4).
4. The magnetoreceptors may be oriented in an unfortunate way so the magnetic field does not affect them. To guard against this, simply move the magnet to test a different field orientation and take another measurement.

Analysis

To derive neural signals from the measurements, apply your standard preprocessing routines, such as spike sorting to extract action potentials of neurons, or motion-correction and ROI analysis to extract calcium signals of neurons, or signal processing to isolate local field potentials. This serves several purposes: (1) Data compression. The preprocessed data sets are much more compact than the raw data, and can be transported and shared more effectively. (2) Application-specific data treatment: Your team knows better than anyone else how to extract reliable measures of neural activity from your measurements. (3) Continuity with other results derived in the same experiment. For example, you may have gathered hours worth of recordings from a set of hippocampal neurons before starting magnetic stimulation at the end of the day. For each of the cells in the magnetic recording one would like to know how that same neuron acted in the other context.

The search for responses locked to the magnetic field depends of course on the nature of the magnetic stimulus. If the stimulus was a constant frequency sinusoid, the simplest approach is a Fourier transform of the response variable, looking for response components

at the stimulus frequency and its harmonics. If no such signal is evident by “just looking” one can proceed with more serious statistical analysis: e.g. compare to components at nearby frequencies, or to a measurement taken in absence of magnetic stimulation.

Data sharing

Every recording we take as part of this collaboration should be documented and archived, regardless of outcome. Of course we will get excited about positive results, but a large collection of negative results can be very informative as well and will influence the direction of this research field.

To limit data volume, we will archive only preprocessed data that emerge from your analysis, for example spike trains or calcium activity tied to a set of neurons, or filtered LFP data. We will use a DropBox folder until other means become necessary. Archive your experiment with these steps:

1. Go to the Dropbox folder for the collaboration: <https://www.dropbox.com/home/MagnetSearch>.
2. Create a subfolder named `TeamName_ExperimentDate`, e.g. `meister_20170928`.
3. Create a `readme.txt` file that contains information about the experiment and explains the data format.
4. Create data files as needed to report the time course of the stimulus and the preprocessed recordings.
5. We have prepared a Jupyter Notebook which is meant to serve as a template for you to structure and visualize your experimental data before sending it back to us. The structure and programming environment presented in the notebook are suggestions - you may choose the programming language and formatting you deem best suited for your data. Note that you should create a structured JSON file within the notebook to save formatted experimental metadata.

Some comments:

1. Use the `metadata.json` from `meister_20170928` as a template to input your meta-data in JSON format. It should include information at the level of the methods section in a publication. If the experiment is just like one you have already published, simply cite the source article. Be sure to include species, sex, age, brain region, and more specific recording location if available. Spell out units of measurement (where useful) and sampling intervals (for evenly sampled time series data).
2. Use text or binary files for the data; no proprietary data formats please. Other than that, choose your own organization and document it clearly in the `metadata.json` (point 3). Finally, to save space and facilitate download, compress all the data files – but not your `readme.txt` or `metadata.json` – into one archive. Consult our experiment `meister_20170928` as an example.

Future options

We will obviously learn a lot as soon as experiments begin, and there is every expectation that our collective science project will evolve flexibly and with input from all teams. Here are a few ideas for what may happen down the line.

In case of a positive report

We will try to avoid celebrating immediately. The tortured history of the magnetosensation field suggests it will be prudent to insist on replication before making a public announcement. We can make use of the collective effort here for blinded analysis: For example, the team who does the replication experiment could share data on neural responses while withholding the frequency of the stimulus, challenging the other teams to identify it from the data.

Publication

We will publish results from the collective magnetoreceptor search, possibly in several iterations. Within a year of the project's start we should have gathered a volume of recordings that vastly exceeds previously available data on magnetic neurophysiology, so that publishing even negative reports becomes meaningful. All teams that contribute data by that point will be on the author list.

Moving to a public project

We are starting the project in a quiet phase, with a small number of teams participating by invitation. After gaining some experience and adjusting operations it may make sense to publicize the project and invite other laboratories to contribute. This could come with a more overt web presence and public outreach components. Fortunately we are not burdened by any federal funding, so the degree and time table for this is very flexible.

Long-term impact of our collaboration

Beyond invigorating magnetic neuroscience, this project will also test a new model for collective science. There has been much discussion of a “national brain observatory”, usually envisioned as a large central facility to which investigators travel for their work (Alivisatos et al., 2015). Our vision is complementary to this model: Many small but exquisite brain observatories for neural recording have already been built by individual investigators. As a rule, each of these sophisticated laboratories is focused on a different scientific problem. Here we focus them all on the same question for a small portion of the available experimental time. When combined with effective project management and data sharing, this approach may solve a vexing scientific problem rather quickly. Perhaps this model of a distributed observatory will prove attractive for other research problems in neuroscience and elsewhere.

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