

# TRPA channels distinguish gravity sensing from hearing in Johnston's organ

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Contributed by Michael J. Welsh, June 15, 2009 (sent for review April 11, 2009)

Although many animal species sense gravity for spatial orientation, the molecular bases remain uncertain. Therefore, we studied *Drosophila melanogaster*, which possess an inherent upward movement against gravity-negative geotaxis. Negative geotaxis requires Johnston's organ, a mechanosensory structure located in the antenna that also detects near-field sound. Because channels of the transient receptor potential (TRP) superfamily can contribute to mechanosensory signaling, we asked whether they are important for negative geotaxis. We identified distinct expression patterns for 5 TRP genes; the TRPV genes *nanchung* and *inactive* were present in most Johnston's organ neurons, the TRPN gene *nompC* and the TRPA gene *painless* were localized to 2 subpopulations of neurons, and the TRPA gene *pyrexia* was expressed in cap cells that may interact with the neurons. Likewise, mutating specific TRP genes produced distinct phenotypes, disrupting negative geotaxis (*painless* and *pyrexia*), hearing (*nompC*), or both (*nanchung* and *inactive*). Our genetic, physiological and behavioral data indicate that the sensory component of negative geotaxis involves multiple TRP genes. The results also distinguish between different mechanosensory modalities and set the stage for understanding how TRP channels contribute to mechanosensation.

*Drosophila* | transient receptor potential | geotaxis

The primary mechanosensory organ that detects gravity in *Drosophila* appears to be Johnston's organ (1). This organ is located in the second antennal segment. It consists of over 200 scolopidia arrayed in a bowl shape (2), with each scolopidium containing mechanosensory chordotonal neurons and their support cells (3–5) (Fig. 1A). Johnston's organ is well known as a detector of near-field sound (3–6). Air particle displacement vibrates the third antennal segment, deforming the cuticle at the joint between segments 2 and 3 where the sensory units of Johnston's organ attach. It was proposed that the third segment may also be deflected by gravity (7), and the geometry of Johnston's organ suggests it could respond to gravity irrespective of head orientation (2). Indeed, recent work indicates that Johnston's organ can also respond to gravity, as well as to wind (1, 8). Thus, Johnston's organ may detect multiple different mechanosensory stimuli, and investigations of specific molecular mechanisms underlying these sensory functions may benefit our understanding of other polymodal sensory structures such as the inner ear and dorsal root ganglion in mammals.

Almost 50 years ago, Hirsch and colleagues demonstrated that negative geotaxis is genetically encoded in *Drosophila* (9, 10). Since then, several genes influencing this behavior have been identified (11–13). However, those genes are expressed in both central and peripheral nervous systems, and the nature of their role in the sensory organ that detects gravity remains unknown. The goal of this work was to identify genes involved in sensory aspects of negative geotaxis and in so doing to obtain genetic data to discriminate between the structural and functional components of Johnston's organ involved in negative geotaxis and hearing.

## Results

**Johnston's Organ Is Essential for Negative Geotaxis.** To verify that Johnston's organ is essential for negative geotaxis, we restricted

movement of the third antennal segments by fixing them to the head with nontoxic glue. To assess negative geotaxis, we used a tube-climbing assay. Flies were tapped to the bottom of a vertical tube, and the number of flies climbing above a 15-cm mark within 15 s was counted as a climbing score (Fig. 1B). This assay was done first with illumination and then repeated in the dark. The "Light" condition tests general locomotion driven by both phototaxis and negative geotaxis, and the "Dark" condition tests locomotion due to negative geotaxis only. Wild-type flies showed upward movement in both Light (L) and Dark (D) conditions, producing similar climbing scores and a D/L Ratio close to 1 (Fig. 1C and D). This behavior reflects negative geotaxis. Gluing the antenna reduced the preference for upward movement. Injuring Johnston's organ by pinching the second segment with fine forceps had a similar effect (Fig. 1E and F). An independent assay that does not depend on tapping-initiated locomotion (the vertical choice maze) also showed that gluing the antenna disrupted the preference for upward movement (Fig. S1). These data suggest that Johnston's organ is essential for negative geotaxis and are consistent with a recent publication (1).

**TRP Channels Are Expressed in Specific Populations of Johnston's Organ Cells.** Previous data indicate that TRP superfamily ion channels may be involved in mechanosensation (14–18). Of note, the TRPN gene *no mechanoreceptor potential C* (*nompC*) (19, 20) and the TRPV genes *nanchung* (*nan*) and *inactive* (*iav*) (21, 22) are expressed in Johnston's organ chordotonal neurons and are required for normal hearing. Moreover, a *nan* mutant reduced the Ca<sup>2+</sup> elevations in Johnston's organ neurons that occur with antennal movement (1). The TRPA genes *painless* (*pain*) (23) and *pyrexia* (*pyx*) (24) are also expressed in Johnston's organ, although their function there is unknown. Outside Johnston's organ, Pain channels are required for avoidance behaviors in response to harsh touch, noxious heat ( $\geq 38^\circ\text{C}$ ), and aversive chemicals (23, 25–27), and Pyx channels contribute to protection from noxious heat ( $\geq 40^\circ\text{C}$ ) (24).

We hypothesized that some of these TRP channels are involved in gravity sensing, and we therefore tested their expression in Johnston's organ using TRP gene promoters linked to *Gal4* to drive *UAS*-fluorescent reporters. With *nan*, *iav*, *nompC*, and *pain* promoters, the GFP reporter filled sensory dendrites and cilia of chordotonal neurons (Fig. 2A Upper). With a nuclear-localized DsRed as the reporter, we localized the cell

Author contributions: Y.S., L.L., Y.B.-S., J.S.J., D.F.E., and M.J.W. designed research; Y.S., L.L., Y.B.-S., and J.S.J. performed research; Y.S. and L.L. contributed new reagents/analytic tools; Y.S., J.S.J., D.F.E., and M.J.W. analyzed data; and Y.S., L.L., Y.B.-S., D.F.E., and M.J.W. wrote the paper.

The authors declare no conflict of interest.

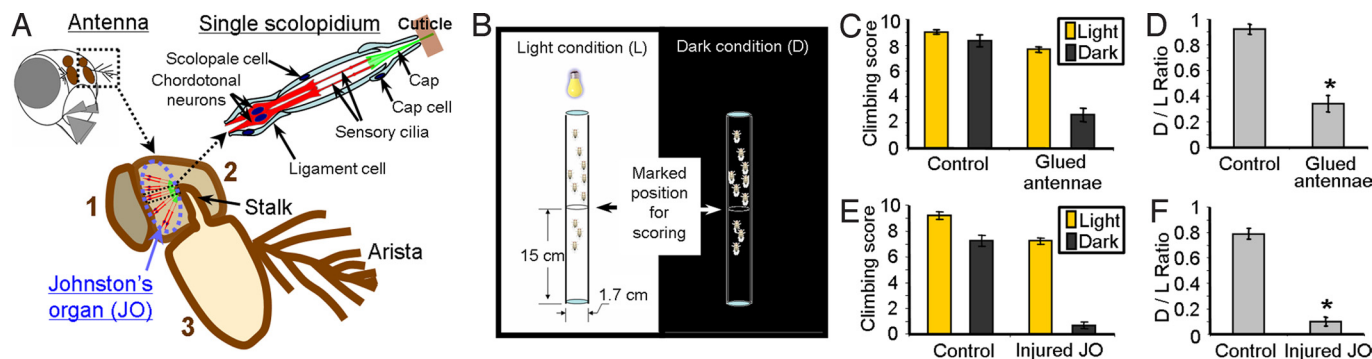
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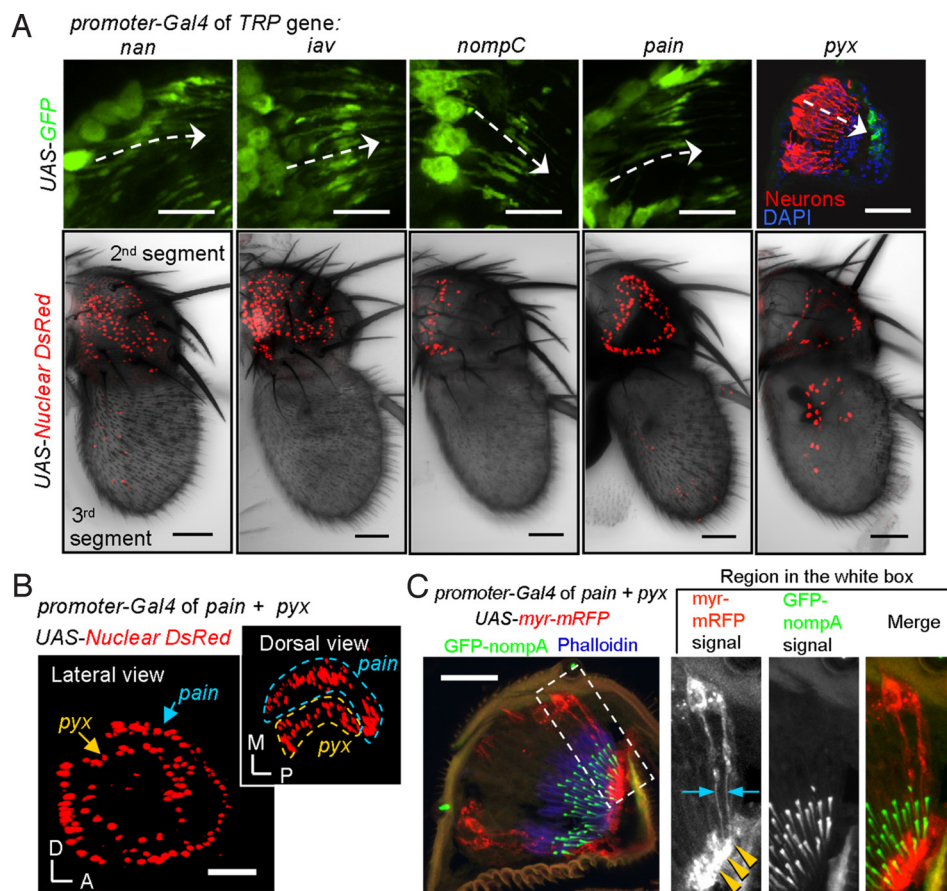


**Fig. 1.** Johnstone's organ is required for negative geotaxis in *Drosophila*. (A) Schematic showing the fly head, antenna, Johnstone's organ, and a single scolopidium of Johnstone's organ. The numbers 1, 2, and 3 refer to the 3 antennal segments. (B) Schematic of the tube-climbing test. In each trial, a group of 10 flies were tapped to the bottom of the tube, and we counted the number of flies crossing the 15-cm threshold line within 15 s as the climbing score. (C) Climbing scores in Light (L) and Dark (D) conditions of control *Canton-S* (CS) flies ( $n = 10$  trials) and CS flies with the third antennal segment glued to head ( $n = 12$  trials). (D) Ratios of climbing scores (D/L Ratios) for flies in C. (E) Climbing scores of control  $w^{1118-WLS}$  flies ( $n = 10$  trials) and  $w^{1118-WLS}$  flies with injured Johnstone's organ because of second segment pinching ( $n = 10$  trials). (F) D/L Ratios for flies in E. Data are mean  $\pm$  SEM. \*,  $P < 0.05$  by unpaired *t* test.

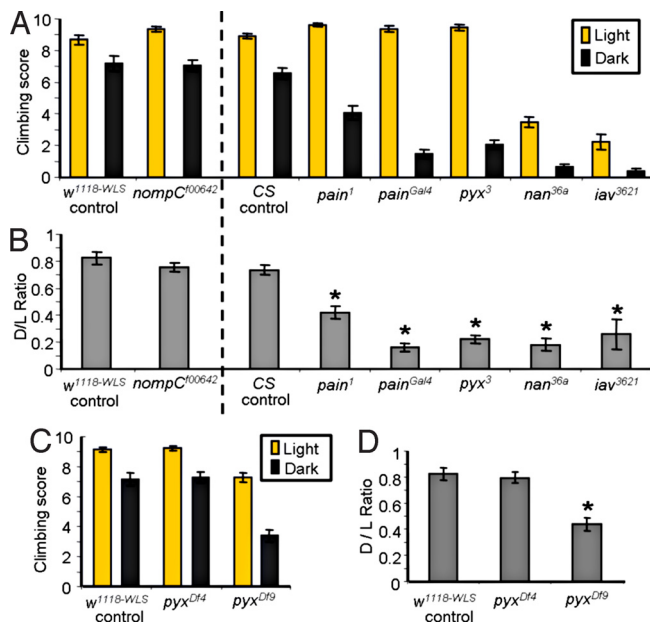
bodies of these neurons (Fig. 2A Lower). *nan-Gal4* and *iav-Gal4* expressed in chordotonal neurons throughout Johnstone's organ. In contrast, *nompC-Gal4* expressed in a cluster of  $\approx 60$ – $70$  medial chordotonal neurons, and *pain<sup>Gal4</sup>* expressed in  $\approx 100$  chordotonal neurons arranged in a bent ring. Identification of distinct populations of Johnstone's organ neurons expressing *nompC* and *pain* are consistent with recent reports that Johnstone's organ contains different cell types that project to discrete portions of the brain (1, 2, 8).

In contrast to the neuronal expression patterns, *pyx-Gal4* expressed in  $\approx 50$  Johnstone's organ cells, with the cell bodies

forming a ring (Fig. 2A Lower) that was located at the distal end of the scolopidium array (Fig. 2A Upper). This site receives projections of the apical tips of the chordotonal cilia and includes the caps that cover the ciliary tips (Fig. 1A). In flies carrying both *pain<sup>Gal4</sup>* and *pyx-Gal4* driving the *UAS-Nuclear-DsRed* reporter, the labeled nuclei formed 2 concentric rings (Fig. 2B and Movie S1); the outer ring was in the position of *pain<sup>Gal4</sup>*, and the inner ring was in the position of *pyx-Gal4*. Using a cell membrane tethered mRFP (*UAS-myr-mRFP*) as a reporter, we were able to trace the projection of *pain<sup>Gal4</sup>*-expressing chordotonal cilia to *pyx-Gal4*-expressing cells (Fig. 2C). These observations suggest



**Fig. 2.** TRP genes have distinct expression patterns in Johnstone's organ. (A) Expression of *nan-Gal4*, *iav-Gal4*, *nompC-Gal4*, *pain<sup>Gal4</sup>*, and *pyx-Gal4* in Johnstone's organ visualized by *UAS-GFP*, which labels the cytoplasm (Upper, scale bars, 10  $\mu$ m), except for *pyx-Gal4* where scale bar, 30  $\mu$ m), and *UAS-Nuclear DsRed*, which labels cell nuclei (Lower, fluorescence images are overlaid on DIC images, scale bars, 30  $\mu$ m). In the second antennal segment, all labeling appeared to be in Johnstone's organ; *nan-Gal4*, *pain<sup>Gal4</sup>* and *pyx-Gal4* also labeled some cells in the third segment. White dashed arrows indicate direction from cell body to dendrite and cilium. (B) Confocal reconstruction of Johnstone's organ expressing both *pain<sup>Gal4</sup>* and *pyx-Gal4* visualized by *UAS-Nuclear DsRed* (red). Inset shows a different view of the structure with *pain* and *pyx* expressing cell nuclei indicated. D, dorsal; A, anterior; M, medial; P, posterior. (C) Expression of both *pain<sup>Gal4</sup>* and *pyx-Gal4* visualized by *UAS-myr-mRFP* (red), which labels the plasma membrane. Scolopale rods are stained with Alexa633-phalloidin (blue). Dendritic caps are labeled with transgenic GFP-NompA (green). (Scale bar, 20  $\mu$ m.) The boxed region highlights 2 *pain<sup>Gal4</sup>*-expressing neurons projecting their cilia to *pyx-Gal4*-expressing cap cells. Three enlarged views of this region are displayed on the right. Arrows indicate cilia and arrowheads indicate a cluster of cap cells.



**Fig. 3.** Specific TRP mutants impair negative geotaxis behavior in the tube-climbing test. (A) Climbing scores of TRP mutants. *nompC<sup>f00642</sup>* was backcrossed to the *w<sup>1118-WLS</sup>* control strain. *pain<sup>1</sup>*, *pain<sup>Gal4</sup>*, and *pyx<sup>3</sup>* were backcrossed to the *Canton-S* (CS) control strain. *nan<sup>36a</sup>* and *iav<sup>3621</sup>* were in their original genetic backgrounds. (B) D/L Ratios for flies in A. *w<sup>1118-WLS</sup>* ( $n = 14$  trials) and *nompC<sup>f00642</sup>* ( $n = 27$  trials) were compared with unpaired *t* test. CS ( $n = 25$  trials) and mutants (*pain<sup>1</sup>*,  $n = 25$  trials; *pain<sup>Gal4</sup>*,  $n = 10$  trials; *pyx<sup>3</sup>*,  $n = 9$  trials; *nan<sup>36a</sup>*, 10 trials; *iav<sup>3621</sup>*,  $n = 10$  trials) were compared by ANOVA ( $\alpha$  level = 0.05) and post hoc test of Games-Howell. (C and D) *pyx<sup>Df4</sup>* flies ( $n = 22$  trials) had normal behavior, but *pyx<sup>Df9</sup>* ( $n = 24$  trials) flies were defective compared with the *w<sup>1118-WLS</sup>* control ( $n = 15$  trials). D/L Ratios were analyzed by ANOVA ( $\alpha$  level = 0.05) and post hoc test of Games-Howell. Data are mean  $\pm$  SEM. \*, significant difference from the control.

that *pain<sup>Gal4</sup>*-labeled neurons and *pyx-Gal4*-labeled cap cells might assemble in the same scolopidia (4).

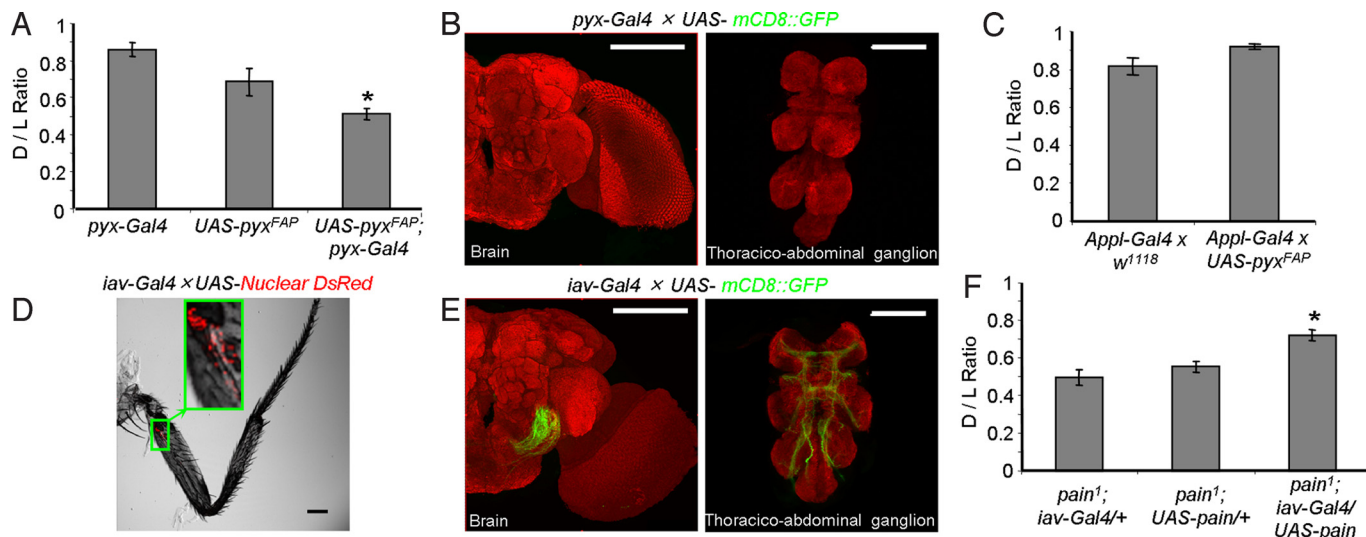
**Specific TRP Channels Are Required for Normal Negative Geotaxis Behavior.** To determine whether any of these TRP channels contribute to gravity sensing, we tested mutants (Table S1) for negative geotaxis. We found that a *nompC* mutant (*nompC<sup>f00642</sup>*, Note S1 and Fig. S2), behaved like wild-type controls, showing a preference for upward movement in the tube-climbing assay (Fig. 3 A and B and Fig. S3) and vertical choice maze (Fig. S1). These data suggest that this TRP channel is not required for negative geotaxis behavior and are consistent with the prediction that gravity detection would be independent of *nompC* (1). In contrast, mutants of *pain* and *pyx* were impaired in negative geotaxis (Fig. 3 A and B and Fig. S1). Likewise, *nan* and *iav* mutants showed defective negative geotaxis, although their general locomotion was reduced as indicated by a reduced climbing score in light conditions (Fig. 3 A and B, Note S2, and Fig. S1).

The *pyx* gene has at least 2 transcripts of differing length—both encode channel subunits, but only the long transcript encodes 9 ankyrin repeats at the N terminus (24). The *pyx<sup>3</sup>* mutation eliminates both transcripts and disrupted negative geotaxis (Fig. 3 A and B). *pyx<sup>Df9</sup>* selectively eliminates the long transcript disrupted negative geotaxis (Fig. 3 C and D). In contrast, the *pyx<sup>Df4</sup>* mutation, which only eliminates the short transcript, had normal behavior. These data suggest that the Pyx channel requires the ankyrin repeats for its role in negative geotaxis.

We tested whether the geotaxis defect of *pyx* and *pain* mutants arose from loss of their function in Johnston's organ. When *pyx-Gal4* drove expression of a dominant-negative *pyx* (*UAS-pyx<sup>FAP</sup>*, Note S3), it mimicked the geotaxis defect of *pyx<sup>3</sup>* mutants (Fig. 4A). Of note, *pyx-Gal4* labeled cap cells in Johnston's organ (Fig. 2A and C), but it did not label the CNS (Fig. 4B). In contrast, expressing *pyx<sup>FAP</sup>* with the pan-neuronal *Appl-Gal4* driver failed to alter negative geotaxis (Fig. 4C). These results imply that Pyx channel function in Johnston's organ cap cells is important for negative geotaxis. To manipulate *pain* function specifically in the sensory system, we used *iav-Gal4* whose expression was restricted to chordotonal neurons of Johnston's organ (Fig. 2A) and femoral chordotonal organs (Fig. 4D). These sensory neurons project their axons to the CNS (Fig. 4E), but we did not detect *iav-Gal4* activity in CNS neurons with the *UAS-Nuclear DsRed* reporter. When *iav-Gal4* drove expression of *UAS-pain* (encoding the *pain* cDNA), we rescued the *pain<sup>1</sup>* geotaxis defect (Fig. 4F). Although these data cannot exclude a role for *pain* in femoral chordotonal organs, they indicate that the geotaxis defect of *pain* mutants is due to abnormal transduction in the sensory system.

**TRP Mutations Impaired the Electrophysiological Response of the Antenna to Changes in Body Position.** To more directly test whether these TRP channels contribute to gravity detection in Johnston's organ, we developed an electrophysiological assay. Prior studies in insects have shown that controlled body rotations mimic gravity-sensing experiences (28, 29). We therefore recorded neural activity of Johnston's organ while the fly body was rotated (Fig. 5A and Fig. S4). The extracellular recording electrode was placed at the junction between the first and second antennal segments (Fig. 5A Inset), a position allowing us to capture most of the action potentials generated by Johnston's organ chordotonal neurons (19). Rotating the fly in all 3 orthogonal axes—pitch, roll, and yaw—triggered spiking activity in wild-type flies (Fig. 5B–D). Reverse movements that returned the body to its original orientation evoked similar spiking activity. The responses were transient, coincided with the rotation event ( $\approx 0.5$  s), and were reproducible with repeated rotations (Fig. 5B). A pitch of 90° from horizontal to an upward vertical orientation evoked  $\approx 20$  spikes in *Canton-S* flies (Fig. 5B expanded trace and G). When we restricted antennal movements by gluing the third antennal segment, we abolished rotation-evoked spikes (Fig. 5E). Removing the glue partially reversed the effect. *iav<sup>3621</sup>* and *nan<sup>36a</sup>* mutations eliminated most of the electrophysiological response to a 90° pitch (Fig. 5F and G). These results suggest that *Iav* and *Nan* channels may serve an essential function in the response of chordotonal neurons to gravity. Likewise, body rotations elicited fewer spikes in *pain<sup>Gal4</sup>* and *pyx<sup>3</sup>* flies than in congenic controls (Fig. 5F and G). *pain<sup>1</sup>* flies were statistically indistinguishable from *Canton-S* flies, consistent with the observation that they were also less defective than *pain<sup>Gal4</sup>* flies in the climbing tube assay (Fig. 3 A and B).

***pain* and *pyx* Mutations Do Not Impair Hearing.** Earlier data showing that *nan* and *iav* mutations impair hearing (21, 22) combined with our results indicate that these channels are essential for both geotaxis and hearing. To learn whether the other TRP channels that are expressed in Johnston's organ also influence both mechanosensory modalities, we assayed hearing (Fig. 6A). The *nompC<sup>f00642</sup>* mutation greatly reduced sound-evoked antennal responses (Fig. 6B). These results were expected based on data from other *nompC* mutants (19) and serve as a positive control. Conversely, whereas *pain* mutants manifest defective negative geotaxis, their sound-evoked potentials were normal. Likewise, the *pyx<sup>3</sup>* mutation had only minimal effects on sound-evoked potentials. These results suggest that specific TRP channels play distinct roles in signaling sound and gravity.



**Fig. 4.** Function of *pain* and *pyx* in peripheral sensory tissues is key for negative geotaxis. (A) Expression of the dominant negative *UAS-pyx<sup>FAP</sup>* driven by *pyx-Gal4* disrupted negative geotaxis in the climbing assay. Note that in the 3 genotypes each transgene was in homozygous state. \*, significant difference from *pyx-Gal4* based on ANOVA ( $\alpha$  level = 0.05) and post hoc test of Games Howell (ANOVA).  $n = 10$  trials in each group. (B) *pyx-Gal4* did not drive *mCD8::GFP* reporter expression in the brain or thoraco-abdominal ganglion. CNS tissues were stained with the nc82 antibody to visualize neuropil (red) and anti-GFP antibody to visualize the reporter. (Scale bars, 100  $\mu$ m.) (C) Expression of *UAS-pyx<sup>FAP</sup>* driven by *Appl-Gal4* (a pan-neuronal Gal4 driver) had no effect on negative geotaxis.  $n = 9$  trials in each group. (D) In the leg, nuclear *DsRed* expression driven by *iav-Gal4* was only detected in femoral chordotonal organs. Inset shows a cluster of nuclei of chordotonal neurons. (E) *iav-Gal4* did not drive *mCD8::GFP* expression in central neurons, but anti-GFP staining was present in projections of sensory afferents from Johnston's organ and leg chordotonal neurons (green). (Scale bars, 100  $\mu$ m.) (F) Expression of *UAS-pain* under the control of *iav-Gal4* restored negative geotaxis. \*, significant difference from each of the 2 controls, based on ANOVA ( $\alpha$  level = 0.05) and post hoc test of Games Howell.  $n = 39$  trials for *pain<sup>1</sup>; iav-Gal4/+*,  $n = 35$  trials for *pain<sup>1</sup>; UAS-pain/+*, and  $n = 39$  trials for *pain<sup>1</sup>; iav-Gal4/UAS-pain*. Data are mean  $\pm$  SEM.

## Discussion

Our data show that several TRP superfamily channels in Johnston's organ are required for *Drosophila* to respond to gravity. *nan* and *iav* are expressed in chordotonal neurons throughout Johnston's organ, and their mutation disrupted both hearing and geotaxis. Previous studies of hearing suggested that Nan and Iav form a TRPV channel that acts downstream of the primary mechanotransducer and enhances the relay of excitatory signals toward the cell body (20). We speculate that they may serve a similar role in gravity sensing.

In contrast to *nan* and *iav*, *pain* expression was limited to a subset of chordotonal neurons, and its mutation disrupted negative geotaxis, but not hearing. Might the Pain channel be a mechanosensor? This possibility is intriguing because the Pain channel also mediates mechanical nociception in fly larvae (25). However, *pain* also contributes to thermal and chemical nociception in larvae (25) and heat-induced currents when expressed in heterologous cells (30), and thus a specific role in detecting mechanical stimuli remains speculative.

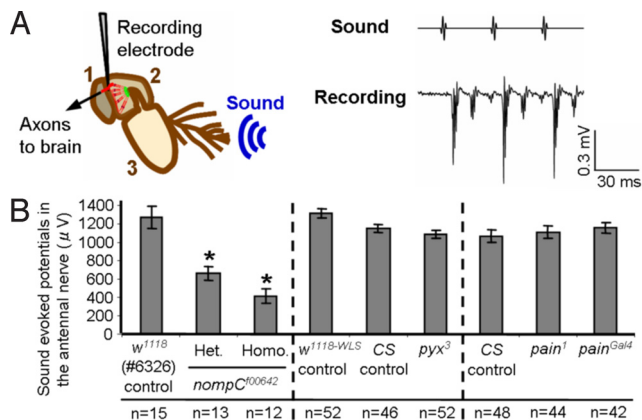
The contribution of *pyx* to geotaxis was distinct from the other TRP subunits by its expression in cap cells rather than neurons. Cap cells form structural links between chordotonal neurons and the moving joint between second and third antennal segments. Interestingly, in the cap cells of the katydid *Caedicia simplex* auditory sensilla, acoustic stimuli generate a slow, graded hyperpolarizing membrane potential and a train of fast, biphasic spikes, and the spikes correlated temporally with depolarizing spikes in the chordotonal sensory neurons (31). Those results raise the possibility that in *Drosophila* gravity perception cap cells and their Pyx channels might actively respond to motion and participate in mechanosensory signaling. In addition, cap cells in *Drosophila* larval chordotonal organs contain numerous aligned microtubules (6, 32–34), and motility of those microtubules is thought to modulate tension in the chordotonal organ (4). Thus, another possibility is that Pyx channels trigger contraction or

microtubule motility in Johnston's organ cap cells to influence gravity signals.

We found that pitch, roll, and yaw in either direction triggered action potential firing from Johnston's organ. Once the rotation stopped, the electrical activity returned to basal levels. Interestingly, recent data indicated that continuous mechanical displacement of the arista to mimic the effect of gravity (or wind) induced a tonic increase in intracellular  $Ca^{2+}$  concentration,  $[Ca^{2+}]_i$ , in some Johnston's organ neurons (1, 8). Methodological factors likely explain the apparent difference in phasic vs. tonic responses. First, the dynamic level of  $[Ca^{2+}]_i$  may not precisely predict the timing of action potentials in a neuron, and thus phasic action potential firing is not necessarily inconsistent with a tonic elevation of  $[Ca^{2+}]_i$ . Second, in our study body rotations mimicked real-life experience of a fly moving in the gravitational field, and the antennal receiver (third segment including the arista) was free to respond to transient angular accelerations accompanying body rotations. Elastic properties of the antenna and muscle control of antennal movement might have played a role in our studies. In studies measuring  $[Ca^{2+}]_i$  (1, 8), a probe statically controlled the position of the antennal receiver, and the first and second antennal segments were immobilized to prevent muscle-based antennal movement. In the future, a comparison between  $[Ca^{2+}]_i$  signals and action potential firing in the same experimental setting may yield a better understanding of how Johnston's organ codes sensory information of gravity, acceleration, and orientation.

Our study also has limitations. First, a caveat to our gene expression data are use of promoter-Gal4 transgenic constructs. A putative enhancer/promoter fragment may not contain the complete information to precisely reproduce endogenous gene expression. Moreover, the location of a transgene in the fly genome may affect expression patterns and levels. In the future, it will be desirable to examine the anatomical and subcellular localization of these TRP channels with specific antibodies. Second, the *nompC* and *pain* lines were hypomorphs rather than





**Fig. 6.** The *nompC* but not the *pain* and *pyx* mutations disrupted auditory responses. (A) Schematic of the auditory recording method. Near-field sound, mimicking the *Drosophila* courtship song was delivered to the antenna. An extracellular electrode positioned as in Fig. 5A recorded sound-evoked potentials (SEP) in the axons of Johnston's organ. A sample trace from a wild-type fly is shown. (B) Amplitude of SEPs in *pain*<sup>1</sup>, *pain*<sup>Gal4</sup>, *pyx*<sup>3</sup>, and *nompC*<sup>F00642</sup> flies. *nompC*<sup>F00642</sup> heterozygous (het.) and homozygous (homo.) flies are shown. \*, difference from control by ANOVA ( $\alpha$  level = 0.05) and post hoc test of Games-Howell. The number (n) of antennae recorded in each group is shown underneath the corresponding genotype. Data are mean  $\pm$  SEM.

extracellularly from the antennal nerve. A similar, reference electrode was inserted into the dorsal side of the head. Raw differential signals were amplified and digitized as previously described. Each antenna was given 10 consecutive auditory stimuli. Amplitudes are the average of the 10 recordings. Flies of different genotypes were recorded in alternate order to minimize systematic variations.

Responses of Johnston's organ to body rotations were also recorded ex-

tracellularly (see Fig. 5A for a diagram for the recording apparatus). A living female fly ( $\approx$ 1 week old) was placed into a cut plastic pipette tip, with the fly head protruding from the tip. A piece of wet cotton was placed next to the fly abdomen and connected to an AgCl-coated silver wire as the reference electrode. A sharp tungsten electrode was placed between the first and second antennal segments. Voltage differences between the reference and recording electrodes were amplified by an Axopatch-1D (Axon Instruments). Clampfit 9.0 software (Axon Instruments) was used to analyze spike frequency. The amplitude threshold was set at 2 times the baseline noise level and the number of spikes was counted automatically. Each antenna was stimulated with multiple, manually controlled 90° rotations. The spiking responses occurred immediately after initiation of the rotation and stopped as soon as the rotation ended. The number of spikes was scored as the average of the 2 strongest responses of  $\approx$ 6 trials. Flies of different genotypes were recorded in alternate order. The experimenter was blinded to the genotypes during the recording and subsequent data analysis. To control for possible stimulation by airflow, we placed a chamber over the fly and electrode; we observed no difference in electrophysiological response in the presence or absence of the chamber. In addition, the manually controlled rotation is not accompanied by noise, so contribution of an auditory response to the recording should be negligible (Note S4).

See SI Text for additional materials and methods.

**ACKNOWLEDGMENTS.** We thank T. Kitamoto (University of Iowa) for valuable discussions and sharing fly strains, C. Reddy for help with electrophysiology, Y. Li for invaluable support, and T. O. Moninger for assistance with microscopy. We are also grateful to the following investigators for fly strains: C. Kim (Chonnam National University, Gwangju, Korea) for *nan*<sup>36a</sup>, *jav*<sup>3621</sup>, and *nan-Gal4*; J. Kim (Korea Advanced Institute of Science and Technology, Daejeon, Korea) for *pyx*<sup>3</sup>, *pyx*<sup>Df4</sup>, *pyx*<sup>Df9</sup>, and *pyx*<sup>Ge4</sup> *pyx*<sup>3</sup>; S. Benzer (California Institute of Technology, Pasadena, CA) W. D. Tracey, Jr. (Duke University, Durham, NC) and B. Al-Anzi (California Institute of Technology, Pasadena, CA) for *pain*<sup>1</sup>, *pain*<sup>Gal4</sup>, and *UAS-pain*; M. J. Kernan (Stony Brook University, Stony Brook, NY) for *GFP-nompA*; and R. S. Hewes (University of Oklahoma, Norman) for *Appl-Gal4*. We thank J. Hoang, K. Crose, and J. Berge for help with fly husbandry and general laboratory assistance. This work was partially supported by National Institutes of Health Grant DC04848 (to D.F.E.). Y.B.S. was an Associate and M.J.W. is an Investigator of the Howard Hughes Medical Institute.

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