Mechanosensory Systems and Sensory Integration Mediate *C. elegans* Negative Gravitaxis

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Summary

The ability to sense Earth's gravitational pull is essential for orientation, navigation, and proprioception in most organisms. We report here that *C. elegans* dauer larvae and adults exhibit pronounced negative gravitaxis. This behavior is antagonized by light and electromagnetic fields, suggesting that it is integrated with other sensory inputs. We found that the MEC-7 and MEC-12 microtubule components of the mechanosensory transduction system involved in gentle touch sensation are essential for negative gravitaxis. Further, TRPA-1, a channel protein associated with cold and mechanosensation in both mammals and invertebrates, is required for this behavior. However, the MEC-4/10 DEG/ENaC channels and other components that transduce gentle touch sensation are not required, suggesting that the sensory system for detecting and responding to gravity is separable from the touch sensation system. We found that the PVD neurons, which use TRPA-1 to detect harsh cold, but neither their quaternary processes nor the touch receptor neurons (TRNs), are essential for negative gravitaxis. These findings implicate an interconnected mechanism for gravity sensation involving an ion channel that is also present in the mammalian vestibular system, suggesting possible homology in gravity sensing across animal phylogeny.

Keywords

gravitaxis, mechanosensation, sensory biology, sensory integration

Introduction

Gravity sensation is a common trait among most Eukaryotes. Members of the protists, fungi, plants, and animals depend on gravity sensation for survival. Small changes in the position or orientation of these organisms result in a mechanical force that is transduced by graviperceptive organelles or organs. This force is often conveyed through dense organelles or mineral-rich structures whose displacement triggers a signaling pathway that ultimately results in a behavioral output[1]. A common characteristic of gravity transduction pathways in animals is the use of ciliated neurons, in which deflection of hair-like "stereocilia" opens mechanically gated ion channels[2,3]. Although this general mechanism has been well characterized, less is known about how mechanoreceptors and mechanosensory cells transduce such minute forces – estimated to be as small as 0.57-1.13 pN in *Euglena*, for example – into a robust signal[4].

Given the availability of a detailed connectome of the entire nervous system and powerful genetic and optogenetic tools, *C. elegans* has been a highly effective model organism for elucidating the neural circuitry and molecular mechanisms governing sensory perception, learning, memory, and behavior[5–12]. However, because they are traditionally studied in a two-dimensional environment on the surface of Petri dishes containing agar, perpendicular to the vector of gravity, few studies have examined complex behavior exhibited by *C. elegans* in three-dimensions. In the wild, *C. elegans* is typically found in moist compost, such as under shrubs or along riverbanks, which can change drastically from season to season[13,14]. These animals have evolved an adaptive alternative larval phase, the dauer larvae, that prioritizes dispersal over reproduction during adverse environmental conditions. Dauer larvae exhibit characteristic nictation, in which they "stand" on their tails and wave their heads. This behavior is believed to facilitate dispersal, possibly by allowing worms to "hitch a ride" on larger animals that pass by, such as isopods[15]. Therefore, gravitational force may be a critical input that *C. elegans* use in combination with other cues to navigate to the surface before traveling longer distances (Fig. 1A).

In previous studies, *C. elegans* dauer larvae showed no gravitactic preference, in contrast with *C. japonica*, which negatively gravitax on vertically oriented Petri plates[16]. Other studies reported that *C. elegans* adults show a tendency to orient downwards when swimming in liquid, suggesting potential positive gravitaxis[17]. However, as in *Drosophila* and the ascidian *Ciona*, the ability of nematodes to undergo gravitaxis is likely context-dependent[18,19]. *C. elegans* shows a strong aversive response to light[6] and also responds to electromagnetic fields[20–22]. Integration with these or other sensory modalities may influence or mask gravitactic behavior and experiments to test for gravitaxis in the absence of either stimulus have not been reported with *C. elegans*. In this study we report that in the absence of light, *C. elegans* dauers exhibit pronounced negative gravitaxis, which is strongly enhanced when they are shielded from ambient electromagnetic fields and light.

While little is known about their behavioral responses to gravity, the effects of hyper- and microgravity on *C. elegans* physiology has been documented[23–27]. In response to hypergravitational forces, signaling by the mechanosensory DEG/ENaC sodium channel proteins, MEC-4/MEC-10, leads to nuclear localization of the DAF-16 FoxO transcription factor, which also transduces insulin-like growth factor signaling and stress responses[28]. The *mec-4/10* genes in *C. elegans*, which encode channel proteins, and several other "*mec*" genes involved in gentle touch sensation, are expressed in the six touch receptive neurons (TRNs), which specifically mediate gentle touch sensation[29]. While the MEC-4 and -10 proteins function together in the TRNs, they also show non-overlapping expression in some cells: MEC-10 in the proprioceptive/multimodal PVDL/R and FLPL/R neurons and MEC-4 in the FLP neuron pair (Fig. 1B). Mechanosensation in many animals is also mediated by TRP (transient receptor potential) cation channels, which are common across metazoan phylogeny[30–33]. The TRPA-1 receptor is a polymodal sensor, capable of conveying either high or low temperatures as well as light and noxious stimuli[34]. In worms, TRPA-1 confers sensitivity to noxious cold in PVD neurons as well as mechanosensation in OLQ and IL1 neurons[32]. The *trpa-1* homologs *pyx* and *pain* are necessary for gravitaxis in *Drosophila*[35].

In this study, we sought to determine whether *C. elegans* possesses a system for detecting normal gravitational force and whether known mechanosensory molecular components and neurons participate in response to gravity. We discovered that *C. elegans* prefers to migrate vertically against the force of gravity – i.e., to undergo negative gravitaxis. Further, we found that gravitaxis is profoundly influenced by environmental cues of light and background electromagnetic fields, revealing sensory integration in the behavior. We also report that the MEC-7/12 microtubule components, but not the associated MEC-4/10 DEG/ENaC channel proteins or several other components involved in TRN function and touch sensation, are required for gravitactic behavior. Further, we found that the TRPA-1 channel is essential for negative gravitaxis in *C. elegans*. Finally, we identified the polymodal PVD neurons, which span the length of the worm, but not their quaternary processes, as essential for this directional bias. Based on these findings, we propose a model of gravity transduction that involves the unique action of MEC-7/12, TRPA-1, and PVD neurons. Our findings suggest that even in a diminutive animal with low mass, in which the force of gravity is much weaker than many other forces it experiences, a homologous system for gravity sensing integrated with other sensory inputs operates to optimize responses to environmental cues and its orientation on the planet.

Results

C. elegans exhibits pronounced negative gravitactic behavior

We investigated whether *C. elegans* can detect and respond to the force of gravity by initially focusing on the behavior of the dispersal state, the dauer larva. When first stage (L1) larvae experience stressful conditions, including overcrowding, lack of resources, and extreme temperatures, they subsequently develop into an alternative third stage larva, the dauer larva. Through pronounced physical, metabolic, and behavioral changes, dauers become efficient vectors for dispersal that can survive for months without food[36]. Dauer larvae of many nematodes show nictation behavior, in which they raise their heads at a 90° angle to the surface plane, raising the possibility that they can orient in the gravitational field, though this behavior may simply reflect orientation perpendicular to the surface.

Under normal laboratory growth conditions in which *C. elegans* are cultivated on flat agar surfaces in Petri dishes, dauers of the laboratory N2 strain of *C. elegans* do not nictate unless contaminated by fungi or when grown on three dimensional habitable scaffolds[37]; however, 3-D scaffolds are not convenient for studying migratory behavior. To address this issue, we adapted a setup used to study neuromuscular integrity in *C. elegans*[38] to investigate whether *C. elegans* dauers exhibit directional bias in response to gravity (see Methods). We injected dauers into the gravitaxis assay chambers comprised of two serological pipettes that, when stacked end-to-end, allow for ~25 cm of movement in either direction from the injection site (Fig.1C). Chambers loaded with worms were oriented either vertically to test for movement in the gravitational field or horizontally as a control for general migratory behavior that is not influenced by gravitational force. The migration of the populations was scored 12-24 hours after loading. Horizontal and vertical chambers were constructed and generally assayed simultaneously to control for any directional preference attributable to the construction and design of the chamber itself; in the absence of other cues, worms are not expected to exhibit a migratory bias in either direction in such chambers. However, if worms perceive and respond to the force of gravity, they would be

expected to exhibit a directional bias specifically in the vertically oriented chamber, showing a migratory bias toward the bottom with positive gravitaxis (average migration value of <0 to -7.0) or the top with negative gravitaxis (average migration value of >0 to 7.0; see Methods).

In contrast to prior assays conducted on Petri plates, which failed to reveal gravitactic preference with *C. elegans*[16], we found that N2 dauer larvae showed a weak but significant directional bias in migration toward the top of the vertical chamber under normal laboratory conditions when compared to the horizontal controls (average vertical location = 0.89, as defined in Methods, n =2,222 worms over 10 trials; horizontal = 0.47, n = 1,594 worms over 4 trials; $p < 0.0001$, Kruskal-Wallis test) (Fig. 2A, B). This difference was not statistically significant when the results were analyzed using a "Gravitaxis Index" (GI; analogous to the Chemotaxis Index[39,40]), in which the fraction of worms moving to either side is compared (GI vertical = 0.43 +/-0.17, SEM; GI horizontal 0.20 +/-0.16; $p > 0.05$, two-tailed t test). These observations suggest that *C. elegans* dauer larvae show a mild preference for upward migration under normal laboratory conditions when the migration field is >5x larger than that of previous studies[16].

Evidence for sensory integration: negative gravitaxis is attenuated by light and electromagnetic fields

External and internal sensory cues that provide important context alter behavioral responses in animals[41,42]. As *C. elegans* exhibits negative phototactic behavior[6], we posited that negative gravitaxis might be adversely influenced by this response to light (Fig. 1A). To address this possibility, we repeated the gravitaxis assay in the dark using a light-blocking blackout cloth. In sharp contrast to the lack of discernible directional preference in the horizontal chambers, we found that N2 dauer larvae demonstrated an enhanced and highly significant preference for upward migration in the vertical chambers under these conditions (average vertical location = 1.42, n = 4,276, 11 trials; horizontal = 0.008, n = 1,939, 6 trials; $p < 0.0001$) (Fig. 2A, B). This striking preference for upward migration was significantly greater ($p < 0.01$) than was seen

under normal laboratory lighting conditions (Fig. 2B), suggesting that light attenuates the ability of the animals to sense or respond to the force of gravity.

C. elegans has also been found to respond to electromagnetic fields (EMF)[20]. To test whether EMF, as with light, influences the gravitactic behavior of dauers, we analyzed their movement in chambers that were shielded from the ambient EMF present in the lab using a Faraday cage (see Methods). We found that shielding the chambers from both EMF and light resulted in dramatically increased negative gravitactic behavior: in such chambers, dauers showed strong negative gravitaxis, with highly significant directional bias compared to horizontal controls (average vertical position = 2.76, n = 28,011, 76 trials; horizontal = 0.48, n = 11,626, 33 trials; $p < 0.0001$) (Fig. 2A, B). Further, the animals showed significantly stronger negative gravitaxis under these conditions than either the -light +EMF or +light +EMF conditions ($p < 0.0001$ in both cases). Over the course of several months, we noticed that gravitaxis in our Faraday cage gradually decreased as a result of diminished EMF blocking, as confirmed by restoration of cell phone reception (see Methods). Strong negative gravitatic behavior was restored after reinforcing the cage (Fig. S1). Thus, the ability of *C. elegans* to undergo negative gravitaxis is inhibited by both light and EMF, suggesting that gravitaxis behavior in the animal is integrated with other sensory inputs. In all subsequent experiments, we used a Faraday cage in the dark to block these other sensory inputs.

Our findings that *C. elegans* dauer larvae exhibit pronounced negative gravitaxis contrasts with prior studies which found no gravitactic behavior in dauers, or that reported positive reorientation toward the gravitational vector in adults[16,17]. These differences are likely attributable to several parameters of assay design. The previous experiments were performed on Petri dishes, which limit the distance worms can travel. Owing to the long distances traversed in our chambers, we were able to find robust and highly significant differences between horizontal and vertically oriented chambers. In addition, the environment of a worm crawling in the thin space between the agar substrate and the wall of the chamber may be more similar to the act of crawling through a

column of soil than the experience of moving along the agar surface at the interface with the atmosphere on a plate. Finally, earlier experiments on gravitaxis in *C. elegans* made no mention of lighting conditions used or shielding against EMF, leading us to propose that signal integration with gravity and these other sensory inputs may have attenuated the behavior in those studies, as we have demonstrated here.

Elimination of the neural-expressed MEC-7/12 microtubule subunits, but not other components involved in gentle touch sensation, abolishes negative gravitaxis

Gravitational force is exceedingly weak compared with other mechanical stimuli, and particularly so in organisms with low mass. Diminutive organisms must therefore evolve extremely sensitive mechanisms for perceiving this force. In some small invertebrates, stretch receptors in the cuticle generate an ion influx upon deformation in a manner analogous to stretch and pressure sensors in mammals[43]. The comprehensive set of molecular components that mediate gentle touch sensation in *C. elegans* have been identified through extensive genetic analysis[44]. Because the gentle touch mechanosensory channel proteins MEC-4 and MEC-10 are also essential for transducing hypergravitational force in worms, we sought to determine whether these known mechanosensory components function in sensing Earth's gravity.

We found that mutations that eliminate most components in the gentle touch response (Fig. 3A) did not prevent gravitaxis in dauer larvae, demonstrating that gravity sensing is separable from touch reception. Gentle touch in *C. elegans* is perceived by the mechanosensory DEG/ENaC channels MEC-4 and MEC-10[29], which form a pressure-sensitive channel in the set of Touch Receptor Neurons (TRNs). We found that neither *mec-4(-)* nor *mec-10(-)* mutants were significantly defective for gravitaxis compared with wildtype controls (Fig. 3B, C): in both mutants, animals in vertically oriented chambers exhibited a strong directional bias compared to the horizontal controls (average vertical location = 2.96, n = 1,340, 7 trials; horizontal = 0.83, n = 407, 4 trials; $p < 0.0001$ for the *mec-4(e1339)* missense mutation and average vertical location = 2.77,

n = 2,110, 12 trials; horizontal = 0.81, n = 2,292, 9 trials; $p < 0.0001$ for the *mec-10(tm1552)* null mutation (Fig. 3B, C), implying that the MEC-4/MEC-10 mechanosensory channels are not required for response to gravitational fields. Analysis of *mec-4(-)* mutants is complicated by the observation that *mec-4*(*tu253)* null mutant animals are sluggish[45]; this impaired movement could confound conclusions about the requirement for MEC-4 in gravitaxis. Indeed, when tested in our assay, *mec-4(tu253)* worms showed severely defective movement and did not show clear gravitaxis (Fig. S2). Nonetheless, our findings with the *mec-4*(*e1339*) missense mutant, which is touch-defective, and the *mec-10(tm1552)* null mutant suggest that the MEC-4/10 channel are not required for gravitaxis.

We found that several other genes in the canonical gentle touch sensing system are similarly largely dispensable for gravitaxis. *mec-2* encodes a stomatin-like protein required for MEC-4/MEC-10 channel activity[46]. MEC-2 binds cholesterol[47] and likely facilitates gentle touch transduction by altering the composition of the plasma membrane surrounding MEC-4/MEC-10 ion channels[46]. We found that *mec-2(e75)* dauers were not significantly impaired in their ability to gravitax when compared with the horizontal controls (average vertical location = 2.29, n = 2,621, 10 trials; horizontal = -1.13, n = 1,526, 10 trials; $p < 0.0001$) (Fig. 3B, C). MEC-18 is required for gentle touch sensation, although its exact function has not been well characterized[44,48]. *mec-18(u228)* mutants showed a strong gravitactic preference compared to horizontal controls (average vertical location = 1.11, n =1,645, 4 trials; horizontal = -0.19 n = 4,175, 5 trials; $p < 0.0001$). While both mutants showed a significantly reduced vertical preference compared to N2, these experiments point to at most a minor role for these components in gravitaxis.

The specialized MEC-12 alpha-tubulin and MEC-7 beta-tubulin proteins form unique 15-protofilament microtubules that are found only in the six TRNs and that are essential for mechanosensation. These microtubule bundles are thicker than the typical 11 protofilament microtubules found in most cells, including in other neurons[49]. These unique 15 protofilament structures may provide intracellular resistance required for mechanotransduction; however, their

exact function in this process is unknown[50,51]. In contrast to our findings with other gentle touch sensation components, we found that removal of either MEC-7 or MEC-12 abolished negative gravitaxis, resulting in a random distribution in the chambers similar to that seen in the horizontal controls (average vertical location = 0.60, n = 1,034, 4 trials; horizontal = 0.26, n = 407, 4 trials; $p >> 0.05$ for *mec-7(ok2152)* and average vertical location = 0.83, n = 5,063, 10 trials; horizontal = 0.92, n = 5,058, 10 trials; $p >> 0.05$ for *mec-12(e1605)*) (Fig. 3B, C).

MEC-7 and MEC-12 are required for normal axonal outgrowth[49,50,52] and it is therefore conceivable that the *mec-7(-)* and *mec-12(-)* mutations might block gravitaxis by altering neuronal development or structure. We were able to separate the role of MEC-7/12 in neuronal structure from its other functions by taking advantage of the *mec-12(e1605)* allele, an H192Y missense mutation that eliminates gentle touch sensation without detectably altering TRN development or structure[53,54]. We found that gravitaxis is abolished in *mec-12(e1605)* mutants (Fig. 3 B, C; $p >> 0.05$), suggesting that MEC-12 requirement in gravitaxis is separable from its role in neuronal structure.

MEC-12 is the only *C. elegans* alpha-tubulin subunit known to be acetylated at K40[53,55]. Mutations that modify K40 or that eliminate the MEC-17 transacetylase required for microtubule acetylation also prevent mechanosensation[56–58]. We found that *mec-17(u265)* mutants, which lack functional MEC-17, undergo normal gravitaxis behavior (average vertical location = 3.10, n = 856, 5 trials; horizontal = -0.90, n = 245, 2 trials; $p < 0.0001$) (Fig. 3B, C) that did not significantly differ ($p >> 0.05$) from that of N2 animals, indicating that this modification, which is essential for stabilizing the MEC-7/12 microtubules in TRNs, is not required for sensation or response to gravity.

Taken together, these results support the notion that MEC-7/12 microtubules perform a role in gravity perception that is distinct from their structural roles or action in conferring gentle touch sensitivity.

The TRPA-1 channel is essential for gravity sensation

Our finding that MEC-4 and MEC-10 are not required for gravitaxis suggests that other types of mechanosensory channels may be involved in gravity perception. Prime candidates for such channels are members of the superfamily of TRP (transient receptor potential) proteins, which are implicated in many sensory modalities including sensitivity to touch, hot and cold temperatures, noxious chemicals, and light[31,34]. Orthologs of these channels have been found across metazoan phylogeny, including in all triploblast and diploblast animals, sponges, and even unicellular choanoflagellates, which are believed to be the closest surviving relatives of all metazoans [59]. Mouse TRPA-1 is expressed in the vestibular system, the primary organ where gravity sensation occurs in mammals[60]. In *C. elegans*, *trpa-1* is expressed in the proprioceptive/nociceptive PVDL/R neuron pair, among others. Although TRPA-1 is known to play a role in noxious cold sensation in these neurons, *Drosophila* homologs of TRPA channels, *pain* and *pyx,* are required for gravity sensation[35]. Additionally, *trpa-1* knockout mice show impaired mechanosensation and perception of noxious cold[33]. *C. elegans* TRPA-1 is likely to perform mechanosensory functions in addition to its known role in cold sensation[33].

We found that removal of TRPA-1 in the *trpa-1(ok999)* null mutant abolishes gravitaxis in dauer larvae (average vertical location = 0.93, n = 1,392, 10 trials; horizontal = 0.84, n = 1,300, 7 trials; $p >> 0.05$) (Fig. 4A, B). As *trpa-1(ok999)* mutant adult worms exhibit several movement defects, including decreased forward locomotion and several variations in the sinusoidal movement typical of wildtype worms[61], it was conceivable that elimination of net upward biased movement of the animals might reflect diminished locomotory capacity rather than defects in gravity sensing *per se*. However, we found that *trpa-1(ok999)* dauer larvae distributed broadly across the chambers comparable to that observed with N2 animals in both the horizontal and vertical conditions, demonstrating that these mutants are capable of traveling long distances regardless of orientation. These findings suggest that TRPA-1 channels are essential for, and may mediate, *C. elegans* gravitaxis.

PVD neurons, but not TRNs, are required for gravitaxis

Our observations that apart from the TRN-expressed MEC-7/12 microtubules many components that are essential for TRN function in gentle touch sensation are not required for gravitaxis led us to ask whether TRNs themselves are required in the response. The LIM homeodomain transcription factor MEC-3 mediates differentiation of several sensory neurons including the TRNs by activating a battery of genes required for touch sensation. TRNs in *mec-3(-)* mutants have smaller-than-normal cell bodies and shorter processes, leading to touch insensitivity[29]. We found that elimination of MEC-3 function in a *mec-3(e1338)* mutant did not significantly alter the ability of the animals to undergo gravitaxis (average vertical location = 3.8, n = 1,225, 5 trials; horizontal = 2.47, n = 1,657, 7 trials; $p < 0.0001$). Thus, normal TRN differentiation is not essential for negative gravitaxis.

The requirement in gravitaxis for *trpa-1, mec-7,* and *mec-12*, but not *mec-4/10*, other genes required for gentle touch response, or normal TRN differentiation, suggested that neurons other than the TRNs are required for this behavior. Although MEC-7 and MEC-12 are present in many neurons, their function beyond the TRNs is poorly understood. Further, there is no reported connection between MEC-7/12 microtubules and TRPA-1 function. Of the six TRNs, only one expresses *trpa-1*[62]; however, available expression data[31,62] indicates that PVDs, which detect muscle movement in addition to a host of other stimuli, express all three genes. We hypothesized, therefore, that PVD neurons might be integration centers for two proprioceptive inputs: muscle movement and position relative to the vector of Earth's gravitational field.

To test this hypothesis, we first investigated the requirement for TRNs in gravity sensing by genetically ablating them. The *mec-4(u253d)* gain-of-function mutation results in constitutive MEC-4/10 activation, which triggers degeneration and necrotic death specifically of the TRNs. We confirmed loss of TRNs using a TRN-expressed *mec-3p::*RFP reporter (Fig. 5A-F). Ablation of the TRNs did not alter the ability of the animals to perceive and respond to gravity: the gravitactic

behavior of worms lacking TRNs was not significantly different from that of N2 animals in our gravitaxis assay ($p >> 0.05$) (average vertical location = 2.68, n = 1,955, 12 trials; horizontal = -0.57, n = 711, 5 trials; $p < 0.0001$) (Fig. 6C,D); hence, efficient gravitaxis of dauer larvae does not require the TRNs.

We then ablated the PVD neurons by expressing a constitutively active version of the nicotinic acetylcholine receptor (nAChR) channel subunit, *deg-3(u662)/*DEG-3, under the *ser2prom3* promoter[63]. Elimination of PVD neurons using this construct has been confirmed with a PVD-specific fluorescent reporter[63]. We found that animals lacking PVD neurons completely failed to undergo negative gravitaxis, showing a relatively even distribution between upward and downward movement, with a slight downward bias (average vertical location = -0.14, n = 2,323, 9 trials; horizontal = 0.95, n = 2,532, 6 trials; $p < 0.0001$) (Fig. 7A, B). Based on this finding, it is conceivable that removal of PVDs results in a reversal of gravitactic preference, albeit with a weak effect. This behavior is likely attributable to *bona fide* inability of the PVD-ablated animals to undergo negative gravitaxis, rather than defects in motility, as they are capable of effectively reaching both ends of the chamber in either the horizontal or vertical orientations. These findings strongly support an essential requirement for PVD neurons in sensing and responding to the gravitational fields.

Our observation that *mec-3(e1338)* mutants exhibit negative gravitaxis, while dauers lacking PVD neurons do not, led us to probe how PVDs may be responding to gravity. MEC-3 expression is required for TRN development[64,65] and low levels of this transcription factor are required for specification of PVD neurons[64,66]. In adulthood, PVD neurons are highly branched, with 2°, 3°, and 4° processes that have been described as “menorahs” (Fig. 5). In the absence of MEC-3, PVD neurons develop 1° axons, but lack 2°-4° branches[66,67]. The observation that PVD neurons, but not MEC-3, are required in gravitaxis may be explained by the role of these processes in sensing polymodal information. Harsh touch and proprioceptive signaling are compartmentalized to the axon and 3° branches, respectively, within PVDs[68]. Thus, the finding

that *mec-3* mutants are not impaired in their gravitational response implies that 2°-4° processes are not essential for this behavior. Moreover, while 1°-3° processes are observed in dauer larvae (Fig. 5A, inset)[69], 4° processes do not appear until L4 or adulthood (Fig. 5C, inset)[70], further substantiating that 4° branches are not involved in gravity sensation.

The difference in PVD architecture between dauers and adults led us to ask whether adults behave differently in response to gravity. Changes in gravitational response – or even in the ability to sense gravity – throughout development could also be important for the worms' ecology, particularly given the importance of the dauer stage, but not adults, in dispersal of the animal. We found that, like dauer larvae, N2 adults also undergo significant negative gravitaxis in the absence of light and EMF (average vertical location = 2.00, n = 520, 5 trials; horizontal = -0.50; n = 777, 6 trials; $p < 0.0001$) (Fig. 7). While overall gravitactic preference was reduced somewhat in adults compared with dauers in our vertical assay ($p < 0.0001$), we note that the distances travelled by adults and dauers may not be directly comparable as adults are significantly larger, and therefore travel shorter distances relative to their body length in this assay. Evidence of gravitactic behavior by adults suggests that gravity sensation may have a strong influence on behavior throughout *C. elegans* development.

Discussion

In this study, we report four major advances. 1) We discovered that *C. elegans* dauer larvae and adults show pronounced negative gravitaxis. This drive toward movement away from the center of the earth may direct the animals toward food sources, typically decomposing vegetative matter at the surface[14]. 2) We found that the ability of the animals to sense and respond to the force of gravity is attenuated by light and electromagnetic fields, suggesting that they integrate sensation of these other influences to make decisions about whether to undergo negative gravitaxis. 3) The ability of the animals to sense and/or respond to gravity does not require DEG/ENaC channels or other factors involved in touch sensation but does require the MEC-7/12 microtubule components and TRPA-1 channels. Thus, gravity sensing involves a previously unknown system involving specialized microtubules, which also participate in touch sensation and may function with TRP sensory channels. 4) Both immature PVD neurons in dauer larvae, and those in adults, but not TRNs, are essential for response to gravity. Moreover, adult PVDs lacking quaternary processes ("menorahs") appear fully functional for this response.

Underground habitats are generally ill-suited for *C. elegans* and such environments may cause dauer larvae to find their way to the surface where decaying vegetation might be present. However, the animals also avoid light[6]. Further, some studies suggest that they may navigate using Earth's magnetic field[20–22]. Our finding that ambient light and EMF in the laboratory setting strongly attenuate gravitactic behavior – which may explain why negative gravitaxis has not been reported previously – suggests that the animals integrate sensory inputs from a variety of environmental signals, including the force of gravity, to optimize behavioral decisions[71]. The extent to which light, EM fields, and potentially other inputs override the response to gravity, as well as the circuits required to calculate their respective influence on behavior, are important outstanding questions.

Although it was not detected in earlier studies, our altered assay revealed that both dauers and adults of *C. elegans* exhibit pronounced negative gravitaxis when properly shielded from other environmental stimuli. The reduced ability of N2 dauers to exhibit negative gravitaxis compared to that observed with *C. japonica* dauers, might be an outcome of myriad adaptations to laboratory cultivation, as has been observed for several other traits, including social feeding, egg laying behavior, oxygen tolerance, and nictation[11,14,15,72–74]. We found that negative gravitactic behavior, albeit weakened, occurs in the LSJ1 sister strain, which has been raised in liquid media over many decades, and is also observed in *C. briggsae*[75]. It remains an open question as to whether these strains show negative gravitaxis when shielded from light and EMF when swimming in liquid rather than crawling on solid media.

Our finding that *trpa-1* and MEC-7/12 microtubule functions are both required for gravitaxis reveals an unexplored mechanism for transducing gravitational force. Unlike the DEG/ENaC channels, TRPA-1 receptors do not require internal or external machinery to function[33]. Additionally, *trpa-1* is expressed in only one of six TRNs, which are the only neurons containing the 15 protofilament structures formed by MEC-7/MEC-12 tubulins. MEC-7/MEC-12 microtubules may perform more than a structural role in mechanosensation; whether this function is independent of their assembly into 15 protofilament structures has not been determined.

We obtained evidence pointing to a pair of neurons, PVDL/R, as potential gravity-sensors. Unlike animals lacking TRN function or cells, which show normal behavior in our gravitaxis assay, those lacking the PVDs show no detectable response to gravity. These results, in conjunction with our *trpa-1* findings, suggest a sketch of how gravity may be transduced at a molecular and cellular level. PVD neurons may use TRPA-1 to provide information about the direction of gravity. However, in the absence of additional information, it is not clear whether TRPA-1 is the primary gravity receptor in worms. As with TRP channels generally, TRPA-1 is known to have primary and secondary roles in a variety of sensory transduction pathways[30]. It is not clear from our studies, or other reports, how MEC-7 and MEC-12 microtubule subunits function in

mechanosensation. While these tubulins may perform a vital function within PVD neurons that is required to transmit a signal, it is also possible that they play a role in other neurons involved in the response. It is noteworthy that ciliated neurons are frequently used in gravity sensation including in humans[2,3]. There are 60 ciliated neurons in *C. elegans*[63], 38 of which express MEC-12 and TRPA-1[76]. Of these, 3 (OLQ, AQR, and PHA) also express an orthologue of the likely gravity-sensitive mechanoreceptor in mammals, TMC-1[77–79]. Thus, it is conceivable that PVDs may be secondary neurons that act within a neural circuit to make decisions about movements in response to the direction of the gravitational field.

Understanding how *C. elegans* perceives gravity has implications for both invertebrate and mammalian biology. *C. elegans* has been used for decades as a model for understanding the effects of microgravity on animal physiology in space[23,24,26,80–84], and yet scant information is available regarding their ability to sense gravity. Moreover, gravity perception is linked evolutionarily and developmentally with hearing[85,86]: the vestibular and auditory systems in mammals share similar sensory structures, transduction machinery, and gene expression[87]. A role for TRPA1 has been identified in sensory transduction within inner ear hair cells in mice[88]. The similarities between these systems makes the study of gravity sensation important not only for understanding mammalian vestibular sensation, but also auditory sensation. A recent study showed that *C. elegans* can sense sound through mechanical perturbations of the epidermis and that this sense involves the multimodal PVD neurons and the FLP mechanosensory neurons[89]. Thus, uncovering the role of TRPA-1 as a mechanoreceptor raises the possibility that *C. elegans* could be used as a model for studying both vestibular sensation and audition.

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Author contributions

CA: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Visualization, Supervision. **NZK:** Methodology, Validation, Investigation. **LW:** Methodology, Validation, Investigation. **RK:** Investigation, Formal Analysis. PJ: Conceptualization, Writing – Review & Editing. **JR:** Conceptualization, Writing – Review & Editing, Project administration, Funding acquisition.

Declaration of Interests

The authors declare no competing interests.

Figure Legends

Figure 1. Behavioral model, wiring diagram, and gravitaxis assay design

(A) Graphic depicting environmental stimuli that inform *C. elegans* behavior in a natural setting. **(B)** Diagram of key neurons mentioned in this study. TRNs are labeled in red, while FLP and PVD neurons are green (for neurons with left and right pairs, only the left neuron is displayed). Adapted from individual neuron illustrations available on WormAtlas [90]. **(C)** Gravitaxis chamber and experimental design used in this study. Figures **(B)** and **(C)** created with BioRender.com.

Figure 2. *C. elegans* negatively gravitax under different environmental conditions

(A) Histograms depicting cumulative distribution of *C. elegans* worms over multiple trials in vertical (blue) and horizontal (orange) assays. Presence or absence of normal overhead light and background EMF (no Faraday cage) are denoted with +/-. **(B)** Boxplots depicting data shown in A. Dauers tested in a dark Faraday cage are used in future comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n.s. is not significant using Kruskal-Wallis followed by Dunn's test with Bonferroni correction. Notches on boxplots represent 95% confidence intervals; mean values are indicated with a diamond.

Figure 3. MEC-7/12 microtubule subunits, but not several other components in the gentle touch mechanosensory system, are necessary for gravitaxis

(A) MEC-4 and MEC-10 subunits form a sodium channel that is required for gentle touch sensation in TRNs. MEC-1, -5, and -9 components of the extracellular matrix (ECM) form a structure between TRNs and the cuticle [91,92]. MEC-7/12 microtubules form 15 protofilament structures in the TRNs only; however, these β and α subunits (respectively) are found in other neuronal 11 protofilament structures[50]. MEC-2 proteins associate with both the Deg/EnaC channels and TRN microtubules and enhance channel activity, likely through interactions with cholesterol in the plasma membrane [46,93,94]. MEC-17 is a transacetylase that stabilizes MEC-7/12 microtubules [56–58]. MEC-18 (starred) performs a poorly understood role the transduction of gentle touch. Based on its proposed function as an acetyl CoA ligase, we propose it may act in conjunction with MEC-17 to acetylate tubulin proteins. (Adapted from images by *Tavernarakis et. Al 1997* [95] and others). Image was created with BioRender.com. **(B-C)** Gravitaxis assays of N2 dauers and dauers carrying mutations in genes involved in mechanosensory transduction. **(B)** Histograms comparing horizontal (orange) and vertical (blue) distributions of each strain over multiple trials. **(C)** Boxplots summarizing data shown in **A**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n.s. is not significant using Kruskall-Wallis followed by Dunn's test with Bonferroni correction. Notches on boxplots represent 95% confidence intervals; mean values are indicated with a diamond.

Figure 4. TRPA-1 channels are required for gravitaxis

(A-B) *trpa-1* mutants in vertical (blue) and horizontal (assays) compared with N2 controls. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n.s. is not significant using Kruskall-Wallis followed by Dunn's

test with Bonferroni correction. Notches on boxplots represent 95% confidence intervals; mean values are indicated with a diamond.

Figure 5. PVDs and TRNs in dauer and adult worms

(A, C, and E) Confocal fluorescent micrographs of *mec-3p::RFP* and *ser-2prom-3::GFP* expression labelling the TRN and AWC neurons and PVD and OLL neurons, respectively. Brightfield images shown in **B, D, and F**. **(A-B)** Wildtype dauer worms; developing 3° processes of PVD neurons are indicated with a white arrow in inset. **(C-D)** Wildtype adult hermaphrodites; 4° process are indicated with white arrowhead in inset. **(E-F)** *mec-4(e1611)* gain of function dauers, in which TRNs are ablated. Scale bar 100µm, 10µm in insets.

Figure 6. Genetic ablation of PVDs but not TRNs affects gravitaxis

(A) *mec-3(e1338)* mutant worms have small TRN cell bodies that lack axons. **(B)** PVD neurons were ablated genetically, leaving TRNs intact. **(C-D)** Gravitaxis behavior of *mec-3(e1338)* dauers, dauers lacking TRNs only (*mec-4* gof), dauers lacking PVD neurons only (*ser2prom-3::deg-3*), and N2 dauer controls. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n.s. is not significant using Kruskall-Wallis followed by Dunn's test with Bonferroni correction. Notches on boxplots represent 95% confidence intervals; mean values are indicated with a diamond.

Figure 7. Adult worms negatively gravitax

(A-B) Adult worms demonstrate a strong negative gravitaxis preference similar to dauers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n.s. is not significant using Kruskall-Wallis followed by Dunn's test with Bonferroni correction. Notches on boxplots represent 95% confidence intervals; mean values are indicated with a diamond.

Methods

Worm rearing

C. elegans worms were grown and maintained on OP50 *E.coli* at room temperature (23°C) using standard methods[96]. Dauer formation was induced by overcrowding, which occurred 8-12 days after chunking onto seeded NGM plates. Adults used for gravitaxis assays and imaging were taken from synchronized populations 2 days after hatching. Crosses were performed as described previously[97], using fluorescence microscopy and/or PCR to confirm the outcome of each cross.

A list of strains is available in the Supplementary Material.

Dauer isolation

Dauer larvae were isolated from 1-5 starved NGM plates using 1% SDS and a 30% sucrose gradient as described previously [98,99]. Briefly, worms were rinsed and collected in M9, then pelleted and resuspended in 7mL 1% SDS in a 15 mL falcon tube. Worms were left rotating for 30 minutes in SDS to kill all non-dauer life stages. After 3-5 rinses with M9, worms were resuspended in 10 mL 30% sucrose solution and centrifuged for 5 minutes. Surviving dauers were collected at the interface of the water-sucrose gradient with a large bore glass Pasteur pipette and rinsed 3-5X in M9. Dauers were used immediately or after rotating in solution overnight.

Synchronization

Mixed stage worms were rinsed from confluent plates with M9 and collected in 1.5 mL tubes. Worms were then rinsed and pelleted. A 1 mL bleach solution containing 150 µL bleach and 50 µL 5N KOH was added and worms were lysed in this solution for 3 minutes, or until most corpses were dissolved. The embryo pellet was then rinsed 3-5X in M9 and plated onto OP50 NGM plates until adulthood (~2 days at 23°C).

Assay preparation

Gravitaxis assay chambers were created using 5 mL serological pipettes and 4% NGM agar as described previously [75]. To assemble each chamber, a heated blade was used to cut the tapered end of one pipette and the cotton plug was removed from the end of a second pipette. The two pipettes were then joined end-to-end by melting the two ends slightly over a Bunsen burner and fusing them with moderate pressure, ensuring that no gaps remained. The NGM media was then autoclaved and then taken up by each double pipette using a standard serological pipettor while the agar was still molten. Best results were achieved by orienting each pipette parallel with the benchtop as much as possible during the procedure and as the agar solidified. Pipettes were used within 24 hours of construction or stored at 4°C to be used within 48 hrs.

Once solidified, a heated 3mm Allen key was used to punch a hole through one wall of the pipette about 5mm below the fusion point (for reference, the cotton-plug end is the “top”). Each end of the pipette was removed using a heated blade and sealed with parafilm.

Gravitaxis assay

Worms were pelleted in M9 and were either extracted directly from the pellet or pipetted onto a square of parafilm. After cutting the tip to increase the bore, a pipette was used to aspirate 0.5-2 uL of the

concentrated worm pellet, letting in a small volume of air at the end to facilitate injection. Worms were injected into the agar at the opening created by the Allen key. This opening was then sealed with parafilm, and the chamber was immediately oriented vertically or horizontally. Unless indicated otherwise, all experiments were performed in a custom-manufactured Faraday cage. The effectiveness of the cage in blocking EMF was tested by placing a cell phone within the cage and calling it to determine if it received a signal. Worms were allowed to gravitax overnight and were scored the following day (typically 12-24 hrs after injection).

Pipettes were examined under a dissecting microscope and a marker was used to mark the location of each worm. Pipettes were scored immediately after removal from the assay location. Worms were only scored if they appeared alive, healthy, and were not swimming (chambers were only scored if a majority of worms were alive and crawling). Worms were not scored within 2.5 cm to either side of the injection point.

To tally the location of each worm, pipettes were divided into 7 sections on each side of the 5 cm injection site. Each section was 3.5 cm long (the same length as a 1 mL volume indicated on the pipettes). The number of marker points in each section was then recorded. Data collection was validated with blinded replicates.

Imaging

Fluorescent micrographs were taken using a Leica SP8 Resonant Scanning Confocal microscope at 10-40x. Worms were prepared for imaging by mounting on 2% agarose pads in 10mM levamisole. Brightness and contrast were adjusted and overlays added using ImageJ.

Quantification and Statistical Analysis

Data were collected in Excel and analyzed using Rstudio. Statistical tests, p values, sample sizes, and replicates are detailed in the results and figure legends.

References

1. Ross, M.D., Pote, K.G., Miller, A., Phillips, D.C., and Williams, R.J.P. (1984). Some properties of otoconia. Philos. Trans. R. Soc. Lond. B Biol. Sci. *304*, 445–452.

2. Bezares-Calderón, L.A., Berger, J., and Jékely, G. (2020). Diversity of cilia-based mechanosensory systems and their functions in marine animal behaviour. Philos. Trans. R. Soc. Lond. B. Biol. Sci. *375*, 20190376.

3. Lacquaniti, F., Bosco, G., Gravano, S., Indovina, I., La Scaleia, B., Maffei, V., and Zago, M. (2014). Multisensory Integration and Internal Models for Sensing Gravity Effects in Primates. BioMed Res. Int. *2014*, 1–10.

4. Häder, D.-P., and Hemmersbach, R. (2017). Gravitaxis in Euglena. In Euglena: Biochemistry, Cell and Molecular Biology Advances in Experimental Medicine and Biology., S. D. Schwartzbach and S. Shigeoka, eds. (Springer International Publishing), pp. 237–266.

5. Riddle, D.L., Blumenthal, T., Meyer, B.J., and Priess, J.R. (1997). C. elegans Responds to a Variety of Chemicals (Cold Spring Harbor Laboratory Press).

6. Ward, A., Liu, J., Feng, Z., and Xu, X.Z.S. (2008). Light-sensitive neurons and channels mediate phototaxis in C. elegans. Nat. Neurosci. *11*, 916–922.

7. Bargmann, C.I., Hartwieg, E., and Horvitz, H.R. (1993). Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell *74*, 515–527.

8. Ward, S. (1973). Chemotaxis by the Nematode Caenorhabditis elegans: Identification of Attractants and Analysis of the Response by Use of Mutants. Proc. Natl. Acad. Sci. U. S. A. *70*, 817–821.

9. Ramot, D., MacInnis, B.L., Lee, H.-C., and Goodman, M.B. (2008). Thermotaxis is a Robust Mechanism for Thermoregulation in C. elegans Nematodes. J. Neurosci. Off. J. Soc. Neurosci. *28*, 12546–12557.

10. Russell, J., Vidal-Gadea, A.G., Makay, A., Lanam, C., and Pierce-Shimomura, J.T. (2014). Humidity sensation requires both mechanosensory and thermosensory pathways in Caenorhabditis elegans. Proc. Natl. Acad. Sci. *111*, 8269–8274.

11. Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., Marletta, M.A., and Bargmann, C.I. (2004). Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Nature *430*, 317–322.

12. Bretscher, A.J., Busch, K.E., and Bono, M. de (2008). A carbon dioxide avoidance behavior is integrated with responses to ambient oxygen and food in Caenorhabditis elegans. Proc. Natl. Acad. Sci. *105*, 8044–8049.

13. Kodama-Namba, E., Fenk, L.A., Bretscher, A.J., Gross, E., Busch, K.E., and de Bono, M. (2013). Cross-Modulation of Homeostatic Responses to Temperature, Oxygen and Carbon Dioxide in C. elegans. PLoS Genet. *9*.

14. Frézal, L., and Félix, M.-A. (2015). C. elegans outside the Petri dish. eLife *4*.

15. Lee, H., Choi, M., Lee, D., Kim, H., Hwang, H., Kim, H., Park, S., Paik, Y., and Lee, J. (2012). Nictation, a dispersal behavior of the nematode Caenorhabditis elegans, is regulated by IL2 neurons. Nat. Neurosci. *15*, 107–112.

16. Okumura, E., Tanaka, R., and Yoshiga, T. (2013). Negative gravitactic behavior of Caenorhabditis japonica dauer larvae. J. Exp. Biol. *216*, 1470–1474.

17. Chen, W.-L., Ko, H., Chuang, H.-S., Raizen, D.M., and Bau, H.H. (2021). Caenorhabditis elegans exhibits positive gravitaxis. BMC Biol. *19*, 186.

18. Bostwick, M., Smith, E.L., Borba, C., Newman-Smith, E., Guleria, I., Kourakis, M.J., and Smith, W.C. (2020). Antagonistic Inhibitory Circuits Integrate Visual and Gravitactic Behaviors. Curr. Biol. *30*, 600-609.e2.

19. Bae, J.-E., Bang, S., Min, S., Lee, S.-H., Kwon, S.-H., Lee, Y., Lee, Y.-H., Chung, J., and Chae, K.-S. (2016). Positive geotactic behaviors induced by geomagnetic field in Drosophila. Mol. Brain *9*, 55.

20. Vidal-Gadea, A., Ward, K., Beron, C., Ghorashian, N., Gokce, S., Russell, J., Truong, N., Parikh, A., Gadea, O., Ben-Yakar, A., et al. (2015). Magnetosensitive neurons mediate geomagnetic orientation in Caenorhabditis elegans. eLife *4*.

21. Landler, L., Nimpf, S., Hochstoeger, T., Nordmann, G.C., Papadaki-Anastasopoulou, A., and Keays, D.A. (2018). Comment on "Magnetosensitive neurons..." eLife *7*.

22. Vidal-Gadea, A., Bainbridge, C., Clites, B., Palacios, B.E., Bakhtiari, L., Gordon, V., and Pierce-Shimomura, J. (2018). Response to comment on "Magnetosensitive neurons mediate geomagnetic orientation in Caenorhabditis elegans." eLife *7*, 1–12.

23. Gao, Y., Xu, D., Zhao, L., and Sun, Y. (2017). The DNA damage response of C. elegans affected by gravity sensing and radiosensitivity during the Shenzhou-8 spaceflight. Mutat. Res. Mol. Mech. Mutagen. *795*, 15–26.

24. Gao, Y., Xu, D., Zhao, L., Zhang, M., and Sun, Y. (2015). Effects of microgravity on DNA damage response in Caenorhabditis elegans during Shenzhou-8 spaceflight. Int. J. Radiat. Biol. *91*, 531–539.

25. Saldanha, J.N., Pandey, S., and Powell-Coffman, J.A. (2016). The effects of short-term hypergravity on Caenorhabditis elegans. Life Sci. Space Res. *10*, 38–46.

26. Xu, D., Gao, Y., Huang, L., and Sun, Y. (2014). Changes in miRNA expression profile of space-flown Caenorhabditis elegans during Shenzhou-8 mission. Life Sci. Space Res. *1*, 44–52.

27. Kalichamy, S.S., Lee, T.Y., Yoon, K., and Lee, J.I. (2016). Hypergravity hinders axonal development of motor neurons in Caenorhabditis elegans. PeerJ *4*, e2666.

28. Kim, N., Dempsey, C.M., Kuan, C.-J., Zoval, J.V., O'Rourke, E., Ruvkun, G., Madou, M.J., and Sze, J.Y. (2007). Gravity Force Transduced by the MEC-4/MEC-10 DEG/ENaC Channel Modulates DAF-16/FoxO Activity in Caenorhabditis elegans. Genetics *177*, 835–845.

29. Chalfie, M., and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of Caenorhabditis elegans. Dev. Biol. *82*, 358–370.

30. Montell, C. (2003). The venerable inveterate invertebrate TRP channels. Cell Calcium *33*, 409–417.

31. Chatzigeorgiou, M., Yoo, S., Watson, J.D., Lee, W.-H., Spencer, W.C., Kindt, K.S., Hwang, S.W., Miller, D.M., Treinin, M., Driscoll, M., et al. (2010). Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. Nat. Neurosci. *13*, 861–868.

32. Han, L., Wang, Y., Sangaletti, R., D'Urso, G., Lu, Y., Shaham, S., and Bianchi, L. (2013). Two novel DEG/ENaC channel subunits expressed in glia are needed for nose-touch sensitivity in Caenorhabditis elegans. J. Neurosci. Off. J. Soc. Neurosci. *33*, 936–949.

33. Kindt, K.S., Viswanath, V., Macpherson, L., Quast, K., Hu, H., Patapoutian, A., and Schafer, W.R. (2007). Caenorhabditis elegans TRPA-1 functions in mechanosensation. Nat. Neurosci. *10*, 568–577.

34. Venkatachalam, K., Luo, J., and Montell, C. (2014). Evolutionarily conserved, multitasking TRP channels: lessons from worms and flies. Handb. Exp. Pharmacol. *223*, 937–962.

35. Sun, Y., Liu, L., Ben-Shahar, Y., Jacobs, J.S., Eberl, D.F., and Welsh, M.J. (2009). TRPA channels distinguish gravity sensing from hearing in Johnston's organ. Proc. Natl. Acad. Sci. *106*, 13606–13611.

36. Wang, Y., Ezemaduka, A.N., Tang, Y., and Chang, Z. (2009). Understanding the mechanism of the dormant dauer formation of C. elegans: From genetics to biochemistry. IUBMB Life *61*, 607–612.

37. Guisnet, A., Maitra, M., Pradhan, S., and Hendricks, M. (2021). A three-dimensional habitat for C. elegans environmental enrichment. PLOS ONE *16*, e0245139.

38. Bainbridge, C., Schuler, A., and Vidal-Gadea, A.G. (2016). Method for the assessment of neuromuscular integrity and burrowing choice in vermiform animals. J. Neurosci. Methods *264*, 40–46.

39. Srinivasan, J., Reuss, S.H. von, Bose, N., Zaslaver, A., Mahanti, P., Ho, M.C., O'Doherty, O.G., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2012). A Modular Library of Small Molecule Signals Regulates Social Behaviors in Caenorhabditis elegans. PLOS Biol. *10*, e1001237.

40. Larsch, J., Flavell, S.W., Liu, Q., Gordus, A., Albrecht, D.R., and Bargmann, C.I. (2015). A circuit for gradient climbing in C. elegans chemotaxis. Cell Rep. *12*, 1748–1760.

41. Chen, X., and Chalfie, M. (2014). Modulation of C. elegans Touch Sensitivity Is Integrated at Multiple Levels. J. Neurosci. *34*, 6522–6536.

42. Sengupta, P. (2013). The belly rules the nose: feeding state-dependent modulation of peripheral chemosensory responses. Curr. Opin. Neurobiol. *23*, 68–75.

43. Bender, J.A., and Frye, M.A. (2009). Invertebrate solutions for sensing gravity. Curr. Biol. *19*, R186–R190.

44. Gu, G., Caldwell, G.A., and Chalfie, M. (1996). Genetic interactions affecting touch sensitivity in Caenorhabditis elegans. Proc. Natl. Acad. Sci. *93*, 6577–6582.

45. Maguire, S.M., Clark, C.M., Nunnari, J., Pirri, J.K., and Alkema, M.J. (2011). The C. elegans Touch Response Facilitates Escape from Predacious Fungi. Curr. Biol. *21*, 1326–1330.

46. Brown, A.L., Liao, Z., and Goodman, M.B. (2008). MEC-2 and MEC-6 in the Caenorhabditis elegans Sensory Mechanotransduction Complex: Auxiliary Subunits that Enable Channel Activity. J. Gen. Physiol. *131*, 605–616.

47. Huber, T.B., Schermer, B., Müller, R.U., Höhne, M., Bartram, M., Calixto, A., Hagmann, H., Reinhardt, C., Koos, F., Kunzelmann, K., et al. (2006). Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. Proc. Natl. Acad. Sci. U. S. A. *103*, 17079–17086.

48. Zhang, Y., and Chalfie, M. (2002). MTD-1, a touch-cell-specific membrane protein with a subtle effect on touch sensitivity. Mech. Dev. *119*, 3–7.

49. Chalfie, M., and Thomson, J.N. (1982). Structural and functional diversity in the neuronal microtubules of Caenorhabditis elegans. J. Cell Biol. *93*, 15–23.

50. Bounoutas, A., O'Hagan, R., and Chalfie, M. (2009). The multipurpose 15-protofilament microtubules in C. elegans have specific roles in mechanosensation. Curr. Biol. CB *19*, 1362–1367.

51. Krieg, M., Dunn, A.R., and Goodman, M.B. (2015). Mechanical systems biology of C. elegans touch sensation. BioEssays *37*, 335–344.

52. Bounoutas, A., Kratz, J., Emtage, L., Ma, C., Nguyen, K.C., and Chalfie, M. (2011). Microtubule depolymerization in Caenorhabditis elegans touch receptor neurons reduces gene expression through a p38 MAPK pathway. Proc. Natl. Acad. Sci. *108*, 3982–3987.

53. Lockhead, D., Schwarz, E.M., O'Hagan, R., Bellotti, S., Krieg, M., Barr, M.M., Dunn, A.R., Sternberg, P.W., and Goodman, M.B. (2016). The tubulin repertoire of Caenorhabditis elegans sensory neurons and its context-dependent role in process outgrowth. Mol. Biol. Cell *27*, 3717–3728.

54. Chen, C.-H., Lee, A., Liao, C.-P., Liu, Y.-W., and Pan, C.-L. (2014). RHGF-1/PDZ-RhoGEF and retrograde DLK-1 signaling drive neuronal remodeling on microtubule disassembly. Proc. Natl. Acad. Sci. U. S. A. *111*, 16568–16573.

55. Akella, J.S., Wloga, D., Kim, J., Starostina, N.G., Lyons-Abbott, S., Morrissette, N.S., Dougan, S.T., Kipreos, E.T., and Gaertig, J. (2010). MEC-17 is an α-tubulin acetyltransferase. Nature *467*, 218–222.

56. Shida, T., Cueva, J.G., Xu, Z., Goodman, M.B., and Nachury, M.V. (2010). The major α-tubulin K40 acetyltransferase αTAT1 promotes rapid ciliogenesis and efficient mechanosensation. Proc. Natl. Acad. Sci. U. S. A. *107*, 21517–21522.

57. Topalidou, I., Keller, C., Kalebic, N., Nguyen, K.C., Somhegyi, H., Politi, K.A., Heppenstall, P., Hall, D.H., and Chalfie, M. (2012). Enzymatic and non-enzymatic activities of the tubulin acetyltransferase MEC-17 are required for microtubule organization and mechanosensation in C. elegans. Curr. Biol. *22*, 1057–1065.

58. Neumann, B., and Hilliard, M.A. (2014). Loss of MEC-17 leads to microtubule instability and axonal degeneration. Cell Rep. *6*, 93–103.

59. Carr, M., Leadbeater, B.S.C., Hassan, R., Nelson, M., and Baldauf, S.L. (2008). Molecular phylogeny of choanoflagellates, the sister group to Metazoa. Proc. Natl. Acad. Sci. *105*, 16641–16646.

60. Kamakura, T., Ishida, Y., Nakamura, Y., Yamada, T., Kitahara, T., Takimoto, Y., Horii, A., Uno, A., Imai, T., Okazaki, S., et al. (2013). Functional expression of TRPV1 and TRPA1 in rat vestibular ganglia. Neurosci. Lett. *552*, 92–97.

61. Yemini, E., Jucikas, T., Grundy, L.J., Brown, A.E.X., and Schafer, W.R. (2013). A database of Caenorhabditis elegans behavioral phenotypes. Nat. Methods *10*, 877–879.

62. Taylor, S.R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M.B., Xu, C., Varol, E., Oikonomou, P., Glenwinkel, L., McWhirter, R., et al. (2020). Molecular topography of an entire nervous system (Neuroscience).

63. Albeg, A., Smith, C.J., Chatzigeorgiou, M., Feitelson, D.G., Hall, D.H., Schafer, W.R., Miller, D.M., and Treinin, M. (2011). C. elegans multi-dendritic sensory neurons: Morphology and function. Mol. Cell. Neurosci. *46*, 308–317.

64. Smith, C.J., O'Brien, T., Chatzigeorgiou, M., Spencer, W.C., Feingold-Link, E., Husson, S.J., Hori, S., Mitani, S., Gottschalk, A., Schafer, W.R., et al. (2013). Sensory Neuron Fates Are Distinguished by a Transcriptional Switch that Regulates Dendrite Branch Stabilization. Neuron *79*, 266–280.

65. Wang, L., and Way, J.C. (1996). Activation of the mec-3 promoter in two classes of stereotyped lineages in Caenorhabditis elegans. Mech. Dev. *56*, 165–181.

66. Smith, C.J., Watson, J.D., Spencer, W.C., O'Brien, T., Cha, B., Albeg, A., Treinin, M., and Miller, D.M. (2010). Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in C. elegans. Dev. Biol. *345*, 18–33.

67. Tsalik, E.L., Niacaris, T., Wenick, A.S., Pau, K., Avery, L., and Hobert, O. (2003). LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. Dev. Biol. *263*, 81–102.

68. Tao, L., Porto, D., Li, Z., Fechner, S., Lee, S.A., Goodman, M.B., Xu, X.Z.S., Lu, H., and Shen, K. (2019). Parallel Processing of Two Mechanosensory Modalities by a Single Neuron in C. elegans. Dev. Cell, S1534580719308524.

69. Richardson, C.E., Yee, C., and Shen, K. (2019). A hormone receptor pathway cell-autonomously delays neuron morphological aging by suppressing endocytosis. PLoS Biol. *17*, e3000452.

70. Sundararajan, L., Stern, J., and Miller, D.M. (2019). Mechanisms that regulate morphogenesis of a highly branched neuron in C. elegans. Dev. Biol. *451*, 53–67.

71. Metaxakis, A., Petratou, D., and Tavernarakis, N. (2018). Multimodal sensory processing in Caenorhabditis elegans. Open Biol. *8*, 180049.

72. Félix, M.-A., and Braendle, C. (2010). The natural history of Caenorhabditis elegans. Curr. Biol. *20*, R965–R969.

73. de Bono, M., and Bargmann, C.I. (1998). Natural Variation in a Neuropeptide Y Receptor Homolog Modifies Social Behavior and Food Response in C. elegans. Cell *94*, 679–689.

74. Large, E.E., Padmanabhan, R., Watkins, K.L., Campbell, R.F., Xu, W., and McGrath, P.T. (2017). Modeling of a negative feedback mechanism explains antagonistic pleiotropy in reproduction in domesticated Caenorhabditis elegans strains. PLoS Genet. *13*, e1006769.

75. Ackley, C., Washiashi, L., Krishnamurthy, R., and Rothman, J.H. (2022). Large-Scale Gravitaxis Assay of Caenorhabditis Dauer Larvae. JoVE J. Vis. Exp., e64062.

76. Hammarlund, M., Hobert, O., Miller, D.M., and Sestan, N. (2018). The CeNGEN Project: The Complete Gene Expression Map of an Entire Nervous System. Neuron *99*, 430–433.

77. Corey, D.P., and Holt, J.R. (2016). Are TMCs the Mechanotransduction Channels of Vertebrate Hair Cells? J. Neurosci. Off. J. Soc. Neurosci. *36*, 10921–10926.

78. Nist-Lund, C.A., Pan, B., Patterson, A., Asai, Y., Chen, T., Zhou, W., Zhu, H., Romero, S., Resnik, J., Polley, D.B., et al. (2019). Improved TMC1 gene therapy restores hearing and balance in mice with genetic inner ear disorders. Nat. Commun. *10*, 236.

79. Chatzigeorgiou, M., Bang, S., Hwang, S.W., and Schafer, W.R. (2013). tmc-1 encodes a sodium-sensitive channel required for salt chemosensation in C. elegans. Nature *494*, 95–99.

80. Honda, Y., Higashibata, A., Matsunaga, Y., Yonezawa, Y., Kawano, T., Higashitani, A., Kuriyama, K., Shimazu, T., Tanaka, M., Szewczyk, N.J., et al. (2012). Genes down-regulated in spaceflight are involved in the control of longevity in *Caenorhabditis elegans*. Sci. Rep. *2*, 487.

81. Selch, F., Higashibata, A., Imamizo-Sato, M., Higashitani, A., Ishioka, N., Szewczyk, N.J., and Conley, C.A. (2008). Genomic response of the nematode Caenorhabditis elegans to spaceflight. Adv. Space Res. Off. J. Comm. Space Res. COSPAR *41*, 807–815.

82. Zhao, L., Rui, Q., and Wang, D. (2017). Molecular basis for oxidative stress induced by simulated microgravity in nematode Caenorhabditis elegans. Sci. Total Environ. *607–608*, 1381–1390.

83. Qiao, L., Luo, S., Liu, Y., Li, X., Wang, G., and Huang, Z. (2013). Reproductive and Locomotory Capacities of Caenorhabditis elegans Were Not Affected by Simulated Variable Gravities and Spaceflight During the Shenzhou-8 Mission. Astrobiology *13*, 617–625.

84. Honda, Y., Honda, S., Narici, M., and Szewczyk, N.J. (2014). Spaceflight and Ageing: Reflecting on Caenorhabditis elegans in Space. Gerontology *60*, 138–142.

85. Fritzsch, B., Kopecky, B.J., and Duncan, J.S. (2014). Chapter 12 - Development of the Mammalian 'Vestibular' System: Evolution of Form to Detect Angular and Gravity Acceleration. In Development of Auditory and Vestibular Systems, R. Romand and I. Varela-Nieto, eds. (Academic Press), pp. 339–367.

86. Lipovsek, M., and Wingate, R.J. (2018). Conserved and divergent development of brainstem vestibular and auditory nuclei. eLife *7*, e40232.

87. Scheffer, D.I., Shen, J., Corey, D.P., and Chen, Z.-Y. (2015). Gene Expression by Mouse Inner Ear Hair Cells during Development. J. Neurosci. *35*, 6366–6380.

88. Corey, D.P., García-Añoveros, J., Holt, J.R., Kwan, K.Y., Lin, S.-Y., Vollrath, M.A., Amalfitano, A., Cheung, E.L.-M., Derfler, B.H., Duggan, A., et al. (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature *432*, 723–730.

89. Iliff, A.J., Wang, C., Ronan, E.A., Hake, A.E., Guo, Y., Li, X., Zhang, X., Zheng, M., Liu, J., Grosh, K., et al. (2021). The nematode C. elegans senses airborne sound. Neuron *109*, 3633-3646.e7.

90. Altun, Z. Individual Neuron List. WormAtlas. https://www.wormatlas.org/neurons/Individual%20Neurons/Neuronframeset.html.

91. Emtage, L., Gu, G., Hartwieg, E., and Chalfie, M. (2004). Extracellular Proteins Organize the Mechanosensory Channel Complex in C. elegans Touch Receptor Neurons. Neuron *44*, 795–807.

92. Cueva, J.G., Mulholland, A., and Goodman, M.B. (2007). Nanoscale Organization of the MEC-4 DEG/ENaC Sensory Mechanotransduction Channel in Caenorhabditis elegans Touch Receptor Neurons. J. Neurosci. *27*, 14089–14098.

93. Goodman, M.B., and Schwarz, E.M. (2003). Transducing touch in Caenorhabditis elegans. Annu. Rev. Physiol. *65*, 429–452.

94. Chen, X., Cuadros, M.D., and Chalfie, M. (2015). Identification of Nonviable Genes Affecting Touch Sensitivity in Caenorhabditis elegans Using Neuronally Enhanced Feeding RNA Interference. G3 GenesGenomesGenetics *5*, 467–475.

95. Tavernarakis and, N., and Driscoll, M. (1997). Molecular Modeling of Mechanotransduction in the Nematode Caenorhabditis Elegans. Annu. Rev. Physiol. *59*, 659–689.

96. Stiernagle, T. (2006). Maintenance of C. elegans. WormBook.

97. Fay, D. (2006). Genetic mapping and manipulation: Chapter 1-Introduction and basics. WormBook.

98. Karp, X. (2018). Working with dauer larvae. WormBook, 1–19.

99. Ow, M.C., and Hall, S.E. (2015). A Method for Obtaining Large Populations of Synchronized Caenorhabditis elegans Dauer Larvae. In Methods in Molecular Biology (Clifton, N.J.), pp. 209–219.

Figure 1. Behavioral model, wiring diagram, and gravitaxis assay design

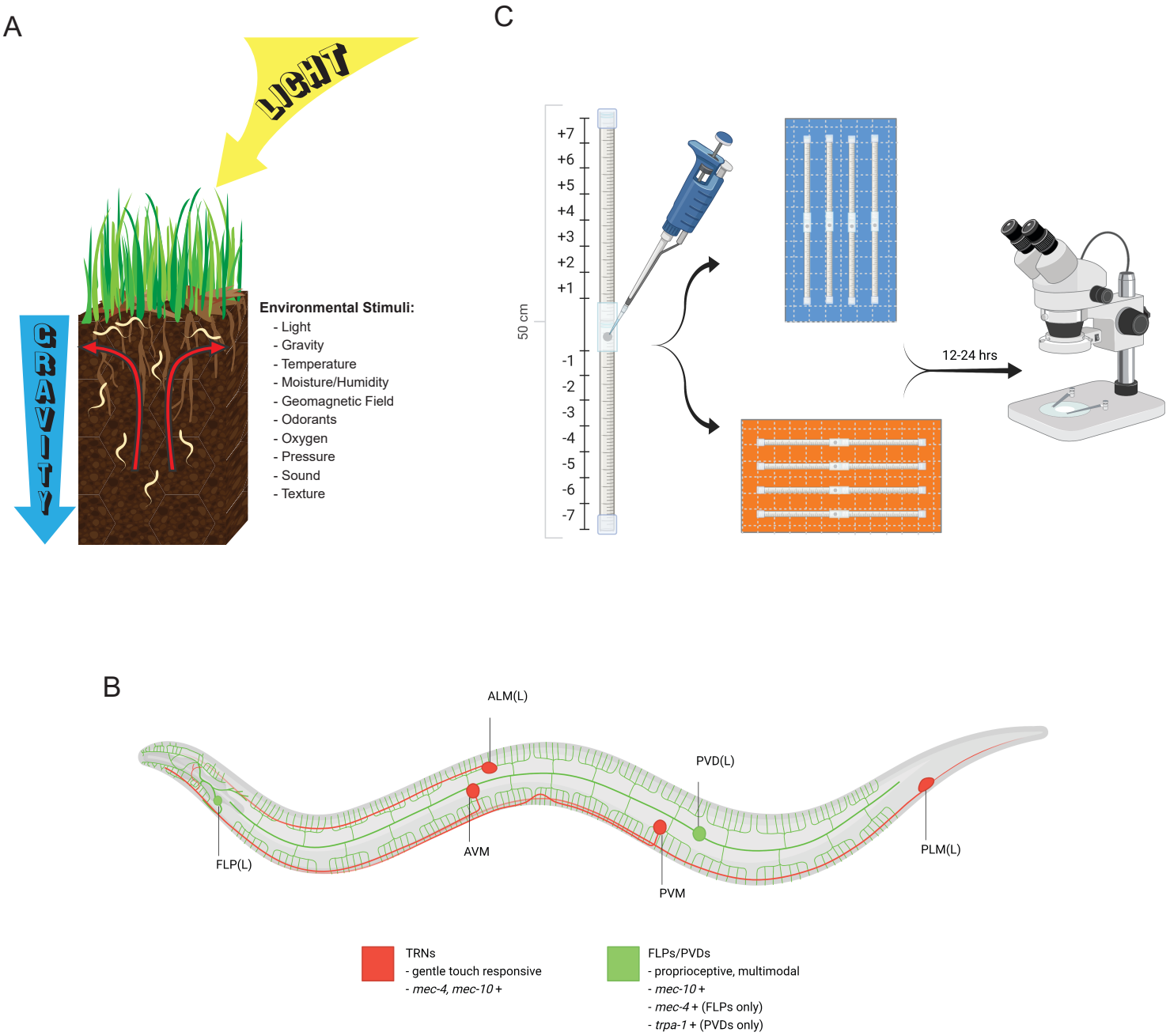


Figure 2. *C. elegans* negatively gravitax under different environmental conditions

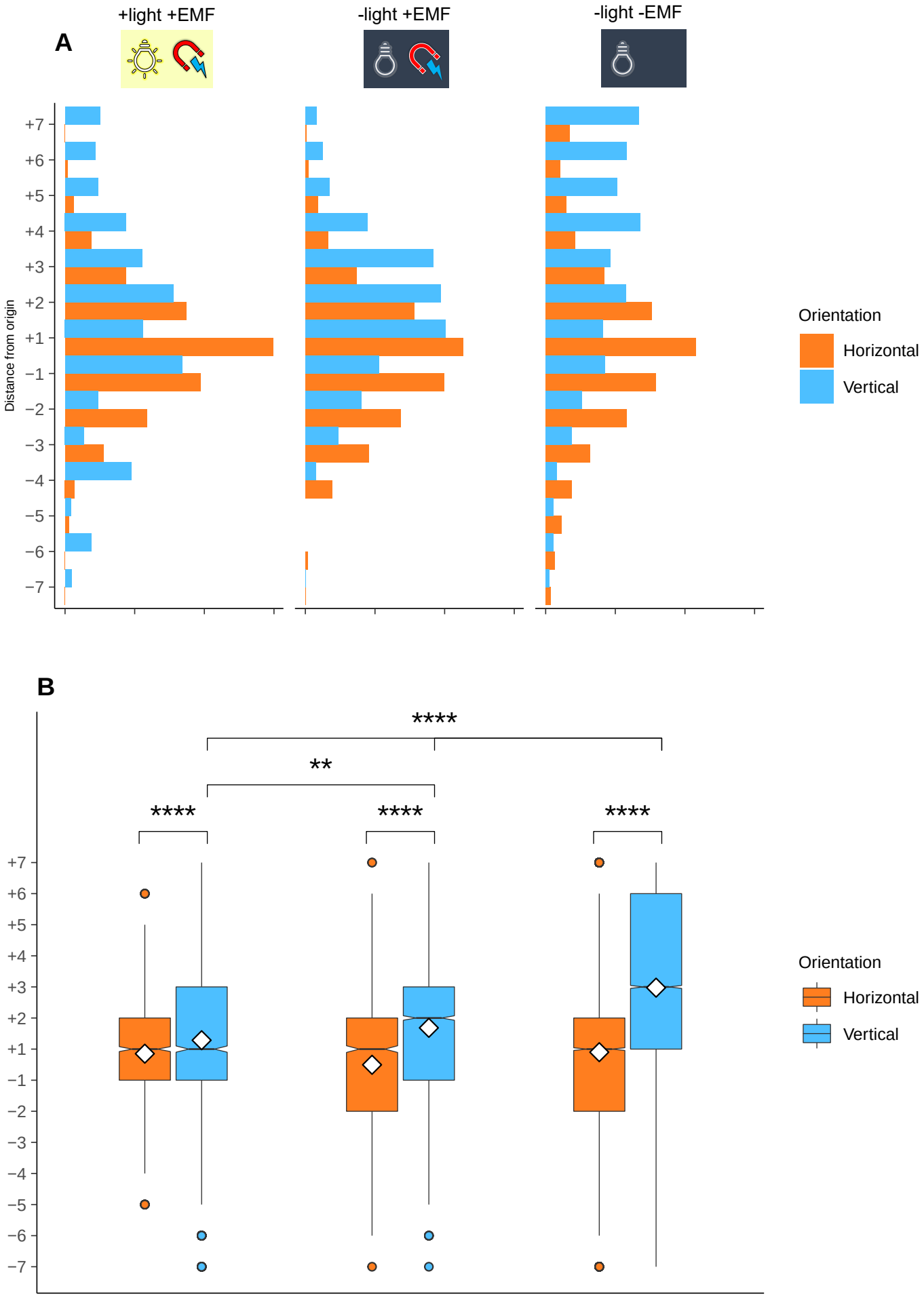
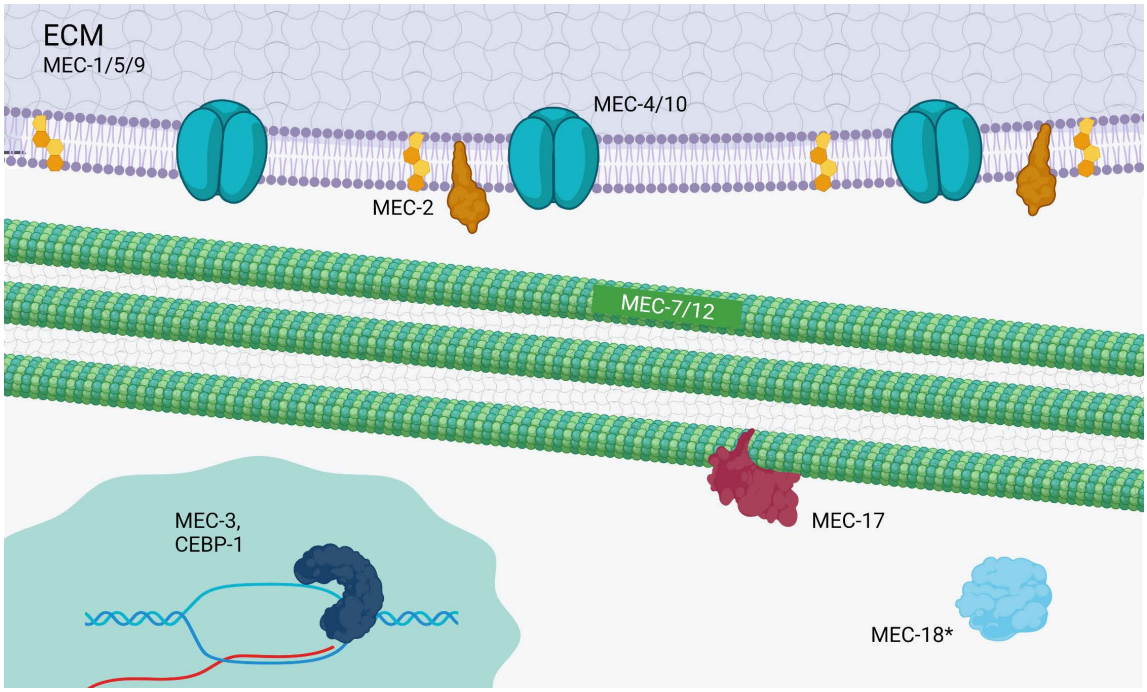


Figure 3. MEC-7/12 microtubule subunits, but not several other components in the gentle touch mechanosensory system, are necessary for gravitaxis

A



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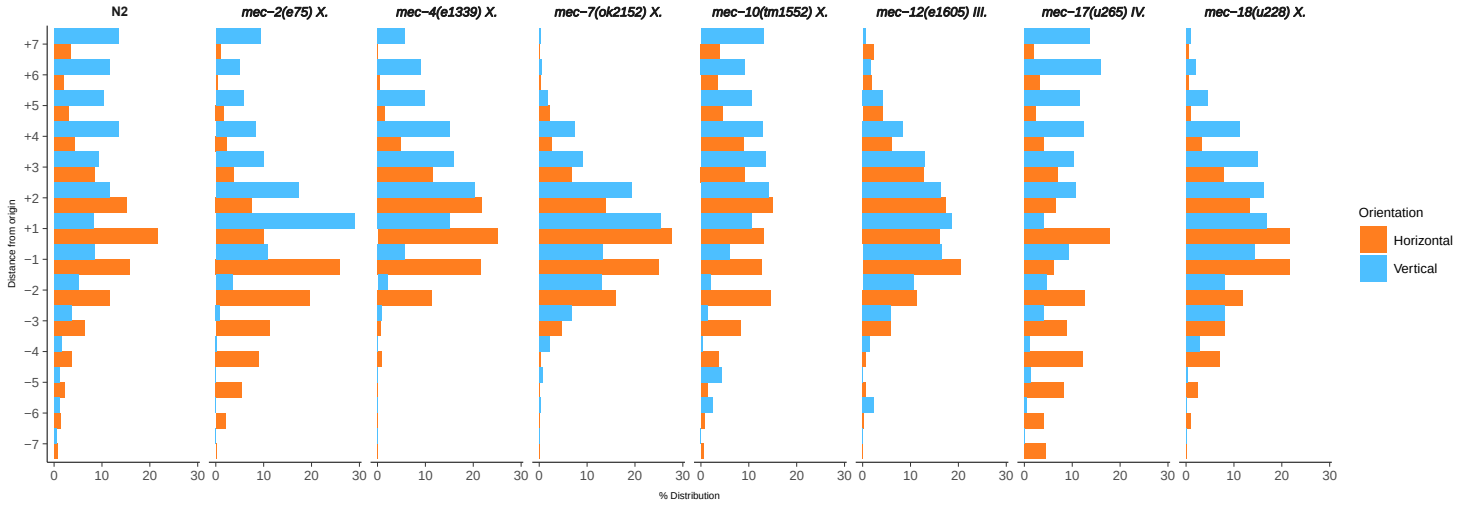

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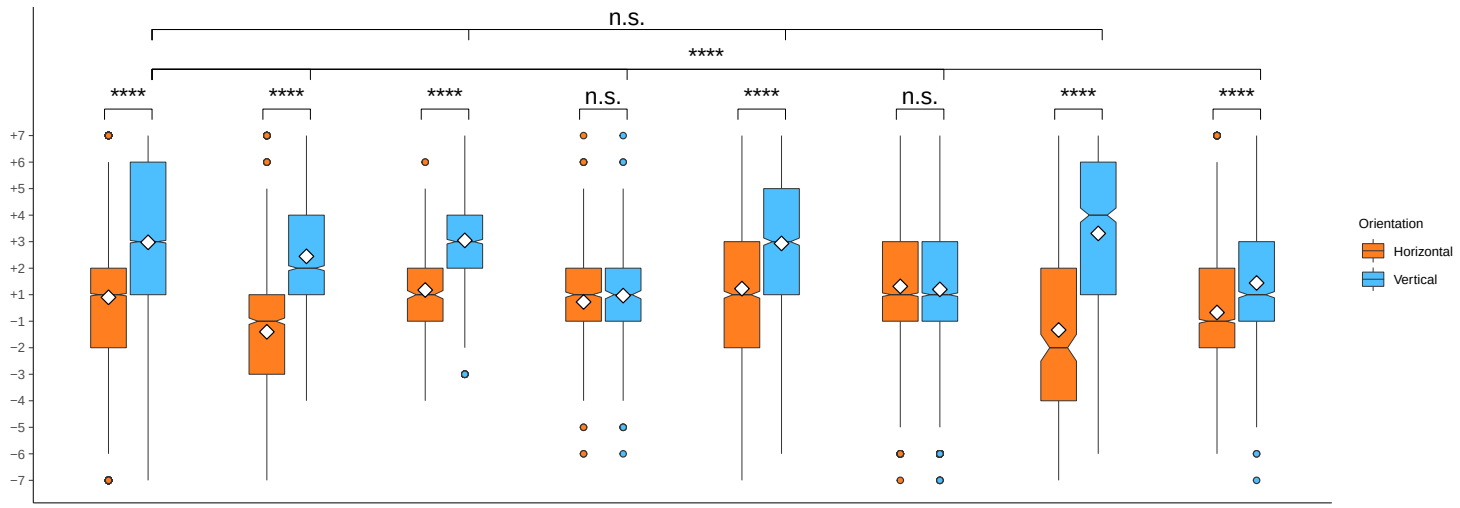


Figure 4. TRPA-1 channels are required for gravitaxis

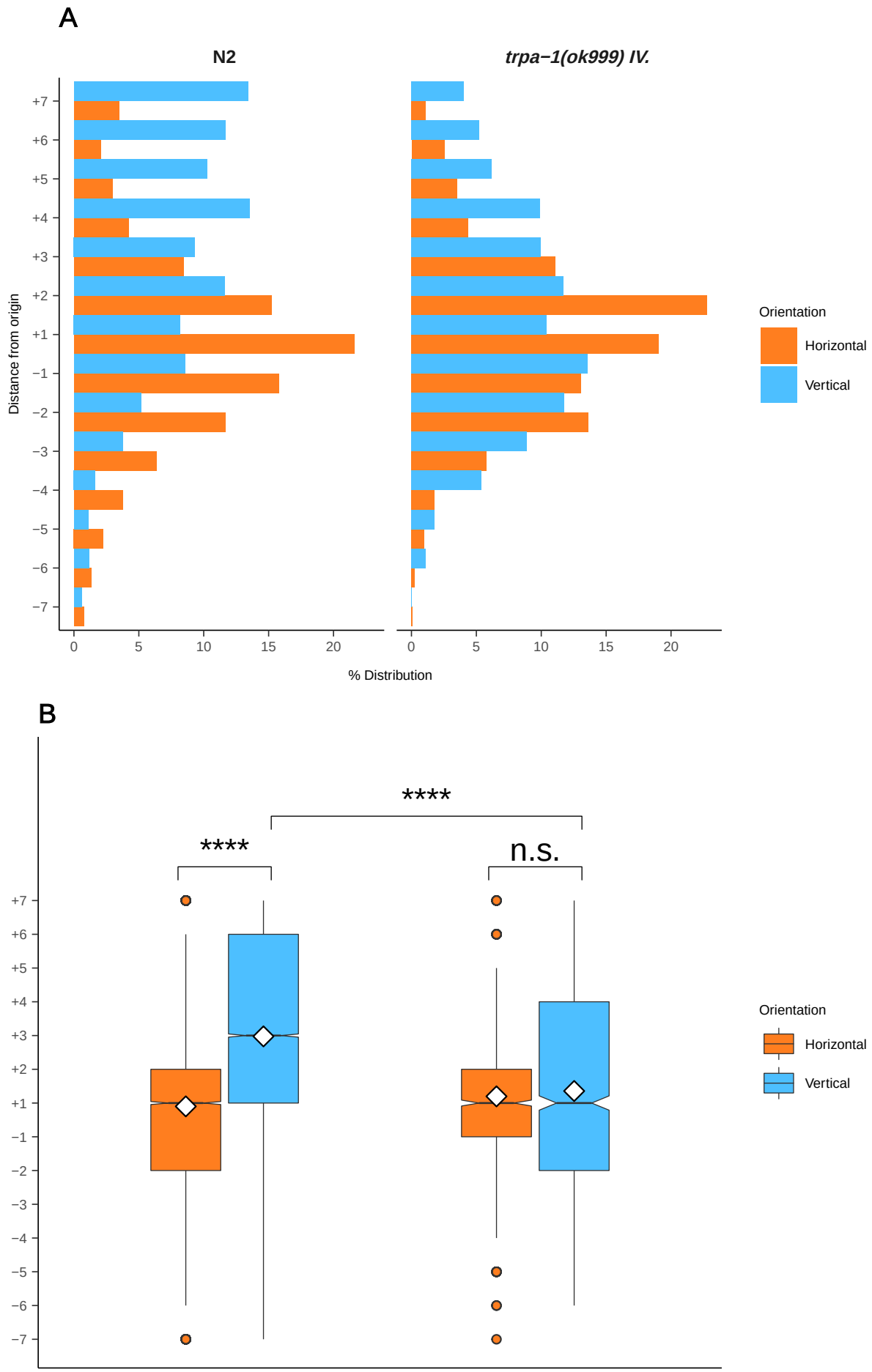


Figure 5. PVDs and TRNs in dauer and adult worms

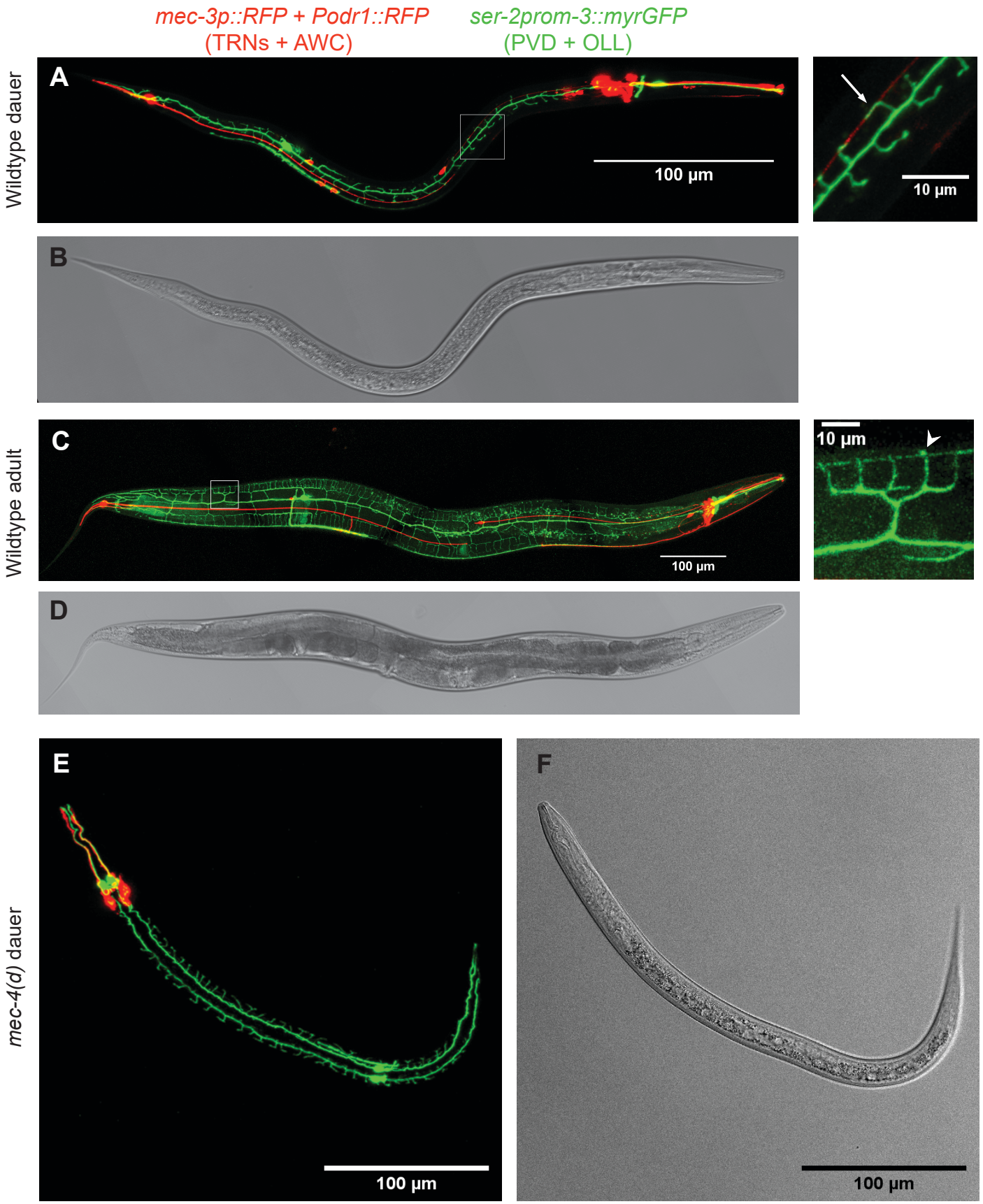


Figure 6. Genetic ablation of PVDs but not TRNs affects gravitaxis

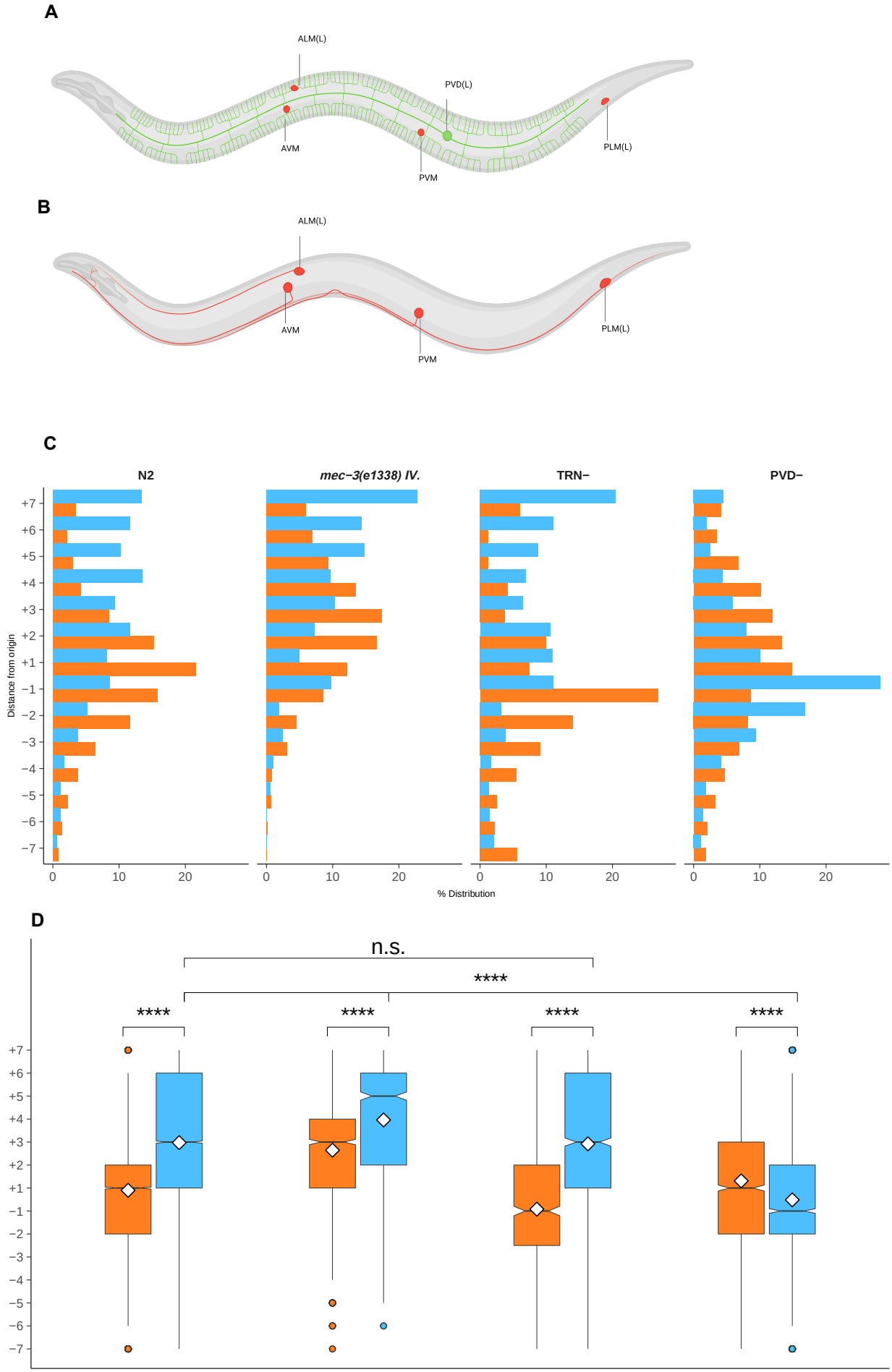
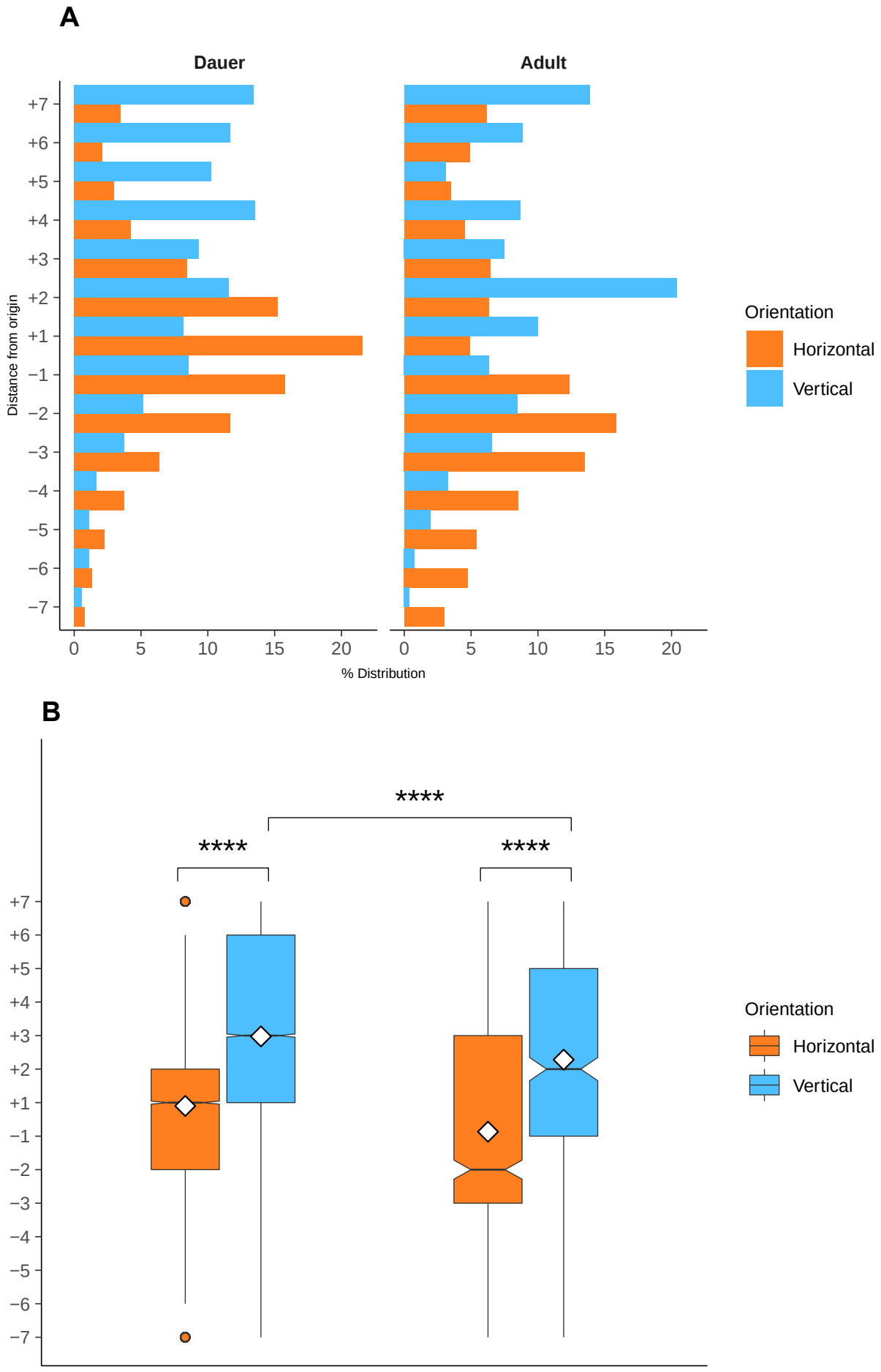
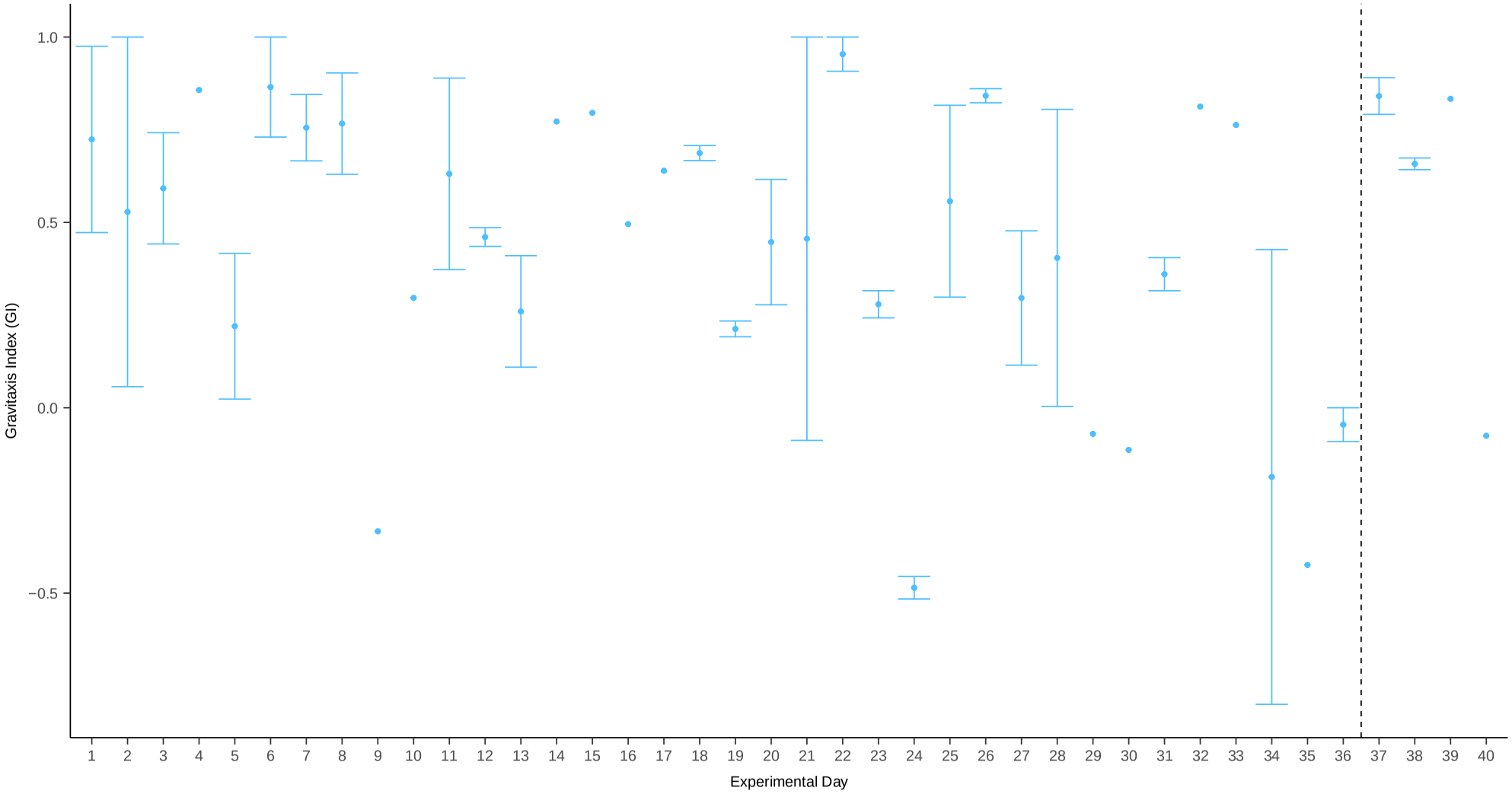


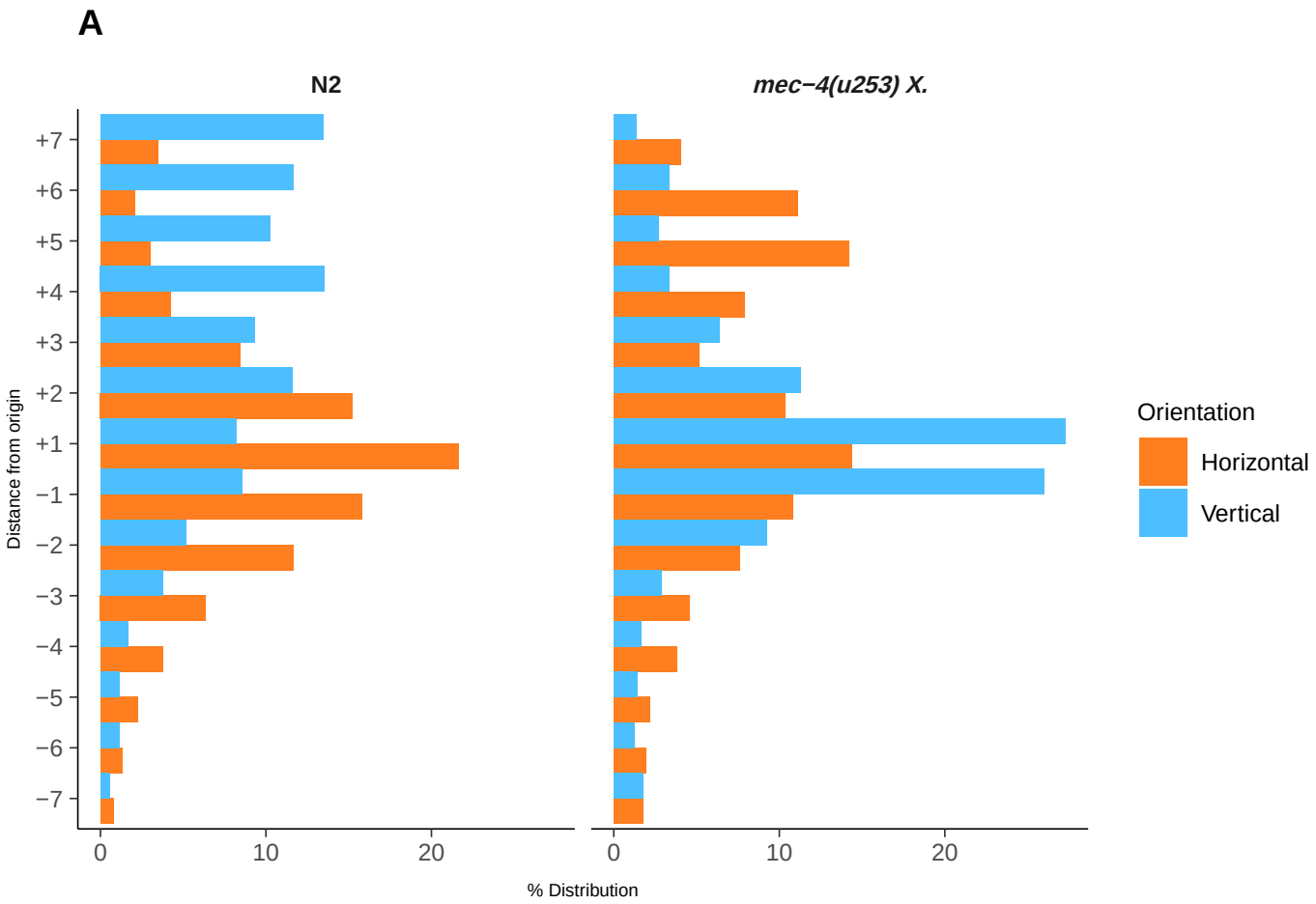
Figure 7. Adult worms negatively gravitax

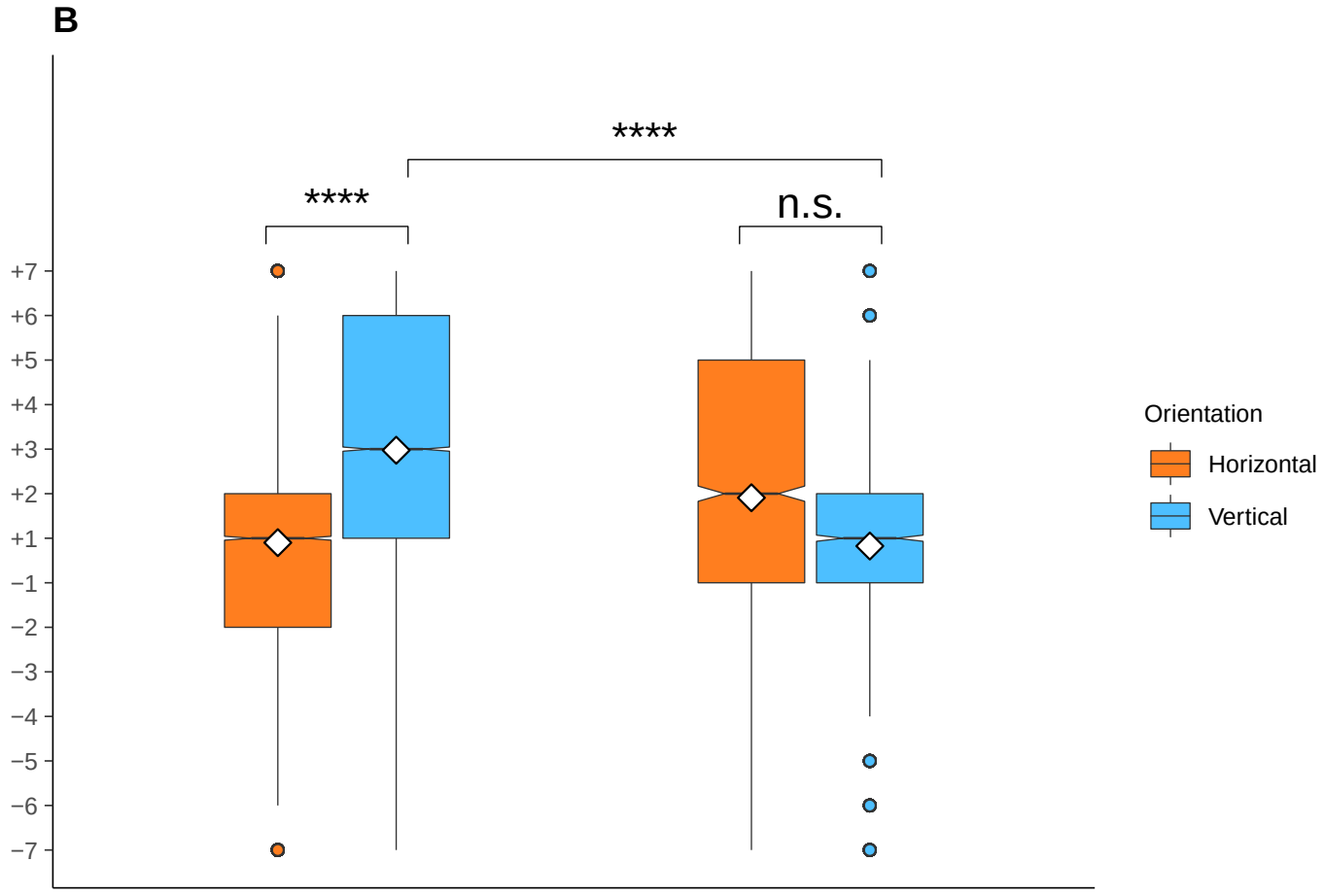
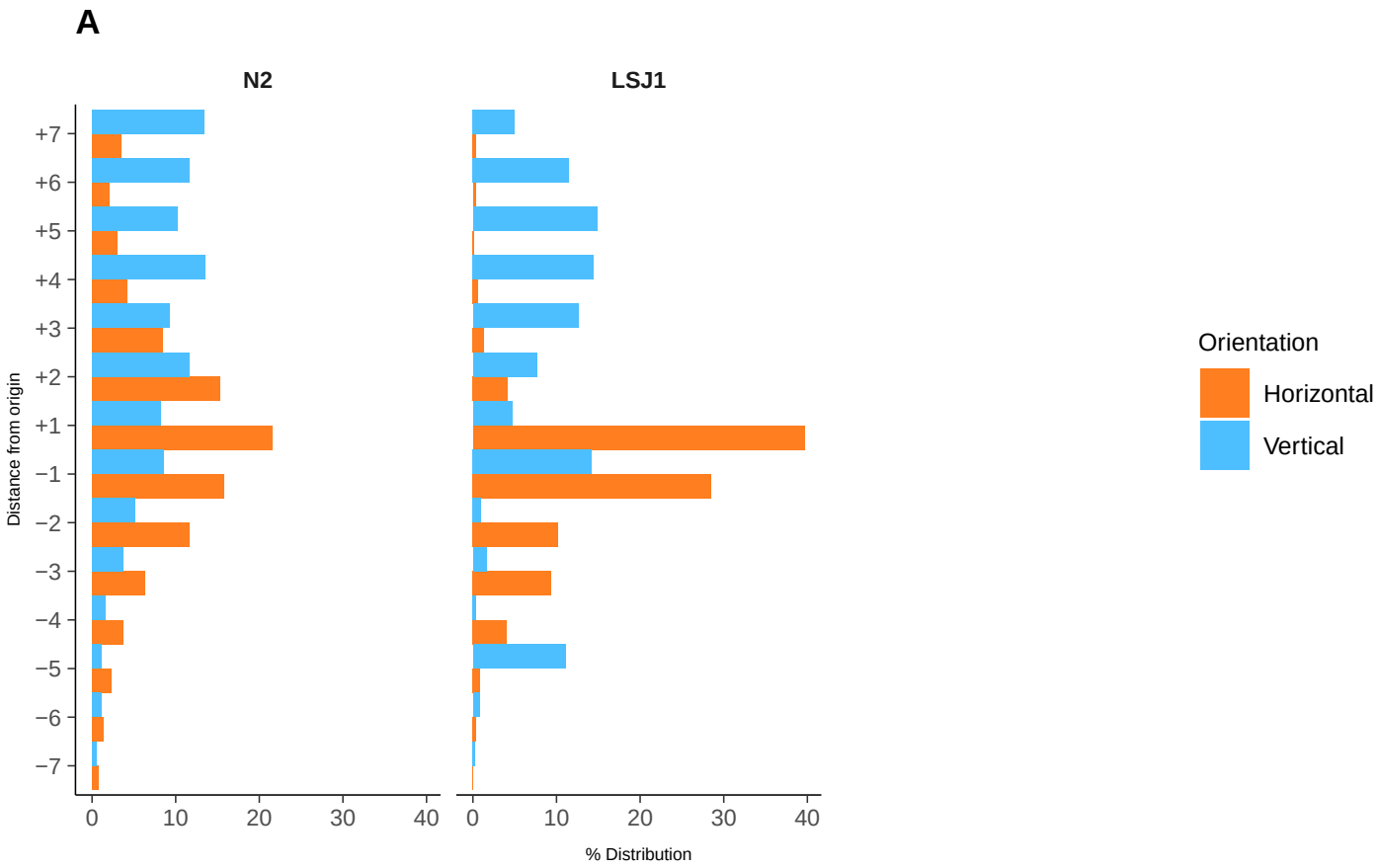
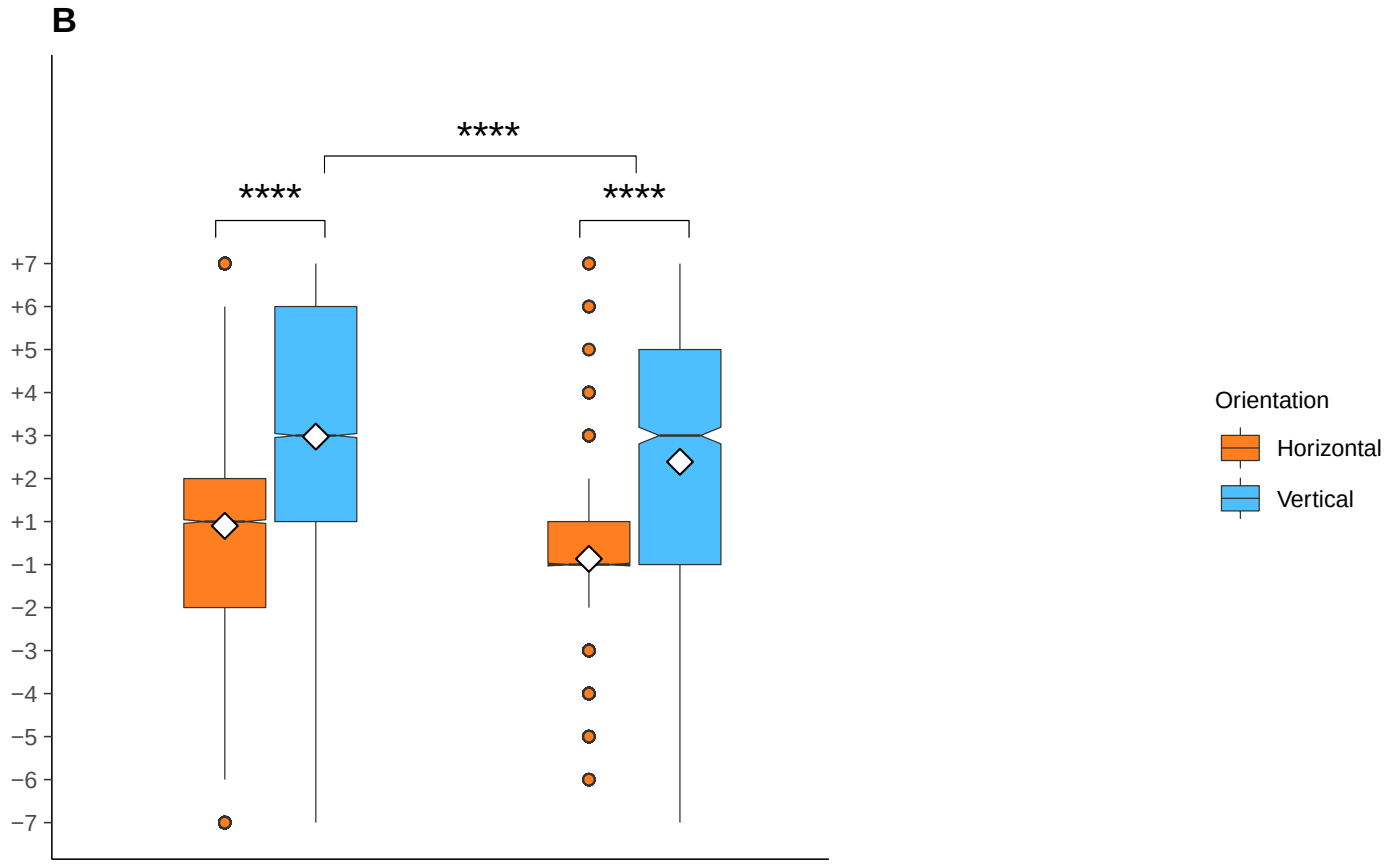


S1. Faraday cage integrity influences gravitactic behavior



S2. *mec-4* null mutants do not gravitax





S3. *C. elegans* worms maintained in liquid media for several generations also negatively gravitax