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RESEARCH ARTICLE

Vibration sensitivity found in *Caenorhabditis elegans*

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ABSTRACT

Mechanical sensing is important for all organisms, but is the least understood of the senses. As mechanical stimuli come in diverse forms, organisms often have sensors or sensory systems that specialise in a form of mechanical stimuli, such as touch or vibration. Here, we tested the hypothesis that the nematode worm *Caenorhabditis elegans* exhibits a behavioural response to vibration that is distinct from its responses to touch. We show that wild-type strain worms respond to sustained low-frequency vibration in a manner distinct from the known responses to non-localised mechanical stimuli. Furthermore, the behavioural responses of mutant strains suggest different roles for ciliated versus non-ciliated neurons in mediating the response. Although further study is required to identify the vibration-sensing pathway, our data support that *C. elegans* can sense substrate-borne vibrations using cells distinct from those used in gentle touch.

KEY WORDS: Biotremology, Mechanosensation, Nematode, Tactile

INTRODUCTION

Mechanical stimuli encode important information for both biological and technological use (Hill, 2008; Tiwana et al., 2012). There are many forms of mechanical stimuli, from localised touch, to texture and vibration. Animals often have specific sensory systems or sensors that specialise in detecting a particular form of mechanical stimulus. For example in mammalian skin, there are multiple sub-types of mechanical sensors that respond to many aspects of touch, including one for vibration (Abraira and Ginty, 2013). Of course, mammals also possess ears that act as specialised organs to respond to vibrational stimuli, whether detected from the air or transferred to the ear via bone conduction (Hill, 2008). Unlike other mechanical senses, the vibration sense provides high-resolution temporal information that can be used to determine the identity, status or location of a mechanical stimulus source (Mortimer, 2017), providing richer information from fewer sensors. As such, there are many applications of bioinspired vibration sense technologies, particularly in robotics (Kim et al., 2013; Tiwana et al., 2012), but also within structural health monitoring (Carden and Fanning, 2004).

This study tested the hypothesis that the well-studied nematode worm *Caenorhabditis elegans* exhibits a vibration sense distinct from its known touch senses (Kaplan and Horvitz, 1993; Sawin et al., 2000; Way and Chalfie, 1989; Wicks et al., 1996) through studying behavioural responses of worms to vibrational stimuli. There are more than 30 mechanosensory cells in *C. elegans* hermaphrodites (Goodman, 2006; Li et al., 2011), including ciliated and non-ciliated neurons both along the body and at the worm's head (Perkins et al., 1986; Tsalik et al., 2003; Ward et al., 1975; Ware et al., 1975). Previous studies have shown that worms respond to non-localised mechanical stimuli (e.g. substrate texture, short-term high-frequency vibration pulses and plate tap) by increasing the incidence of reversal behaviour and slowing down (Sawin et al., 2000; Sugi et al., 2016; Wicks et al., 1996). The response is identical following localised touch to the head, known as the touch response (Chalfie and Sulston, 1981; Wicks et al., 1996). Here, we presented different forms of vibrational stimuli to the worms, using long-term low-frequency vibrations, which are most likely to be encountered in the worm's environment as a result of the movement of potential predators (Catania, 2008).

As a model organism, *C. elegans* provides a powerful tool for exploring the genetic basis underlying a particular sense and probing the cellular-level mechanism of a response. We therefore also investigated the responses of two mutant strains deficient in two different broad groups of mechanosensory neurons. Our aim was to narrow down which types of neurons may be involved in vibration sensitivity, as preliminary evidence to aid future research on the topic.

The first mutant, *mec-3*(*e1338*) (strain CB1338), was deficient in eight mechanosensory non-ciliated neurons (AVM, 2 ALM, 2 PVD, PVM and 2 PLM) and two ciliated neurons (2 FLP) (Way et al., 1992). In the *mec-3* mutant, non-ciliated mechanosensory neurons do not differentiate properly (Tsalik et al., 2003; Way and Chalfie, 1988), so this strain has no touch response (Way and Chalfie, 1989).

The second mutant was deficient in two genes, *daf-19*(*m86*) and *daf-12*(*sa204*) (strain JT6924). The double mutant was chosen as *daf-12* supresses dauer formation, which allows mutants with the *daf-19*(*m86*) mutation to reach the adult life stage (Senti and Swoboda, 2008). The *daf-12* mutants are defective in developmental pathways (Antebi et al., 2000). The *daf-19* locus encodes four gene products in *C. elegans* (Craig et al., 2013). DAF-19C controls ciliogenesis, so *daf-19* mutants show no signs of cilia (Perkins et al., 1986; Swoboda et al., 2000). Thus, the 22 ciliated neurons involved in mechanosensation are defective (including 2 FLP), but mutants still show a response to head touch (Perkins et al., 1986). DAF-19A and B are expressed in non-ciliated neurons (Saito et al., 2013) and hypodermis and body wall muscle (Craig et al., 2013). Mutants defective in these gene products show abnormal dwelling and roaming behaviour (Senti and Swoboda, 2008), as a result of its role in synaptic protein maintenance (De Stasio et al., 2018). *daf-19* also influences other signalling pathways, such as the innate immune response (Xie et al., 2013).

Combined, these two mutants provide a robust starting point for further studies making use of mutant strains of *C. elegans*, including narrowing down the combinations of neurons involved in the vibration-specific sensory pathway.

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MATERIALS AND METHODS

Caenorhabditis elegans husbandry

Populations of the three strains of *C. elegans* used in this experiment [N2 wild isolate, CB1338 (hereafter C strain) and JT6924 (hereafter J strain); all obtained from the *Caenorhabditis* Genetics Center, St Paul, MN, USA] were maintained in large numbers on standard 5.5 cm diameter NGM (nematode growth medium) plates (Brenner, 1974) seeded with the OP50 strain of *Escherichia coli*. All worms were kept in an incubator at a constant 20°C. No institutional or governmental animal welfare authorities were required to approve the research on nematodes, and the experiments complied with UK and EU animal welfare protocols.

Vibration generation

Audio tracks were played through a speaker to generate the 12 vibration treatments on the plates (see below). The tracks were played through the headphone jack on a laptop; the headphone jack was plugged into an amplifier (Pyle PLMRA400 400 W), which connected to a speaker (Seas CA12RCY H1152-08 Woofer). A metal rod (2 mm diameter) was glued onto the centre of the speaker so that it was orthogonal to the speaker membrane plane. The speaker was sealed in a custom-made 5 mm thick plastic box to limit the acoustic energy emitted, such that only the rod and input cables emerged from the box. The speaker was placed on a vibration-dampened optic table. A custom-built Petri dish holder was built to hold the standard 5.5 cm diameter NGM plates. This holder was vibrationally separated from the speaker, as it was not in contact with the optic table. The Petri dish holder had a hole for the metal rod, which contacted the plate in the centre. The whole set-up was designed so that position and contact between the speaker rod and worm plate were identical for each experiment.

Twelve sound files of 90 s were created (Table 1). All tracks were calibrated using a laser vibrometer (Polytec PDV-100) to record plate vibration in response to each audio track. For the calibration, the laser was focused at the centre of the Petri dish, which contained agar. Fast Fourier transform mode was used with a 0.5 Hz resolution from 0 to 200 Hz, collecting 15 magnitude averages. Audio tracks were generated using Audacity (freeware). The first and last 30 s of each track was silent, i.e. with just background vibrations present. Pure tone sine wave tracks were created at five frequencies: 23, 31, 61, 86 and 111 Hz, avoiding resonant frequencies of the speaker, frequencies and multiples of background vibration noise, and multiples of mains power noise at 50 Hz. The absolute displacements of the different frequencies were chosen based on the level of that frequency in the background noise, which was calibrated to be 58.5 dB (±0.5 dB s.d. between frequencies) above background level at the frequency of interest. Lower frequencies at a comparable amplification level above background noise were not possible with the modified speaker used, as the speaker cannot generate sufficiently high amplitude in this range and background noise is higher at lower frequencies.

Table 1. Details of the 12 vibration treatments

Frequency (Hz)	Mean amplification relative to background (dB)	s.d. of the mean amplification between repeated treatments (dB)
PT: 23	High (58.7)	0.02
PT: 31	High (59.0)	0.01
PT: 61	High (58.7)	0.01
PT: 86	High (57.8)	0.01
PT: 111	High (58.5)	0.02
PT: 31	Low (51.5)	0.01
PT: 61	Low (51.7)	0.02
PT: 111	Low (51.1)	0.44
PT: 61	AM (51.7 to 58.7)	0.78
FM: 23, 31, 61, 86, 111	High (58.5)	0.15*
Background (all)	Amplified (13.8)	1.23*
Background (all)	Background (0.0)	3.32*

Frequency and amplitude of treatments, including pure-tone (PT), frequency-modulated (FM), amplified, background and amplitude-modulated (AM) tracks. *s.d. of the mean is given across all frequencies present (thus increasing error compared with a single frequency).

In order to investigate the effect of amplitude, as well as frequency, three frequencies were additionally played at lower amplitude, picking a low (31 Hz), medium (61 Hz) and high (111 Hz) frequency within our tested range, calibrated at 51.4 dB (±0.3 dB s.d. between frequencies) above background level at that frequency. Another track ramped between the low and high amplitude at 61 Hz at a rate of 0.29 Hz (one cycle every 3.5 s). Another track ramped from low to high frequency: from 23 Hz to 31, 61, 86 and 111 Hz, with 10 cycles of each frequency at the 58.5 dB amplitude level. A sound file of no additional energy (i.e. just background noise) was used as a 'silent' control. For the final track, the background noise on the Petri dish was recorded by the laser vibrometer, and was then amplified in Audacity to the maximum level without clipping. The resulting track had similar flat frequency spectra to the 'silent' track, but amplified on average by 13.8 dB (±3.5 dB s.d. between all frequencies, N=400).

Recording behaviour

Ten hermaphrodite worms at the young adult stage were transferred using a platinum wire worm pick from the population maintenance plates to unseeded standard 5.5 cm diameter NGM plates, which were kept at 20°C. Unseeded plates were used to limit behavioural variability over time: transitions from food searching to feeding behaviours affect behavioural responses and variability (Moy et al., 2015). For experiments, the plates were transferred to the custom-made plate holder. Worms were transferred quickly to the experimental plate, and experimental observations were made as soon as possible, within 10 min. This was to avoid starvation effects that can start to alter worm behaviour (transition from dwelling to roaming state) any time between 10 and 45 min after transfer (Gray et al., 2005). Worms in all trials had equal treatment in terms of time since transfer, manner of transfer and clean plates for experiments.

A 4.2 megapixel camera (2048×2048 pixels) attached to a 5× zoom lens captured the whole plate in view and recorded at 25 frames s^{-1}. Darkfield red LED lighting (LATAB SAH3 4166, Lambda Photometrics, Harpenden, UK) provided illumination. Worms were recorded moving for the full 90 s, where a stimulus (including the silent control) was applied between 30 and 60 s. The vibration treatment was picked at random (randomisation was performed using Matlab, MathWorks, Natick, MA, USA) and run for three separate, identical *C. elegans* 10-worm plates of the same strain. A total of three strains of *C. elegans* were investigated (N2, C strain and J strain). Not all worms on each plate were successfully tracked. Each frequency and amplitude combination had at least 50 worms that had been successfully tracked.

Data analysis

A custom-written multi-worm tracker was used to track worm behaviour frame-by-frame. The C++ code for the multi-worm tracker is freely available online at: https://bitbucket.org/leedswormlab/multi-worm-thingy. The worm tracker first generated a background image of the plate. As darkfield lighting was used, worms appeared white on a dark background. For each

Journal of Experimental Biology (2018) 221, jeb178947. doi:10.1242/jeb.178947

pixel in the image, the darkest shade over the movie length was used to generate the background image. This background image was then subtracted from each frame in the movie, clearly highlighting the worms. A threshold for these images was calculated using Otsu's method to identify the worms on the plate (Otsu, 1979). To reduce noise from the light source, a circular region of interest with a 2.5 cm diameter was set around the vibration pin (at the plate centre) and objects identified as worms had to be within this area to be included in the analysis.

Contiguous sets of 100 pixels or greater were classified initially as worms. The mean coordinates of each contiguous set of pixels were calculated to represent an approximation of the centre of mass of each worm. The trajectories for each worm were calculated by taking each worm identified in the first frame and finding whether there was a worm identified in the subsequent frame within 50 pixels of its previous position. This was continued for all frames throughout the 90 s period. If no worm was found within this distance in any subsequent frame, the individual worm was removed from the dataset. This meant only worms that were tracked moving for the full 90 s period within the region of interest were analysed.

The trajectories for the remaining worms were filtered using a third-order Butterworth filter at a cut-off frequency of 1.7 Hz (see Bomphrey et al., 2009, for details). Example tracks following this analysis can be seen for N2 strain worms under 23 Hz treatment in Fig. 1 and Movie 1. We calculated the total distance travelled during the treatment and two control periods, as well as the number of reversals the worm performed (see Table S1). A reversal was defined as a movement greater than a right angle from the previous direction of travel.

Statistics

A stringent three-pass approach using non-parametric statistics was used for statistical analysis, which was performed in Matlab. A paired Mann–Whitney ranked sum test was applied to the silent treatment (ST, middle 30–60 s of silent treatment) versus other treatment periods (30–60 s of all other treatments, pass 1). If treatment had a significant effect, a similar test was applied to the treatment period versus its corresponding control period 1 (CP1; 0–30 s, pass 2). The direction of the response was then compared with the control, where an opposite direction was required to determine a significant response of our treatment. As discussed, the behaviour over time was used as a final measure of vibration sensitivity. As an optional third pass, a similar test was applied to the treatment period versus its control period 2 (CP2; 60–90 s, pass 3). *P*-values from all Mann–Whitney ranked sum tests are given in Table S1.

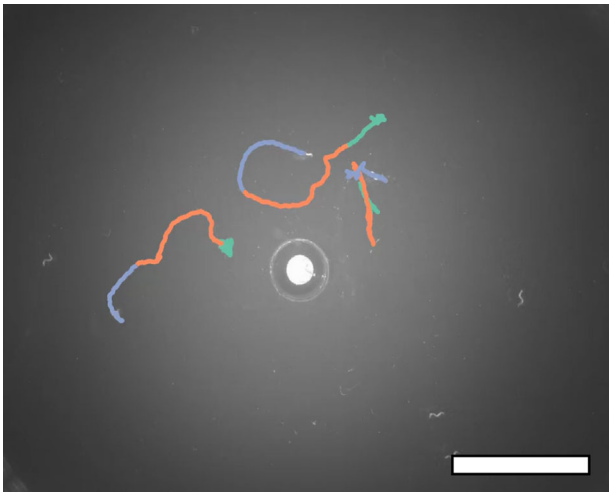

Fig. 1. Behavioural tracks of wild-type (N2) strain worms under 23 Hz treatment. Three of the 74 worms tracked under the 23 Hz treatment are shown. Control period 1 (CP1, 0–30 s) is in green, vibration treatment at 23 Hz (30–60 s) is in orange and control period 2 (CP2, 60–90 s) is in purple. Only worms that moved within the area of interest for the full 90 s were tracked. Scale bar denotes 10 mm.

We validated the use of non-parametric statistics using a GLMM on data that took plate and date into account as random factors. The results confirmed the findings of the non-parametric tests. We confirmed the normality of the data by plotting a *Q*–*Q* plot in R (R Foundation for Statistical Computing, Vienna, Austria), which showed that the data were more tightly distributed than a normal distribution, meaning that any significance found would be conservative. We then generated a range of linear models with mixed effects with different interactions between the random factors of plate and date and the fixed factors of frequency and strain for the data from the vibration treatment period. An Akaike's information criterion (AIC) value was used to determine the best model for each of total distance and number of reversals, which were nested interactions between the random factors and an interaction between the fixed factors. Because of the nature of the model, a conservative alpha value of 0.001 was chosen.

RESULTS

We applied 12 vibration treatments for 30 s each, with 30 s of silence either side of the treatment (CP1 and CP2). This included a silent treatment (ST) that was used as an additional control (i.e. 90 s of silence). Our statistical analysis involved comparing behavioural responses during vibration treatment periods with both the ST during the same time period and both control periods before and after the treatment (CP1 and CP2). Our treatments varied in frequency content over the range 23–111 Hz, using sinusoidal, frequency-modulated and broadband vibration forms (Table 1). We therefore tested for the ability to discriminate frequencies, which allowed us to probe the physical mechanism of any vibration sensor.

Wild-type responses

Wild-type (N2 strain) worms changed behaviour in response to vibrational stimuli of 23 Hz, which was the lowest frequency treatment applied (Figs 1, 2; Fig. S1, Movie 1). They exhibited significantly fewer reversals during 23 Hz treatment (ST Wilcoxon rank sum=5588, N_{ST}=70, $N_{23\ Hz}$=74, P=0.0399; CP1 Wilcoxon rank sum=6465, N=74, P<0.001), and a significantly longer total distance travelled (ST Wilcoxon rank sum=3588, N_{ST}=70, $N_{23\ Hz}$=74, P<0.001; CP1 Wilcoxon rank sum=4712, N=74, P=0.0021). None of the other 10 treatments applied to N2 animals caused significant changes in behaviour (see Table S1), including the frequency-modulated treatment of 23–111 Hz and broadband noise of amplified background recordings (Fig. S1). The parametric statistics confirmed that both total distance and number of reversals were significantly different for the wild-type worms at 23 Hz versus ST during the treatment period (t=−4.384, P<0.001 for number of reversals, t=5.095, P<0.001 for total distance).

Without vibration treatment, worms also significantly changed behaviour over time. Worms under the silent treatment showed a significantly higher number of reversals (Wilcoxon rank sum=4122.5, N=70, P<0.001) and a significantly lower total distance over the three 30 s periods (Wilcoxon rank sum=5576, N=70, P=0.0076). This is consistent with the transition from a

Journal of Experimental Biology (2018) 221, jeb178947. doi:10.1242/jeb.178947

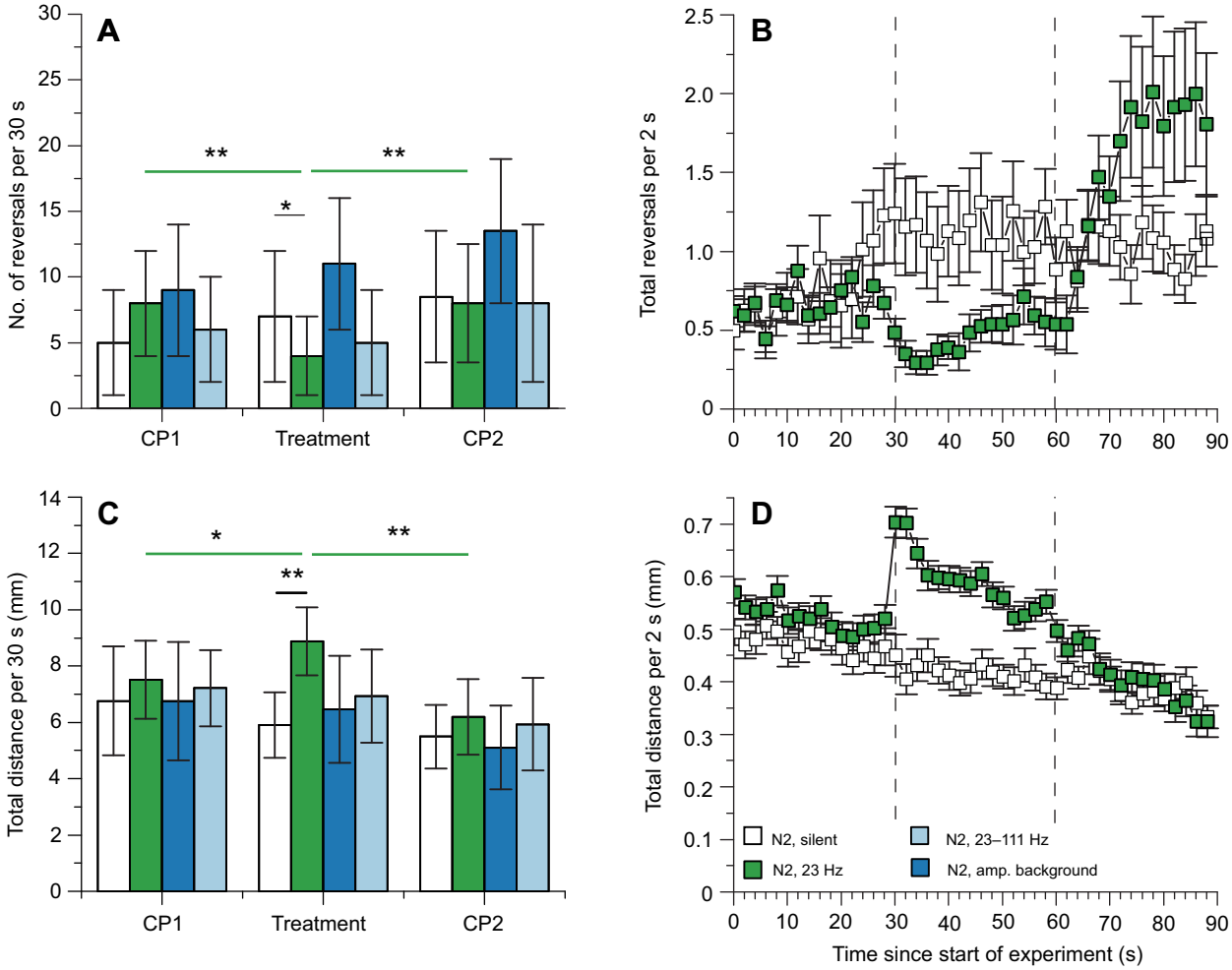

Fig. 2. Behavioural responses of wild-type (N2) strain worms. (A) Median number of reversals in a 30 s period. (B) Mean number of reversals in a 2 s period. (C) Median total distance in a 30 s period. (D) Mean total distance in a 2 s period. White is the silent treatment (*N*=70), green is the 23 Hz treatment (*N*=74), dark blue is the amplified background treatment (*N*=75) and light blue is the frequency-modulated 23–111 Hz treatment (*N*=76). Error bars in A and C give the median absolute deviation, and those in B and D give s.e.m. Two types of control were used: a silent treatment (ST, 90 s of silence) and silent periods before and after the treatments (CP1, 0–30 s and CP2, 60–90 s). Dashed lines in B and D separate treatment (30–60 s) from control periods. Lines and asterisks above bars denote a statistically significant change due to vibration treatment: *0.05>*P*>0.001, **P<0.001. Black lines denote a significant difference between the silent and 23 Hz treatment, and green lines denote a significant difference between the 23 Hz treatment period and control periods. See also Fig. S1.

roaming to a dwelling state due to the noxious effects of transfer with a wire pick (Cohen et al., 2012), but not due to starvation effects, which would cause an opposite transition (Gray et al., 2005). Importantly, the 23 Hz treatment showed the opposite trend in behaviour, so counteracted the trend in behaviour seen under the silent control treatment. This further supports a significant difference from the control treatment (Fig. 2).

Behaviour changed over the 30 s treatment period of 23 Hz (Fig. 2B,D, with a 2 s resolution). The number of reversals was at its minimum within the first 8 s of applied vibration and total distance travelled peaked within the first 4 s. There was also evidence of habituation to vibration, as the total number of reversals increased and total distance decreased over the 30 s treatment period. The number of reversals also increased in CP2, where the longer error bars indicate that a few worms in the population responded by dramatically increasing the number of reversals once the vibration stimulus was removed, with little change in the median. The increase in the number of reversals in CP2 further supports a significant difference in behaviour during the treatment period, where the number of reversals was reduced.

Mutant strain responses

The C strain [*mec-3*(*e1338*)], which has deficient non-ciliated mechanosensory neurons, also showed a significant behavioural response to the 23 Hz treatment (Fig. 3). This was manifested by a significant increase in total distance travelled during this 30 s period (Fig. 3D; Fig. S2; ST Wilcoxon rank sum=3493, N_{ST}=66, $N_{23\ Hz}$=65, P<0.001; CP1 Wilcoxon rank sum=3779, N=65, P=0.026), which is similar to the wild-type strain. However, unlike the wild-type strain, there was no significant decrease in the number of reversals over the same time period for the 23 Hz treatment. In addition, the frequency-modulated 23–111 Hz treatment caused a significant decrease in the number of reversals (Fig. 3A; ST Wilcoxon rank sum=5229.5, N_{ST}=66, $N_{23–111\ Hz}$=66, P<0.001; CP1 Wilcoxon rank sum=4887.5, N=66, P=0.0232), but with no significant increase in total distance. These paired statistical tests therefore reveal subtle differences between the wild-type and C strains. However, parametric statistics indicated that the mechanosensory neuron-deficient C strain worms were not overall significantly different from wild-type worms in any of the model outputs (when responses to all vibration treatments are analysed).

Journal of Experimental Biology (2018) 221, jeb178947. doi:10.1242/jeb.178947

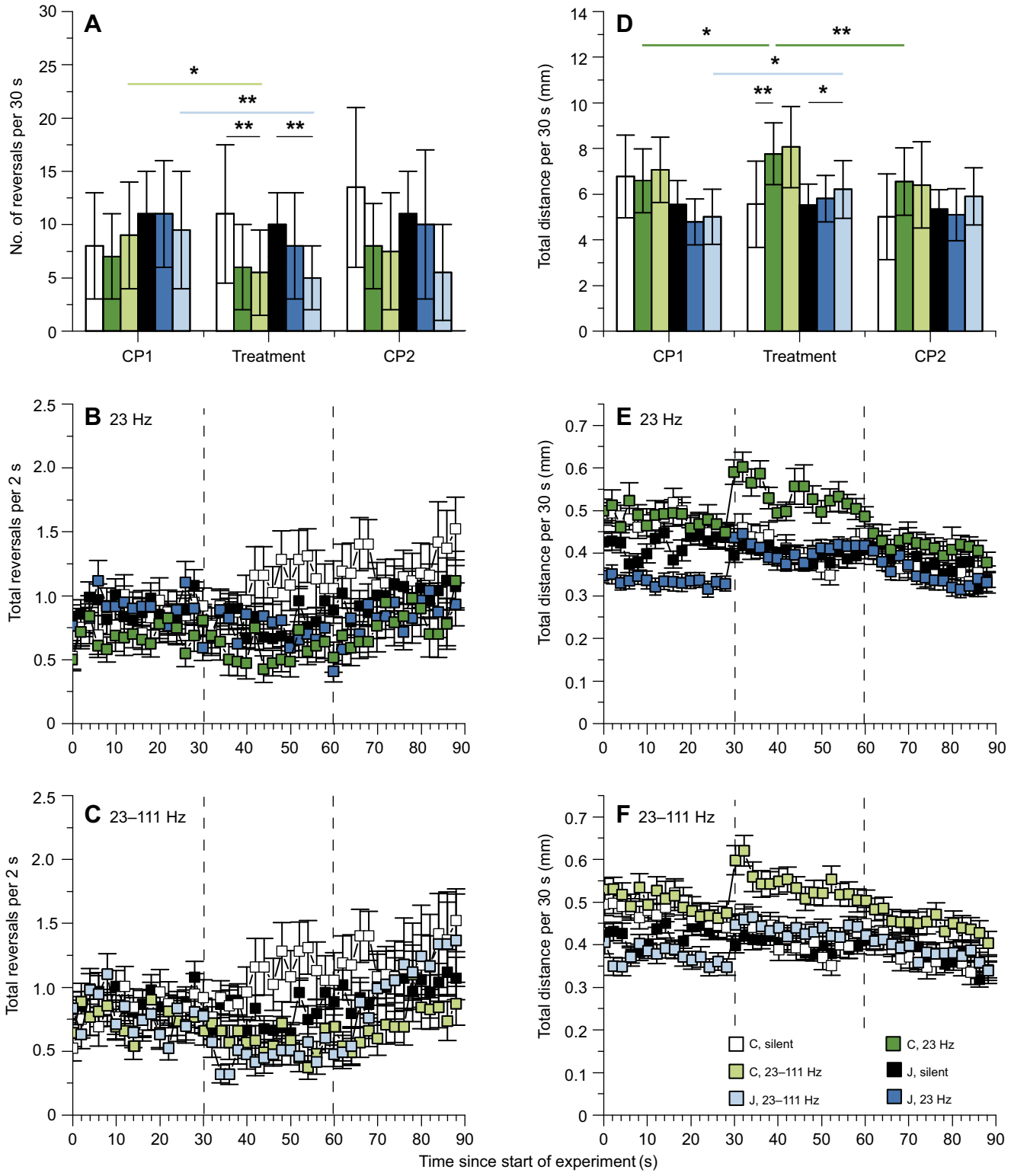

Fig. 3. Behavioural responses of mechanosensory neuron-deficient [C strain, *mec-3*(*e1338*)] and ciliated neuron-deficient [J strain, *daf-19*(*m86*);*daf-12*(*sa204*)] worms. (A) Median number of reversals in a 30 s period. (B,C) Mean number of reversals in a 2 s period for the 23 Hz and frequency-modulated 23–111 Hz stimulation. (D) Median total distance in a 30 s period. (E,F) Mean total distance in a 2 s period for the 23 Hz and frequency-modulated 23–111 Hz stimulation. White and black are silent treatments for C (*N*=66) and J (*N*=99) strains, respectively. Dark green is the 23 Hz treatment for C strain (*N*=65); light green is the frequency-modulated 23–111 Hz treatment for C strain (*N*=66); dark blue is the 23 Hz treatment for J strain (*N*=65); and light blue is the frequency-modulated 23–111 Hz treatment for J strain (*N*=64). Error bars in A and D give the median absolute deviation and those in B, C, E and F give s.e.m. Two types of control were used, a silent treatment (ST, 90 s of silence) and silent periods before and after the treatments (CP1, 0–30 s and CP2, 60–90 s). Dashed lines in B, C, E and F separate treatment (30–60 s) from control periods. Lines and asterisks above bars denote a statistically significant change due to vibration treatment: *0.05>*P*>0.001, **P*<0.001. Black lines denote a significant difference between the silent and vibration treatment, and coloured lines denote a significant difference between vibration treatment periods and control periods. Fig. S2 compares worms' responses over time for all strains.

The J strain [*daf-19*(*m86*);*daf-12*(*sa204*)], which has functionally deficient ciliated neurons, showed no changes in behaviour for the 23 Hz treatment. However, the frequency-modulated 23–111 Hz treatment caused a significant reduction in the number of reversals (Fig. 3A; ST Wilcoxon rank sum=9697.5, N_{ST}=99, $N_{23–111\ Hz}$=64, P<0.001; CP1 Wilcoxon rank sum=4926, N=64, P<0.001) and a significant increase in total distance travelled (Fig. 3D; ST Wilcoxon rank sum=7372, N_{ST}=99, $N_{23–111\ Hz}$=64, P=0.0113; CP1 Wilcoxon rank sum=3488, N=64, P=0.0023). Our parametric statistics indicated that the J strain worms showed a significant difference in the difference between 23 Hz and the silent treatment during the treatment period compared with N2 worms for both total distance (t=−4.074, P<0.001) and number of reversals (t=3.819, P<0.001).

None of the other treatments caused significant changes in behaviour for either the ciliated neuron-deficient J strain or the mechanosensory neuron-deficient C strain (see Table S1). The vibration treatments that led to significant changes in behaviour showed similar trends in behaviour over the 30 s treatment period to that of the wild-type strain (Fig. 3B,C,E,F; Fig. S2). The exception was that the C strain did not show a notable transition at the start of the treatment period of the frequency-modulated 23–111 Hz treatment.

DISCUSSION

Putting these findings in context, we can accept our proposed hypothesis as wild-type (N2 strain) *C. elegans* worms showed a distinct behavioural response to sustained 23 Hz vibration compared with short-term non-localised mechanical stimuli. For the former, worms decreased the incidence of reversals and increased total distance travelled, whereas for the latter, the opposite direction of behaviours was shown. This separate behavioural outcome means that an identical circuit cannot be used to modulate the behaviour to both stimuli – the vibrational stimuli applied here must be modulated by a distinct circuit. The vibration circuit is probably a modified version of the known touch or navigation circuits (Chalfie et al., 1985; Gray et al., 2005).

The different responses to sustained vibration versus short-term non-localised stimuli imply that either the time over which the vibration is applied or their amplitude, or both, is important for the type of behavioural response. However, not all sustained vibrations elicited a response. This suggests that *C. elegans* can discriminate both the frequency content (i.e. 23 Hz, but not 31, 61, 86 or 111 Hz) and how the frequency is presented (i.e. 23 Hz pure tone, but not in frequency-modulated or broadband forms that also contain 23 Hz), and that both are important for eliciting the behavioural response. Response to frequency will be amplitude dependent, so future studies should investigate more detailed frequency thresholds over a variety of amplitude ranges.

There are two non-mutually exclusive mechanisms for frequency discrimination, which determine when transduction will occur (Yack, 2004). Either physical filtering mechanisms – for example, during propagation through the cuticle – modify spectral content before the sensor(s) or physiological filtering mechanisms utilise temporal and amplitude thresholds for mechanotransduction. A low-frequency range including 23 Hz would be expected to be biologically relevant for detecting movements of predators and/or the physical environment (Catania, 2008). The specific response to sinusoidal vibration gives insight into how transduction might occur, as periodic vibration will induce more stable movement of the body wall than broadband forms, which will be more likely to promote mechanotransduction when the duration and/or amplitude of the stimulus is important, as there is evidence for here.

The behavioural responses of the mutant strains suggest that ciliated and non-ciliated neurons may play different roles in modulating the response to vibrational stimuli. The worms functionally deficient in ciliated neurons (J strain) exhibited vibration sensitivity in response to the frequency-modulated vibration (23–111 Hz), rather than to 23 Hz treatment that was seen in wild-type worms. This suggests a role for *daf-19* or *daf-12* in frequency discrimination. The most likely explanation is that ciliated mechanosensory neurons are involved in frequency discrimination; however, any ciliated neurons could be involved. DAF-19 proteins are also involved in synaptic protein maintenance in non-ciliated neurons (De Stasio et al., 2018). Indeed, the shorter total distance travelled by these mutants compared with the wild-type was consistent with the abnormal roaming phenotype (Senti and Swoboda, 2008). DAF-19 proteins are also expressed in the hypodermis (Craig et al., 2013), and as such may be involved in physical filtering as vibrations propagate through the body wall. Finally, *daf-12* has links to developmental pathways (Antebi et al., 2000), making it less likely to be involved, but it cannot be ruled out. Future studies should investigate vibration sensitivity in other mutant strains of *C. elegans* to narrow down how *daf-19* or *daf-12* influences the ability of the worms to discriminate vibrational stimuli.

In contrast, the worms that were functionally deficient in mechanosensory neurons (C strain) increased total distance travelled in response to 23 Hz, but did not increase the number of reversals under the same treatment, revealing a subtle difference between wild-type and *mec-3* mutant strains. Although a significant increase in reversals was seen in response to the frequency-modulated 23–111 Hz vibration, the timing of the response was not comparable with that of the wild-type. These *mec-3* mutants also do not show reversal behaviour associated with localised touch (Way and Chalfie, 1989), so our findings are consistent with the importance of non-ciliated neurons for the reversal behaviour. Evidence from this mutant therefore suggests a different role for *mec-3* compared with *daf-19;daf-12*, as frequency discrimination was unaffected but the behavioural outcome was altered compared with that of the wild-type strain. Using our described behavioural assay, future studies should be able to map the sensory pathway of vibrational sensitivity, which will provide further insight into the biological and mechanistic differences between sustained vibration, short-term vibration and touch as mechanical stimuli.

In terms of why the worms react differently to vibrational stimuli, we suggest that long-term vibration represents a different form of information compared with short-term non-localised stimuli, where the latter represent a one-off event. However, it remains to be seen what the biological relevance of vibrational sensitivity in *C. elegans* might be. Vibration is often not used in isolation. For example, Chen and Chalfie (2014) have shown that the presence of a vibration stimulus can increase the speed of chemotaxis behaviour of *C. elegans* dauer larvae (Chen and Chalfie, 2014). The response to vibration is therefore likely to change with the presence and strength of stimuli from other modes, as part of a multimodal sensory integration within each worm.

In other nematodes, vibrations are used as one cue to enable host location, and three species of parasitic nematodes show a similar increased forward movement in response to vibrational stimuli (Torr et al., 2004). The question of whether vibrational sensitivity and sensory mechanism are conserved across the phylum remains to be investigated. Exploring vibration sensing in parasitic nematodes will probably reveal many important insights for disease management strategies (Kennedy and Harnett, 2013).

If the location or mechanism of any vibration sensors are evolutionarily conserved, the potential applications in bioinspired

technologies will be extremely promising. Robotics research has recently been shifting focus away from the use of hard metallic materials towards the use of non-linear pliant materials – the field of soft robotics (Kim et al., 2013). Integration of body morphology and sensors of external stimuli is vital for robotic design, so a biomimetic approach based on the soft-bodied *C. elegans* has enormous potential to inform and direct this branch of engineering.

Taken together, we propose that *C. elegans* has a dedicated sensory system for detecting and processing vibrational stimuli, responding to substrate-borne vibrations in the worm's environment without utilising hard material components usually found in vibrational organs. This sensory system remains to be identified in full, but the downstream behavioural outcome provides a useful assay for fully elucidating its components and connections. Given the simple morphology and limited number of neurons present in *C. elegans*, this sensory system has one of the simplest anatomies yet identified in the animal kingdom.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: R.I.H., B.M.; Methodology: R.I.H., B.M.; Software: R.I.H.; Formal analysis: R.I.H., B.M.; Investigation: B.M.; Resources: R.I.H., B.M.; Data curation: R.I.H.; Writing - original draft: B.M.; Writing - review & editing: R.I.H., B.M.; Visualization: R.I.H., B.M.; Project administration: R.I.H.; Funding acquisition: B.M.

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Data availability
The code for the multi-worm tracker is freely available online at: https://bitbucket.org/leedswormlab/multi-worm-thingy

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.178947.supplemental

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Supplementary Information

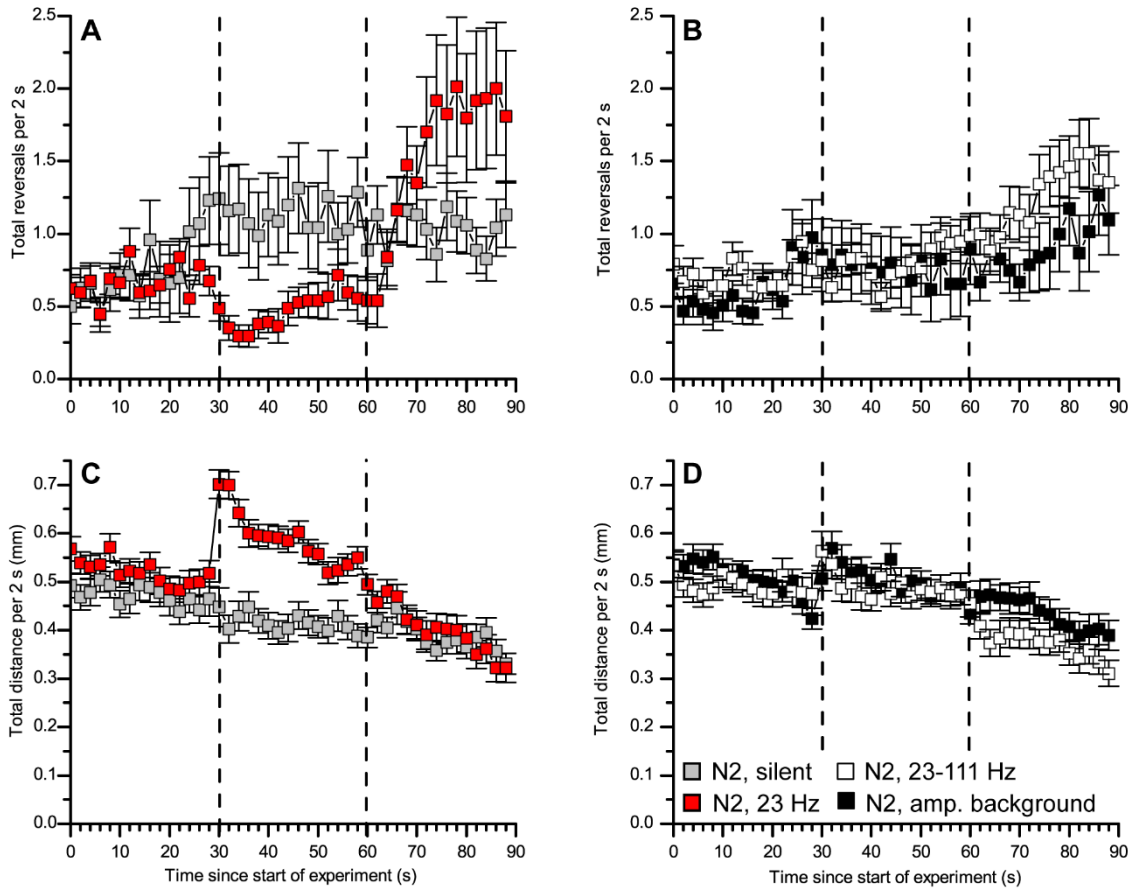

Fig. S1. Responses over time of wild-type (N2) strain worms to four vibration treatments. (A) and (B) mean number of reversals in a 2 second period, (C) and (D) mean total distance in a 2 second period. Grey is silent treatment (also shown in Fig. 2B and D, N=70), red is the 23 Hz treatment (also shown in Fig. 2B and D, N=74), black is the amplified background treatment (N=75) and white is the frequency-modulated 23-111 Hz treatment (N=76). Error bars give s.e.m. Dashed lines separate treatment period (30-60 seconds) from control periods.

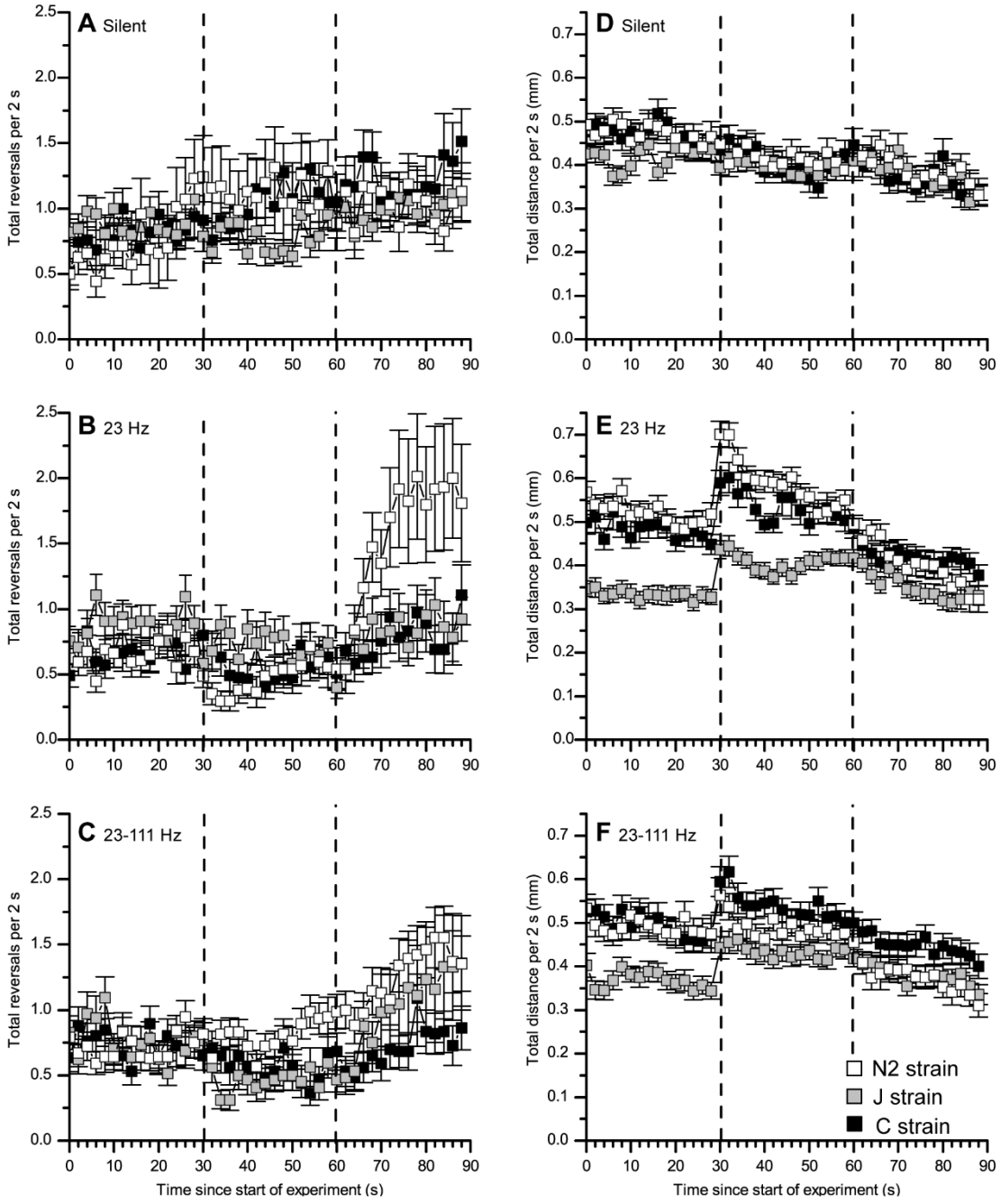

Fig. S2. Responses over time of wild-type (N2) and mechanosensory neurons deficient [C strain, *mec-3*(e1338)] and the ciliated neurons deficient [J strain, *daf-19*(m86);*daf-12*(sa204)] strain worms to three vibration treatments. (A-C) Mean number of reversals in a 2 second period, (D-F) mean total distance in a 2 second period. (A) and (D) silent treatment, (B) and (E) 23 Hz treatment, (C) and (F) frequency-modulated 23-111 Hz treatment. White gives wild-type (N2) strain, grey gives J strain (*daf-19;daf-12* mutant) and black gives C strain (*mec-3* mutant). Error bars give s.e.m. Dashed lines separate treatment period (30-60 seconds) from control periods. All data are also shown in Fig. 2B and D and Fig. 3B, C, E and F.

Table S1. Full data table from this study. The number of reversals and total distance travelled by all tracked worms over all 12 vibration treatments, three 30 second periods and three strains are given. A summary table of the average number of reversals and average total distance for each 30 second period is also given, as well as the outputs from the Mann-Whitney ranked sum tests.

Click here to Download Table S1

Movie 1. Behavioural tracks of wild-type (N2) strain worm under 23 Hz treatment. Movie shows tracks of four of the 74 worms tracked under the 23 Hz vibration treatment. First 30 seconds is control period 1 (no vibration, green), middle 30 seconds is the vibration treatment at 23 Hz (orange) and last 30 seconds is control period 2 (no vibration, purple). Only worms that moved within the area of interest for the full 90 seconds were tracked. White circle in middle has 2 mm diameter.