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Report

NompC TRP Channel Is Essential for *Drosophila* Sound Receptor Function

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Summary

The idea that the NompC TRPN1 channel is the *Drosophila* transducer for hearing has been challenged by remnant sound-evoked nerve potentials in *nompC* nulls [1–5]. We now report that NompC is essential for the function of *Drosophila* sound receptors and that the remnant nerve potentials of *nompC* mutants are contributed by gravity/wind receptor cells. Ablating the sound receptors reduces the amplitude and sensitivity of sound-evoked nerve responses, and the same effects ensued from mutations in *nompC*. Ablating the sound receptors also suffices to abolish mechanical amplification, which arises from active receptor motility [6, 7], is linked to transduction [8], and also requires NompC [9]. Calcium imaging shows that the remnant nerve potentials in *nompC* mutants are associated with the activity of gravity/wind receptors and that the sound receptors of the mutants fail to respond to sound. Hence, *Drosophila* sound receptors require NompC for mechanical signal detection and amplification, demonstrating the importance of this transient receptor potential channel for hearing and reviving the idea that the fly's auditory transducer might be NompC.

Results and Discussion

Ever since NompC (also known as TRPN1) was implicated in *Drosophila* touch sensation [10], it has been speculated that this transient receptor potential (TRP) channel could be one of the elusive transduction channels for hearing [2–5, 11]. First, bearing a predicted pore region and an N-terminal ankyrin spring, NompC seems structurally qualified for being a gating spring-operated ion channel as implicated in auditory transduction [12–17]. And second, though displaying a rather spotty phylogenetic appearance [18], NompC is required for the function of certain *Drosophila* and nematode mechanoreceptors [10, 19–21] and zebrafish hair cells [22]. NompC is also expressed in hair cells of frogs [23] and in mechanoreceptors of the *Drosophila* ear [24–27], but even though NompC demonstrably can serve as mechanotransduction channel [21], its importance for auditory transduction and hearing remains uncertain: in frog hair cells, NompC localizes to kinocilia [23] that are dispensable for transduction [28]. And in the *Drosophila* ear, loss of *nompC* function reduces the amplitude of sound-evoked afferent nerve responses by only approximately one-half [1, 25].

A possible explanation for the mild latter effect has emerged with the recent discovery that the antennal hearing organ of *Drosophila*, Johnston's organ (JO), houses sound and gravity/wind receptors: about half of the fly's approximately 480 JO receptor cells preferentially respond to dynamic antennal vibrations and serve sound detection, whereas the other half preferentially respond to static antennal deflections and mediate the detection of gravity and wind [24, 25, 29]. Driving reporter genes via a *nompC-Gal4* promoter fusion construct only labeled the sound receptors [24], suggesting that the sound-evoked nerve potentials that persist in *nompC* mutants may be contributed by *nompC*-independent JO gravity/wind receptor cells [5, 24, 30]. *nompC-Gal4*, however, reproduces endogenous *nompC* expression only partially, and an antibody detected NompC protein in virtually all receptors of JO [26]. To explore whether the two JO receptor types nonetheless differ in their *nompC* dependence, we here analyzed JO function in *nompC* mutants and in flies with ablated sound or gravity/wind receptor cells.

To selectively ablate JO sound or gravity/wind receptors, we expressed *UAS-ricin toxin A* [31] in these cells using receptor type-specific GAL4 drivers [24, 32, 33] in conjunction with the ey-FLP/FRT system [34] to restrict toxin expression to GAL4-expressing cells in the antenna and eye. To assess JO function, we exposed the flies to pure tones of different intensities and simultaneously monitored the resulting mechanical input and electrical output of JO. The mechanical input was measured as sound-induced displacement of the antenna's arista [35, 36], whereas the electrical output was recorded in the form of sound-evoked compound action potentials (CAPs) from the receptor axons in the antennal nerve [37]. The frequency of the tones was adjusted to the mechanical best frequency of the antenna, which was deduced from the power spectrum of the antenna's free fluctuations [9, 36] (see Figure S1 available online). The intensity of the tones was measured as the sound particle velocity at the position of the fly [35, 36].

Residual Sound-Evoked Nerve Potentials in *nompC* Mutants and Flies with Ablated Sound Receptor Cells

In accord with previous observations [1], we found that remnant sound-evoked nerve potentials persist in *nompC* nulls: varying the sound particle velocity between approximately 0.001 and 10 mm/s evoked CAPs in $nompC^{2}$ and $nompC^{3}$ null mutants whose maximum amplitudes were ~6 times smaller than those of the wild-type and controls (Figure 1A). Mutant flies carrying the weaker allele $nompC^{4}$ displayed equally reduced CAP amplitudes, but the amplitudes were normal when we expressed a *UAS-nompC-L* rescue construct [20] in all JO receptors of $nompC^{3}$ nulls (Figure 1A). Reduced CAP amplitudes as observed in *nompC* mutants also ensued from the targeted ablation of JO sound receptors (Figure 1A). When JO gravity/wind receptors were ablated, however, CAP amplitudes remained normal, resembling those of wild-type flies and controls (Figure 1A). Hence, sound-evoked potentials in the fly's antennal nerve are not only contributed by JO sound receptors: if these receptors are ablated, residual CAPs persist whose amplitudes resemble those of *nompC* nulls.

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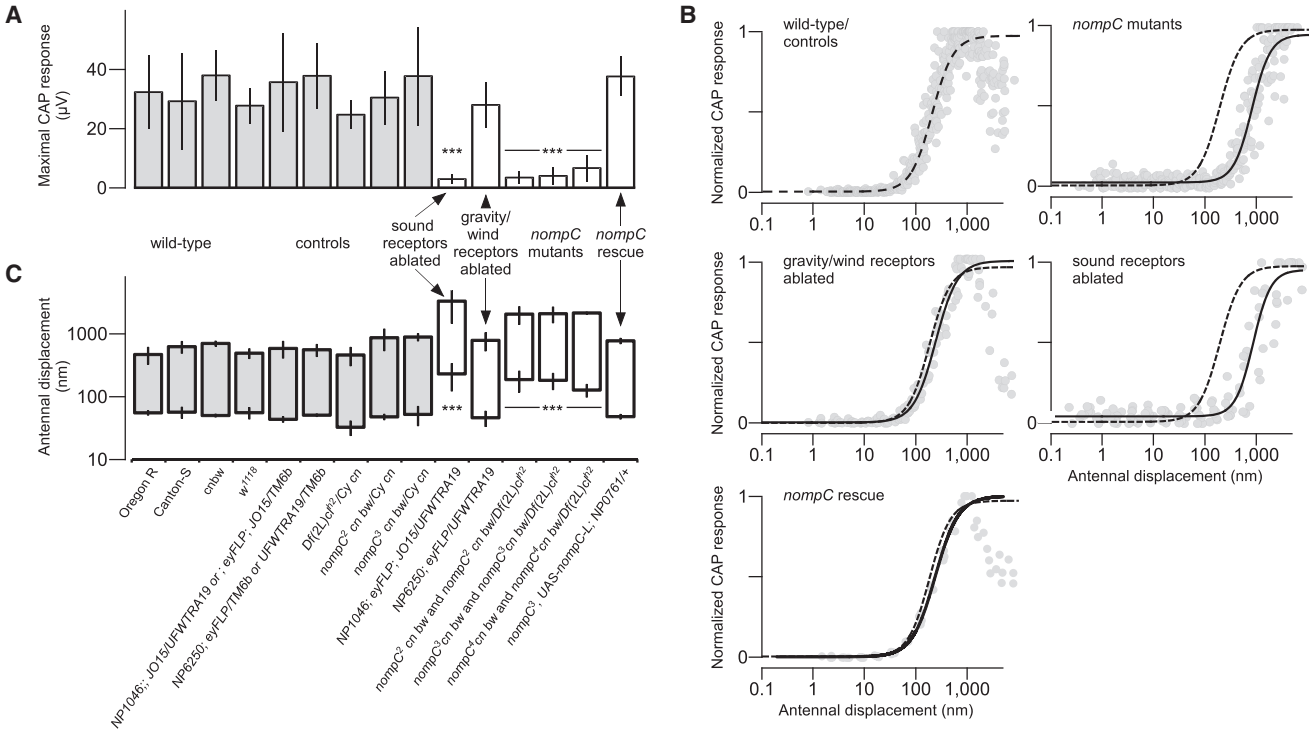

Figure 1. Sound-Evoked Antennal Nerve Potentials

(A) Maximum compound action potential (CAP) amplitudes (means ± 1 standard deviation [SD]) evoked by pure-tone stimulation at the antenna's mechanical best frequency with particle velocities between approximately 0.001 and 10 mm/s (Figure S2).

(B) Relative CAP amplitudes as functions of the tone-induced antennal displacement (lin-log). Above threshold, CAP amplitudes monotonously increase with the antennal displacement and, upon reaching a maximum, redecrease (for corresponding simulations, see [8]). Relative voltage (V) amplitudes of the CAPs were calculated as $(V - V_{min})/(V_{max} - V_{min})$. Solid lines represent Hill functions fitted to the increasing slope of the CAPs. The fit obtained for wild-type and controls flies (dashed line) is repeated in each panel to facilitate comparisons.

(C) Dynamic ranges of the CAPs, deduced from the fits in (B) (means ± 1 SD, log scaling). Dynamic ranges are given as antennal displacements spanning 5%–95% of the maximum values of the fits in (B).

***$p < 0.05$ by two-tailed Mann-Whitney U test against control and wild-type strains (n = 4–20 receivers per strain).

Flies with Ablated Sound Receptors and *nompC* Mutants Lack Sensitive Hearing

Mutations in *nompC*, in addition to reducing sound-evoked nerve potentials, impair sensitive hearing. This reduction in auditory sensitivity became apparent when we plotted the relative CAP amplitudes against the corresponding sound-induced antennal displacement (Figure 1B). In wild-type and control flies, antennal displacements equal to or greater than ~50 nm were sufficient to elicit CAPs, and the CAP amplitude increased monotonously for displacements between approximately 50 and 600 nm (Figures 1B and 1C). In *nompC* mutants, this dynamic range of the CAP response consistently shifted up to antennal displacements between approximately 160 and 2000 nm, corresponding to an ~3-fold sensitivity drop (Figures 1B and 1C). This sensitivity drop, which was rescued by expressing *UAS-nompC-L* in the JO receptors of $nompC^3$ mutants, was also observed in flies with ablated JO sound receptor cells (Figures 1B and 1C). When the gravity/wind receptors were ablated, however, auditory sensitivity remained unchanged (Figures 1B and 1C).

When we plotted the relative CAP amplitudes against the sound particle velocity instead of the antennal displacement, the sensitivity drop observed in *nompC* mutants and flies with ablated sound receptors was even more pronounced, assuming figures around 10 (Figure S2): in these flies, the dynamic range of the CAPs spanned particle velocities between approximately 0.4 and 6 mm/s, whereas it spanned between approximately 0.03 and 1 mm/s in flies with ablated gravity/wind receptors, wild-type flies, and controls. Accordingly, loss of *nompC* function and loss of sound receptor function reduce both the sensitivity of JO to antennal displacements and, in addition, the mechanical sensitivity of the antenna to sound.

Mechanical Amplification of Antennal Vibrations Requires NompC and Sound Receptor Cells

To assess the mechanical sensitivity of the antenna, we determined how its displacement varies with sound intensity. In wild-type and control flies, the antenna's displacement nonlinearly increased with sound particle velocity (Figure 2A), displaying a compressive nonlinearity that, arising from mechanical activity of JO receptors [8, 9], enhanced the mechanical sensitivity ~8-fold when sound was faint (Figure 2B). Consistent with previous observations [9], we found that this nonlinear mechanical amplification was lost in *nompC* mutants, rendering their antennae mechanically less sensitive to acoustic stimuli so that louder sounds were required to displace their antennae by a given distance, in addition to the larger antennal displacements that were required to elicit CAPs in their antennal nerves (Figures 1B and 1C; Figure S2). We also found that this nonlinear amplification could be rescued by expressing *UAS-nompC-L* in JO receptors and

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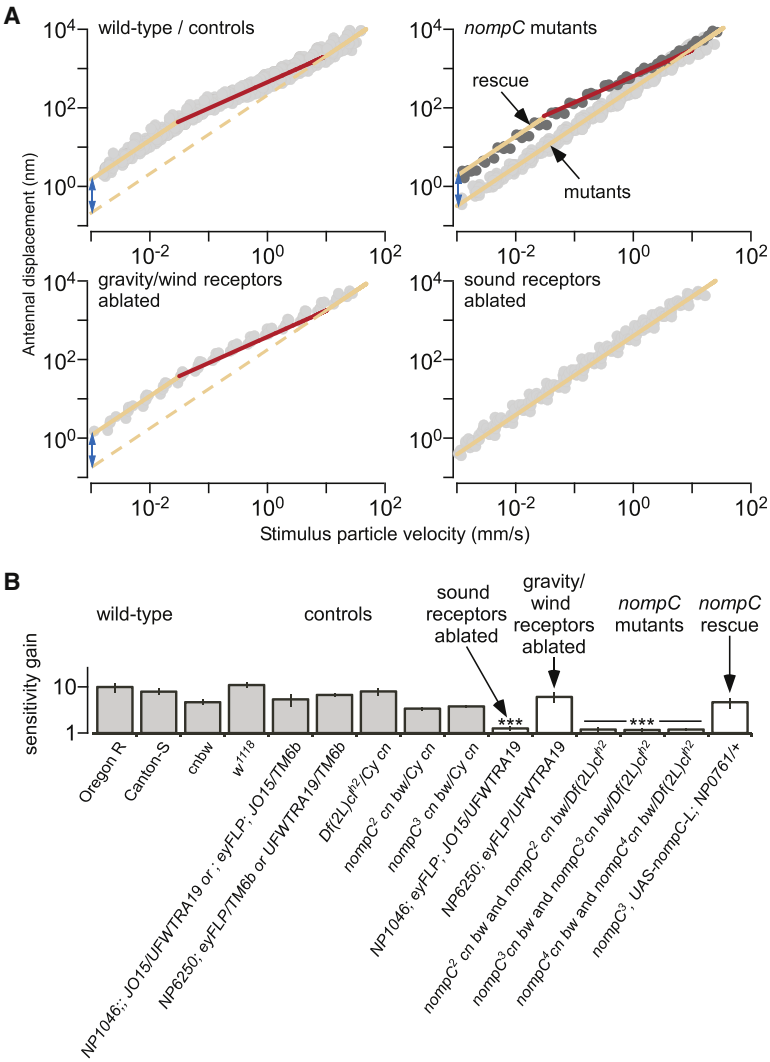

Figure 2. Nonlinear Amplification of Antennal Vibrations

(A) Tone-evoked antennal displacement as a function of the particle velocity of the tones (log-log). Lines indicate linear (peach) and nonlinear (red) regimes. Blue arrows indicate nonlinear sensitivity gain, determined as the amplitude ratio between the upper and lower linear regimes.

(B) Nonlinear sensitivity gain (means ± 1 SD, log scaling) deduced from the data in (A). A gain of 1 indicates the absence of amplification. ***$p < 0.05$ by two-tailed Mann-Whitney U test against control and wild-type strains (n = 4–20 receivers per strain).

that it specifically required JO sound receptor cells: ablating only the sound receptors abolished mechanical amplification, and the same effect was caused by mutations in *nompC* (Figures 2A and 2B). In *nompC* mutants, this loss of amplification was associated with alterations of the antenna's tuning and fluctuation power that were quantitatively mimicked in flies with ablated sound receptor cells (Figure S1). If the gravity/wind receptors were ablated, however, mechanical amplification remained normal, with the antenna's compressive nonlinearity, its tuning, and its fluctuation power resembling those of wild-type, *nompC-L* rescue, and control flies (Figure 2A; Figure S1). Hence, nonlinear mechanical amplification in the *Drosophila* ear requires both the NompC channel and JO sound receptors but is independent of JO gravity/wind receptor cells.

Sound Receptors, but Not Gravity/Wind Receptors, Need NompC

Ablating JO sound receptors phenocopies the auditory defects of *nompC* mutants (Figure 1; Figure 2), suggesting that NompC is essential for the mechanosensory function of these cells. To test this hypothesis, we monitored mechanically evoked calcium signals in the somata of JO receptors of $nompC^3$ null mutants and controls while simultaneously recording the displacement of the antenna and the ensuing CAPs from the antennal nerve. Calcium signals were measured through the cuticle of the antenna using the genetically encoded ratiometric calcium sensor Cameleon2.1 (Cam2.1) [24, 38, 39]. To evoke calcium signals, we sinusoidally actuated the antenna at its mechanical best frequency with electrostatic force (for the equivalence of electrostatic and acoustic actuation, see [37]).

When we expressed Cam2.1 in either the sound receptors alone or all JO receptors, antennal vibrations evoked robust calcium signals in controls (Figure 3A). The calcium signals of the sound receptors were entirely abolished in $nompC^3$ mutants, but when we expressed Cam2.1 in all of their JO receptors, small calcium signals were detected that closely resembled those of the gravity/wind receptors of controls (Figure 3A). To assess the relation between JO calcium signals and antennal nerve potentials, we plotted their respective amplitudes against the antennal displacement (Figures 3B and 3C). The large calcium signals of the sound receptors of controls superimposed with the relative amplitudes of the simultaneously recorded CAPs and the CAPs of flies with ablated gravity/wind receptor cells (Figure 3B). The small calcium signals of the gravity/wind receptors were shifted to larger antennal displacements and superimposed with the CAPs of flies with ablated sound receptor cells (Figure 3B). Calcium signals obtained from all JO receptors of controls had intermediate amplitudes (Figure 3B), identifying them as mixed signals contributed by sound and gravity/wind receptor cells (Figure S3A). The residual CAPs of $nompC^3$ mutants did not associate with calcium signals in their sound receptors, yet they superimposed with the small calcium signals obtained from all JO receptors of the mutants and from JO gravity/wind receptors of the controls (Figure 3C). Although unsuccessful recombination prevented us from selectively expressing Cam2.1 in the gravity/wind receptors of the mutants, the above findings show that calcium signals that can be ascribed to these receptors are associated with the residual CAPs in *nompC* nulls. Additional evidence that the calcium signals in the mutants arise from gravity/wind receptors was obtained when we inspected the time course of these signals (Figure 3D): in controls, the onset of the calcium signals of all JO receptors followed two exponentials. The exponential with the larger time constant well fitted the calcium signals of their sound receptors. The exponential with the smaller time

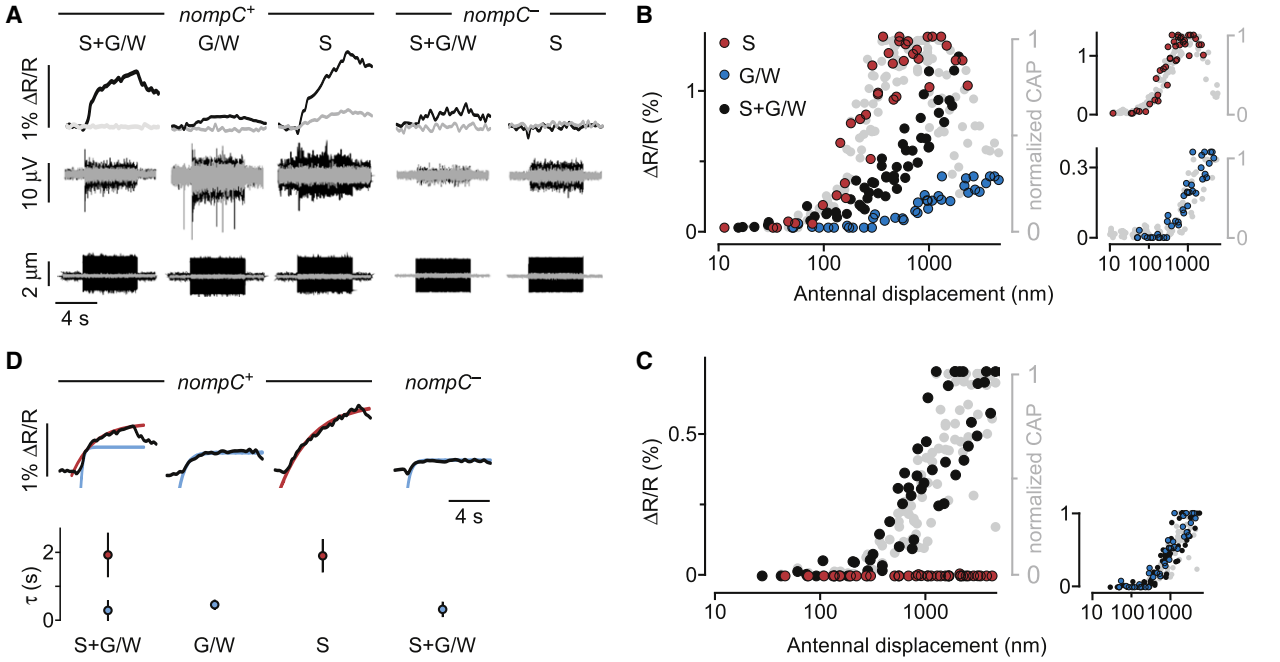

Figure 3. Mechanically Evoked Calcium Responses Obtained by Expressing Cameleon2.1 in JO Sound Receptors, JO Gravity/Wind Receptors, and All JO Receptors

The following abbreviations are used: S, JO sound receptors; G/W, JO gravity/wind receptors; S+G/W, all JO receptors.

(A) Antennal vibrations (bottom), CAPs (middle), and calcium signals (top) evoked by weak (gray traces) and strong (black traces) sinusoidal stimulation at the antenna's mechanical best frequency in *nompC*^{3} mutants (*nompC*$^{-}$) and controls (*nompC*$^{+}$). Calcium signal amplitudes represent changes in the Cam2.1 eYFP/eCFP fluorescence ratio, where R is the average ratio before the stimulus and ΔR is the deviation from R (means of ten repetitions).

(B) Left: calcium signal amplitudes as a function of antennal displacement in *nompC*$^{+}$ flies. Calcium signal amplitudes were measured as asymptotic values of exponential fits. For an explanation of the intermediate calcium signal amplitudes of all JO receptors, see Figure S3A. Gray symbols indicate relative amplitudes $[(V - V_{min})/(V_{max} - V_{min})]$ of the simultaneously measured CAPs. Upper right: calcium signals of the sound receptors superimposed with the CAPs (gray symbols) of flies with ablated gravity/wind receptor cells. Bottom right: calcium signals of the gravity/wind receptors superimposed with the CAPs of flies with ablated sound receptor cells.

(C) Left: calcium signal amplitudes as a function of antennal displacement in *nompC*$^{-}$ flies (color code as in B). Gray symbols indicate relative amplitudes $[(V - V_{min})/(V_{max} - V_{min})]$ of the simultaneously measured CAPs. Right: relative calcium signal amplitudes of all JO receptors of the mutants superimposed with those of JO gravity/wind receptors of controls and the normalized CAPs (gray symbols) of flies with ablated sound receptor cells.

(D) Exponentials fitted to the calcium signals (top) and corresponding time constants (bottom).

For (B)–(D), n = 4–20 receivers per strain.

constant well fitted the calcium signals of their gravity/wind receptors and also those of *nompC*^{3} nulls. Hence, instead of being contributed by JO sound receptors, the residual CAPs of *nompC* mutants are deemed to reflect the activity of JO gravity/wind receptor cells.

Judged from the intracellular calcium signals, the responses of JO gravity/wind receptors to sinusoidal forcing are independent of NompC. Because these receptors preferentially respond to static forcing [24, 29], we statically deflected the flies' antennae and measured the ensuing calcium signals (Figure S3B). In accord with previous observations [24, 29], JO sound receptors hardly responded to antennal deflections, and the calcium signals obtained from all of the JO receptors of *nompC*^{3} mutants were indistinguishable from those of controls (Figure S3B). Hence, whereas NompC is essential for the mechanosensory function of JO sound receptors, the mechanosensory function of JO gravity/wind receptors seems independent of NompC. Because NompC is detectable in the dendritic tips of virtually all JO receptors [26], other proteins may compensate for the loss of NompC in JO gravity/wind receptors. Possibly, both JO receptor types also use different NompC isoforms, which could also explain why certain *nompC* promoter fusion constructs are selectively expressed in JO sound receptor cells [24]. The isoform NompC-L [20] rescues the auditory defects of *nompC* mutants and accordingly seems crucial for JO sound receptor function. Determining NompC isoform patterns in JO may help understanding why gravity/wind receptors express, but apparently do not need, this TRP.

Conclusions

We have shown that NompC is essential for the mechanosensory function of *Drosophila* sound receptors, making this TRP channel a strong candidate for the fly's auditory mechanotransducer. Precedence that NompC can serve as a mechanotransduction channel comes from work on *C. elegans* [21], and the importance of NompC for *Drosophila* auditory transduction is supported by its requirement for nonlinear mechanical amplification: in the *Drosophila* ear, the source of this amplification has been traced down to mechanotransducers [8] that, judged from the present study, reside in the sound receptors. Loss of amplification in flies with ablated sound receptors and in *nompC* mutants indicates that these auditory transducers require NompC. Clearly, more work is needed to dissect the specific roles of NompC in auditory transduction, and such dissection now seems most worthwhile given the auditory importance of this TRP.

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Experimental Procedures

Flies

The following GAL4 strains were used: JO15 [32] and JO2 (also known as NP1046) [33] for targeting sound receptors, JO31 (also known as NP6250) [33] for targeting gravity/wind receptors, and JO1 (also known as NP0761) [33] for targeting sound and gravity/wind receptors. Other strains used included *UAS-cam2.1* [24, 38, 39] for calcium imaging; *eyFLP* [34] and *UFWTRA19* [31] for ricin-mediated cell ablation; *UAS-nompC-L* [20] for ectopic NompC expression; the deficiency strain *Df(2L)cl^{h2}*; and the *nompC* alleles *nompC^{2}*, *nompC^{3}*, and *nompC^{4}* [10]. Genotypes of the experimental flies were *nompC^{2} cn bw/Df(2L)cl^{h2}* or *nompC^{2} cn bw* (*nompC^{2}* mutants); *nompC^{2} cn bw/Cy cn* (*nompC^{2}* controls); *nompC^{3} cn bw/Df(2L)cl^{h2}* or *nompC^{3} cn bw* (*nompC^{3}* mutants); *nompC^{3} cn bw/Cy cn* (*nompC^{3}* controls); *nompC^{4} cnbw/Df(2L)cl^{h2}* or *nompC^{4} cn bw* (*nompC^{4}* mutants); *nompC^{3};UAS-NompC-L/nompC^{3};NP0761/+* (*nompC* rescue); *NP1046; eyFLP/+;JO15/UFWTRA19* (ablation of sound receptors); *NP6250/+;eyFLP/ UFWTRA19* (ablation of gravity/wind receptors); *UAS-cam2.1;JO15/TM6b* (*cam2.1* expression in sound receptors); *NP6250;UAS-cam2.1* (*cam2.1* expression in gravity/wind receptors); *UAS-cam2.1;NP0761/TM6b* (*cam2.1* expression in all JO receptors); *nompC^{3};JO15/UAS-cam2.1* (*cam2.1* expression in JO sound receptors in *nompC^{3}* background); and *nompC^{3};NP0761/ UAS-cam2.1* (*cam2.1* expression in all JO receptors in *nompC^{3}* background). Cell ablations were confirmed by coexpressing a *UAS-GFP* reporter as described previously [24].

Antennal Vibrations and CAPs

Antennal vibrations were evoked acoustically or electrostatically via an external electrode placed behind the tip of the antennal arista [8, 24, 37]. Sound particle velocities were accessed with an Emkay NR 3158 pressure-gradient microphone as described previously [36]. Antennal displacements were monitored at the tip of the antenna's arista with a Polytec PSV-400 laser Doppler vibrometer equipped with an OFV-700 closeup unit (70 mm focal length) [36]. CAPs were recorded with an electrolytically tapered tungsten electrode inserted between antenna and head, with the indifferent electrode being placed in the thorax [37]. Signals were digitized at a rate of 12.1 kHz and subjected to fast Fourier transforms (1 Hz frequency resolution). Signal amplitudes were measured as Fourier amplitudes at the frequency of stimulation. Only CAP amplitudes were measured at twice the stimulus frequency because of their frequency doubling [37]. Data analysis and statistical data evaluation were performed using PSV-VIB (Polytec), Spike 2 (Cambridge Electronic Design), Excel 2004 (Microsoft), and SigmaPlot 10 (Systat Software).

Calcium Signals

Transcuticular imaging of intracellular calcium signals was performed as described previously [39]. A Cameleon two-filter set (455 nm DCLP, 515 nm DCLP, 535/30 nm emission filter, 485/40 nm emission filter; Chroma Technology) and a dual view beam splitter (Photometrics DV2) were used for detecting the eYFP and eCFP images simultaneous with a charge-coupled device camera (Photometrics Cascade II:512).

Supplemental Information

Supplemental Information includes three figures and can be found with this article online at doi:10.1016/j.cub.2011.02.048.

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