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Identification of magnetically responsive neurons in the marine mollusc *Tritonia diomedea*

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Summary

Behavioral experiments have demonstrated that the marine mollusc *Tritonia diomedea* can use the Earth's magnetic field as an orientation cue. Little is known, however, about the neural mechanisms that underlie magnetic orientation behavior in this or any other animal. In previous studies, two neurons in the brain of *Tritonia*, known as LPd5 and RPd5, were shown to respond with enhanced electrical activity to changes in earth-strength magnetic fields. We report evidence that two additional neurons, known as LPd6 and RPd6, also respond with increases in electrical activity when the magnetic field around the animal is altered.

Anatomical analyses revealed that prominent neurites from the Pd6 cells are located within two ipsilateral nerves, pedal nerves 1 and 2. These nerves extend to the periphery of the animal and innervate tissues of the anterior ipsilateral foot and body wall. Electrophysiological recordings demonstrated that action potentials generated by the Pd6 cells propagate from the central ganglia toward the periphery. These results imply that the Pd6 cells play an efferent role in the magnetic orientation circuitry. Given that these cells contain cilio-excitatory peptides and that *Tritonia* crawls using ciliary locomotion, the Pd6 neurons may control or modulate cilia used in crawling, turning, or both.

Key words: orientation, navigation, magnetoreception, magnetic, neuroethology, mollusc, *Tritonia diomedea*, TPep, neuropeptide, cilia.

Introduction

Behavioral studies have demonstrated that many organisms orient and navigate using the Earth's magnetic field (reviewed by Wiltschko and Wiltschko, 1995). Despite the interest in this behavioral phenomenon, comparatively few electrophysiological studies have addressed the neural mechanisms that underlie magnetic orientation behavior (Semm et al., 1984; Beason and Semm, 1987; Semm and Beason, 1990; Walker et al., 1997). As a result, the way in which the nervous system detects magnetic cues, processes them, and produces appropriate motor responses has remained poorly understood (reviewed by Lohmann and Johnsen, 2000). A significant obstacle to understanding these neural mechanisms is the difficulty of identifying magnetically responsive neurons and then determining their role (e.g. sensory, processing, motor) in magnetic orientation behavior.

A promising model system that can be used to investigate the neural circuitry underlying magnetic orientation is the marine mollusc *Tritonia diomedea*. This species has been used extensively in neuroethological studies because it possesses individually identifiable neurons that are easily accessible while the animal performs a wide repertoire of behaviors (Willows, 1967; Willows et al., 1973). Laboratory experiments have demonstrated that *Tritonia* can use the Earth's magnetic field as an orientation cue (Lohmann and Willows, 1987) while field studies have suggested that this sensory ability may help guide the animal between offshore and inshore areas (Willows, 1999). In addition, electrophysiological experiments have demonstrated that a pair of neurons, left pedal 5 (LPd5) and right pedal 5 (RPd5) (Fig. 1), respond to changes in earth-strength magnetic fields with increased spiking (Lohmann et al., 1991; Popescu and Willows, 1999; Cain, 2001). Studies have also indicated that the Pd5 neurons influence ciliary locomotion by modulating the beating rates of cilia on the foot of the animal (Popescu and Willows, 1999; Cain, 2001).

While the Pd5 cells are some of the most thoroughly characterized magnetically sensitive neurons, other cells involved in the neural circuitry underlying magnetic orientation behavior in *Tritonia* have yet to be identified. One pair of bilaterally symmetrical neurons, the pedal 6 (Pd6) neurons, shares many characteristics with Pd5. Among these are similarities in coloration and location, the production of neuropeptides known as TPeps (Lloyd et al., 1996; Willows et al., 1997), and common synaptic inputs (Snow, 1982). In this study, we present evidence that the Pd6 neurons also respond to magnetic field changes with increased electrical activity and,

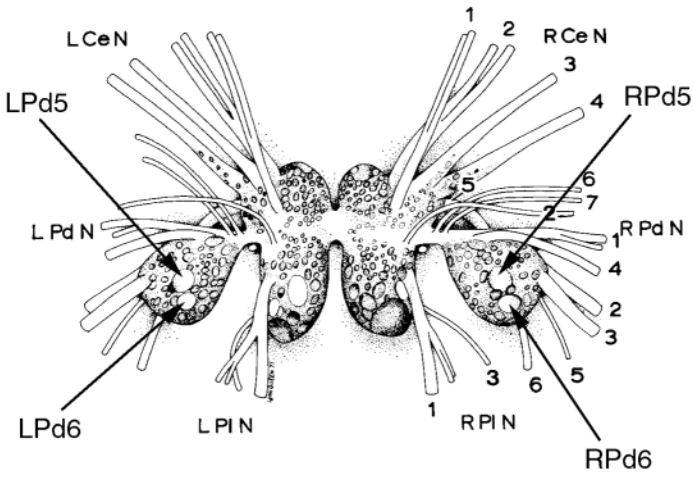

Fig. 1. The dorsal surface of the central nervous system of *Tritonia diomedea* showing the location of the bilaterally symmetric, visually identifiable neurons: left pedal 6 (LPd6), right pedal 6 (RPd6), left pedal 5 (LPd5) and right pedal 5 (RPd5). L, left; R, right; PdN, pedal nerve; CeN, cerebral nerve; PlN, pleural nerve.

like the Pd5 neurons, play efferent roles in the neural circuitry underlying magnetic orientation.

Materials and methods

Animals

Tritonia diomedea Bergh were collected from Dash Point, Washington, USA by SCUBA divers and shipped overnight to Chapel Hill, North Carolina, USA. The animals were housed in a 750 litre Plexiglas® aquarium filled with recirculating Instant Ocean® artificial seawater maintained at 11°C (Coolflow CFT-33 chiller). Animals were kept for 6–42 days and fed sea pens (*Ptilosarcus gurneyi*) *ad libitum*. The magnetic field in the aquarium had a total field intensity of 48.1 μT and an inclination angle of 66.4° (measured with a Schonstedt DM2220 single-axis magnetometer). For dissections and electrophysiological experiments, animals were transferred to a dissection chamber that contained circulating artificial seawater at 11°C.

Semi-intact animal preparations

Intracellular recordings from neurons were carried out in semi-intact preparations (Willows et al., 1973). A small incision was made directly above the brain on the animal's dorsal surface. Non-magnetic tungsten hooks were used to retract the body wall, support the animal in the seawater bath, and expose the central nervous system (CNS). A wax-covered platform was placed beneath the brain and tungsten pins were used to immobilize the central ganglia. This preparation allowed access to specific individual neurons while giving the animal freedom to perform many of its behaviors (e.g. escape swimming). Animals were allowed to recover for at least 1 h after the brain had been immobilized. Neurons were then identified by their size, coloration and location within the CNS. Targeted cells were impaled with glass microelectrodes filled with 3 mol l^{-1} KCl (15–30 MΩ). Electrical signals were amplified, monitored on an oscilloscope, digitized using a CED 1401 A-D board, and analyzed using CED Spike 2 software (Cambridge Electronics Design, Cambridge, UK).

Magnetic field measurements and magnetic field manipulations

The dissection chamber was located in the center of a computer-controlled magnetic coil system consisting of three orthogonally arranged Alldred–Scollar coils (Alldred and Scollar, 1967). The two outer wraps of each Alldred–Scollar coil measured 2.1 m on a side and the two inner wraps measured 2.2 m on a side. The coil system was used to replicate the magnetic field in which the animals were kept and to rotate the magnetic field 60° clockwise during experiments. This field rotation occurred in one step and took less than 1 s. During the rotated field condition, alterations to the total magnetic intensity and inclination angles were minimal (the total intensity of the magnetic field was only altered by +0.6% and the inclination angle was altered by +0.8°).

Magnetic experiments with left pedal 6

Because the Pd6 neurons share many characteristics with the magnetically sensitive Pd5 neurons, we investigated whether the Pd6 neurons also respond to rotations of the magnetic field. We focused our experiments on left pedal 6 (LPd6). LPd6 could be easily distinguished from other large neighboring cells based on location and size (Willows et al., 1973; Murray et al., 1992).

Animals were placed in the magnetic coil system so that they initially faced magnetic 240°. We used a magnetic test protocol similar to that of Lohmann et al. (1991). This test protocol consisted of a 20 min baseline followed by a 26 min magnetic stimulus period. At the beginning of each baseline period, hyperpolarizing or depolarizing current was injected into LPd6 to ensure that the cell produced at least one action potential and no more than ten action potentials during the ensuing 20 min period. If the activity of the cell was not in this range, the current injected into the cell was adjusted and a completely new 20 min baseline period was recorded.

Once the baseline was obtained, the computerized coil system produced a 26 min magnetic stimulus period in which the magnetic field was rotated 60° every minute. The magnetic field was first rotated 60° clockwise. After 1 min, the magnetic field was rotated 60° counterclockwise back to its original position. This alternating exposure of a clockwise 60° and counterclockwise 60° magnetic field rotation was repeated for the 26 min magnetic stimulus period (Lohmann et al., 1991).

After each trial, the animal was allowed to recover for 1 h before another was conducted. Trials were continued as long as stable recordings of LPd6 could be maintained. For each trial, the number of spikes occurring during the 20 min baseline period and the final 20 min of the magnetic stimulus were counted. The LPd6 neurons of 11 different animals were tested using these procedures. When possible, recordings from RPd6

and other neurons were made simultaneously with those of LPd6.

To confirm that the increased spiking observed in LPd6 (see Results) was due to the magnetic stimulus, we performed a second series of trials that controlled for potential spontaneous increases. In these experiments, each trial began with a 20 min baseline period. Once the baseline was recorded, the animal was exposed to either the 26 min magnetic stimulus period or to a 26 min control period in which the magnetic field remained unaltered (Lohmann et al., 1991). After a 1 h recovery period, another 20 min baseline was recorded and the alternative treatment was applied. The animal was exposed to these alternating magnetic and control treatments as long as electrophysiological recordings remained stable. The LPd6 of 10 animals were tested using this procedure.

Single rotation experiments

We also tested the response of LPd6 to single rotations of the magnetic field. Such rotations may simulate approximately what the animal experiences as it turns. These experiments were performed at Friday Harbor, Washington, USA. Animals were trawled at Bellingham Bay from depths of 20–30 m and were maintained in flow-through seawater tanks at the University of Washington Friday Harbor Laboratories for 1–3 weeks before use.

During experiments, a semi-intact preparation was placed into a dissection chamber with the anterior end directed toward a magnetic heading of 300°. The dissection chamber was placed in the center of a 1 m × 1 m single-axis Merritt 4-coil system (Merritt et al., 1983) and attached to a flow-through water system. At this location, the magnetic field had an inclination angle of 76.9° and an intensity of 53.0 μT. A BK Precision 1760 Triple output d.c. power supply was used to power the Merritt 4-coil system causing the magnetic field to rotate 60° clockwise from the Earth's field. The intensity and inclination of the field remained nearly constant (intensity changed by +0.2% and inclination by +0.4°).

An initial 15 min baseline was recorded from LPd6 and other pedal neurons without adjusting their resting potentials, using a procedure adapted from Popescu and Willows (1999) and Cain (2001). The magnetic field was then rotated 60° clockwise once. After a 6 min adjustment period, the number of spikes was counted for 15 min and compared to the number of spikes during the baseline period.

Neuroanatomy of the Pd6 neurons

To visualize the morphology of the Pd6 neurons, 500 mmol l^{-1} $CoCl_2$ was pressure-injected into their somata (for LPd6, N=5; for RPd6, N=4) using a PV820 Picopump (World Precision Instruments). After the $CoCl_2$ was allowed to diffuse throughout the neuron for 12–24 h the CNS was removed from the animal. The brain was incubated in 11°C ASW with several drops of concentrated ammonium sulfide (Croll, 1986). After 15 min, the CNS was washed with fresh ASW, fixed with 10% formalin in ASW for 24 h, dehydrated with an ascending ethanol series, cleared with methyl salicylate, and mounted onto a glass slide. The Pd6 somata and neurites were visualized using light microscopy.

The $CoCl_2$ fills indicated that Pd6 neurons had neurites located in two ipsilateral pedal nerves, pedal nerves 1 and 2 (see Results), which innervated the foot. These two nerves were dissected to determine their gross areas of innervation. Semi-intact preparations (N=5) were incubated in 1% Methylene Blue for 1 h at 4°C. Each preparation was washed with ASW and the pedal nerves were carefully followed from the CNS. At the point where the nerves entered the foot and body wall, they were dissected from the musculature. To facilitate dissecting the pedal nerves from the foot musculature, the buccal mass and viscera were removed and additional 1% Methylene Blue incubations were used as needed. Pedal nerve 1 (PdN1) and pedal nerve 2 (PdN2) were traced to determine branching patterns and innervation areas. This procedure continued until the nerves and subsequent branching neurites became too small to visualize.

Determining the direction of LPd6 action potentials

The direction of action potential propagation in LPd6 was determined by recording intracellularly from this cell while simultaneously recording extracellularly from the pedal nerves, LPdN1 and LPdN2. Extracellular signals were recorded using *en passant* suction electrodes in differential recording mode and amplified with an A-M Systems Differential AC Amplifier (Carlsborg, Washington, USA). Single units corresponding to LPd6 were identified in LPdN1 and LPdN2 by evoking action potentials in the soma of LPd6 (with current injection through the intracellular electrode). LPd6 units in the nerves were located during stimulation of LPd6 by determining which spikes corresponded one-to-one with the evoked potentials in the soma. Spontaneous LPd6 spikes were then used to determine the direction of action potential propagation within LPdN1 and LPdN2.

Results

Magnetic experiments with the LPd6 neurons

In the initial electrophysiological experiments in which the magnetic field around the animal was changed, the Pd6 neurons from 11 different animals were tested. Recordings were maintained as long as possible and multiple trials on the same animal could sometimes be completed. Thus, of the eleven animals, seven were subjected to a single trial, three were tested twice, and one was tested 3 times for a total of 16 trials. Because multiple trials conducted on the same cell are not statistically independent, the mean number of action potentials occurring during baseline periods and the mean number occurring in the imposed field periods were calculated for each LPd6 neuron. Analysis with the Wilcoxon ranked-signs test indicated that the rate of spiking was significantly higher during the magnetic stimulus periods (P<0.005, N=11). These data are summarized in Fig. 2.

Although the statistical analysis demonstrated that increased spiking occurred during the imposed field periods, responses

in individual trials were variable. Increases in spike frequency occurred in 10 of the 16 individual trials (62.5%). When increases occurred, they ranged in magnitude from 1 to 44 spikes. Responses of the LPd6 were characterized by a gradual increase in spike frequency occurring with a latency of approximately 4–12 min after the first field change (Fig. 2A).

Experiments with constant-field controls

A second experiment was conducted to confirm that the increased spiking observed in LPd6 during magnetic stimuli was attributable to the change in magnetic field and not to spontaneous changes in electrical activity. For each LPd6 tested, we compared its electrical activity during magnetic stimuli periods with control periods of equal length (N=10, Fig. 3).

For each animal, the mean change in action potentials between the imposed magnetic field period and preceding baseline period was compared to the mean change between the control period and preceding baseline period (Fig. 4). As a group, significantly more action potentials were produced in the imposed magnetic field periods than in the control periods (Wilcoxon ranked-signs test, P<0.01, N=10).

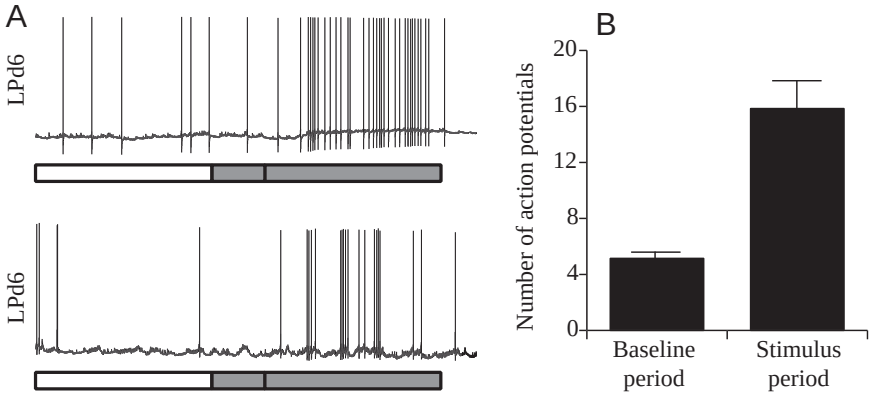

Fig. 2. The electrical responses of LPd6 to an earth-strength magnetic stimulus. (A) The electrical activity of two different LPd6 neurons during initial experiments. The white bar beneath each trace indicates the 20 min baseline period. The gray bars beneath each trace indicate the 26 min period in which the magnetic field was rotated 60° every minute (see text for details). The first gray bar represents the first 6 min while the subsequent gray bar represents the last 20 min of the magnetic stimulus (the period when data was collected). Action potentials are between 90 and 100 mV in amplitude. (B) Summary of results from initial magnetic experiments (see text). The numbers of action potentials during the baseline period and during the last 20 min of the stimulus period are plotted. Values are means ± S.E.M. (N=11).

Magnetic experiments with other neurons

The electrical activity of RPd6 (the bilateral mate of LPd6) was monitored in six animals while the magnetic field around the animal was changed (Fig. 5). The mean change in number of spikes during the baseline period from the magnetic stimulus period was 16.3±7.8 action potentials (N=6, mean ± S.E.M.). In 4 of the 6 trials, RPd6 increased spiking during the magnetic stimulus period, with increases ranging from 9 to 46 spikes.

During the course of our experiments, intracellular recordings were also made from 13 other cells during rotations of the magnetic field. With the exception of the

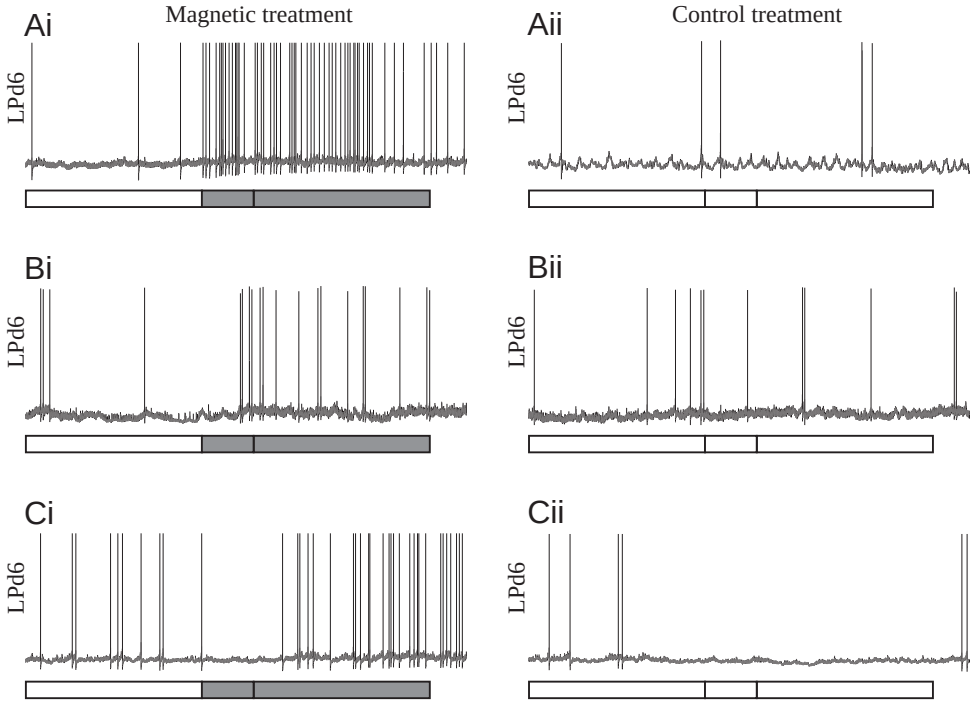

Fig. 3. Electrophysiological recordings of LPd6 from three different animals (A–C) during magnetic treatments (Ai,Bi,Ci) and during control treatments (Aii,Bii,Cii) in which the magnetic field was not changed. The bars beneath the magnetic treatment traces follow the convention in Fig. 2. The white bar beneath the control treatment indicates that the magnetic field remained unchanged. Action potentials are between 90 and 100 mV.

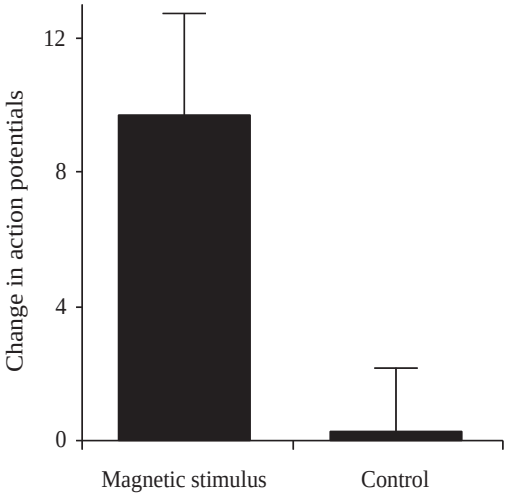

Fig. 4. Summary of results from magnetic stimulus treatments and control treatments in which the magnetic field was not changed (see text). The change in action potentials between the baseline period and the magnetic stimulus period or the control period are plotted. Values are means ± S.E.M. (*N*=10).

Pd5 neurons, which have previously been shown to respond to magnetic stimuli (Lohmann et al., 1991; Popescu and Willows, 1999; Cain, 2001), no other cells showed evidence of magnetic sensitivity (Fig. 6).

Electrophysiological responses to single field rotations

Although the majority of our tests relied on a magnetic stimulus involving multiple field rotations (Lohmann et al., 1991; Popescu and Willows, 1999; Cain, 2001), the response of the LPd6 cell to a single 60° clockwise rotation of the field was also monitored in four animals. In 3 of 4 trials, spiking in the LPd6 increased after the field had been rotated, with increases ranging from 4 to 76 action potentials. Simultaneous recordings of LPd6, LPd5, RPd6 and RPd5 indicated that all four cells responded to a single rotation of the magnetic field (Fig. 7). In addition, the postsynaptic potentials of all four of

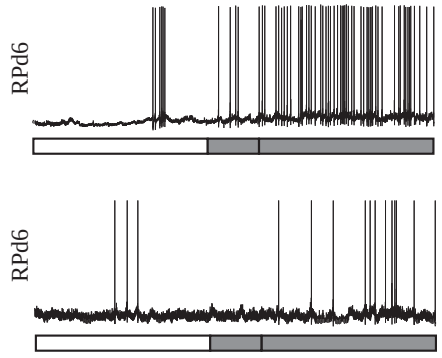

Fig. 5. Electrophysiological recordings of RPd6 from two different animals showing that this cell responds to rotations of the magnetic field with increased spiking. The bar beneath the traces follows the convention in Fig. 2.

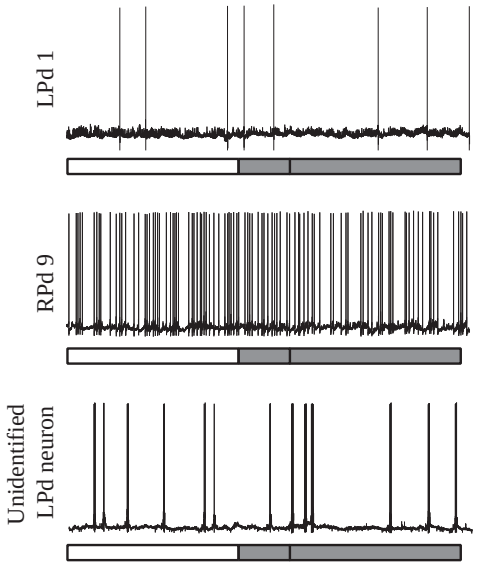

Fig. 6. Electrophysiological recordings from three cells (LPd1, RPd9, and an unidentified LPd neuron) that do not respond to rotations of the magnetic field. The third trace is from a small (50 μm) left pedal neuron located anterior of the LPdN2 trunk. The bars beneath the traces follow the conventions in Fig. 2.

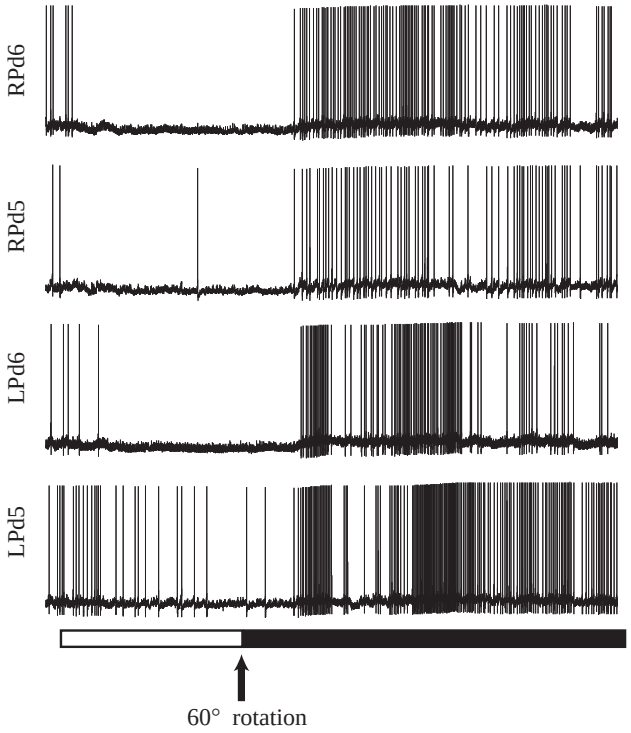

Fig. 7. Simultaneous electrophysiological recording of Pd6 and Pd5 neurons during a single 60° rotation. The white bar indicates a 15 min baseline before the magnetic field was rotated 60° clockwise. The arrow shows the point at which the field was rotated (see text for details). Action potentials are between 90 and 100 mV.

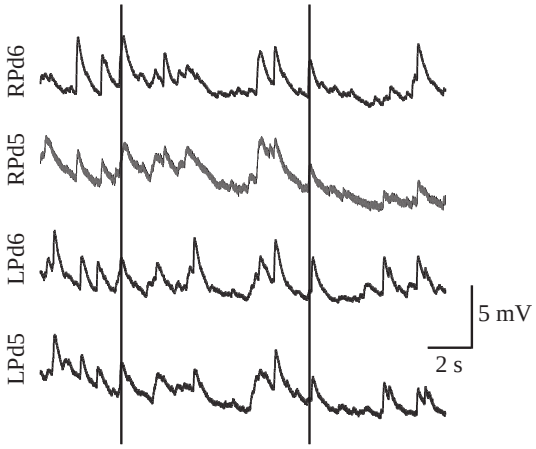

Fig. 8. Simultaneous intracellular recording of LPd6, RPd6, LPd5 and RPd5 showing synchronous postsynaptic potentials over time.

these neurons were often synchronous (Fig. 8), implying that they have one or more presynaptic cells in common.

Neuroanatomy of LPd6

The somata of the Pd6 neurons are located in the dorsal, posterior region of the pedal ganglia and often measure approximately 350 μm in diameter (Fig. 9A,B). Cobalt fills revealed that each Pd6 cell possesses a large, primary neurite, which emerges from the soma and bifurcates within the pedal ganglion (Fig. 9A,B). One process enters ipsilateral pedal nerve 1 and the other enters ipsilateral pedal nerve 2.

Gross dissection and Methylene Blue staining (*N*=5) indicated that LPdN1 and LPdN2 project to distinct, non-overlapping regions of the anterior ipsilateral foot and body wall (Fig. 9C). These nerves emerge from the ganglion laterally, pass around the buccal mass (which lies just ventral to the fused CNS) and then extend toward the ipsilateral body wall before innervating the foot. PdN1 innervates the anterior portion of the foot, while PdN2 innervates a region of the foot immediately posterior to that of PdN1. The innervation areas of these two nerves did not appear to overlap. Innervation patterns of the bilaterally symmetric RPdN1 and 2 appeared to be similar (data not shown).

Direction of LPd6 action potential propagation

Simultaneous extracellular and intracellular recordings of LPd6 were performed to determine the direction of action potential propagation. In all preparations (*N*=5), spontaneous action potentials were observed in the LPd6 soma before the corresponding extracellular units were recorded in LPdN1 or LPdN2 (Fig. 10A,B). This demonstrated that action potentials in LPd6 neurons propagate from the central ganglia to the periphery.

Discussion

The results of this study indicate that the electrical activity of the Pd6 neurons increased when *Tritonia* was subjected to various changes in earth-strength magnetic fields. Increased spiking could be elicited either by a series of field rotations (Figs 2–5) or by a single change in the direction of the field (Fig. 7). Magnetic stimuli, however, had no apparent effect on the spiking rates of other neurons tested in this study (e.g. Fig. 6) or the 50–60 neurons tested by Lohmann et al. (1991); the only exceptions were the Pd5 neurons (Fig. 7), which responded to magnetic stimuli as reported previously (Lohmann et al., 1991;

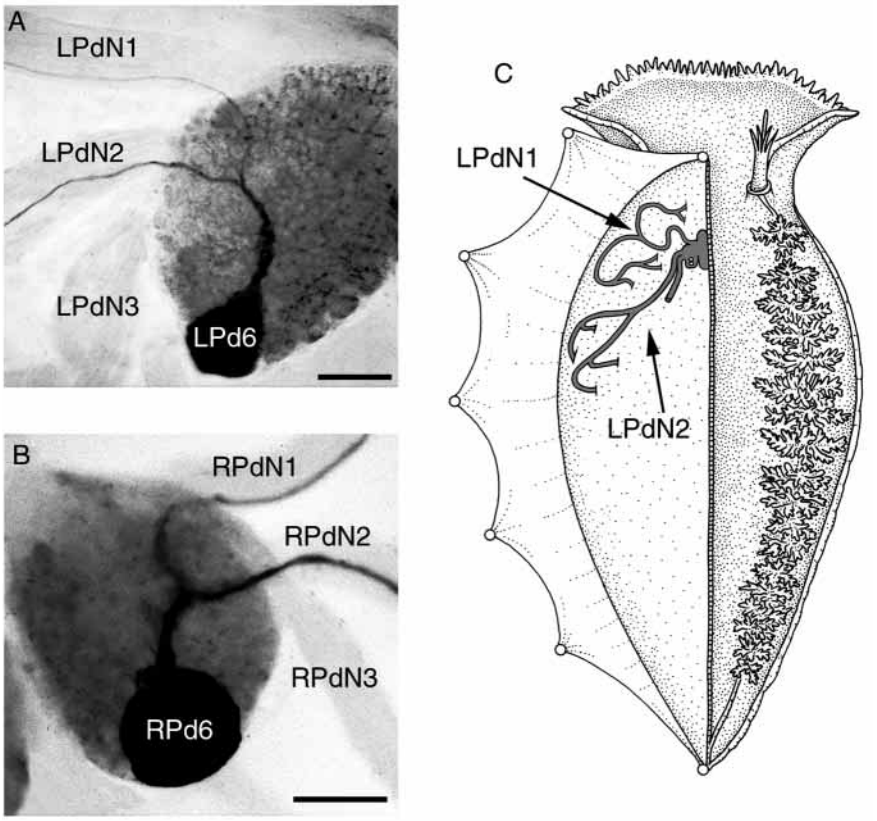

Fig. 9. Morphology and anatomy of Pd6 neurons. (A) Cobalt fill of LPd6 showing the large soma and primary neurite within the left pedal ganglion. Branches from the LPd6 primary neurite enter left pedal nerve 1 (LPdN1) and left pedal nerve 2 (LPdN2). Scale bar, 300 μm. (B) Cobalt fill of RPd6 showing branches from the primary neurite entering right pedal nerve 1 (RPdN1) and right pedal nerve 2 (RPdN2). Scale bar, 350 μm. (C) A schematic diagram of the innervation pattern LPdN1 and LPdN2 showing that these two nerves innervate the anterior regions of the foot. LPdN1 innervates the most anterior region of the foot, while LPdN2 innervates a more posterior region. There was little or no overlap between the areas of the foot innervated by the two nerves.

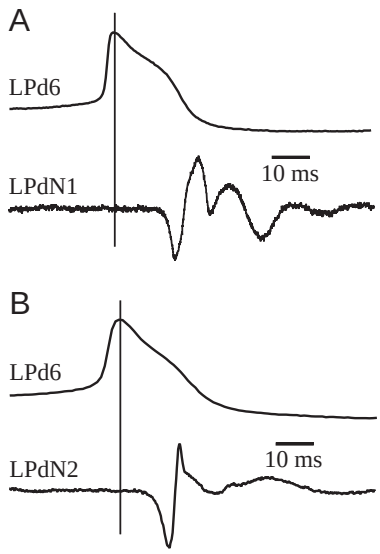

Fig. 10. Action potential propagation in LPd6 through LPdN1 and LPdN2. Single units corresponding to LPd6 were identified in LPdN1 and LPdN2 (see text for details) (A,B) Simultaneous intracellular (LPd6) and extracellular (LPdN1 or LPdN2) recordings show spontaneous action potentials occurring in the cell soma before being recorded by the extracellular electrode.

Popescu and Willows, 1999; Cain, 2001). Detailed measurements and analyses indicated that the responses of the Pd6 and Pd5 neurons are unlikely to be due to electric field artifacts, warming effects of the coil, or any factor other than the magnetic field itself (Lohmann et al., 1991).

Behavioral experiments demonstrated that *Tritonia* can orient using the Earth's magnetic field and that rotating, reversing or eliminating the horizontal component of the ambient field alters the orientation and turning responses of these animals (Lohmann and Willows, 1987). We therefore hypothesize that the Pd6 and Pd5 neurons are components of the neural circuitry that underlies magnetic orientation behavior. This circuitry presumably enables the animal to detect the Earth's magnetic field, orient its body relative to this cue, and locomote along a specific magnetic heading.

Anatomical and electrophysiological characterization of the Pd6 neurons

In principle, the Pd6 neurons might function in any part of the magnetic orientation circuitry, including: (i) a sensory role; (ii) an integrative or processing role; (iii) a motor role in controlling or modulating effector cells such as muscles or cilia. However, the anatomical and electrophysiological evidence strongly suggest a motor role for the Pd6 neurons. First, these neurons appear to innervate the foot. Each Pd6 neuron possesses one neurite, which then divides and enters each of the ipsilateral Pd nerves 1 and 2 (Fig. 9A,B). These pedal nerves in turn project to non-overlapping regions of the foot. Second, action potentials in the LPd6 propagate from the central ganglia through LPdN1 and LPdN2 to the anterior foot (Fig. 10A,B). Such a pattern, from CNS to periphery, is typical of motor neurons (Bullock and Horridge, 1965). We conclude, therefore, that LPd6 has the anatomical features, projection patterns and pattern of action potential propagation that are characteristic of many molluscan motor neurons (Willows et al., 1973; Dorsett, 1986). Thus, the results suggest that the Pd6 neurons play an efferent role in magnetic orientation by regulating the activity of unknown effector cells in the anterior part of the foot.

An enigmatic yet consistent feature of the Pd6 neurons' response to magnetic stimuli was a long latency (approximately 1–15 min) between the onset of the stimulus and the onset of the response (Figs 2, 3, 5, 7). Similar latencies were also observed in the responses of the Pd5 neurons (Fig. 7) and have been reported in all previous studies involving magnetic responses of the Pd5 neurons (Lohmann et al., 1991; Popescu, 1999; Cain, 2001). In other animals, reported latencies of physiological responses to magnetic stimuli range from 20 to 40 min in honeybee bristle cell sensilla (Korall and Martin, 1987) to approximately 2 min in guinea pig pineal cells (Semm et al., 1980; Semm, 1983) to milliseconds in birds and fish (Semm, 1983; Beason and Semm, 1987; Walker et al., 1997). In addition, behavioral responses to magnetic field changes can have lengthy latencies: from 3 min in spiny lobsters during reversals of the horizontal magnetic field (Lohmann et al., 1995) to latencies of up to 5 days in bobolinks after reversals of the vertical magnetic field (Beason, 1989). The reasons for the variability in latency between species, and the cause of the unusually long delays observed in *Tritonia* and in some other animals, remain to be determined. Among several interesting possibilities are that the receptor mechanism or neural processing of the magnetic information may require a significant period of averaging in some animals, or that the nervous system may update magnetic field information only periodically (Beason, 1989; Walcott, 1996; Wiltschko et al., 1998).

Possible function of the Pd5 and Pd6 neurons in magnetic orientation behavior

With the finding that the Pd6 neurons respond to changes in earth-strength magnetic fields, four magnetically sensitive neurons in *Tritonia* have now been identified (LPd6, RPd6, LPd5, RPd5). The Pd6 and Pd5 neurons share several characteristics that suggest that these cells may have a similar function. First, simultaneous recordings from these four neurons during the presentation of magnetic field stimuli indicate that the responses in each are qualitatively similar (Fig. 7). Second, these neurons often have synchronous postsynaptic potentials (Fig. 8), implying that they receive common synaptic input from presynaptic cells; both also receive common sensory input (Murray et al., 1992). Third, both bilaterally symmetric pairs of neurons have action potentials propagating to the periphery through nerves that appear to innervate the foot (Fig. 9; Cain, 2001).

An additional similarity is that the Pd6 and Pd5 neurons both synthesize a trio of 15-amino-acid neuropeptides known

collectively as TPeps (Lloyd et al., 1996). Immunohistological studies localized TPep in ciliated structures such as the foot epithelium and oviduct (Willows et al., 1997). Ultrastructural studies further localized TPep to dense-cored vesicles within the cell bodies of the Pd6 and Pd5 neurons and to neurites adjacent to the ciliated cells of the foot epithelium (Cain, 2001). TPeps have been shown to increase the ciliary beating frequency of isolated foot epithelial cells as well as the ciliary transport rates of foot patches (Willows et al., 1997). Given that *Tritonia* locomotes using cilia on the ventral surface of its foot, the Pd5 cells have been hypothesized to control or modulate cilia involved in locomotion, turning, or both (Willows et al., 1997; Popescu and Willows, 1999). Our results suggest that a similar function is possible for the Pd6 neurons, although alternative functions (e.g. the control of muscles or the release of mucus for facilitating ciliary locomotion) cannot be ruled out at present.

In summary, our results demonstrate for the first time that the Pd6 neurons respond with enhanced electrical activity when earth-strength magnetic fields around the animal are changed. They also suggest that these neurons play efferent (motor) roles in the magnetic orientation circuitry. Thus, these advances represent another step in the difficult task of identifying and unraveling the sensory, processing and motor elements that enable *Tritonia* and other animals to orient to the Earth's magnetic field.

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