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AN IDENTIFIABLE MOLLUSCAN NEURON RESPONDS TO CHANGES IN EARTH-STRENGTH MAGNETIC FIELDS

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Summary

Diverse animals can orient using geomagnetic cues, but little is known about the neurophysiological mechanisms that underlie magnetic field detection. The marine mollusc *Tritonia diomedea* (Bergh) has a magnetic sense and its nervous system is amenable to cellular-level electrophysiological analysis. In a semi-intact whole-animal preparation, intracellular recordings from the large, visually identifiable neurons left pedal 5 (LPe5) and right pedal 5 (RPe5) in the brain of *Tritonia* revealed enhanced electrical activity in response to changes in ambient earth-strength magnetic fields. No such changes in activity were observed in approximately 50 other neurons subjected to identical magnetic stimuli. The responses of LPe5 were characterized by increases in spiking frequency occurring about 6–16 min after the ambient magnetic field had been rotated to a new position. The response was abolished when the brain had been isolated from the periphery of the animal by severing nerves, a procedure that also transected prominent neurites of LPe5. We hypothesize that LPe5 is one component of a neural circuit mediating detection of the earth's magnetic field or orientation to it.

Introduction

The magnetic field of the earth influences the orientation of diverse organisms, including various bacteria (Blakemore and Frankel, 1981), molluscs (Lohmann and Willows, 1987), arthropods (Walker and Bitterman, 1989), fish (Quinn, 1980; Quinn and Brannon, 1982), amphibians (Phillips, 1986), reptiles (Lohmann, 1991), birds (reviewed by Wiltschko and Wiltschko, 1988) and mammals (Mather

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and Baker, 1981). Little is known, however, about the neural mechanisms that underlie magnetic field detection in animals (reviewed by Gould, 1984; Lohmann and Willows, 1989; Semm and Beason, 1990). Numerous hypotheses of magnetoreception have been proposed, including transduction processes mediated by magnetite (Kirschvink and Gould, 1981), photopigments (Leask, 1978), electromagnetic induction (Kalmijn, 1978; Rosenblum *et al.* 1985), melanin (Leucht, 1987) and biological free radicals (Schulten and Windemuth, 1986). Compelling neurobiological evidence for any of these mechanisms, however, has not yet been obtained.

One impediment to determining the neural basis of magnetoreception is the scarcity of animal model systems in which neural correlates of magnetic field detection can be easily and reliably elicited. The nudibranch mollusc *Tritonia diomedea* can orient to the geomagnetic field (Lohmann and Willows, 1987). This animal also possesses large, individually identifiable neurons and a relatively simple central nervous system accessible to cellular-level electrophysiological analysis (Willows, 1971; Willows *et al.* 1973). We present evidence that a large, identifiable neuron in the brain of *Tritonia* responds with enhanced electrical activity to changes in earth-strength magnetic fields. We hypothesize that this neuron, known as left pedal 5, is part of a neural circuit underlying magnetic field detection or geomagnetic orientation.

Materials and methods

Animals

Animals were trawled from East Sound (Orcas Island) or Bellingham Bay in Washington, USA. Nudibranchs were maintained at the University of Washington Friday Harbor Laboratories in fiberglass, concrete or Plexiglas flow-through sea water tanks located in areas free of major magnetic distortions (i.e. where the total field intensity was ±5 % of the natural geomagnetic field intensity). Animals were kept in captivity for 3–90 days before they were used in experiments; those injured by the trawl or showing symptoms of poor health were not used. Captive nudibranchs were fed sea pens [*Ptilosarcus gurneyi* (Gray)].

Semi-intact whole-animal preparation

Semi-intact whole-animal preparations (Willows *et al.* 1973) were used in initial experiments. A small incision on the anterior dorsal surface of the animal provided access to the paired cerebral, pedal and pleural ganglia that constitute the brain. The brain was gently maneuvered onto a wax-covered platform where it was pinned in place with cactus needles or stainless-steel minuten pins. Although using minuten pins had no apparent effect on the outcome of experiments, cactus needles were nearly always used to avoid the possibility of placing a weakly magnetized pin in the vicinity of the brain. The semi-intact *Tritonia* preparation is described in detail by Willows *et al.* (1973).

Once the brain had been immobilized, individual neurons could be impaled with

microelectrodes filled with 3 mol l^{-1} KCl. Intracellular recording procedures were conventional.

Coil systems

Two different coil systems were used to generate magnetic fields in the experiments. In some experiments, a Rubens cube coil (Rubens, 1945) 1.0 m on each side was placed around a wooden table which supported the preparation. When connected to a d.c. power source, the coil generated a weak, essentially uniform magnetic field throughout the space it enclosed. The field generated by the coil combined with the geomagnetic field to rotate the resultant field in a new direction. The Rubens coil was used in initial experiments to rotate the ambient field either 20° or 90° clockwise while keeping the intensity of the field approximately constant [the new horizontal field was ±5 % of the natural geomagnetic horizontal component of about 0.016 mT, while the geomagnetic vertical component of about 0.045 mT remained unchanged]. All magnetic field intensities were measured with a Schonstedt single-axis digital magnetometer (model DM 2220).

In other series of experiments the preparation was surrounded by a Helmholtz coil 33 cm on each side (Fig. 1). Each of the two square coils consisted of 35 loops of 26-gauge copper wire. Helmholtz coil experiments were carried out at a location where the ambient horizontal field component at the level of the brain was 0.025 mT and the vertical component was 0.043 mT (inclination angle was 59°). These magnetic parameters varied slightly from those measured at other locations at the Friday Harbor Laboratories (where the geomagnetic horizontal component was about 0.016–0.018 mT, the vertical component 0.045–0.047 mT and the inclination angle about 70°); the difference was attributable to steel in the laboratory walls and to a nearby grounding plate. However, the background field was constant throughout the experiments and was of earth-strength intensity (total background field intensity was 0.050 mT). When the Helmholtz coil was gradually turned on, magnetic north was rotated 60° clockwise; the horizontal component of the ambient field at the new magnetic north was 0.019 mT, the vertical component was 0.043 mT and the inclination angle was 66°. During Helmholtz coil experiments all animals were oriented so that their anterior ends were directed approximately (±15°) towards geomagnetic 240° (approximately southwest).

The power supplies used in these initial experiments both had residual a.c. 'ripples' superimposed on the d.c. current shift. The power supply used with the Rubens coil to rotate the field 20° clockwise (see Fig. 3), for example, produced a current ripple of about 35 mA (peak-to-peak) superimposed on a 170 mA average; the power supply used to rotate the field 60° clockwise with the Helmholtz coil (see Figs 4, 6A–C) had a 50 mA peak-to-peak ripple on a 110 mA average. However, because neuronal responses like those reported here have been elicited in other experiments using a different d.c. power supply with a ripple 0.0–0.5 % of the d.c. shift (Lohmann and Willows, 1991), the time-varying component does not appear to be a necessary part of the stimulus. The electric field induced by the ripple was

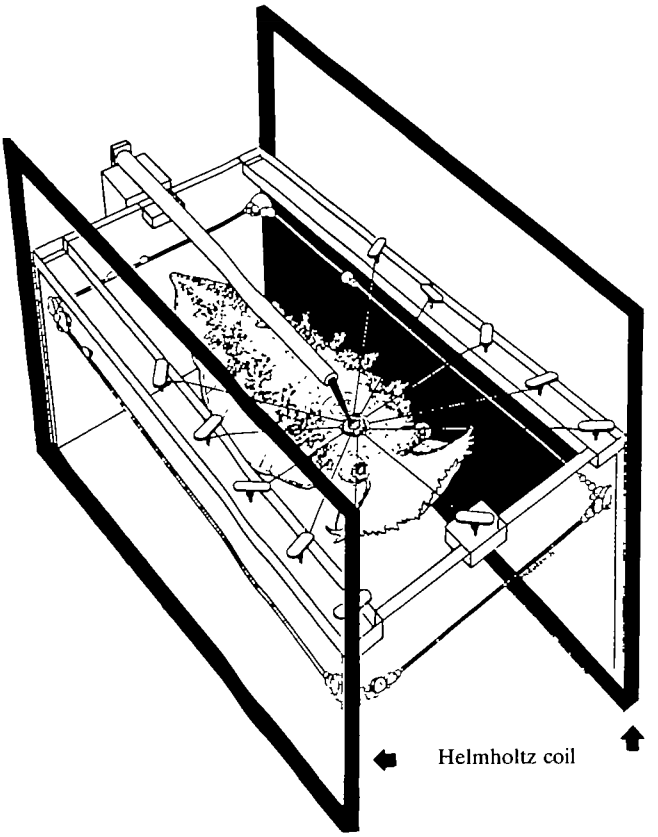

Fig. 1. Diagrammatic representation of the semi-intact whole-animal preparation (not to scale). An incision on the anterior dorsal surface of the animal exposed the brain, which was immobilized on a wax platform for intracellular recording while the nervous system remained intact. A Helmholtz coil (33 cm on each side) surrounded the entire preparation but did not contact the sides of the preparation chamber (see text). Thus, the magnetic field around the animal could be controlled while intracellular recordings were made from neurons in the cerebral, pedal and pleural ganglia.

less than the $0.00026\,V\,m^{-1}$ (maximum) resulting from magnetic field rotation (see Measurement of induced electric fields and Appendix).

Temperature stability and coil heat

Throughout the dissection and all experiments the preparation was completely submerged in flowing sea water inside a Plexiglas dissection chamber that held 7.0 liters of water. Fresh sea water from the Friday Harbor Laboratory system continuously flowed into the dissection chamber at a rate of approximately $600\,ml\,min^{-1}$ (range $500–780\,ml\,min^{-1}$). Water draining from the dissection chamber was not recirculated. The continuously flowing sea water kept the preparation at constant temperature (usually $\pm 0.4°C$ over a 10-h experiment).

Because the laboratory seawater system drew water from offshore, the precise temperature at which different experiments were conducted varied depending upon the season and daily weather (range 7–13 °C).

Because the wires of the coil systems warmed very slightly when the coil was on for a period of minutes, care was taken to ensure that the heat could not be transferred to the waterbath. During experiments with the Rubens coil, the dissection chamber was separated from the nearest wires by about 40 cm of air, making the possibility of heat transfer to the continuously running seawater system remote. During the Helmholtz coil experiments, however, the chamber was much closer to the coil. To minimize the possibility of heat transfer in experiments with the Helmholtz coil, the dissection chamber was placed on a layer of styrofoam so that it was not in contact with the wire; the smallest distance separating the chamber from any part of the coil was 2.5 cm. When sea water was flowing through the tank at normal rates (about 500 ml min^{-1}), leaving either coil system (Rubens or Helmholtz) on for 2 h (which was four times the longest period either coil was on in any experiment) did not result in any change in water bath temperature detectable with standard laboratory thermometers (resolution about 0.1 °C).

We also calculated the resistive heating for the Helmholtz coil under the most common experimental conditions (with the coil set up to rotate magnetic north 60° clockwise). Assuming the coil was on for a period of 1 min before it was turned off (as in most experiments; see below), the total resistive heating of the coil (about 0.15 J s^{-1}) would be sufficient to raise the temperature of a 7-l water bath by 3.1×10^{-4} °C, but only if (1) 100 % of the energy were transferred to the water bath (perfect efficiency of transfer), and (2) there were no water exchange in the chamber. In reality, efficiency of transfer was unlikely to have been even 1 % because of the insulating air and Plexiglas between the coil and bath. Moreover, fresh sea water continuously flowed through the tank, further reducing the likelihood of heat building up. We conclude that heating from the coil systems was unlikely to influence the experiments.

Preliminary experiments

Because nothing was known about the neurons involved in magnetic field detection in *Tritonia,* initial experiments consisted of searching the central nervous system cell by cell in an effort to identify neurons responsive to changes in earth-strength magnetic fields. Randomly selected cells on the dorsal surfaces of the paired cerebral, pedal and pleural ganglia were impaled, and their electrical activity monitored while the ambient magnetic field was altered. The precise stimulus used varied from experiment to experiment but always consisted of a 20°, 60° or 90° clockwise or counterclockwise rotation of the horizontal magnetic field. The transient electric fields induced during the magnetic field rotations were found to be several orders of magnitude weaker than those known to influence molluscan neurons (see Measurements of induced electric fields and Appendix). During preliminary experiments animals remained in the rotated magnetic field for 15–200 s while neuronal electrical activity was monitored.

Long-term recordings

Electrophysiological recordings from honeybee bristle-field sensilla (Korall and Martin, 1987) and guinea pig pinealocytes (Semm, 1983) have suggested that latencies of 2–40 min may occur between magnetic stimuli and changes in electrical activity of some cells. In a second series of experiments longer recording periods were therefore used. The procedure for these experiments was identical to that used previously, except that the electrical activity of the cells was monitored for about 30 min after turning the coil on and for an additional 30 min after turning the coil off.

Quantitative experiments with left pedal 5 (LPe5)

Only two neurons (LPe5 and RPe5) showed evidence of altered electrical activity in response to magnetic stimuli (see Results). Because these two cells are apparently bilaterally symmetrical homologs, judging by available structural and functional criteria, and because it was difficult to maintain stable, long-term recordings from both cells simultaneously, we arbitrarily chose to focus subsequent experiments on LPe5.

We reasoned that because a single 20°, 60° or 90° clockwise rotation of an earth-strength field was sufficient to elicit enhanced electrical activity from LPe5, a series of field rotations might be an even more effective stimulus. The semi-intact preparation and Helmholtz coil (Fig. 1) were used in two ensuing experiments. In both, the coil was aligned so that activating it rotated magnetic north 60° clockwise.

Monitoring LPe5 in the imposed magnetic field

Long-term intracellular recordings from LPe5 in the unaltered geomagnetic field indicated that, once spiking frequencies had stabilized (following electrode impalement), the rate of spiking usually remained relatively constant for periods of several hours or more. In some experiments the baseline became stable almost immediately after the cell had been impaled with the electrode; in other cases several hours or more elapsed before an acceptable baseline could be obtained.

In 10 different animals the electrical activity of LPe5 was monitored until a stable 20 min baseline had been obtained. The Helmholtz coil was then turned on and off at 1-min intervals for 20 min. Animals were thus subjected to 1 min in the rotated field followed by 1 min in the geomagnetic field followed by 1 min in the rotated field, and so on. The number of action potentials occurring in the 20-min baseline period was compared to the number occurring during the 20-min imposed magnetic field period. If the electrode remained in the cell long enough, the procedure was repeated after 1 h or more had elapsed. As many as six trials were successfully conducted on a single cell using these procedures.

Experiments on LPe5 with geomagnetic controls

A second experiment was conducted to confirm that the magnetic stimulus elicited greater electrical activity from LPe5 than would normally occur in the

unaltered geomagnetic field. A computer was interfaced with the Helmholtz coil and programmed to turn the coil on and off. LPe5 neurons were impaled as before. To facilitate detection of increases in neuronal electrical activity, hyperpolarizing current was injected into the cells to bring the resting potential just below the threshold for spiking. In addition, the imposed field period was lengthened from 20 to 26 min because spiking in the previous experiments rarely began until at least 6 min after the first field change and often persisted after the stimulus had been turned off (see Results).

Each LPe5 neuron was subjected to a series of trials. In each trial, the electrical activity of LPe5 was monitored until a stable baseline of 20 min or more had been obtained. The baseline was considered to be stable when the hyperpolarizing current was on and two conditions were met: (1) between 0 and 10 action potentials were produced over the 20-min baseline period (if more were produced, additional hyperpolarizing current was injected and the start of the trial delayed until an acceptable 20-min baseline period was obtained), and (2) the distribution of spikes was relatively uniform over the baseline period (i.e. the rate of spiking was approximately constant).

When these conditions were satisfied we started the computer program. The outcome of a random number generator at the start of the program determined whether the coil was activated. In half of the trials, the computer automatically turned the coil on and off at 1-min intervals for 26 min; in the other half, the coil remained off and the animal remained in the unaltered geomagnetic field for 26 min as a control. Thus, both imposed field and control periods lasted 26 min, but only action potentials occurring in the final 20 min of each period were counted for analysis.

After each trial the animal was given a recovery period of at least 1 h before the entire process (baseline followed by imposed field or control period) was repeated. Trials were conducted for as long as the recording could be maintained (up to 20 h in some cases). The LPe5 neurons of 28 different animals were tested using these procedures.

Isolated brain experiments

We reasoned that if LPe5 detects magnetic fields directly or receives input from one or more primary magnetoreceptors located in the brain, the neuron might continue to respond to magnetic stimuli even when the brain was isolated from the rest of the body. In 20 experiments, we therefore removed the brain from a nudibranch and pinned it out on the wax-covered platform inside the Plexiglas dissection chamber. Care was taken to orient the anterior edge of the brain towards 240°, as in the previous experiments, and recordings were obtained from LPe5 following the same procedure used before.

Cobalt fills

To examine the morphology of LPe5, 500 mmol l^{-1} cobalt chloride solution was pressure-injected into LPe5 neurons using a Picospritzer. LPe5 neurons selected

for cobalt fills were unambiguously identifiable on the basis of visual criteria. Once the $CoCl_2$ had been injected, the fill was developed by adding a drop of concentrated ammonium sulfide, following the procedures of Croll (1986).

Measurement of induced electric fields

Moving a magnetic field induces an electric field $\bar{E}$ in accordance with Faraday's Law, which is:

$$\epsilon = -\frac{d\phi_B(t)}{dt},$$

where ϵ is the induced electromotive force (EMF) as a result of the field $\bar{E}$, ϕ_B is the magnetic flux and t is time. Thus a weak, transient electric field was induced in our experiments during the time a coil was being turned on or off; no such field would have been present, however, during the time the ambient magnetic field was stationary. In view of possible electric field transients, it was important to consider whether the observed changes in spiking frequency of LPe5 might be attributable to effects of transiently induced electric fields rather than to magnetic field effects.

The electric fields induced during magnetic field rotations were measured for both the Rubens coil and the Helmholtz coil based on the principles outlined in the Appendix. Measurements for the Rubens coil were made while the coil was set to rotate the horizontal field 20° clockwise; measurements on the Helmholtz coil were made when the coil was set to rotate the field 60° clockwise. These conditions corresponded to those under which the neuronal responses shown in Fig. 3 (using the Rubens coil) and Figs 4–8 (using the Helmholtz coil) were recorded.

An oscilloscope was first used to determine the maximum rate of change of current through each of the coils when it was turned on as in the experiments. Maximum rates were found to be approximately 25 mA ms^{-1} for the Helmholtz coil and 50 mA ms^{-1} for the Rubens coil.

To determine the strength of the induced electric field generated by the changing magnetic flux through the region in the center of the Helmholtz coil, a 20-turn circular test coil constructed of 26-gauge copper wire was placed in the plane halfway between the two square coils (see Fig. 10 in Appendix). The test coil was 17.5 cm in diameter; the area within the circle was thus considerably larger than the cross-sectional area of a nudibranch but slightly smaller than that of the water bath (and much smaller than that of the Helmholtz coil). The test coil was arranged so that it was parallel to the Helmholtz coil to maximize the electric field caused by the changing magnetic flux through it.

We then recorded the voltage induced in the test coil when the Helmholtz coil was driven with a 170 Hz, 35 mA RMS sinusoidal wave. This frequency and amplitude provided a rate of current change (dI/dt) matching or exceeding the maximum rate of 25 mA ms^{-1} that occurred in experiments when the Helmholtz coil was turned on. The Helmholtz coil was driven by a power amplifier with a 1-A capability d.c.-coupled to an Exact function generator (model 7260). The frequency of the sinusoidal wave was confirmed with a Fluke universal counter/

timer model 7261A and the 35 mA RMS amplitude measured with a true RMS reading Fluke 8840A multimeter. The sinusoidal wave form was also confirmed by oscilloscope.

Maximum voltages induced in the 20-turn test coil were measured at 2.5 mV with an oscilloscope and 2.91 mV (±0.02 mV) with a true RMS reading Fluke model 8840A multimeter. Accounting for the length of wire in the coil (see Appendix), the 2.91 mV measurement indicated that the maximum amplitude of the transient electric field that could be generated by turning on the Helmholtz coil was 2.6×10^{-4} V m^{-1}. This value is nearly four orders of magnitude less than the minimum voltage gradient known to induce spiking in molluscan neurons (Ierusalimsky and Balaban, 1987).

An identical procedure was carried out with the Rubens coil, except that it was driven with a sinusoidal wave of 170 Hz and 45 mA RMS to match the maximum rate of current increase for this coil. The maximum voltage that could be induced in the test coil by turning on the Rubens coil was 0.836 mV (±0.002 mV), indicating that the maximum electric field that could have been produced around the preparation in a 'worst-case scenario' was about 8.4×10^{-5} V m^{-1} (see Appendix). Thus, the electric field induced by the Rubens coil was even weaker than that produced by the Helmholtz coil.

The rates of current change (dI/dt) in the current 'ripples' produced by the power supplies (see Coil systems) were considerably slower than the rates of current change when each coil was turned on. Thus, any induced electric field resulting from the ripples would have been even weaker than the values calculated above.

Results

Preliminary experiments

The preliminary experiments failed to identify any neuron that showed rapid changes (within 15 s) in spiking activity in response to a 20°, 60° or 90° change in field direction. In addition, none of the neurons tested showed a response in this time period when the coil was turned off and the field rotated back to its original position. Because of the exploratory nature of these experiments, no attempt was made to search the ganglia systematically or to subject all neurons tested to all of the field rotations. An estimated 160 trials were carried out on approximately 80 impaled cells in 30 different animals. Because many of these neurons were not individually identifiable, the precise number of different cells tested at least once with one stimulus could not be determined but is estimated to be 50–60. These results demonstrated that none of the magnetic stimuli elicited a general (non-specific) excitatory or inhibitory effect on the electrical activity of neurons, at least within 15 s of the field change.

Experiments with long-term recordings

Of approximately 50 different neurons on the dorsal surface of the brain that

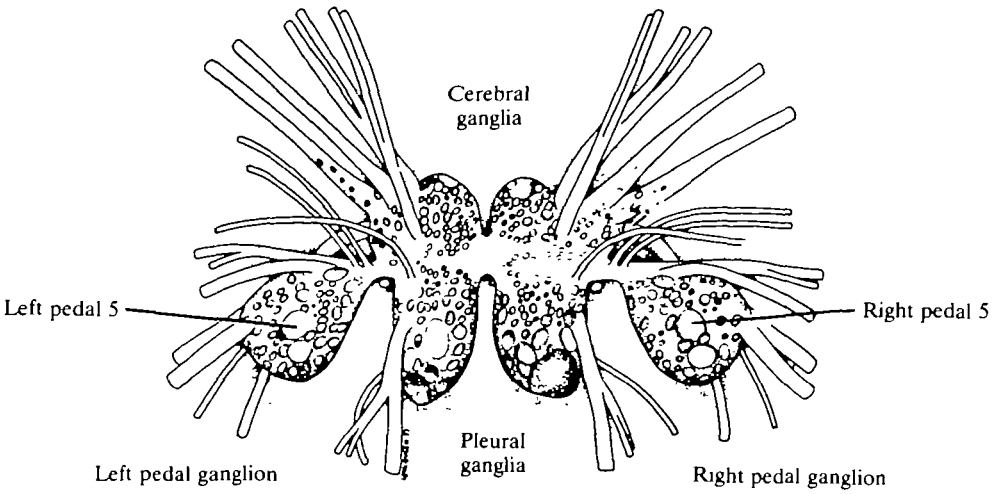

Fig. 2. The dorsal surface of the brain of *Tritonia diomedea*, showing the paired cerebral, pedal and pleural ganglia and the large, visually identifiable neurons left pedal 5 and right pedal 5.

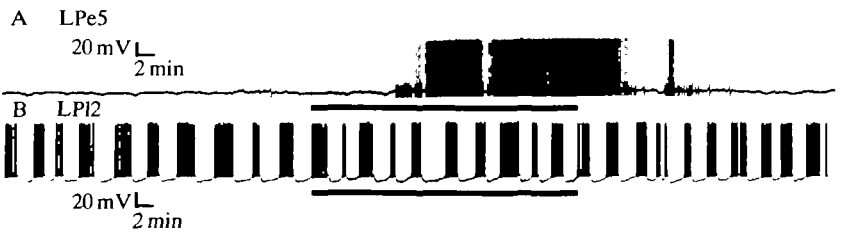

Fig. 3. Electrophysiological recordings from an experiment in which the Rubens coil was used to rotate magnetic north 20° clockwise. During this experiment the animal was oriented so that its anterior end was directed towards geomagnetic east. The long black bar beneath each trace indicates a 30-min period during which the Rubens coil was on and the ambient field was rotated 20° clockwise. (A) A recording from LPe5. The neuron began to spike approximately 9 min after the field had been rotated. (B) A recording simultaneously obtained from left pleural 2 (LPl2), another large, identifiable neuron. This cell has a characteristic pattern of bursting, and the magnetic stimulus had no apparent effect on its spiking frequency.

were selected for long-term recordings, only two cells showed evidence of altered electrical activity in response to the magnetic stimulus. These neurons were left pedal 5 (LPe5) and right pedal 5 (RPe5), an apparently bilaterally symmetrical pair of large, white, visually identifiable neurons (Fig. 2).

Responses of the neurons LPe5 and RPe5 were characterized by a gradual increase in spiking frequency occurring with a latency of 6–16 min after the field change (Fig. 3). Although increases were clear in some trials (Fig. 3A), no clear increase in spiking occurred in nearly 40 % of the trials.

Table 1. *Responses of the neuron LPe5 to a 20-min series of 60° earth-strength magnetic field rotations*

Trials	Number of animals	Number of trials	Percentage increasing	Mean increase	S.D.
All trials	10	28	82.1	15.9	17.1
First	10	10	90.0	27.9	21.3
Second	6	6	50.0	16.0	11.5
Third	5	5	100.0	8.6	5.7
Fourth	4	4	100.0	3.8	2.1

In each trial the animal was exposed to 1 min in the rotated field, 1 min in the geomagnetic field, 1 min in the rotated field, and so on (see text).

Data are from 10 different animals. In six cases the recording could be maintained long enough to expose the animal to the field rotations more than once (with a minimum recovery period of 1 h between trials).

'First' refers to responses obtained during the first exposure to the magnetic stimulus, 'second' to results obtained during second exposures, and so on. Responses obtained during fifth and sixth exposures (N=3 total) are included in the 'All trials' calculation but are not listed separately.

'Percentage increasing' refers to the percentage of trials in which an increase was observed (regardless of magnitude).

'Mean increase' is the mean difference between the number of action potentials in the baseline period and the number in the imposed magnetic field period in trials where an increase occurred. Baseline spiking rates in these trials ranged from 0 to 19 action potentials per 20 min (mean 5.6).

Quantitative experiments with LPe5

Increases in spiking frequency were observed during the imposed magnetic field period in 82.1% of all trials (Table 1); one such recording is shown in Fig. 4. Increases in different trials ranged from 1 to 73 action potentials, with a mean increase of 15.9 for all trials, but the data suggest that responses decreased in magnitude with each stimulus presentation when animals were exposed to the stimulus more than once (Table 1). Whether this apparent decline reflected a feature of the neural response (e.g. adaptation) or was merely due to the decreasing viability of the preparations could not be determined. Because multiple trials conducted on the same cell are not statistically independent, the mean number of action potentials occurring in baseline periods and the mean number occurring in the imposed field periods were calculated for each LPe5 neuron. All 10 LPe5 neurons had higher mean spiking frequencies in the imposed field periods than during baseline periods. Analysis with the Wilcoxon ranked-signs test (Siegel, 1956) indicated that the rate of spiking was significantly higher during the magnetic stimulus periods (P=0.005).

Experiments on LPe5 with geomagnetic controls

Because imposed-field and control trials in this experiment occurred in random order determined by the computer, animals were often subjected to different

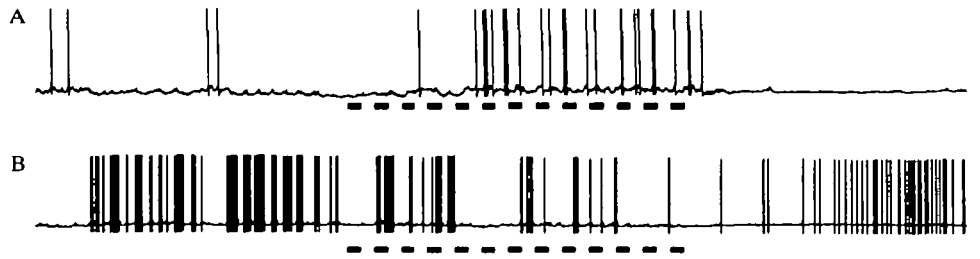

Fig. 4. Recordings from two neurons during a series of 60° magnetic field rotations. Each black bar beneath the trace represents a 1-min period during which the Helmholtz coil was switched on, rotating magnetic north 60° clockwise. During the intervening minutes, the Helmholtz coil was off and the animal was in the earth's magnetic field. Action potentials in both traces were 85–100 mV in amplitude. (A) A recording from an LPe5 neuron; the cell began spiking consistently about 9–10 min after the first field rotation. (B) A recording simultaneously made from another large, white neuron (tentatively identified as LPe7) adjacent to LPe5 that showed a gradual decline in spiking over the course of the experiment, but did not appear to respond to the stimulus.

numbers of trials under each field condition. All experiments in which at least one trial was successfully completed under each field condition were used in the analysis (N=28 animals).

For each animal, the mean change in number of action potentials between preceding baseline periods and imposed magnetic field periods was compared to the mean change between preceding baseline periods and control periods. Significantly more action potentials were produced in the imposed magnetic field periods than in the control periods (P=0.001, Wilcoxon ranked-signs test). These data confirmed that the neuron LPe5 responded to the magnetic stimulus with enhanced electrical activity. The mean changes in spiking activity for all 28 neurons are plotted in Fig. 5.

Responses of LPe5 to the magnetic stimulus are shown for three different animals in Fig. 6. Responses were characterized by enhanced spiking activity beginning 6–16 min after the first field change. Spiking usually persisted throughout the duration of the stimulus presentation; in some cases it stopped at about the time the stimulus did (Fig. 6A; also Fig. 4A), whereas in others it persisted for minutes afterwards (Fig. 6B,C). Representative control trials are shown in Fig. 6D,E.

Although the statistical analyses demonstrated that an increase in spiking occurred during the imposed field periods, the magnitude of individual responses varied considerably. Two responses weaker than those in Fig. 6A–C are shown in Fig. 7A,B, and a trial in which the magnetic stimulus failed to elicit a response from LPe5 is shown in Fig. 7C. Although some failures and weak responses of LPe5 might have been attributable to excessive hyperpolarization of the cell prior to stimulus presentation, to deteriorating viability of the preparation or to damage

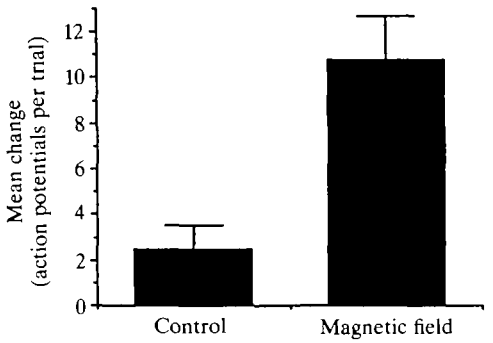

Fig. 5. Summary of results of imposed magnetic field periods and control periods in which the ambient magnetic field was not changed (see text). Mean changes for each animal (N=28) were calculated using all trials, regardless of whether an increase occurred; for example, an animal with an increase of 20 spikes during the first magnetic field trial and 0 spikes during a second trial would have a mean change of 10 spikes per trial. Error bars indicate standard errors.

to the nervous system during dissection, at least a few of the failures occurred when none of these conditions appeared to be met.

Isolated brain experiments

For each of the 20 LPe5 neurons tested, the mean change in the number of action potentials between baseline periods and subsequent imposed magnetic field periods was compared to the mean change between baseline and control periods. These paired comparisons were analyzed with the Wilcoxon ranked-signs test (Siegel, 1956). Responses in the imposed magnetic field and control periods did not differ significantly (P=0.67, N=20 animals). Thus, the magnetic stimulus failed to elicit a detectable change in the electrical activity of LPe5 in an isolated brain. The mean spiking changes of the 20 LPe5 neurons tested in this experiment are summarized in Fig. 8.

Cobalt fills

Cobalt fills from four LPe5 neurons indicated that neurites project from LPe5 through left pedal nerves 2 and 3. One such fill is shown in Fig. 9.

Discussion

The results indicate that electrical activity in neuron LPe5 increases after *Tritonia* has been subjected to various changes in earth-strength magnetic fields (Table 1; Figs 3–7). Increased spiking can be elicited by a single rotation of the ambient field (e.g. Fig. 3A) or by a series of field rotations (e.g. Figs 4A, 6A–C, 7A–B). These same stimuli, however, have no apparent effect on the spiking rates of all other neurons (except RPe5) tested (e.g. Figs 3B, 4B).

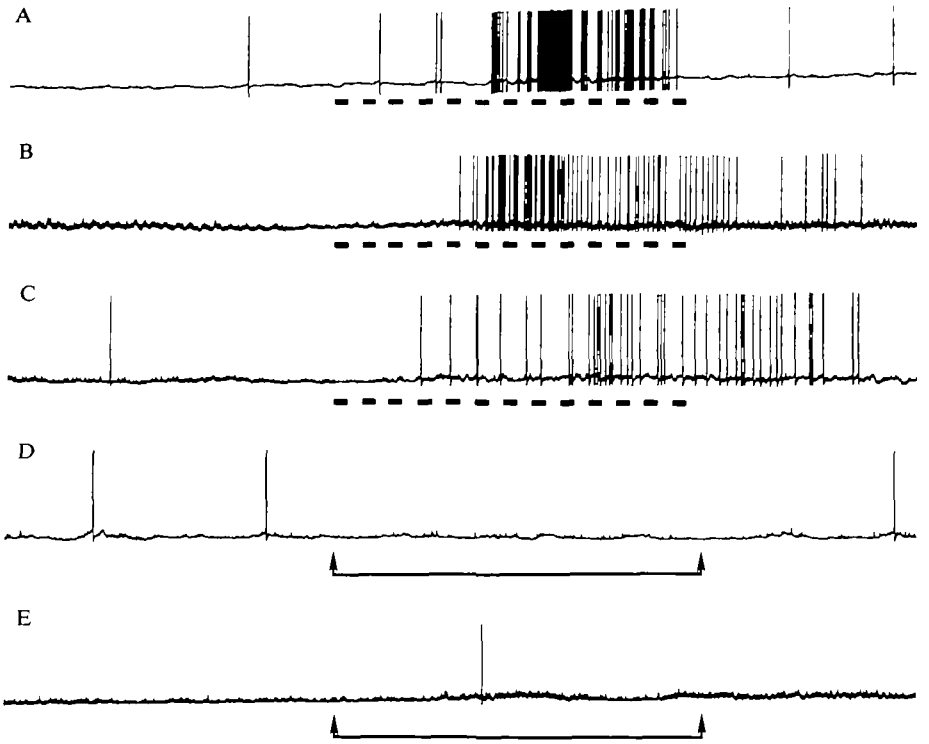

Fig. 6. Electrophysiological recordings of LPe5 during imposed magnetic field trials (A–C) and during control periods (D,E). Each trace was obtained from a different animal. Hyperpolarizing current was injected into each cell to bring the resting potential just below the threshold for spiking. Black bars beneath each trace indicate 1-min periods when the Helmholtz coil system was on, rotating the ambient field 60° clockwise. In some cases the spiking stopped approximately when the stimulus did (A), while in others it persisted for a matter of minutes afterwards (B,C). D and E are representative control trials in which the ambient magnetic field was not altered (at the decision of the computer random number generator) after the baseline had been determined to be stable; in the absence of a change in the ambient magnetic field, spiking frequency remained essentially constant throughout the trial. All action potentials are between 85 and 100 mV in amplitude.

Behavioral experiments have demonstrated that *Tritonia* can orient using the geomagnetic field (Lohmann and Willows, 1987; Lohmann, 1988). Rotating, reversing or eliminating the horizontal component of the earth's field alters the orientation and turning responses of these animals (Lohmann and Willows, 1987). We therefore hypothesize that LPe5 is one component of a neural circuit underlying magnetic field detection or geomagnetic orientation.

Induced electric field effects

Because moving a magnetic field induces an electromotive force (Faraday's Law), it was important to consider whether the observed changes in spiking

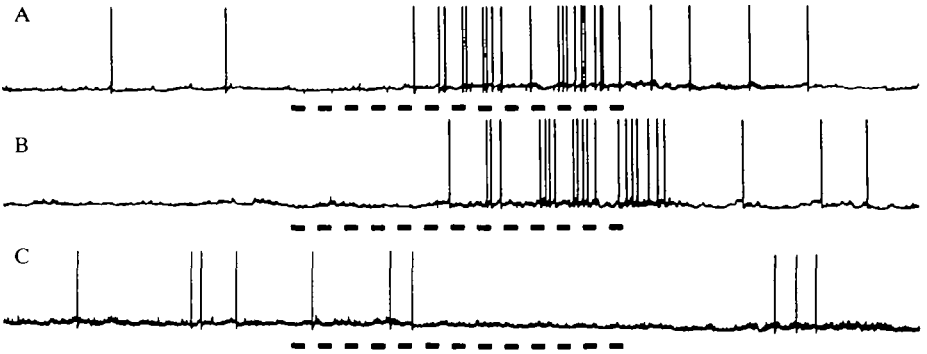

Fig. 7. Examples of variable responses of LPe5 to an imposed earth-strength magnetic field stimulus. A and B are two trials in which moderate increases in electrical activity occurred during the 26-min magnetic field stimulus. C is an example of a trial in which no increase in spiking was observed in response to the stimulus. Black bars beneath each trace indicate 1-min intervals when the Helmholtz coil system was on, rotating the ambient field 60° clockwise. All action potentials are between 85 and 100 mV in amplitude.

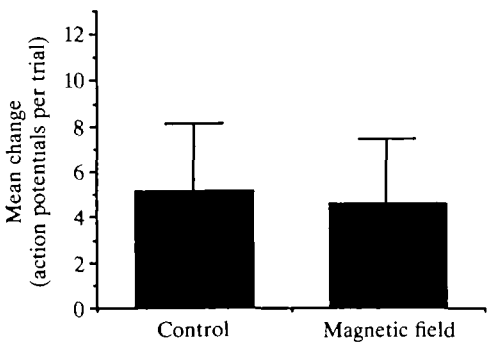

Fig. 8. Summary of results of the isolated brain experiments (see text). Conventions as in Fig. 5; N=20.

frequency of LPe5 might be attributable to effects of transiently induced electric fields rather than to magnetic field effects. Several factors, however, make this unlikely.

In principle, transient induced electric fields could exert an influence on the nervous system in two ways. First, current induced in the recording circuit could pass through the microelectrode and depolarize the neuron directly. Second, neurons could respond to the extracellular electric field (e.g. to current flowing through the water bath).

The possibility that current induced in the recording circuit depolarized LPe5 directly is improbable for several reasons. First, LPe5 rarely began spiking until 6–16 min after the magnetic field was first altered (e.g. Fig. 3). If current induced

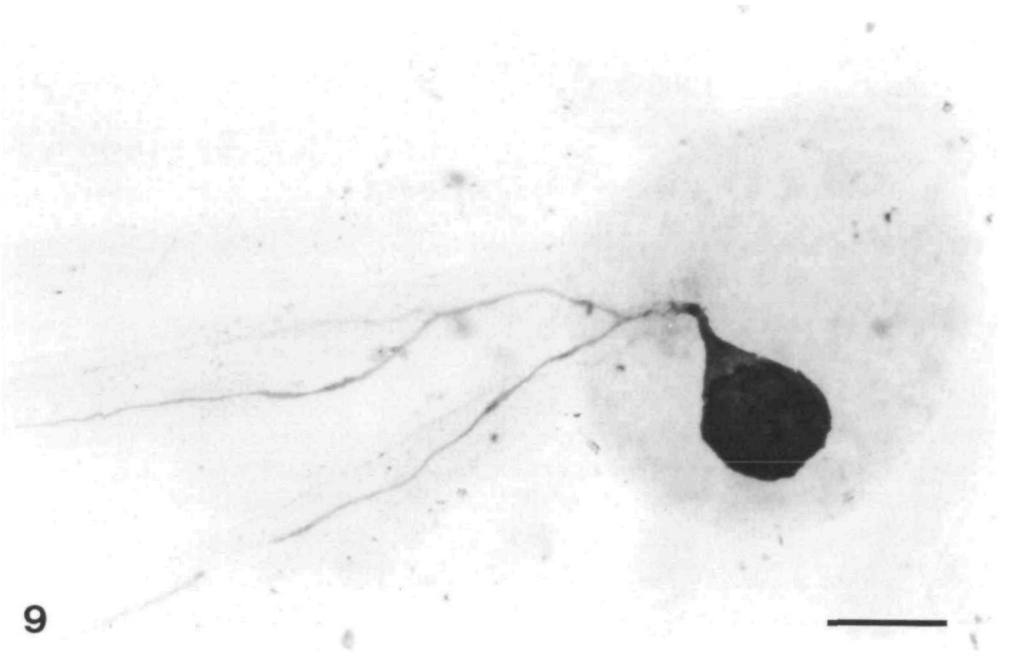

Fig. 9. A cobalt fill of LPe5. The outline of the left pedal ganglion (see Fig. 2) is visible as a shaded ellipse encircling the soma of LPe5. Neurites from LPe5 can be seen projecting through left pedal nerves 2 and 3. Scale bar, 350 μm.

in the recording circuit were sufficient to activate ion channels in the cell membrane, depolarization and spiking would probably have ensued with a latency of milliseconds to seconds, depending upon the channels activated (Hille, 1984). Second, none of the approximately 50 other neurons tested, including at least 40 cells smaller than LPe5, responded in any apparent way to magnetic stimuli. If the increased spiking of LPe5 were attributable to current passing through the microelectrode, smaller cells with a lower capacitance than LPe5 should have been affected more profoundly and would also have showed increased spiking. Finally, a series of field rotations (e.g. Fig. 6A–C) did not induce stronger responses than a single field rotation (e.g. Fig. 3). If current injected through the electrode into LPe5 caused the increased spiking, a series of electric field transients might be expected to elicit more spiking (perhaps also with a shorter latency) than a single field rotation.

In principle, the nervous system of *Tritonia* could also respond to a transient *extracellular* electric field (rather than to current injected intracellularly through the recording circuit). Measurements of the electric fields induced by turning on the Rubens and Helmholtz coils, however, indicated that the maximum voltage gradient that could be induced under the experimental conditions was approximately $2.6\times10^{-4}\,V\,m^{-1}$ (see Appendix). This worst-case value is approximately

four orders of magnitude below the lowest threshold gradient required to elicit spiking in molluscan neurons, even when current is applied directly to a saline bath with macroelectrodes (Ierusalimsky and Balaban, 1987). Moreover, because the seawater bath containing the animal was only part of the volume over which the electric field in our experiments was induced, and because the bath provides numerous shunting paths for current, the current actually reaching the nervous system of the animal would have been reduced still further from the measured values. Thus, we conclude that the electric field induced by the magnetic field rotation was unlikely to have influenced the nervous system of *Tritonia*. The basis for this conclusion is shown quantitatively in the Appendix.

Latency of response

The remarkably long (6–16 min) latency between the time the magnetic field was changed and the time an increase in spiking began in LPe5 was a puzzling, but consistent, feature of the response (e.g. Figs 3, 4, 6, 7). The latency appeared to be similar regardless of whether animals were subjected to a single field rotation (Fig. 3A) or to a series of field rotations (Figs 4A, 6A–C, 7A–B).

Although few electrophysiological responses to earth-strength magnetic stimuli have been recorded in the nervous systems of any animals, there is at least one precedent for a latency of even greater length. Altering weak magnetic fields around a honeybee can reportedly lead to changes in the electrical activity of bristle-field sensilla after a latency of 20–40 min (Korall and Martin, 1987). As in *Tritonia,* the mechanisms underlying the change in spiking frequency and the reason for the long latency are not known.

Considerably shorter latencies have been recorded in birds. Branches of the trigeminal nerve system in bobolinks, for example, reportedly contain fibers with spontaneous electrical activity that changes within a few seconds (or less) after earth-strength magnetic fields have been altered around the animal (Beason and Semm, 1987). Whether birds and nudibranchs detect magnetic fields using the same mechanism, however, is not known.

Because of the slow speed at which it moves, *Tritonia* may have little need to detect the geomagnetic field quickly. During magnetic orientation experiments, for example, nudibranchs commonly required 20–60 min to crawl approximately 0.5 m in a Y-maze and turn towards a specific magnetic direction (Lohmann and Willows, 1987). Thus, a detection process requiring as long as 15–18 min to determine direction cannot be ruled out as inconsistent with the behavioral data obtained so far.

Behavioral relevance of the stimulus

The ambient magnetic field in the natural habitat never abruptly shifts around a stationary nudibranch as it did in our experiments. During escape swimming behavior, however, *Tritonia* does experience rapid and substantial changes in orientation with respect to the earth's magnetic field. In response to a chemical on

the tube feet of predatory starfish, *Tritonia* elongates and flattens its body, pushes off the substratum, and undergoes a series of alternating dorsal and ventral flexions that cause the nudibranch to twist and tumble through the water for a short distance before it drops to the substratum again (Willows, 1971). The swimming does not appear to be oriented; nudibranchs come to rest aligned in random directions and occasionally even land on the starfish that triggered the swim (K. J. Lohmann, unpublished observations). Thus, because the animals usually finish an escape swim oriented towards a significantly different direction from the one they faced while crawling just seconds before, the abrupt field rotations used in the laboratory may have a natural behavioral counterpart.

Function of LPe5

The function of LPe5 is not known. This neuron has no apparent motor function (Willows *et al.* 1973) and is inhibited during escape swimming (Snow, 1982). Its white coloration is characteristic of cells that contain neuroactive peptides and function in modulating behavioral state (Lloyd, 1982; Masinovsky *et al.* 1985). Water currents directed towards the anterior surface of *Tritonia* have recently been demonstrated to elicit EPSPs both in LPe5 and in several neurons presynaptic to it (Murray and Willows, 1990).

If LPe5 is part of a neural circuit underlying magnetic field detection or orientation to the geomagnetic field, it could play one or more functional roles. For example, it could be (1) a primary magnetoreceptor; (2) an interneuron coupled to one or more centrally or peripherally located magnetoreceptors *via* a monosynaptic, polysynaptic or hormonal pathway; (3) a neurosecretory neuron; (4) a motoneuron exerting a subtle, visually undetectable influence on a behavioral response to the imposed field (e.g. a very slight turning response); or (5) a neuron that receives proprioceptive input from muscles in the peripheral body wall and thereby monitors progress in turning in response to a magnetic stimulus.

The results of the cobalt fills do not eliminate any of these possibilities. LPe5 sends neurites to the periphery through left pedal nerves 2 and 3 (Fig. 9), a finding consistent with previous electrophysiological evidence (Willows *et al.* 1973). The basic shape and morphology of LPe5 are similar to those of some cells known to function as motoneurons and interneurons (reviewed by Dorsett, 1985). A few primary sensory (mechanoreceptive) neurons in *Tritonia* (Audesirk, 1979), however, also resemble LPe5 in terms of neurite arrangement and soma morphology, despite being considerably smaller. Thus, the morphology of LPe5 does not provide clear insight into its function.

Variability in LPe5 responses

Although statistical analyses demonstrated that significantly more action

potentials occurred during imposed field periods than during control periods, considerable variability occurred in the responses of LPe5 to the magnetic stimuli (e.g. Figs 6A–C, 7). Moreover, LPe5 did not respond to magnetic stimuli in every trial (Fig. 7C). In initial experiments (Table 1), for example, no increase in spiking activity was observed in 17.9 % of all trials. Although some failures in this and other experiments might have been attributable to the deteriorating condition of the preparation or to damage to the nervous system during dissection, at least some of the failures occurred in apparently viable preparations. These results suggest that other factors, such as the behavioral or physiological state of the animal, influence whether a response in LPe5 can be elicited in a given trial.

Identical sensory stimuli can evoke markedly different neural responses when applied to an animal in different physiological or behavioral states. In locusts, for example, certain interneurons respond to visual cues indicative of flight deviation only when the visual stimulus is accompanied by wind, a secondary stimulus normally indicating that the animal is flying (Reichert *et al.* 1985). Similarly, if *Tritonia* responds to changes in magnetic fields only under specific behavioral conditions (e.g. when crawling), a magnetic stimulus might elicit a response in LPe5 only when the neural circuitry underlying an appropriate behavioral state is activated.

Isolated brain experiments

The somata of several primary sensory neurons are located in the central ganglia of *Tritonia* (Audesirk and Audesirk, 1980) and other molluscs (reviewed by Dorsett, 1985). Because geomagnetic field lines permeate biological tissue, magnetoreceptors need not contact the external environment and could theoretically also reside in the central ganglia. Results from the isolated brain experiments, however, indicated that magnetic stimuli failed to elicit responses from LPe5 when the brain was isolated from the rest of the animal (Fig. 8).

One interpretation of these results is that LPe5 receives synaptic input from one or more magnetoreceptors located outside the central ganglia. Long-lasting or permanent changes in the activity of some neurons, however, are known to occur in response to dissection (Stinnakre and Tauc, 1969; Alevizos *et al.* 1991). Moreover, cutting the nerves projecting from the pedal ganglia severs prominent neurites of LPe5 that project to the periphery through left pedal nerves 2 and 3 (Fig. 9); the physiological consequences of this damage are not known. Thus, the absence of a response to the magnetic stimulus in the isolated brain experiments could be attributable to a dissection artifact rather than to extirpation of the primary receptor(s).

Regardless of the function of LPe5, the discovery of an individually identifiable neuron manifesting electrophysiological correlates of magnetic field detection, combined with the accessibility of the *Tritonia* nervous system to studies of neural circuitry (Getting, 1985), provides a unique opportunity for studying the interactions between earth-strength magnetic fields and the nervous system of an animal.

Appendix

Faraday's law relating the electric field induced by a changing magnetic field is formalized in Maxwell's equation:

$$\nabla \mathbf{x} \bar{\mathbf{E}} = -\frac{\partial \bar{\mathbf{B}}}{\partial t}, \tag{1}$$

where $\bar{\mathbf{B}}$ is the magnetic field vector, $\bar{\mathbf{E}}$ is the electric field vector and $\nabla \mathbf{x}$ the curl operator.

Integrating both sides of equation 1 yields:

$$\int_A (\nabla \mathbf{x} \bar{\mathbf{E}}) \cdot \overline{\mathbf{dA}} = -\int_A \frac{\partial \bar{\mathbf{B}}}{\partial t} \cdot \overline{\mathbf{dA}}, \tag{2}$$

where $\overline{\mathbf{dA}}$ is the vector normal to the differential element of area A.

By Stoke's theorem, the more abstract concept of the curl of the electric field can be replaced by the line integral of the electric field over a specific path denoted by c. In our experiments c represented the circumference of a test coil placed within the Helmholtz coil, as shown in Fig. 10. Physically, the test coil integrated the electric field, resulting in a voltage measurable with a sensitive multimeter. The test coil was placed in the center of the Helmholtz coil, where the largest electric field would be encountered (Fig. 10). Given the structure of the Helmholtz coil, its placement and orientation, the rates of change of the currents and the absence of magnetic materials (of magnetic permeability measurably different from that of air and sea water), it is reasonable to assume that the curl of the electric field is nearly constant in the central region of the plane of the Helmholtz

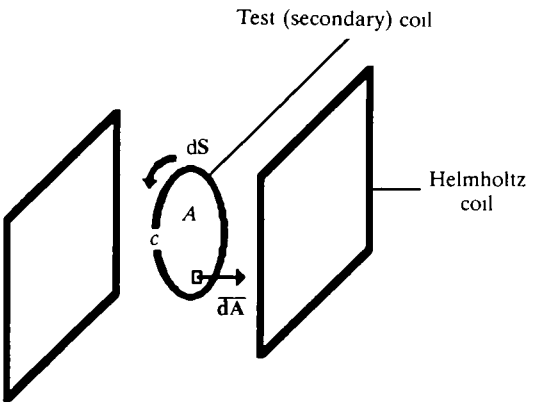

Fig. 10. Diagram of the procedure used to measure the transient electric field induced by switching on the Helmholtz coil. An identical procedure was used to measure the transient electric field induced by the Rubens coil. A test coil 17.5 cm in diameter was placed in the center of the Helmholtz coil and oriented parallel to it. A represents the area in the plane of the test coil that is enclosed by the test coil, c is the circumference of the coil, dS is an increment vector of the circumference of the coil and $\overline{\mathbf{dA}}$ is the vector normal to the differential element of area (see Appendix).

coil. It is also reasonable to assume that the field vector $\bar{\mathbf{E}}$ is nearly constant in magnitude around the circumference of the test coil.

Thus, the left-hand side of equation 2 can be simplified in the following way:

$$\int_A (\nabla \times \bar{\mathbf{E}}) \cdot \overline{\mathbf{dA}} = \oint_C \bar{\mathbf{E}} \cdot \mathbf{ds} = |\bar{\mathbf{E}}| \oint \mathbf{ds} = |\bar{\mathbf{E}}| c\,, \tag{3}$$

where $|\bar{\mathbf{E}}|$ is the magnitude of the electric field vector, **ds** is an increment vector of the circumference of the test coil, $\overline{\mathbf{dA}}$ is the vector normal to the differential element of area, C is the path of integration taken around the circumference of the coil and c is the numerical value of the circumference of the test coil in meters.

In addition, because the magnetic flux $\phi_B = \int \bar{B} \cdot \overline{\mathbf{dA}}$, the right-hand side of equation 2 can be simplified as follows:

$$-\int_A \frac{\partial \bar{B}}{\partial t} \cdot \overline{\mathbf{dA}} = -\frac{\partial}{\partial t} \int \bar{B} \cdot \overline{\mathbf{dA}} = -\frac{\partial \phi_B}{\partial t}. \tag{4}$$

Thus,

$$|\bar{\mathbf{E}}| c = -\frac{\partial \phi_B}{\partial t} \tag{5}$$

or, if the magnetic flux is given by its proportionality constant, the inductance L_m, multiplied by the current I in the Helmholtz coil, then:

$$|\bar{\mathbf{E}}| c = -L_m \frac{dI}{dt}. \tag{6}$$

This equation thus relates the induced electric field magnitude anywhere in the space of the preparation to the rate of change of the current I in the Helmholtz coil.

The induced voltage measured on the test coil with n turns of wire (Fig. 10) will then be V volts on the oscilloscope or voltmeter, and having n turns of wire is equivalent to integrating around the circumference of the coil n times. Therefore:

$$\frac{V}{n} = |\bar{\mathbf{E}}| c = -L_m \frac{dI}{dt} \tag{7}$$

and

$$|\bar{\mathbf{E}}| = \frac{V}{cn}. \tag{8}$$

For a Helmholtz coil current that matched or exceeded the largest experimental magnitude and rate of change (see Materials and methods), the measured voltage yielded a maximum induced electric field magnitude of $|\bar{\mathbf{E}}| = 0.00026\,\mathrm{V\,m^{-1}}$. Measurements using the Rubens coil yielded even lower values (approximately $8.4 \times 10^{-5}\,\mathrm{V\,m^{-1}}$; see text). These values are several orders of magnitude weaker than electric fields commonly encountered by humans in residences and urban environments (Kaune *et al.* 1987), and approximately four orders of magnitude below the $1\,\mathrm{V\,m^{-1}}$ minimum electric field known to influence molluscan neurons (Ierusalimsky and Balaban, 1987).

A consideration of the energy involved provides additional (but less direct) evidence that the weak electric fields did not influence neurons in our experiments.

The self-inductance of the Helmholtz coil system was measured by a bridge method at 2.95 mH, a value close to that calculated using the traditional handbook formula. Given the 49 mA peak-to-peak current in the Helmholtz coil, the maximum possible energy density per cycle of coil current that could be converted to the induced electric fields in the entire space of the Helmholtz coil is approximately 100 $\mu J\,m^{-3}$. The minimum energy for an observed effect of directly imposed electric fields on neuronal electrical activity in the study of Ierusalimsky and Balaban (1987) for the same time period (a cycle of our test coil period, 5.9 ms) was 23.6 $mJ\,m^{-3}$. Because the rates of change of current in our experiments never approached the values where all magnetic field energy is converted to the induced electric fields, even the 100 $\mu J\,m^{-3}$ figure is a high estimate of an upper boundary on our available energy. Moreover, the large volume of shunting parallel conductance paths in our preparation bath compared to that of Ierusalimsky and Balaban (1987) would further weaken the strength of the induced electric fields actually reaching the animals by several orders of magnitude.

Thus, on the basis of both direct measurements of induced electric fields and considerations of energy, the likelihood of significant induced electric field effects on cells in our experiments is very low.

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