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ORIGINAL PAPER

Mechanosensitivity in the model sea anemone *Nematostella vectensis*

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Abstract Tentacles of the sea anemone, *Nematostella vectensis*, are covered with hair bundles. Hair bundles were deflected by water jets to test whether they are mechanoreceptors. Electrophysiological recordings confirm that deflections of hair bundles induce transients in membrane current. In a different species of anemone, hair bundle mechanoreceptors are known to change shape and responsiveness according to the activity of chemoreceptors that bind prey-derived compounds including *N*-acetylated sugars. In *Nematostella*, hair bundles significantly elongate upon exposure to NANA, an *N*-acetylated sugar. Based on a bioassay in which discharged nematocysts are counted in gelatin-coated test probes touched to tentacles, we find that NANA shifts vibration dependent discharge of basitrich nematocysts to lower frequencies overlapping those produced during swimming by known prey including planktonic crustaceans. Furthermore, we find for the first time that vibration detection extends at least 2.5 cm beyond the tentacle tips. Thus, *Nematostella* likely employs its hair bundles to detect swimming movements of nearby prey.

Introduction

The sea anemone, *Nematostella vectensis*, has emerged as a model organism for investigations of the evolutionary origin of genes critical to development of vertebrate animals (Darling et al. 2005; Sullivan et al. 2006). For example, genes for mesodermal structures are present in its genome despite the absence of mesoderm (Martindale et al. 2004). Furthermore, PAX genes employed by vertebrates in eye and brain development are present in the genome of *Nematostella* (Matus et al. 2007). In light of growing scientific interest in *Nematostella*, we wondered about its sensory biology. For more than 20 years, we have studied hair bundle mechanoreceptors in the sea anemone, *Haliplanella lineata* (Watson and Hessinger 1989). Hair bundle mechanoreceptors located on the tentacles of *Haliplanella* are employed to detect the swimming movements of planktonic prey. Hair bundles on tentacles of *Haliplanella* are remarkably similar to those of the acousticolateralis system of vertebrates in terms of their structure and sensitivity to pharmacological agents including aminoglycoside antibiotics (Watson et al. 1997). Nevertheless, the hair bundles of *Haliplanella* have some intriguing features that set them apart from those of the acousticolateralis system of vertebrates. In particular, hair bundles in *Haliplanella* are morphodynamic such that they change shape and responsiveness according to the activity of specific chemoreceptors (Mire-Thibodeaux and Watson 1994). According to the model, *N*-acetylated sugars derived from mucins diffuse from prey and bind to chemoreceptors on the tentacles. The activated chemoreceptors induce the hair bundles to elongate in a process that depends on polymerization of actin (Mire-Thibodeaux and Watson 1994). Presumably, the elongated hair bundles resonate at lower frequencies to shift responsiveness to lower frequencies overlapping those produced by calmly swimming prey such as planktonic crustaceans (Watson and Hessinger 1989; Watson et al. 1998; Watson and Mire 2004). The anemones respond to vibrations with sharp frequency discrimination. Mechanisms other than resonance may contribute to determining sharp frequency specificity (Watson et al. 1998). According

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to the model, properly stimulated hair bundles generate action potentials in the sensory neuron that are transmitted throughout the nerve net. Such neuronal communication sensitizes nematocytes to discharge nematocysts in the event that the prey swims into contact with the tentacles (Watson and Mire 2004). Thus, according to the model, detection of near field vibrations sensitizes the anemone for the possibility of contact between the prey and tentacle. Although the existence of near-field detection of vibrations is well supported for *Haliplanella* (e.g., Watson and Hessinger 1989; Watson et al. 1998; Watson and Mire 2004), the size of the sensory field is unknown. Furthermore, it is unclear to what extent our previous findings using *Haliplanella* can be generalized to other sea anemones. The purpose of the present study is to determine whether tentacles of *Nematostella* feature hair bundle mechanoreceptors that participate in the regulation of nematocyst discharge. In addition, we aim to determine the range of sensitivity of the anemones to near-field vibrations.

Materials and methods

Specimens of *Nematostella vectensis* were obtained from the Marine Biological Laboratory, Woods Hole, MA, USA. The animals were cultured in natural seawater diluted to $16^{o}/_{oo}$ according to the methods described in Hand and Uhlinger (1992). The animals were fed twice weekly with freshly hatched brine shrimp nauplii. Feeding was followed by a change in seawater.

Nematocyst discharge bioassay

Specimens of *Nematostella* spawned in culture. The anemones were raised to a column length of approximately 2–4 cm. These anemones were tested for vibration dependent discharge of nematocysts according to methods previously described (Watson et al. 1998). Briefly, animals were touched with gelatin-coated test probes constructed from 2 cm segments of fishing line (4–6 lb test) dipped at one end in melted 25% gelatin to form a thin coat. The probes were allowed to dry for 24 h to facilitate retention of the gelatin coating. Test probes were briefly hydrated in seawater containing the anemones then touched to tentacles in the presence of vibrations ranging from 1 to 60 Hz. Vibrations were produced by a digital function generator set to the sine-wave function. The output of the function generator was used to vibrate a piezo disc. A glass probe attached to the piezo disc was immersed into the seawater containing the anemones. Linearity of the resulting vibrations was confirmed over the range of 10–60 Hz using a microphone moved into contact with the vibrating glass probe. Peak frequencies were identified by computer after FFT analysis of the recordings (DSSF3 software, Yoshimasa Electronic Inc., Tokyo, Japan). Because the microphone did not respond to vibrations at frequencies lower than 10 Hz, such movements were analyzed by a stroboscope that had been calibrated using a spinning disc and laser tachometer. The stroboscope also was used to confirm that displacement of the test probe was consistent at approximately 40 μm over the range of frequencies tested from 1 to 60 Hz. The anemones were held in a small petri dish (50 mm diameter) filled with 20 ml seawater. The vibrating probe was placed in the center of one quadrant of the dish and the anemones evenly distributed in the other three quadrants. The distance between the vibrating probe and animals ranged from 1.0 to 2.5 cm (probe to the tentacle tips). The tentacles measured 1.5 cm in length. Thus, the distance of the probe to the tentacle base ranged from 2.5 to 4.0 cm. The vibrating probe consisted of a fire polished, 50 μl Drummond microcap tube. Test probes were immersed to allow them to hydrate for 5 s before they were moved into contact with tentacles and then withdrawn in a direction to minimize the possibility that additional tentacles would contact the test probe. The test probes were fixed in 2.5% glutaraldehyde in seawater. Wet mounts were prepared and the test probes were imaged at 600× using phase contrast optics. *Nematostella* has only basitrichous isorhiza (basitrich) nematocysts and spirocysts in the tentacle cnidom (Sebens 1998). Basitrich nematocysts were identified using criteria outlined by Mariscal (1974). Because spirocysts are inadequately contrasted by phase contrast microscopy to permit their quantitation, basitrichs were counted for a single representative field of view for each test probe. Each anemone was touched once. Each replicate consisted of data from five test probes. Final data points consisted of a pooled mean number of basitrichs counted ± SEM for ten replicates (Fig. 6) or 3 replicates (Fig. 7).

Phalloidin cytochemistry

Specimens of *Nematostella* were anesthetized for 1.5 h in high-potassium seawater (KSW) consisting in mM concentrations: NaCl 161; $MgSO_4$ 13; $MgCl_2$ 12; KCl 50; $CaCl_2$ 6; and $NaHCO_3$ 1. Although elevated $MgCl_2$ can be used to anesthetize anemones (e.g., Watson and Mariscal 1983), it causes poor preservation of hair bundles. Here, we suspect that at high concentrations, magnesium ions compete for calcium binding sites in cadherins that are necessary to maintain integrity of hair bundles (Watson et al. 2008). The high-potassium seawater anesthetic was developed by us to allow better preservation of hair bundles in fixed specimens and to allow electrophysiology on functional hair bundles. Although we are not certain why our formulation of high-potassium seawater anesthetizes anemones, we think it likely that the low-sodium content of the 'high potassium'

seawater (reduced 1:1 for added potassium ions) contributes to the effect. Anesthetized specimens were fixed for 30–60 min in 0.05% glutaraldehyde and 4% paraformaldehyde (or sometimes in fixative lacking the glutaraldehyde) prepared in Millonig buffer augmented with 1.5% NaCl. Fixed specimens were washed in PBS, permeabilized for 15 min in 0.05% tween-20, rinsed in PBS and then incubated overnight at 4°C in phalloidin-conjugated to rhodamine or Alexafluor 555 (Molecular Probes, Invitrogen, Eugene, OR, USA) in PBS.

Excised tentacles were mounted in ProLong Gold antifade reagent (Molecular Probes, Invitrogen) and then imaged using a conventional epifluorescence microscope LOMO Lumam (model RP011-T, LOMO America, Prospect Heights, IL, USA). The principal objective used was a 100× oil-immersion fluorite (na = 1.30). Images were obtained using a STL-11000 SBIG cooled CCD camera (SBIG, Santa Barbara, CA, USA) controlled by Maxim-DL software (Diffraction Limited, Ontario, Canada). In addition, images of hair bundles were obtained using transmitted light and oblique contrast optics (Kachar 1985). We were interested in testing whether NANA induced hair bundles to elongate. A specimen of *Nematostella* was anesthetized in KSW for 1 h. The oral disc was subdivided into six segments each bearing several tentacles. Each segment of tissue was incubated in KSW augmented with NANA to contain a final concentration of 10^{-9}–10^{-5} M NANA. The segment of oral disc serving as the untreated control remained in KSW without added NANA. Each experimental segment of the oral disc was exposed to NANA for 5 min before being fixed. The tentacles were examined using oblique contrast microscopy. Hair bundles were measured from micrographs (e.g., Fig. 1a, b). At this magnification, the camera allowed 40 nm/pixel. To avoid sampling errors, measurements were rounded to the nearest 0.1 μm. Statistical analyses were performed using CSS Statistica software (Statsoft, Tulsa, OK, USA). Typically, an ANOVA was performed followed by LSD post hoc tests. In all cases, significance is reported at $P \leq 0.05$.

Electrophysiology

Electrophysiology was performed as described previously (Mire and Watson 1997). Briefly, animals were anesthetized in KSW as described above and then excised tentacles were threaded along a fine human hair. The ends of the hair were glued to a coverslip (Kwiksil, World Precision Instruments, Sarasota, FL, USA) forming the bottom of a plastic Petri dish. The plastic bottom had been previously removed with a Dremel tool and replaced with a sealed glass coverslip. The dish was filled with KSW and recording electrodes were attached to the apical surface of supporting cells (hair cells) using the loose patch configuration.

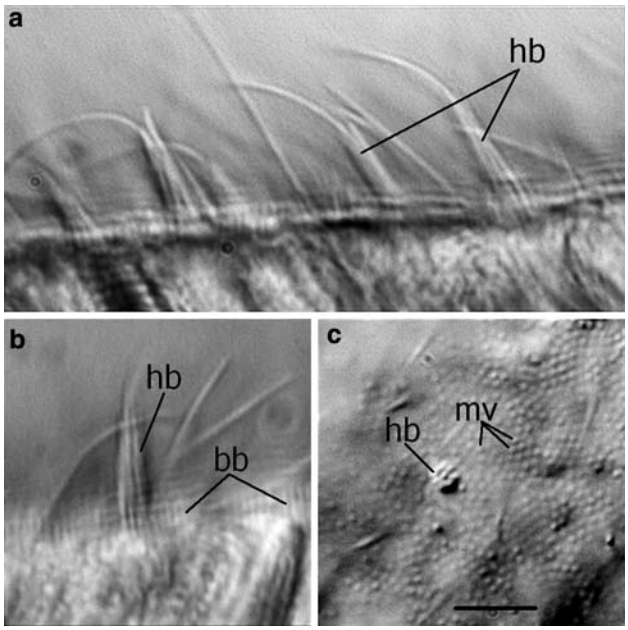

Fig. 1 Light micrograph of anemone hair bundles. A fixed tentacle was imaged using oblique-contrast microscopy. **a** In profile, hair bundles (hb) are abundant at the surface of the tentacle epithelium. **b** A brush border (bb) is visible in profile as indicated by a regular pattern of dark and light bands. **c** In top view, microvilli (mv) of the brush border appear as regularly spaced dots. The tip of a hair bundle (hb) also is shown. *Scale bar* 5 μm

A second 'puffer' pipette was positioned one bundle length away from the tip of the hair bundle of interest. The puffer pipet was used to deflect the hair bundle with a transient jet of KSW for 45 ms controlled by a pneumatic picopump (model 820, World Precision Instruments, Sarasota, FL, USA). Transients in membrane current were recorded through an Axopatch 200A integrating amplifier with CV201 headstage. Data were recorded and analyzed using Pclamp6 software (Axon Instruments, Foster City, CA, USA).

Results

Hair bundle morphology

Hair bundles project from the surface of the tentacle epithelium (Fig. 1a). Otherwise, the surface of the tentacle features a highly ordered array of parallel, microvilli organized into a brush border. In profile, the brush border is suggested by regularly alternating bands at the apical surface of the tentacle (Fig. 1b). The regular spacing of the microvilli is also shown in top views of the tentacle surface (Fig. 1c). Hair bundles of untreated controls had a mean length of 8.26 ± 1.43 μm. The hair bundles significantly elongated in 10^{-8} M NANA to 10.05 ± 1.47 μm (Fig. 2). At higher

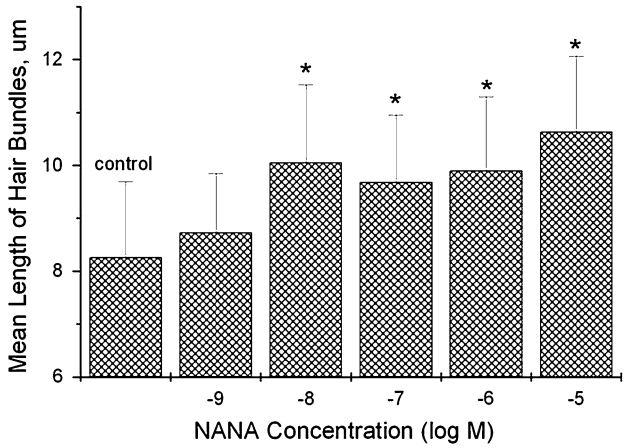

Fig. 2 Lengths of hair bundles imaged in the presence of NANA. Hair bundles (mean ± SD, $n = 15$ for each treatment) were measured from light micrographs obtained in KSW alone (control) or after 5 min in NANA at the concentration indicated. *Asterisks* indicate treatment hair bundles significantly longer than untreated control hair bundles

concentrations of NANA, the hair bundles likewise were significantly longer than in untreated controls (Fig. 2).

Phalloidin cytochemistry

Hair bundles in *Nematostella* consist of six to eight large diameter stereocilia originating from the sensory neuron surrounded by small diameter stereocilia originating from 2 to 4 supporting cells. This assertion is based on observations of the hair bundles using LM and TEM. The large diameter stereocilia and the small diameter stereocilia both are stained by phalloidin indicating that the stereocilia contain F-actin (Fig. 3a). Large diameter stereocilia have long cytoplasmic rootlets that extend approximately 4 um into the cytoplasm (Fig. 3b). From phalloidin preparations, we calculated the density of hair bundle mechanoreceptors on the surface of the tentacle. The surface area of the tentacle was estimated assuming that the tentacle approximates a cone. The mean length (1.49 ± 0.45 mm) of tentacles and diameter (0.55 ± 0.02 mm) at the base of tentacles was determined by measuring tentacles from 10 anesthetized specimens (1 tentacle each). Based on these calculations, and a density of 1 hair bundle per 293 μm^2 (from an analysis of 6 large-format micrographs containing a total of 103 hair bundles), we estimate that each tentacle has approximately 4,341 hair bundles. According to this estimate, a single adult *Nematostella* would have a total of 69, 456 hair bundle mechanoreceptors projecting from the surface of its tentacles.

Electrophysiology

Hair bundles were deflected by jets of KSW delivered by a puffer pipette positioned adjacent to the hair bundle. In

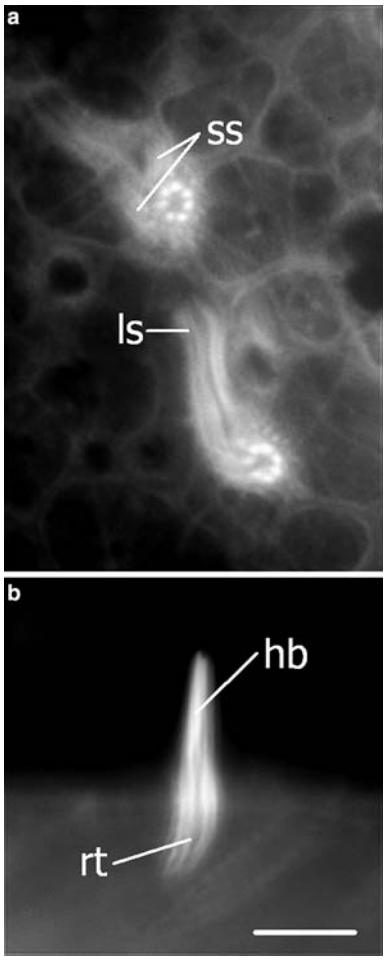

Fig. 3 Phalloidin cytochemistry. Tentacles were fixed and processed for phalloidin cytochemistry. **a** In top view, two hair bundles are pinned down by the coverslip and slightly splayed so that large diameter stereocilia (ls) and small diameter stereocilia (ss) can be observed. The bases of the large diameter stereocila, arranged into a circlet are the brightest objects in the micrograph. **b** In profile, the normal structural integrity of the hair bundle (hb) is maintained. A long rootlet (rt) stained by phalloidin extends into the cytoplasm. *Scale bar* 5 μm

some instances, deflections induced upward current transients consistent with hyperpolarization of the membrane potential. In other cases, deflections induced downward current transients consistent with depolarization of the membrane (Fig. 4). Deflections that produce depolarization of the membrane potential are considered to be 'positive' deflections in which the stereocilia of the hair bundle are deflected towards their taller neighboring stereocilia. Deflections that produce hyperpolarization of the membrane potential are considered to be 'negative' deflections in which the stereocilia of the hair bundle are deflected towards their shorter, neighboring stereocilia. According to the currently favored model for signal transduction of hair cells in vertebrate animals, the gating spring model, positive deflections induce strain on presumptive 'gating springs' to open the transduction channels, leading to

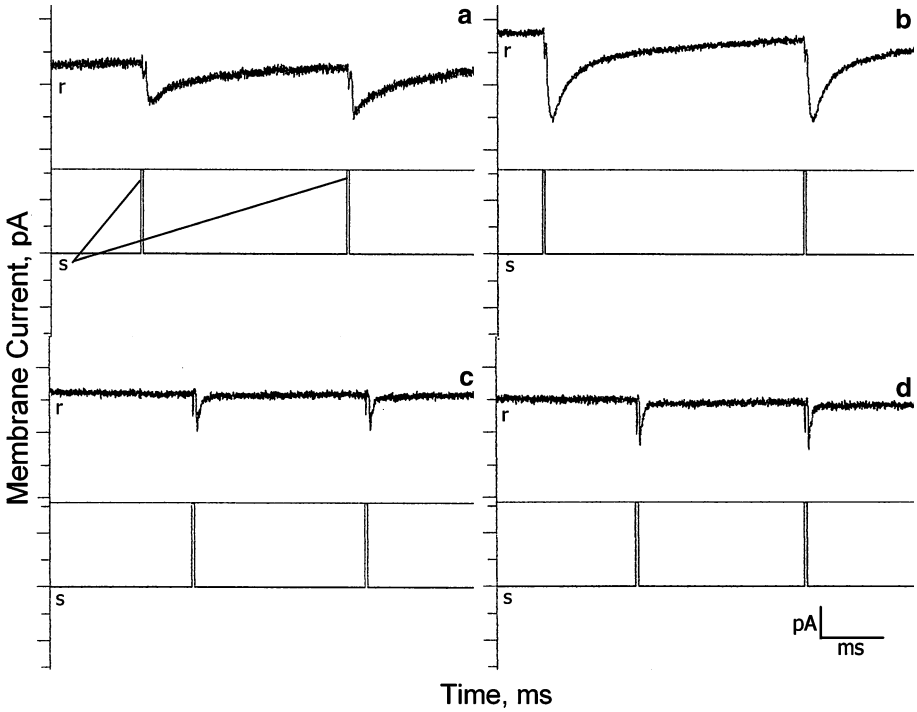

Fig. 4 Paired stimulus/response recordings. Recording pipettes were attached at the bases of hair bundles subjected to deflection by jets of KSW. Membrane current recordings are shown for individual hair bundles stimulated by two sets of jet stimuli. **a** A hair bundle was deflected by two jets in KSW. Membrane current transients are shown in the response curve (r). The timing of the opening of the solenoid valve to release pressure to the puffer pipette is shown in the stimulus curve (s) as *vertical bars*. **b** The same hair bundle shown in panel **a** is again deflected 5 min after the addition of NANA to 10^{-7} M. **c** In KSW alone, a hair bundle was deflected by two jets. **d** The same hair bundle shown in panel **c** was again deflected after the addition of a small volume of KSW. *Scale bar* 20 pA and 1,000 ms

depolarization of the membrane potential. Negative deflections allow slack on the gating springs to allow the transduction channels to close, leading to hyperpolarization of the membrane potential (Howard et al. 1988; Hudspeth 1985, 1997; Ashmore 1991; Lemasurier and Gillespie 2005). In anemone preparations using intact tentacles threaded with human hair, unavoidable ambiguity occurs with respect to the exact position of the recording pipette in relation to the puffing pipette because of abundant microvilli at the surface of the epithelium. Thus, a typical recording session will include a mixture of 'positive' (depolarizing) and 'negative' (hyperpolarizing) current transients. For our purposes here, the direction of the current transient was unimportant. We were interested in testing whether exposure to NANA altered current transients associated with deflection of the hair bundles. Deflections in KSW were followed by an addition of NANA. After 5 min in 10^{-7} M NANA (final concentration), hair bundles were again deflected by jets of KSW (Fig. 4a, b). Controls consisted of deflections in KSW followed by the addition of KSW. After 5 min, hair bundles were again deflected by jets of KSW (Fig. 4c, d). In this example, the membrane currents generated after NANA are noticeably larger in amplitude than before NANA (Fig. 4a, b). The KSW control shows a much smaller increase in current after 5 min (Fig. 4c, d). In other hair bundles, however, the current transients after exposure to NANA were smaller in amplitude than before NANA. Considering only the magnitude of the change in current and disregarding direction, changes in mean current between the initial deflections and the deflections 5 min later were significantly larger after exposure to NANA than in controls (Fig. 5).

Nematocyst discharge bioassay

We were interested in determining whether discharge of nematocysts varied as a function of the frequency of vibration. Discharge of basitrich nematocysts was tested at 1 Hz increments from 1 to 60 Hz. A total of ten test probes (i.e., based on 2 replicate experiments) was obtained for each frequency in seawater alone and in seawater augmented with NANA to 10^{-7} M. The frequency response curves showed an enhancement of discharge by a factor of 2–2.5 at maxima (i.e., 'key' frequencies) as compared to minima. However, noise in the original data set forced us to pool data over 5 Hz increments to reveal more general trends (Fig. 6). Significance was tested using the minima within each frequency response curve as the standard against which data for other ranges of frequencies were compared. In seawater, the frequency response curve was bimodal

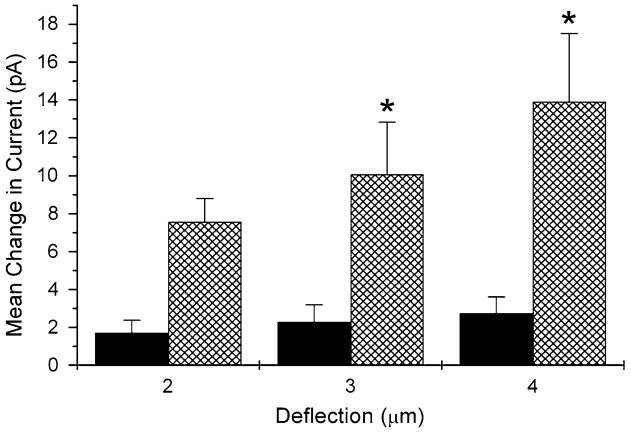

Fig. 5 Mean change in electrophysiological responses of hair bundles to stimuli separated by 5 min. Recording pipettes were attached at the bases of hair bundles subjected to deflection by jets of KSW. Hair bundles were deflected by jets of KSW delivered in pairs. After 5 min, the hair bundles were deflected by a second pair of jet stimuli. The change in the mean responses between the first and second sets of stimuli is plotted as a function of the distance of deflection at the tip of the hair bundle. *Black bars* indicate data obtained in KSW alone. Cross-hatched bars indicate data in which NANA was added to 10^{-7} M after the first set of stimuli. Statistical analyses compared like stimuli (2 μm in KSW vs. 2 μm in NANA). *Asterisks* indicate statistically significant differences

with two significant peaks. One was a narrow peak at 16–20 Hz and the other was a broad peak spanning 36–55 Hz (Fig. 6a). The minima occurred at 21–25 Hz (Fig. 6a). In NANA, a single, significant broad peak occurred at lower frequencies spanning 1–20 Hz. In NANA, the minima occurred at 31–35 Hz (Fig. 6b). We plotted a difference curve in which the data in Fig. 6a for seawater controls were subtracted from the data in Fig. 6b for animals exposed to NANA. The resulting data were fit with a Gaussian function (Fig. 6c). The intercept at zero occurred at 28 Hz. The line suggests that increases in mean discharge of nematocysts occurred at frequencies below 28 Hz at the same time as decreases occurred in discharge of nematocysts at frequencies above 28 Hz. The sum of the data plotted in the difference curve is 0.06.

To determine the sensitivity of the anemones to near-field vibrations, anemones were positioned at increasing distances from the vibrating source set at 56 Hz, a key frequency in seawater alone. The distance was measured from the vibrating probe to the tentacle tips. At the shortest distance, 0.5 cm, discharge of basitrich nematocysts was significantly greater than for non-vibrating controls. Interestingly, levels of discharge remained significantly elevated when tentacle tips were tested at 2.5 cm away from the vibrating source and were nearly significantly elevated at 3.5 cm away from the vibrating source ($P = 0.07$). Thus, the threshold distance for detecting vibrations lies between 2.5 and 3.5 cm away from the tentacle tips. At distances greater than 3.5 cm, discharge was nearly identical to that for non-vibrating controls (Fig. 7).

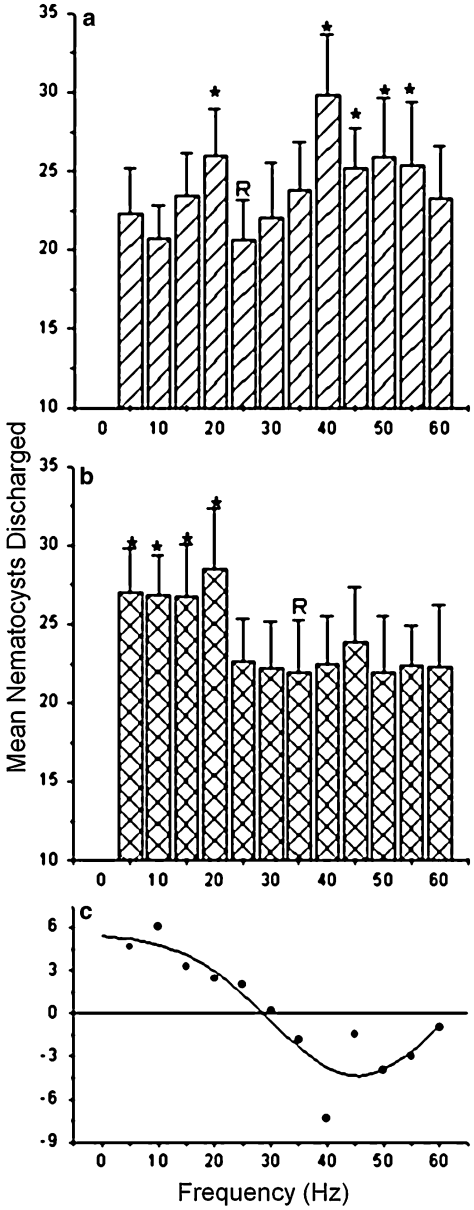

Fig. 6 Discharge of nematocysts in the presence of vibrations. A vibrating source was immersed into the seawater containing the anemones. Tentacles were touched with test probes to elicit discharge of nematocysts into them. Basitrichs were counted for a single field of view for 50 test probes tested over a range of 5 Hz (e.g., 1–5, 6–10, 11–15, etc.) For each range of frequencies, only the upper limit of the range is printed along the X-axis of the graph because of space limitations. Thus, 1–5 Hz is shown as 5 Hz, and so on. Data appear as the mean ± SEM (based on $n = 10$ replicates, each consisting of 5 test probes). **a** In seawater alone, peaks of discharge (significantly larger than the reference minima (R), here at 21–25 Hz) occurred at 16–20 Hz and at 36–55 Hz. **b** In 10^{-7} M NANA, peaks of discharge occurred at lower frequencies ranging from 1 to 20 Hz. The reference minima (R) occurred at 31–35 Hz. **c** A difference curve (NANA minus seawater) for nematocyst discharge tested in the presence of vibrations. Data obtained from anemones tested in seawater (**a**) were subtracted from data obtained form anemones tested in 10^{-7} M NANA (**b**). Positive differences, indicating enhanced discharge in the presence of NANA, appear above the reference line set at zero. The data were fit to a Gaussian function

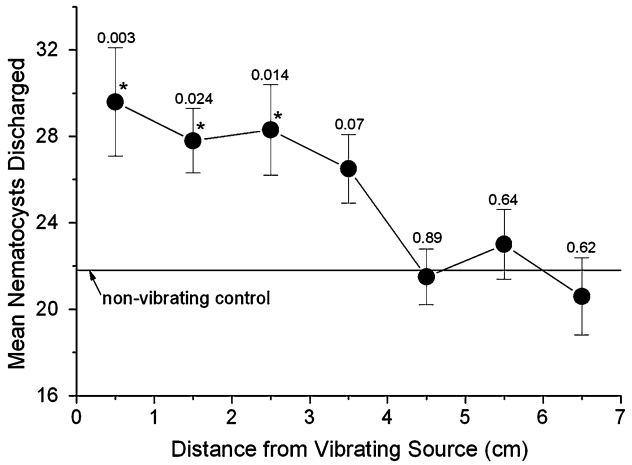

Fig. 7 Discharge of nematocysts as a function of distance from the vibrating source. Discharge was tested in anemones positioned at increasing distances from a source vibrating at 56 Hz, a key frequency in seawater alone. Data indicate the mean number of discharged basitrich nematocysts counted for field of view for $n = 15$ test probes. Data are plotted as the mean ± SEM where the number of replicates = 3, each replicate experiment comprising 5 test probes. Statistical comparisons were made relative to non-vibrating controls. *Asterisks* indicate significant differences

Discussion

In *Nematostella*, hair bundles consist of actin-based stereocilia, as has been reported previously for the sea anemone, *Haliplanella* (Watson and Roberts 1995). Recordings of membrane current confirm that the hair bundles on tentacles of *Nematostella* are mechanoreceptors because deflecting hair bundles induces current transients. Waiting 5 min and then stimulating the hair bundles again can result in modest changes in the magnitude of the response. We believe that such changes result from slight movements of the tentacle caused by muscular contractions or slight rotations of the excised tentacle on the hair strand. Interestingly, if NANA is added just prior to the 5 min waiting period, significantly larger changes in current are observed. In NANA, such changes in responsiveness may result from significant changes to the structure of the hair bundle including: (1) changes in stiffness of the hair bundle; or (2) changes in the orientation of transduction apparatus (transduction channels and associated proteins) into the path of the stimulus (or, alternatively, out of the path of the stimulus). In *Haliplanella*, NANA induces the hair bundles to elongate in a fashion that requires polymerization of actin (Mire-Thibodeaux and Watson 1994; Watson and Roberts 1995). Hence, changes in bundle stiffness might accompany elongation. Furthermore, in *Haliplanella*, NANA induces a repositioning of the small diameter stereocilia such that they converge onto the large diameter stereocilia at a higher position than in seawater alone and a change in membrane currents (Mire and Nasse 2002). Thus, the transduction channels might be moved with respect to the position of the puffing pipette. In *Nematostella*, it is clear that NANA induces hair bundles to elongate, but we do not yet know whether elongation is accompanied by changes in the stiffness of the hair bundle and/or a repositioning of the small diameter stereocilia to a higher position along the length of the large diameter stereocilia of the hair bundle.

Regulation of nematocyst discharge

It appears that discharge of basitrich nematocysts occurs differentially into test probes touched to tentacles in the presence of vibrations according to the frequency of vibration. In seawater alone, the frequency response is bimodal with a sharp peak at 16–20 Hz and a broad peak from 36 to 55 Hz. In the presence of NANA, the peak at 16–20 Hz broadens downward to include 1–20 Hz and the peak from 36 to 55 Hz disappears. Interestingly, a difference curve confirms that increases in discharge at lower frequencies are accompanied by decreases in discharge at higher frequencies. In fact, the sum of the differences approximates zero, indicating that the observed increases in discharge at lower frequencies are essentially balanced by the observed decreases in discharge at higher frequencies. These data confirm that chemoreceptor-mediated, frequency-shifts occur in *Nematostella*. Thus, *Nematostella* becomes only the second species of sea anemone known to exhibit this behavior. However, the discretionary proportion of basitrich nematocysts is relatively small, comprising about 30% of the maximum observed under our experimental conditions. Approximately 70% of the maximum is discharged into test probes touched to tentacles regardless of the frequency of vibration (or in the absence of vibrations). However, in the presence of vibrations at the appropriate frequencies, discharge increases to the maximum (100%). The magnitude of the vibration-dependent component of the response is smaller than that previously noted for microbasic p-mastigophore nematocysts of *Haliplanella* (Watson et al. 1998).

In its natural habitat, *Nematostella* burrows in soft sediment such that its hemispherical array of tentacles, covered with a total of approximately 70,000 hair bundles, lies on top of the sediment exposed to seawater while the body column is buried beneath the sediment. The natural diet of this anemone includes crawling prey such as snails, nematodes and polychaetes in addition to swimming prey such as molluscan larvae, small crustaceans, and insect larvae (Hand and Uhlinger 1994). Touching tentacles with test probes in the absence of vibrations is alone sufficient to trigger discharge of basitrich nematocysts. This response is set to approximately 70% of the maximum response observed into test probes tested in the presence of vibrations at key

frequencies. Perhaps nonvibrating, crawling prey that blunder into the tentacles are subdued by discharge of these basitrich nematocysts. However, in the presence of appropriate vibrations at key frequencies, maximal discharge of basitrichs is achieved (increasing to 100%). Thus, swimming prey may stimulate a larger response, in terms of basitrich discharge, than do crawling prey. In the presence of NANA, key frequencies shift downward to overlap those produced by a variety of calmly swimming animals including small crustaceans such as *Artemia* nauplii and adults (Watson and Hessinger 1989), copepods (Montgomery and MacDonald 1987), and veliger larvae (Gallager 1993). According to our model, in calm water, detection of prey movements predisposes the anemone to discharge nematocysts maximally into the prey in the event that the prey swims into contact with the tentacles.

Sensitivity of vibration detection in Nematostella

Given that the swimming movements of suitable prey have small amplitudes, we wanted to be sure that our tests on vibration sensitivity were reasonable. We modeled the vibrating probe as a sphere having a diameter of 1.6 mm based on a comparable displacement by pre-measured spheres and our vibrating probe inserted into a small volume of water. Particle displacement amplitude was estimated at distances from the vibrating probe using the methods of Tautz and Sandeman (1980). With the vibrating source located 2.5 cm from the tentacle tip, and vibrating with an amplitude of 40 μm, the water particle displacement amplitude at the tentacle tip is estimated to be 40 nm. At this position, the base of the same tentacle is located 4 cm from the vibrating source where the water displacement amplitude is estimated to be 16 nm. Thus, the minimum water displacement to elicit a significant response in the anemone hair bundles is approximately 10^{-8} m, in agreement with that reported for vibration detection in other systems including crayfish (Tautz and Sandeman 1980), cuttlefish (Budelmann and Bleckmann 1988) and fish (Bleckmann and Topp 1981). Our initial investigations of water displacement based on analysis of video recordings suggest that free swimming, freshly hatched, brine shrimp nauplii produce water particle displacement amplitudes approximately 1/3 as large as our vibrating probe (data not shown). Thus, the vibrating probe employed in this study likely reasonably approximates movements produced by suitable prey on the larger end of the spectrum of those captured by *Nematostella*. For such prey, the detection of vibrations extends into the seawater by more than 2.5 cm beyond the tips of the tentacles, giving an adult anemone a hemispherical receptive field having a radius of at least 4 cm with the mouth of the anemone lying at the center of this field. According to our estimates, the hemispherical receptive field would shrink to a radius of 3 cm for freshly hatched *Artemia* nauplii and would be smaller still for veliger larvae of mollusks. Thus, the dimensions of the hemispherical receptive field would vary according to the amplitude of movements produced during swimming by the potential prey organism.

Implications for vibration sensitivity in Nematostella beyond prey capture

Key genes associated with mechanotransduction of hair cells of the acousticolateralis system of vertebrate animals are being identified. For example, cadherin 23 (cdh23) is a polypeptide that forms a part of tip links (Siemens et al. 2004; Sollner et al. 2004). Tip links are extracellular linkages interconnecting stereocilia that are thought to lie in series with the gating spring or to directly attach to the gating spring and/or mechanotransduction channel (Howard et al. 1988; Hudspeth 1985; Ashmore 1991; Lemasurier and Gillespie 2005). In zebrafish, mutants lacking a functional copy of the gene encoding cdh23 lack tip links and functional hair cells (Sollner et al. 2004; Nicolson et al. 1998). In mammals, defects in the gene encoding cdh23 have Usher 1D, a nonsyndromic form of deafness (Bork et al. 2001; Bolz et al. 2001; Di Palma et al. 2001). In *Nematostella* and *Haliplanella*, we recently identified a homolog of cdh23 that is important to vibration sensitivity and may comprise part of the tip link (Watson et al. 2008). Thus, *Nematostella* may constitute an excellent model system in which to investigate the evolution of hair cells.

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