



Magnetic orientation in *C. elegans* relies on the integrity of the villi of the AFD magnetosensory neurons

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ABSTRACT

The magnetic field of the earth provides many organisms with sufficient information to successfully navigate through their environments. While evidence suggests the widespread use of this sensory modality across many taxa, it remains an understudied sensory modality. We have recently showed that the nematode *C. elegans* orients to earth-strength magnetic fields using the first pair of described magnetosensory neurons, AFDs. The AFD cells are a pair of ciliated sensory neurons crowned by fifty villi known to be implicated in temperature sensation. We investigated the potential importance of these subcellular structures for the performance of magnetic orientation. We show that ciliary integrity and villi number are essential for magnetic orientation. Mutants with impairments AFD cilia or villi structure failed to orient to magnetic fields. Similarly, *C. elegans* larvae possessing immature AFD neurons with fewer villi were also unable to orient to magnetic fields. Larvae of every stage however retained the ability to orient to thermal gradients. To our knowledge, this is the first behavioral separation of magnetic and thermal orientation in *C. elegans*. We conclude that magnetic orientation relies on the function of both cilia and villi in the AFD neurons. The role of villi in multiple sensory transduction pathways involved in the sensory transduction of vectorial stimuli further supports the likely role of the villi of the AFD neurons as the site for magnetic field transduction. The genetic and behavioral tractability of *C. elegans* make it a promising system for uncovering potentially conserved molecular mechanisms by which animals across taxa detect and orient to magnetic fields.

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1. Introduction

The magnetic field of the earth provides positional information to many organisms across taxa, from bacteria to mammals (Blakemore, 1975; Wiltschko and Wiltschko, 1995, 2005). There is a wealth of behavioral studies describing how animals like insects and birds use the magnetic field to navigate during seasonal migrations (Mouritsen, 2013; Guerra et al., 2014). While most species so far described use the magnetic field to migrate horizontally, magnetotactic bacteria use the earth's field to migrate vertically in the water column (Blakemore, 1975). Work in the sea slug *Tritonia diomedea* and in the brains of pigeons revealed central neurons integrating magnetic field information (Willows, 1999; Wu and Dickman, 2012). Despite these important milestones, the identification of magnetosensory neurons in any animal has proven challenging. The subcellular site for magneto-transduction remains unknown. Understanding how animals transduce magnetic information pivots on the study of cells capable of magnetic field detection.

We recently identified the first pair of magnetosensitive sensory neurons (the AFDs) in the nematode *C. elegans* (Vidal-Gadea et al., 2015). These neurons terminate in a sensory ending comprised of a cilium crowned by up to 50 microvilli. Sensory cilia and villi are common sensory structures across taxa and sensory modalities (from

the rod and cone cells of the eye, to the hair cells of the ear, and olfactory receptor neurons). For example, hair cells of the inner ear possess sensory cilia that are responsible for transduction of sound, while cilia in the vestibular system are specialized to detect gravity and motion in a mechanism conserved across all vertebrates (Gillespie and Walker, 2001). In nematodes like *C. elegans*, cilia are only found in the nervous system where they specialize as sensory receptors in multiple sensory modalities (Ward et al., 1975). Because of the established role of villi and cilia in sensory transduction we hypothesized that the cilia and villi of AFD are instrumental in magnetic field transduction and orientation.

To determine role of the AFD sensory endings in magnetic field orientation we used complementary approaches combining the study of larval animals with immature villi, as well as the study of ciliary mutants with distinct genetic lesions on AFD's cilium and villi. We show that magnetic orientation requires an intact cilium and villi in the AFD neurons. Previously we showed that the cellular and molecular mechanism for temperature and magnetic sensation in *C. elegans* shows a high degree of overlap. Here we report that while all larval stages of *C. elegans* are capable of thermotaxis, aside from adult animals, only L4 and dauer larvae display magnetotactic behavior. Investigating how cilia and villi function during magnetic orientation and transduction in *C. elegans* will likely help us understand how magnetic transduction is accomplished by many of the organisms capable of this remarkable sensory feat.

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2. Materials and methods

2.1. Animals

Caenorhabditis elegans strains wild-type N2, *che-1(p672)*, *che-3(e1253)*, *che-13(e1805)*, *bbs-8(nx77)*, *bbs-8(ut306)*, *xbx-1(ok279)*, *gcy-18(nj38)*, *nphp-1(ok500)*, *gcy-8(oy44)*, *kcc-3(ok228)*, and *ttx-1(p767)* were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The additional strains **OS4776** = *nsEx2720[pAS117 (Pver-1::rab-1) + F16F9.3::GFP + rol-6]; Podr-1::RFP* **OS-4654** = *nsEx2680[pAS125 (Pver-1::rab-1(S25 N)) + F16F9.3::GFP + rol-6]; Podr-1::RFP* and **OS8595** = *gcy-8(ns35)* (*gain of function*) were kindly provided by Dr. Shai Shaham (Rockefeller University, NY). Animals were cultured on nematode growth media (NGM) agar plates and fed OP50 strain *Escherichia coli* at 20 °C as described (Brenner, 1974) in a humidity controlled environment (37%). To synchronize populations, and to protect plates from infections and overcrowding, all populations tested originated from bleached plates (Stiernagle, 2006). We should note that we observed that infections, overcrowding, nutritional state, as well as temperature and humidity fluctuations permanently and negatively affect the robustness of the magnetic orientation assay. To induce the dauer stage, we moved well fed adult synchronized worms to NGM agar plates containing no food and allowed them to lay eggs for four days as previously described (Ailion and Thomas, 2000).

2.2. AFD villi manipulations

To manipulate the number of villi in the AFD neuron we obtained the OS4654 and OS4776 strains from Dr. Shaham (see above). These strains contain a dominant negative (RAB-1^{S25N}) or control (RAB-1) version of an endoplasmic reticulum-Golgi trafficking regulator that is under the control of the temperature sensitive (and AMsh glia-specific) *ver-1* promoter. To trigger the expression of the dominant negative version of *rab-1* we incubated animals at 25 °C for 24 h which resulted in villi impairments on AFD neurons (please refer to Singhvi et al., 2016 for details). We selected animals where green fluorescence was evident following the 25 °C-induced production of GFP driven by *F16F9.3::GFP*.

2.3. Assay plate preparation

We performed magnetic assays on 10 cm bacteriological petri dishes containing 20 ml of 3% chemotaxis agar (Ward, 1973). Assay plates were made and allowed to cure for one day at test conditions (20 °C, and 37% humidity). To prevent the formation of humidity gradients, plates were kept covered and on a horizontal surface (top side up). A dot was drawn at the center of each plate and equidistant to this, two test dots were drawn at a distance of 34 mm, on opposite sides of the center (Fig. 1). We placed a 2 µl drop of 1 M NaN₃ over each test dot five minutes prior to the start of the assay to immobilize worms at each test area.

2.4. Magnetotaxis assay

Assay plates were placed on top of a 3.5 cm diameter, N42 neodymium magnet (K&J Magnetics Inc., Pennsylvania, USA) so that the center of the magnet coincided with one of the test dots (Fig. 1). This generated a magnetic gradient strongest in the area surrounding the center of the magnet. Twenty day one adult worms were picked from their culture plates and transferred into a 2 µl liquid Nematode Growth Media (NGM) droplet. We took care to minimize the amount of OP50 transported along with the worms by selecting worms crawling outside of the bacterial lawn. Animals were allowed to swim in NGM solution for a minute to rinse themselves of any remaining OP50 bacteria. Excess liquid NGM was removed using a Drummond capillary micropipette to a final volume of <1 µl. Worms were then transferred by micropipette to the center of the assay plate. A small piece of filter paper was used to carefully remove the excess liquid thus allowing the worms to freely crawl on the surface of the assay plate. To prevent animals from becoming starved, we kept the time from culture plate removal to beginning of free crawl on test plate to under five minutes. Larvae were transferred using a slightly different protocol. Culture plates with synchronized larva were gently flooded with 1.5 ml of liquid NGM being careful of not disrupting the bacterial lawn. Worms in suspension were transferred into a centrifuge tube and pelleted by spinning them for one minute at 9000 RPM. Excess NGM was removed to concentrate the worms in the bottom of the tube. Worms were then pipetted to the assay plate using a micropipette as described above.

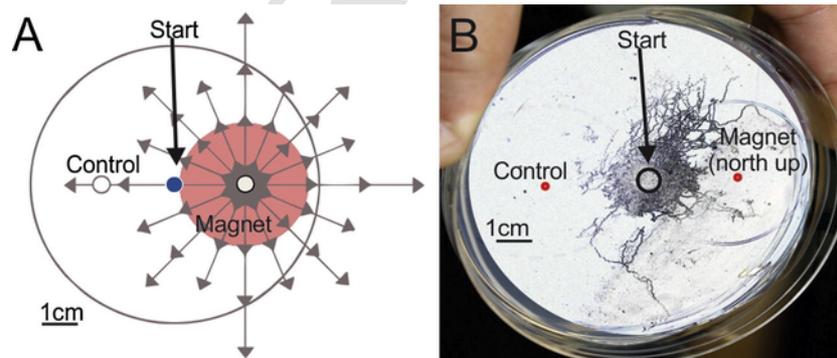


Fig. 1. Setup and performance of magnetic assay. (A) Diagram shows the configuration of the magnetic assay as set up for 10 cm plates. The blue circle labelled start in the center of the plate is where we place the animals and release them from the droplet of liquid NGM. Open circles indicate placement of the 2 µL of 1 M NaN₃ anesthetic to paralyze the worms for scoring. We place the N42 neodymium magnet to one side of the assay which creates a magnetic gradient in the plate that increases in strength as you approach the center of the magnet (as indicated by spacing of arrowheads). (B) Representative plate following a completed magnetic assay. Animals can be easily and reliably scored after an assay. Contrast enhancement was used to reveal the tracks left in the agar surface by migrating worms.

2.5. Magnetotaxis index

Approximately one hour after the start of the assay all worms can be found paralyzed at either the test area (the dot of NaN_3 by the magnet), or the control area (the NaN_3 dot away from the magnet). We calculated the magnetotaxis index (MI) as follows: $\text{MI} = (M - C) / (M + C)$ where M is the number of animals immobilized by the magnet and C is the number of worms immobilized by the control area.

2.6. Thermotaxis assay

Each condition was tested at least ten times using at least twenty animals for each assay. Agar slabs were made with 3% agar chemotaxis agar in 10 cm square plates and allowed to dry as described above. We transferred cured agar slabs using a spatula to 10 cm square test plates scored with slits two of its opposite sides (Fig. 1 Supp.). We used a paintbrush to create a 2 μl , 0.1 M NaN_3 band on each side of the assay plate. Two aluminum rectangles were inserted into the slits so as to come in close proximity at the bottom center of the plates. We established a temperature gradient from 10 °C to 25 °C by heating the sheet aluminum on one side with a hot plate and cooled the other side with dry ice. Temperature across the agar was monitored using high-accuracy electronic thermometers (Fisher Scientific, Waltham MA). Animals were incubated at either 10 °C or 25 °C and then transferred to the middle of the assay plate which was kept at room temperature (20 °C). The worms were then allowed to freely migrate for 45 min. Animals were counted for the thermotaxis index (TI) as follows: $\text{TI} = [(I - C) / (I + C)]$ where I is the number of animals immobilized by the incubation temperature and C is the number of worms immobilized by the control temperature.

2.7. Statistical analysis

Statistical analysis was performed using SigmaPlot 12 (Aspire Software). Comparisons between different experimental groups were done using two-tailed t -tests to compare normally distributed groups. Non-parametric datasets were compared using Mann-Whitney U tests. Throughout this study, p values were reported using the convention: * $p < 0.05$, ** $p < 0.001$. Assay numbers and number of animals for all figures are reported in Table S1.

3. Results

3.1. AFD cilia and villi integrity are critical for magnetotaxis

The sensory ending of the AFD neuron consists of a cilium crowned by up to 50 microvilli. The AFD neuron is surrounded by the amphid sheath glia (AMsh) which supports the development and continuing integrity of the AFD sensory ending (Bacaj et al., 2008; Singhvi et al., 2016). Ablation of AMsh cells abolishes the AFD sensory ending, while leaving behind a viable neuron (Procko et al., 2011). We recently showed that ablation of the AMsh eliminates the ability of the nematode *C. elegans* to detect the magnetic field (Vidal-Gadea et al., 2015). Therefore, we wanted to explore the role of cilia and villi integrity in magneto-transduction.

We began by testing animals with mutations known to affect cilia development in various sensory neurons (including the AFDs, Fig. 2). The intracellular localization and the morphological lesions that result from mutations in these genes have been previously established (Perkins et al., 1986; Nguyen et al., 2014; Fig. 2A). To determine the

impact of general cilia dysfunction on magnetic orientation we tested the abnormal chemosensory mutants *che-1(p672)*, *che-3(e1253)*. These genes are expressed in ciliated sensory neurons with mutations in *che-1* primarily affecting cilia development in ASE, ASH, and AWA sensory neurons, and mutations in *che-3* impairing cilia development primarily in the ADL, ASK, ASH, ASJ, and ASI neurons (Wicks et al., 2000; Uchida et al., 2003). These animals performed at nearly WT levels in our magnetotaxis assays (Fig. 2B). We next turned to test animals with mutations in genes known to result in morphological AFD cilium or villi impairments. The AFD sensory endings have distinct gene expression patterns that are highly compartmentalized to either cilia or villi (Nguyen et al., 2014; Fig. 2A). This allowed us to test the necessity of the cilium for magnetoreception independently from the villi in AFD. Mutations in *bbs-8*, *nphp-1*, *che-13*, and *xbx-1* result in lesions localized to AFD's sensory cilium. BBS-8 expression is localized to the basal body of the cilium at the transition zone at the base of the sensory finger membrane (Jauregui and Barr, 2005; Winkelbauer et al., 2005; Wolf et al., 2005). NPHP-1 localizes to the base of the cilium, as a transition zone protein involved in sensory transduction. XBX-1 is a dynein light intermediate chain subunit involved in cilia development and protein trafficking expressed at the base of the cilium and its axoneme (Schafer et al., 2003; Efimenko et al., 2005; Nguyen et al., 2014). Loss of function mutations in *nphp-1* and *xbx-1* resulted in impaired magnetic orientation (Fig. 2B). We next turned our attention to assess the role of AFD villi in magnetic orientation by testing mutations in *gcy-18*, *ttx-1*, *gcy-8*, and *kcc-3*. GCY-18 is a guanylyl cyclase critical for signaling function with expression solely in the AFD finger membrane (Nguyen et al., 2014). In *C. elegans* *ttx-1* encodes a transcription factor involved in villi development. Similar to GCY-18, GCY-8 is a guanylyl cyclase critical for signaling function with expression solely in the AFD finger membrane. KCC-3 is a K^+/Cl^- cotransporter expressed exclusively in AMsh glia and adjacent to the AFD villi (Singhvi et al., 2016). Loss of function mutations in *ttx-1* result in abnormal AFD villi and thermotaxis (Mori and Ohshima, 1995; Mori, 1999; Satterlee et al., 2001; Okochi et al., 2005). Loss of function in *gcy-18* or in *ttx-1* were both sufficient to abolish the magnetic orientation behavior in *C. elegans*. Loss of function of KCC-3 in the *kcc-3(ok228)* mutant results in loss of villi and similarly resulted in loss of magnetotactic ability. While loss of function in *gcy-8(oy44)* has a similar effect to loss of function in *gcy-18*, the *gcy-8* gain of function allele *gcy-8(ns35)* results in AFD neurons with fewer and shorter than normal villi (12 vs 45 villi, and 1.2 vs 2.5 μm , Singhvi et al., 2016). Surprisingly, these animals displayed negative magnetotaxis under our assay conditions. It therefore appears that both cilia and villi are necessary for the performance of magnetic orientation (Fig. 2B).

3.2. Magnetic orientation requires a minimum number of AFD villi

Based on our preceding results (Section 3.1), we wanted to test how villi development affects magnetic orientation. Normal development in *C. elegans* involves four larval stages (L1 through L4) prior to adulthood. This development can be arrested by adverse circumstances causing L1 worms to molt into L2d animals which quickly enter an alternative larval stage known as dauer (Riddle et al., 1981; Ailion and Thomas, 2000; Frézal and Félix, 2015). Previous work showed that the number of villi in the AFD neuron increases as the worm develops from one larval stage to the next. The AFD of animals prior to the L3 stage have 20 villi or less, L4 animals have about 35 villi, and adults reaching the full complement of about 50 (Albert and Riddle, 1983). The sole exception to this trend is for dauer animals which quickly develop a nearly full complement of AFD villi

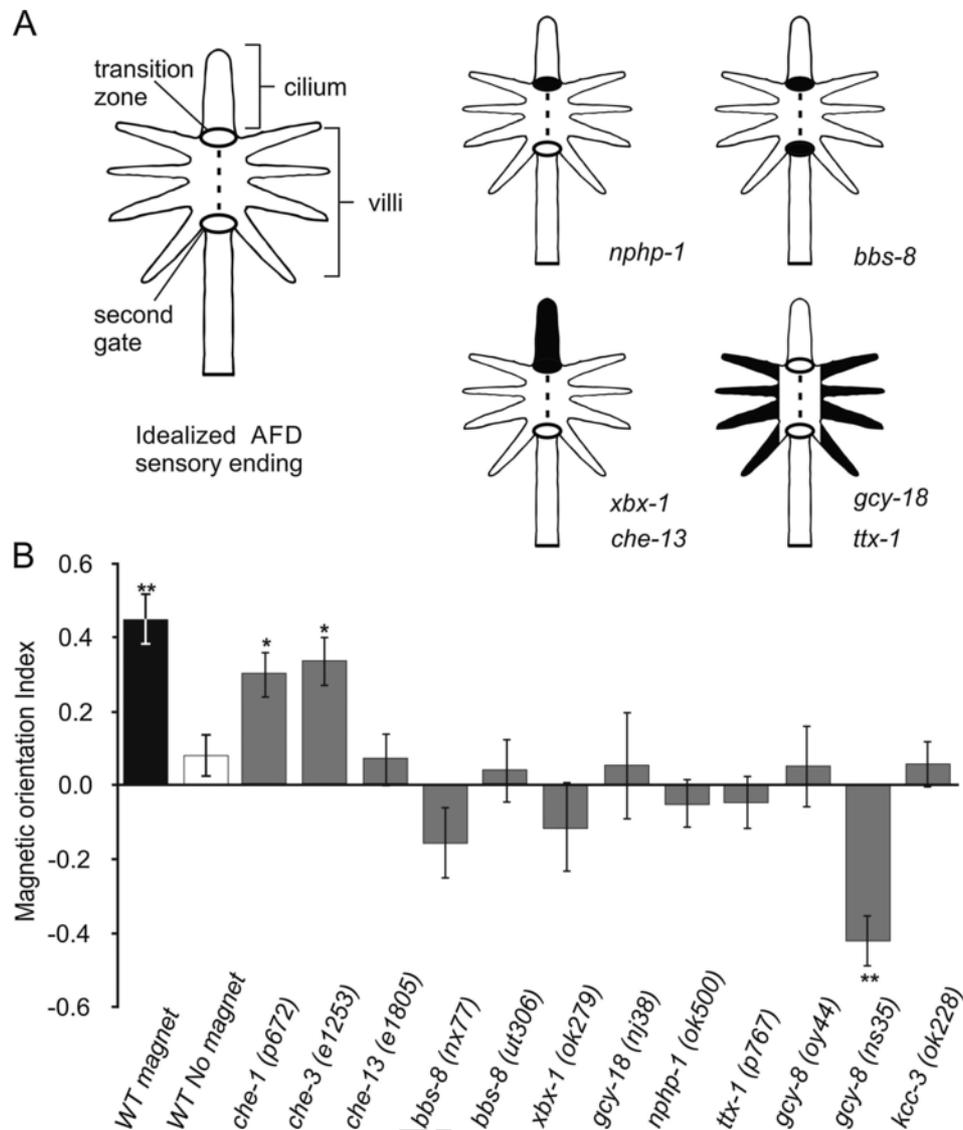


Fig. 2. *AFD* sensory cilia and villi are required for magnetotaxis. (A) Idealized *AFD* cilium shown with villi and cilium compartmental divisions outlined adapted from Nguyen et al., 2014). Transition zone (primary gate) and secondary gate of the cilium are the black rings at the base of the cilium and between the axon and finger membrane compartment. Areas of the neuron that are impacted by genetic lesions in the respective genes are shown in black. (B) Mutations in genes that resulted in lesions to either the villi (*gcy-18*, *ttx-1*, *gcy-8*, *kcc-3*) or the cilium (*che-13*, *bbs-8*, *nphp-1*, *xbx-1*) of the *AFD* neurons impaired the ability of worms to magnetotax. Mutations in *che-1* and *che-3* affect ciliary development primarily in neurons other than *AFD* and showed near normal magnetotaxis. Black bars indicate wild-type adults (orienting to a magnet in B) while white bars correspond to wild-type adults orienting in the absence of a magnet. Error bars are standard error of the means. Statistical comparisons are two-tail *t*-test against adult worms with no magnet treatment. * = $p < 0.05$, ** = $p < 0.001$.

(~45, Albert and Riddle, 1983). To assess the ability of animals to orient to stimuli known to be detected by the *AFD* neurons, we tested the ability of these different larval stages to orient to thermal gradients and magnetic fields. *C. elegans* larvae grown at 10 °C (Fig. 3A) and to 25 °C (Fig. 2 Supp.) were able to orient in a temperature gradient irrespective of their developmental stage. We found that magnetic orientation behavior was only displayed by dauer and L4 larvae (Fig. 3B). The mean magnetic orientation index for L1 ($p = 0.366$), L2 ($p = 0.950$), and L3 ($p = 0.687$) larvae were not significantly different from the adult's no magnet treatment. Interestingly, we found that L4 ($p < 0.001$) and dauer ($p = 0.017$) larvae (both possessing near-adult number of villi) displayed a magnetic orientation bias in the opposite direction as that of adult worms (migrating away from the magnet, Fig. 3B).

To further test the importance of villi in magnetic orientation we obtained two strains (OS4654, and OS4776) from Dr. Shai Shaham (Rockefeller University, NY) where the number and length of villi in *AFD* neurons can be genetically manipulated. Succinctly, these animals contain a dominant-negative version of the endoplasmic reticulum-Golgi trafficking regulator RAB-1^(S25N) that is under the control of the temperature sensitive and AMsh-glia specific *ver-1* promoter (*Pver-1*). Incubation of worms at 25 °C for 24 h blocks exocytic traffic of secreted membrane proteins and results in intact glia and *AFD* neurons displaying shorter and impaired villi in over 30% of the animals (Singhvi et al., 2016). We found that while transgenic animals where the temperature-sensitive promoter driving expression of a control version of *rab-1* (OS4776) did not result in a decrease in magnetotaxis ability, expression of a dominant negative version of the *rab-1* gene (OS4654) significantly impaired worms' ability to

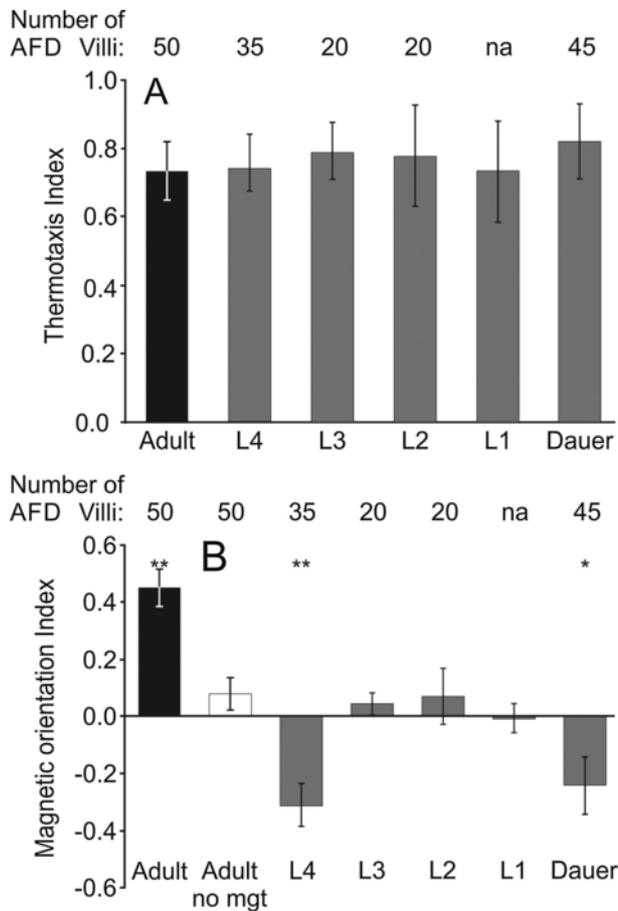


Fig. 3. Development of sensory villi is critical for magnetotaxis but not for thermotaxis. Worms at every larval stage were grown at 10 °C and tested in a thermal gradient (Fig. 1A Supp.). We found that all larval stages were capable of displaying thermotaxis (A). To determine if *C. elegans* larvae were also able to orient to magnetic fields we repeated our magnetic assays with larvae of every developmental stage. We found that besides adult animals, only L4 and dauer larvae are capable of magnetotaxis. The characteristic number of AFD villi present in the AFD neuron of each larval stage is displayed above as reported by Albert and Riddle (1983). Black bars indicate wild-type adults orienting to a magnet while white bars correspond to wild-type orienting in the absence of a magnet. Error bars are standard error of the means. Statistical comparisons are two-tail *t*-test against adult worms with no magnet treatment. * = $p < 0.05$, ** = $p < 0.001$.

magnetotax (Fig. 4). These data are consistent with villi playing a crucial role in magnetic field detection and orientation.

4. Discussion

The magnetic field of the earth provides a constant source of spatial information to any organism able to detect it. The nematode *C. elegans* possesses magnetosensitive AFD neurons that terminate in finger-like cilia and villi projections (Mori and Ohshima, 1997). Removal of the sensory ending of AFD by genetic ablation of AMsh suggested a role for the sensory villi in detecting magnetic fields (Vidal-Gadea et al., 2015). Cilia and villi are recurrent structures for a wide range of sensory modalities (Ward et al., 1975; Bae and Barr, 2008; Berbari et al., 2009). Auditory hair cells rely on cilia deformation to transduce vibrational energy from surrounding fluid into an electrical signal (Lewis and Hudspeth, 1983).

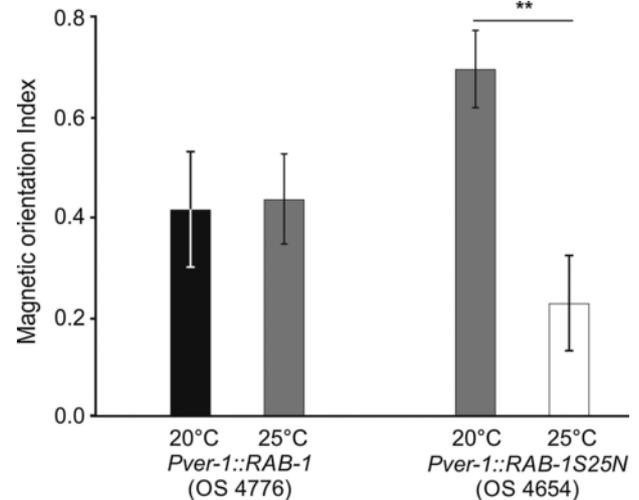


Fig. 4. Genetic ablation of AFD villi impairs magnetotaxis. Animals expressing a dominant negative version of the endoplasmic reticulum-Golgi trafficking regulator (RAB-1^{S25N}) driven by the AMsh glia-specific and temperature-sensitive promoter, *ver-1*, suffer villi impairments and a reduction in magnetotactic ability in response to incubation at 25 °C (OS4654 strain). In contrast, the OS4776 strain carrying a control version of *rab-1* was able to magnetotax at room temperature as well as at 25 °C.

4.1. Thermotaxis and magnetotaxis are differentially mediated by the AFD neurons

We found that animals at every larval stage were able to orient to temperature gradients (irrespective of the number of villi present, Fig. 3A). However, larval stages with reduced villi numbers (L1, L2, and L3) were unable to orient to magnetic fields (Fig. 3B). This represents the first finding potentially separating the temperature and magnetic detection in *C. elegans*, two sensory modalities mediated by the AFD sensory neurons. Our results suggest that a minimum number of villi must be present in the AFD neurons for worms to detect and orient to magnetic fields.

4.2. Animals with reduced villi number perform negative magnetotaxis

L4 and dauer larvae, and the *gcy-8(ns35)* (*gf*) mutant all have fewer villi than adults and displayed negative magnetotaxis under our assay conditions (Figs. 2 and 3). The molecular or behavioral mechanism responsible for this switch is at present not evident. Furthermore, it remains unclear why *gcy-8(ns35)* mutants with fewer villi than L1, L2 or L3 larvae are able to magnetotax (albeit in the opposite direction than WT). One possibility may be that L1, L2 and L3 may have reduced villi length, although an alternative (and more likely) explanation may be that different larval stages are accompanied by distinct patterns of gene expression and development that either permit or interfere with the ability of animals to detect and orient to magnetic fields.

We previously reported that animals allowed to starve for 30 min reversed their magnetotaxis behavior resulting in similarly negative indexes (Vidal-Gadea et al., 2015). Starvation is also capable of flipping other AFD-mediated behaviors such as CO₂ taxis (Bretscher et al., 2008, 2011). Our previous work showed that satiated adult worms migrate vertically up while starved adults prefer to migrate down. While the significance of this behavior is currently not clear, we suggested that well-fed worms might migrate upwards in the soil (from the bacterial food source in the root systems of plants) in search of

more plentiful food sources such as rotting fruit or decaying organic matter at the soil surface where they may establish new populations away from competition (Vidal-Gadea et al., 2015). Under these conditions, starved adults or larvae that failed to locate new food sources could only survive by burrowing down towards (and locating) plant roots harboring bacteria. This could in principle provide a selective mechanism for well-fed adults to migrate up and for starved worms to migrate down.

4.3. The integrity of AFD sensory cilium and villi is critical for magnetotaxis

Within the sensory ending of the AFD neuron, we found that mutations affecting both cilia and/or villi development also resulted in animals unable to orient to a magnetic field (Fig. 2). In contrast with lesions to the villi and cilia of AFD, we found that mutations affecting other ciliated sensory neurons did not impair the ability of worms to magnetotax (i.e. *che-1* and *che-3* mutations). While able to magnetotax, *che-1* mutants displayed below WT ability to orient to magnetic fields (Fig. 1B). A reduction in the number of AFD villi was reported for *che-1* mutants (although variable from animal to animal, Lewis and Hodgkin, 1977) supporting the idea that a minimum number of villi is required for normal magnetotaxis (Fig. 2b). Loss of function mutations in essential genes for cilia and villi function resulted in magnetotactic deficiencies. Ciliary genes *bbs-8*, *nphp-1* and *xbx-1* have been shown to be essential for normal cilium function (Schafer et al., 2003; Blacque et al., 2004; Ou et al., 2005; Nguyen et al., 2014). Disruption of the number and length of AFD villi by mutations in *ttx-1* (Mori, 1999), the guanylyl cyclase encoded by *gcy-18* (Inada et al., 2006), *gcy-8*, and *kcc-3* (Singhvi et al., 2016) also rendered worms unable to magnetotax. Mutations in BBS-8 proteins disrupts localization of GPCRs as well as guanylyl cyclases like GCY-18 (Berbari et al., 2008; Domire et al., 2011; Nguyen et al., 2014). While these findings do not necessarily implicate any of the tested genes in magnetic transduction, they suggest that this phenomenon requires the fine architecture of the cilium and villi of AFD neurons to be intact. This constitutes the first study of the subcellular structures involved in magnetic field transduction in any animal.

Ciliated sensory cells like AFD display structural motifs specialized for sensory transduction that are conserved across taxa (Gillespie and Walker, 2001; Albert and Göpfert, 2015). For example, the vestibular system of all vertebrates employs a sensory structure similar to that of the AFD sensory ending (a taller kinocilium surrounded by smaller villi-like stereocilium) transduce information about motion and balance (Lindeman et al., 1973; Lewis and Hudspeth, 1983). There are many ways in which the cilia and villi of the AFD neurons may transduce magnetic field information. The villi of the AFD do not extend radially from the cilium but rather assume an anterior to posterior alignment (Nguyen et al., 2014). It is therefore possible that these villi may be aligned with some magnetic particle or protein to encode directionality in a fashion resembling how villi encode gravity or sound in the vertebrate ear. Indeed, one of the most promising sites for magnetic transduction in vertebrates is located in the inner ear lagena of pigeons (part of the vestibular system). This structure contains ciliated sensory cells that appear to be important in magneto-transduction (Wu and Dickman, 2011). The finding of a vertebrate magneto-sensory neuron containing cilia and villi would certainly constitute strong support for a conserved mechanism for magnetosensation. Villi are particularly suited for this kind of sensory transduction as they are the only known cellular structures known to encode vectorial sensory information (e.g. gravity and hearing). Uncovering how the villi and cilia of the AFD neurons transduce mag-

netic information could inform the search of similar transduction structures in many organisms capable of navigating the world using this enigmatic sensory modality.

5. Uncited reference

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jphysparis.2016.12.002>.

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