

Force-dependent discharge of nematocysts in the sea anemone *Haliplanella luciae* (Verrill)

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Summary

Sea anemones discharge cnidae ('stinging capsules' including nematocysts) to capture prey and to defend themselves. In the present study, we tested the relationship between the force of test probes striking feeding tentacles and discharge of microbasic p-mastigophore nematocysts into the test probes. In seawater alone, the response curve is bimodal with maximal discharge observed at 0.33 and 1.10 millinewtons (mN) and with minimal discharge at 1.50 mN. Upon activating chemoreceptors for N-acetylated sugars, maximal discharge is observed across a broad range of smaller forces from 0.16 to 0.9 mN before decreasing to a minimum at 1.50 mN. Likewise, in the presence of nearby vibrations at key frequencies, maximal discharge is observed over a broad range of smaller forces before decreasing to a minimum at 1.50 mN. It appears that sensory input indicating proximity of potential prey

expands the range of small forces of impact that stimulate maximal discharge (i.e. to less than 1.10 mN) but not at larger forces of impact (i.e. at approximately 1.50 mN). Thus, contact by small prey would stimulate maximal discharge, and all the more so if such contact is accompanied by specific odorants or by vibrations at specific frequencies. Nevertheless, anemones would not maximally discharge nematocysts into large animals that blunder into contact with their tentacles.

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Key words: Contact-sensitive mechanoreceptor, Cnidaria, Anthozoa

Introduction

Members of the phylum Cnidaria, including jellyfish, hydra, sea anemones and corals, employ cnidae (stinging capsules including nematocysts and spirocysts) to capture prey, to defend themselves, and to attach to substrates (Ewer and Fox, 1947). Cnidae are specialized intracellular capsules containing highly folded tubules that forcibly evert during discharge (Skaer and Picken, 1965). Certain nematocysts function as penetrants such that the everting tubules inject venom into the target organism. Other cnidae function as adherents such that the everting tubules adhere to the surface of the target organism or entangle its appendages (Mariscal, 1974; Mariscal, 1984). Ptychocysts, a third class of cnida, are employed to construct tubes in which cerianthid anemones live (Mariscal et al., 1977). Cnidae are elaborate secretory products of cnidocytes (Holstein, 1981; Watson and Mariscal, 1984a; Watson and Mariscal, 1984b). Once fully mature, cnidae are positioned just beneath the apical plasma membrane of the cnidocytes from which they are discharged. Mechanoreceptors and chemoreceptors participate in the regulation of *in situ* discharge (reviewed by Anderson and Bouchard, 2009). For example, in seawater alone, a clean glass rod touched to tentacles of an anemone triggers baseline discharge of nematocysts. Appropriate chemical stimuli (prey extracts) are alone insufficient to trigger discharge of nematocysts. However, a clean glass rod touched to anemone tentacles in the presence of prey extracts triggers massive discharge of nematocysts (Pantin, 1942a; Pantin, 1942b). Several classes of chemoreceptor that bind prey-derived compounds were identified in anemones that participate in regulating discharge of

cnidae (Thorington and Hessinger, 1988). Using a novel bioassay based on measuring adhesion between the tentacle and test probe in *Aiptasia pallida*, dose-response curves were generated to N-acetylated sugars and to several, specific amino acid ligands including glycine and proline. Here, baseline discharge was observed in seawater alone. As the dose of N-acetylated sugars (or specific amino acids) was experimentally increased, adhesion increased to a maximum at the EC100 dose and then decreased to baseline. Counts of microbasic p-mastigophore nematocysts obtained from the test probes confirmed that adhesion was due, in large part, to discharged microbasic-p mastigophore nematocysts (Muir Giebel et al., 1988). Chemoreceptors for N-acetylated sugars and for proline were localized to the apical plasma membranes of 'supporting cells' adjacent to cnidocytes (Watson and Hessinger, 1987; Watson and Hessinger, 1989a; Watson and Roberts, 1994).

In addition, it was determined that anemones discharge cnidae into vibrating test probes touched to their tentacles. Discharge varies as a function of the frequency and amplitude of the vibrations (Watson and Hessinger, 1989b; Watson and Hudson, 1994; Watson et al., 1998; Watson et al., 2009; Krayesky et al., 2010). For *Haliplanella luciae* tested in seawater alone, maximal discharge of microbasic p-mastigophore nematocysts occurs at several key frequencies higher than 50 Hz. Interestingly, activating chemoreceptors for N-acetylated sugars induces a downward shift in key frequencies such that they overlap frequencies produced by calmly swimming prey (Watson and Hessinger, 1989b; Watson et al., 1998). Moreover, activated receptors for N-acetylated sugars narrow amplitude specificity to relatively small amplitudes

consistent with those produced by small prey (Watson and Hudson, 1994). However, if receptors for proline are activated after those for N-acetylated sugars, maximal discharge shifts to larger amplitudes at low frequencies and to higher frequencies at small amplitudes (Watson and Hudson, 1994).

According to our working model, N-acetylated sugars originating from prey mucins bind to supporting cell chemoreceptors to induce 'tuning' (i.e. the downward shift in frequency responsiveness). Swimming movements produced by the prey are detected by hair bundle mechanoreceptors located on the tentacles. These mechanoreceptors sensitize the anemone to maximally discharge nematocysts. As the prey blunders into contact with the tentacle, it is stung by nematocysts that penetrate its integument to deliver potent toxins. The wounded prey releases amino compounds including proline into the seawater from its hemolymph and struggles to escape. Proline binds chemoreceptors on the tentacle epithelium that now tune the hair bundles such that they respond to movements overlapping those produced by struggling prey. A second round of nematocysts is discharged into the struggling prey. Once the prey is subdued, it is ingested. Thus, chemoreceptors that detect prey-derived compounds alter the responsiveness of hair bundle mechanoreceptors. The activated chemoreceptors induce significant alterations to the morphology of the hair bundles (Mire-Thibodeaux and Watson, 1994; Watson and Roberts, 1995; Mire and Nasse, 2002). Nevertheless, contact between the prey and tentacle is still required in order to trigger discharge. Is it possible that these contact-sensitive mechanoreceptors also are influenced by activated chemoreceptors? In hydra, the answer to this question is 'yes.' It was determined in *Hydra vulgaris* that test probes contacting the cnidocils (modified cilia arising from cnidocytes) trigger nematocyst discharge in a force-dependent fashion (Scappaticci et al., 2010). Here, a piezo bimorph driven by a stimulator delivered impacts to cnidocils over a range of forces depending on the voltage applied to the bimorph. Impact was confirmed by means of microscopic observations and discharge of desmoneme and stenotele nematocysts was recorded. As the force of stimulation was increased, the probability of discharge increased to a maximum and then decreased thereafter. Interestingly, in the presence of 10^{-8} M mucin, the probability of discharge significantly increased at force optima. In addition, the sensitivity of the response increased at smaller forces. It appears in *Hydra*, that activated chemoreceptors may somehow lead to modifications to contact-sensitive mechanoreceptors in cnidocytes in such a way as to regulate *in situ* discharge of nematocysts.

The purpose of this study was to test for the presence of force-dependent discharge in the sea anemone, *Haliplanella luciae*. In addition, we investigated whether force-dependent discharge is altered following activation of chemoreceptors and/or following activation of (vibration sensitive) hair bundle mechanoreceptors.

Materials and Methods

Animal culture

Specimens of the sea anemone *Haliplanella luciae* (Verrill) were cultured in natural seawater at 32 parts per thousand and held at 16–18°C. The animals were fed twice weekly using *Artemia* nauplii. Seawater was changed after feeding. Animals were starved for a minimum of 24 h before they were used in experiments described below.

Piezo-cantilever apparatus

A double-quick-mount piezoceramic bending actuator from Piezo Systems Inc. (model D220-A4-103YB, Woburn, MA, USA) was employed as the force generating motor of the apparatus. The apparatus is diagramed in Fig. 1. The

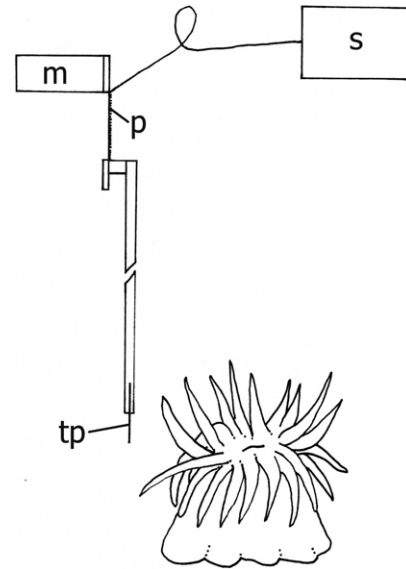


Fig. 1. Diagram of piezoceramic cantilever apparatus employed to contact an anemone tentacle. A three-axis micromanipulator (m) was used to position gelatin-coated test probes (tp) adjacent to a tentacle of a sea anemone. A stimulator (s) induced bending of a piezoceramic wafer (p) in order to bring the test probe into contact with the tentacle.

piezoceramic cantilever was mounted to a manipulator (Narishige, Model M-152, Tokyo, Japan) allowing for controlled positioning of the test probe adjacent to the anemone. A glass capillary tube measuring 100 mm in length was attached to the end of the cantilever. Gelatin coated test probes, prepared as described previously (Watson et al., 1998) were inserted into the end of the capillary tube such that they extended approximately 1 cm from the tip. The piezoceramic actuator was induced to bend by a Phipps & Bird stimulator (model 611, Richmond, VA, USA). The output of the stimulator was adjusted while monitoring a digital multimeter attached to the leads (Sperry Instruments, model DM-210A, Menomonee Falls, WI, USA).

The force generated by the apparatus was measured by orienting the apparatus horizontally, and placing the tip of the capillary extending from the cantilever on the pan of an electronic balance (Sartorius, model H-110, Edgewood, NY, USA). The weight of the cantilever apparatus was digitally subtracted. A stimulator was used to bend the piezoceramic actuator such that the cantilever pushed down on the pan. Thus, the deflection of the cantilever yielded a mass measurement. Conceptually, in this application, the balance functions as a force transducer. Similarly, in its normal function as a balance, the mass of the sample is subjected to the acceleration of gravity to deflect the pan downward. Hence, although the balance actually is measuring the downward force on the mass imposed by gravity, the balance is internally calibrated (i.e. the force is divided by the acceleration due to gravity) so that the balance displays measurements of mass. We here estimated the force applied to the pan by the piezoceramic apparatus by multiplying the mass readings by gravitational acceleration (Measuring mass and force with a balance, A. Reichmuth, Mettler Toledo, 1997 [http://us.mt.com/us/en/home/supportive_content/library/Weigh_Uncertain_Number5.html]). The force produced by the piezoceramic actuator increased as a linear function as the input voltage was increased (Fig. 2, $R=0.999$).

Nematocyst discharge bioassay

Nematocyst discharge was assayed by directly counting microbasic p-mastigophore nematocysts discharged into gelatin-coated test probes using methods modified from those employed previously (Watson and Hudson, 1994; Watson et al., 1998). Briefly, test probes are immersed into the seawater, hydrated and then moved into contact with tentacles. After the test probes are withdrawn, they are fixed in seawater fortified with 2.5% glutaraldehyde. Test probes are prepared as wet mounts and observed using phase contrast optics. Each test probe has one to several patches of discharged cnidae on its surface or penetrating the gelatin coat (Fig. 3A). Each patch corresponds to the imprint of a single tentacle. A single patch is selected (one for each test probe) and then observed at 400× total magnification (40× objective, NA=0.65, Labaroscope, LOMO America, Prospect Heights, IL, USA). Because test probes are cylindrical, nematocysts are counted for a single field of view through several focal planes (Fig. 3B,C). Because the image quality degrades in focal planes beyond the midline of the test probe (Fig. 3B, any focal plane beneath number 4), sampling is restricted to the half of

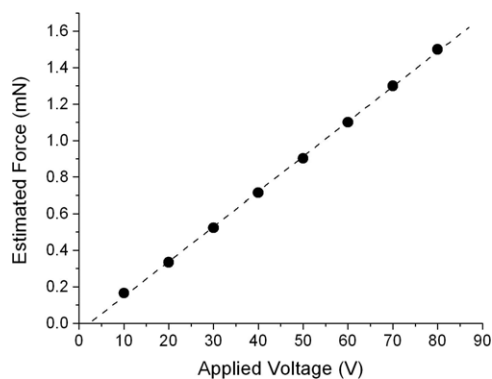


Fig. 2. Effects of varying voltage to the piezoceramic wafer on force produced by the piezoceramic cantilever apparatus. The piezoceramic cantilever apparatus was oriented horizontally so that voltage applied by the stimulator would push down on the pan of an electronic balance. The weight of the apparatus was digitally subtracted. Force was estimated by multiplying the mass reading from the balance by the acceleration due to gravity at sea level. Estimated force (millinewtons) increased as a linear function of applied voltage (volts). The regression line is plotted ($R=0.999$).

the test probe closest to the objective lens. For an upright microscope, the focal planes numbered 1–4 would constitute a typical sample in which nematocysts would be counted. Indeed, probes having the patch of discharged nematocysts on the ‘wrong side’ of the probe must be rolled before the nematocysts are counted. Although this approach has been used successfully for many years in our laboratory and elsewhere (Greenwood et al., 2004), subjectivity is involved in determining whether the midline of the probe has been sampled. In this paper, we took a different approach by counting only the nematocysts within a single focal plane, specifically the one closest to the objective lens (Fig. 3B, number 1), and still within a single field of view. Although the total number of nematocysts counted decreased as compared to those reported in our previous papers, the method was less subjective.

Testing the effects on discharge of varying the force of mechanical stimulation

Discharge of nematocysts was tested in anemones subjected to mechanical stimulation over a range of forces. Anemones were transferred from mass culture

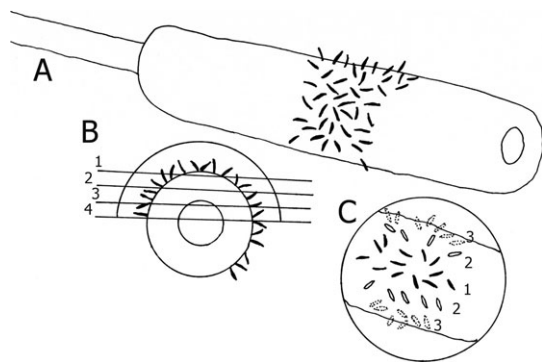


Fig. 3. Diagram of test probe after having touched an anemone tentacle. (A) Test probes are constructed of short strands of fishing line previously dipped in melted gelatin to form a gelatin coat. Test probes moved into contact with tentacles have discrete patches of discharged nematocysts attached to them. For simplicity, only the capsules are drawn. (B) A test probe is viewed from its end. The patch of nematocysts is shown extending partially around the circumference of the gelatin coat. According to methods employed in our previous papers (e.g. Watson et al., 1998), this probe would be sampled through four focal planes (numbered 1–4) using an upright microscope with the objective lens positioned closest to focal plane 1. (C) A representative patch of nematocysts is diagrammed as it would appear through four focal planes. The nematocysts appearing in focal plane 1 are black. Nematocysts appearing in focal plane 2 are clear. Nematocysts appearing in focal plane 3 are stippled. Nematocysts appearing in focal plane 4 are striped.

to 50 ml plastic petri dishes containing fresh seawater. The anemones were allowed a minimum of 1 h to recover from handling. The piezoceramic cantilever apparatus was oriented vertically so that movements induced by the stimulator would be horizontal into contact with the anemone. The manipulator was used to lower the test probes into the petri dish containing the anemones. The test probe was positioned to within 0.5–1.0 mm of the tentacle of interest. Such movements were carried out slowly and gradually such that the anemone did not move the tentacle of interest into contact with the test probe before the piezoceramic actuator was stimulated with electric current. The trial was aborted in the rare instances that the anemone ‘grabbed’ the test probe before the test probe was moved into contact with the tentacle of interest. The piezoceramic was stimulated with a single pulse for 50 ms at a given voltage causing deflection of the cantilever arm of the apparatus. The anemone was subjected to a single impact. The probes were fixed in glutaraldehyde and then viewed using phase contrast optics as was described above. Each anemone was touched once. A total of 25 replicate experiments was performed at each of eight forces ranging from 0.16 to 1.50 mN (Fig. 2).

Testing the effects on discharge of varying the force of mechanical stimulation in the presence of NANA

Discharge of nematocysts was tested in anemones subjected to mechanical stimulation in the presence of N-acetylneuraminic acid (NANA, Sigma, St Louis, MO, USA). Anemones were exposed to fresh seawater 1 h prior to adding NANA to a final concentration of 10^{-7} M, a concentration of NANA previously shown to sensitize discharge of nematocysts into test probes (Watson and Hessinger, 1989a). The specimens were incubated in NANA for 10 minutes followed by mechanical stimulation.

Testing the effects on discharge of varying the force of mechanical stimulation in the presence of nearby vibrations

Discharge of nematocysts was tested in anemones subjected to mechanical stimulation in the presence of nearby vibrations at specific frequencies. For these experiments, two apparatus were inserted into the seawater containing the anemones. The first apparatus was intended to produce vibrations nearby the anemones but not to come into contact with the anemones. Vibrations were produced by a piezoceramic device driven by a digital function generator (Telulex, model SG-100, Mountain View, CA, USA) set to the sine wave function (Watson et al., 1998). The fidelity of the output of the vibrating apparatus was previously tested and confirmed. Moreover, we previously showed that anemones perceive vibrations with the device positioned up to several cm away from the specimen (Watson et al., 2009). For these experiments, the vibrating probe was inserted into the petri dish and positioned 2 cm away from the oral disc of the anemone. Vibrations were produced for 1 min before tentacles were contacted by test probes over a range of forces using the second apparatus in order to trigger nematocyst discharge (Fig. 1).

Data analysis

Statistical analyses were performed using CSS Statistica software (Systat, Tulsa, OK, USA). Data were subjected to a one way ANOVA followed by the LSD post-hoc test. Significant differences were reported at a p-value of ≤ 0.05 . Graphs were prepared using Origin software (Microcal, Northampton, MA, USA). Data points represent the mean number of nematocysts counted \pm standard error. Unless otherwise indicated, statistical comparisons were performed such that each data point within a curve was compared to the data obtained at the highest force (i.e. 1.50 mN) which served as an internal reference. In all cases, the stimulus at 1.50 mN triggered discharge of the fewest nematocysts (i.e. was the minimum). This approach was employed because we found that levels of discharge vary slightly from month to month. We do not understand the basis of such variation, but have found it to be a consistent feature of this bioassay for many years. Data obtained within a four week period tend to be internally consistent. In an attempt to minimize the effects of such temporal variation, replicate experiments were performed sequentially so that the data for each curve were collected before moving on to the next experimental treatment.

Results

Force-dependent discharge of nematocysts

Test probes were moved into contact with tentacles over a range of controlled forces by varying the voltage delivered to a cantilever apparatus driven by a piezoceramic wafer (Fig. 1).

Anemones were assayed for discharge of microbasic p-mastigophore nematocysts. For anemones tested in seawater alone, the response curve was bimodal with discharge maxima observed at 0.33 and 1.10 mN (Fig. 4A). Mean discharge at the maxima was significantly greater than at the minimum observed

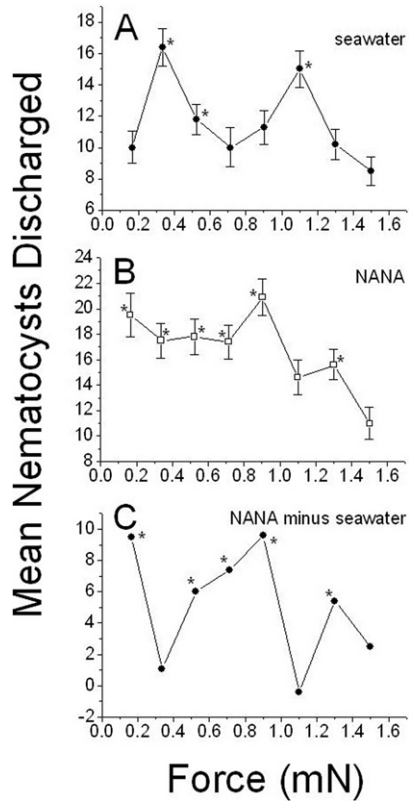


Fig. 4. Effects of varying force of contact on nematocyst discharge in the presence and absence of NANA. (A) Discharge was tested in seawater alone. (B) Discharge was tested at 10 min exposure to 10^{-7} M N-acetylneuraminic acid (NANA), final concentration. Test probes were moved into contact with tentacles over a range of forces. Test probes were examined using phase contrast microscopy. Microbasic p-mastigophore nematocysts were counted for a representative field of view at a single focal plane. Data represent the mean number of nematocysts counted \pm s.e.m ($n=25$). Data were subjected to one way ANOVA followed by the LSD post-hoc test. Data points significantly different from the minimum (at 1.50 mN) are indicated by asterisks. (C) A difference curve is shown. Data for animals tested in seawater alone were subtracted from data for animals tested in NANA. Pair-wise comparisons (NANA versus seawater control) were performed at each force. Asterisks indicate that exposure to NANA significantly affects mean discharge of nematocysts, $p \leq 0.05$.

at 1.50 mN. On average, levels of discharge for data points significantly different from the minimum were approximately 1.7 times higher than that observed at the minimum (Fig. 4A).

Effects of NANA on force-dependent discharge of nematocysts
Discharge of nematocysts was tested over a range of forces after 10 min exposure to 10^{-7} M NANA. In the presence of NANA, levels of discharge were high at the smallest force tested, 0.16 mN, and remained elevated as the force was increased to 0.90 mN above which force, levels of discharge gradually decreased (Fig. 4B). Thus, the response curve was elevated across a range of smaller forces to form a broad maximum and then tailed off at relatively higher forces. On average, levels of discharge for data points significantly different from the minimum were approximately 1.6 times higher than that observed at the minimum.

A difference curve was generated in which the data for seawater controls were subtracted from data for animals tested in the presence of NANA (Fig. 4C). Pair wise comparisons at each

force revealed whether exposure to NANA significantly affected discharge at that force. It appears that NANA significantly increased discharge at forces other than the two maxima observed in seawater alone. Maxima in seawater were not significantly affected by NANA. In addition, the minimum observed at 1.50 mN both in seawater alone and in NANA was not significantly affected by exposure to NANA (Fig. 4C). Thus, NANA sensitizes discharge over a range of smaller forces but not including those that trigger maximal discharge in seawater alone.

Effects of nearby vibrations on force-dependent discharge of nematocysts

In seawater alone, nearby vibrations at 55 Hz stimulate maximal discharge of nematocysts (Watson et al., 1998). For this study, discharge was tested over a range of forces in seawater alone but in the presence of nearby vibrations at 55 Hz. (Fig. 5A). Levels of discharge were high across the range of forces tested although the minimum still occurred at 1.50 mN. Nevertheless, levels of discharge at the other forces tested did not differ significantly from that observed at 1.50 mN. On average, levels of discharge at the other forces tested were approximately 1.2 times that

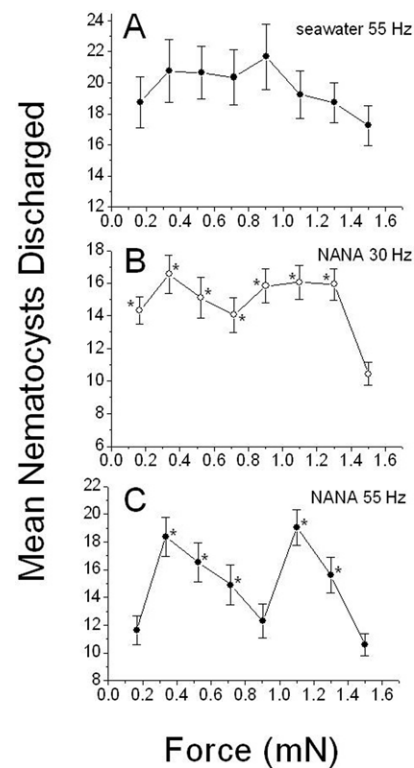


Fig. 5. Effects of varying force of contact on nematocysts discharge in the presence of nearby vibrations. (A) Discharge was tested in seawater alone in the presence of nearby vibrations at 55 Hz, a key frequency in seawater. (B) Discharge was tested in the combined presence of nearby vibrations at 30 Hz and 10^{-7} M NANA, final concentration. In NANA, 30 Hz is a key frequency (Watson et al., 1998). (C) Discharge was tested in the combined presence of nearby vibrations at 55 Hz and 10^{-7} M NANA. In NANA, 55 Hz is not a key frequency. Test probes were moved into contact with tentacles over a range of forces. Test probes were examined using phase contrast microscopy. Microbasic p-mastigophore nematocysts were counted for a representative field of view at a single focal plane. Data represent the mean number of nematocysts counted \pm s.e.m ($n=25$). Data were subjected to one way ANOVA followed by the LSD post-hoc test. Data points significantly different from the minimum (at 1.50 mN) are indicated by asterisks.

observed at the minimum. The curve was shaped as a broad maximum with a modest decrease occurring at relatively higher forces.

Combined effects of NANA and nearby vibrations on force-dependent discharge of nematocysts

In seawater alone, vibrations at several key frequencies higher than 50 Hz stimulate maximal discharge. Exposure to NANA induces a shift in maximal discharge to several key frequencies below 50 Hz (Watson and Hessinger, 1989b; Watson et al., 1998). In the present study, we tested discharge over a range of forces in the combined presence of NANA and nearby vibrations at 30 Hz. Vibrations at 30 Hz are known to stimulate maximal discharge in the presence of NANA (Watson et al., 1998). The response was high at the smallest force tested, 0.16 mN and remained elevated across the other forces tested until 1.50 mN, at which force, discharge sharply decreased to a minimum (Fig. 5C). Thus, the response curve exhibited a broad maximum. On average, levels of discharge for data points significantly different from the minimum were approximately 1.5 times higher than that observed at the minimum.

In addition, we tested discharge over a range of forces in the combined presence of NANA and nearby vibrations at 55 Hz. Because NANA tunes discharge to lower frequencies, minimal discharge was expected. The response curve was bimodal with two maxima observed: one at 0.33 mN; and the other at 1.10 mN (Fig. 5B). The minimum was observed at 1.50 mN. On average, levels of discharge for data points significantly different from the minimum were approximately 1.6 times higher than that observed at the minimum.

Discussion

A re-evaluation of baseline discharge in seawater

It has long been thought that in the absence of chemical stimulation (i.e. in seawater alone), anemones respond to contact of objects to the feeding tentacles with modest, 'baseline' discharge of nematocysts (Pantin, 1942a; Pantin, 1942b). This idea seemed to be confirmed in studies incorporating quantitative bioassays for evaluating discharge (Thorington and Hessinger, 1988; Muir Giebel et al., 1988; Watson and Hessinger, 1989a). However, those studies did not control for possible variation in the force with which test probes contacted tentacles. The results of the present study indicate that, in seawater alone, anemones differentially respond to mechanical stimulation. Depending on the force with which the tentacle is contacted, discharge can be modest (i.e. at baseline), maximal, or in between the two extremes. The increase in discharge above baseline was by a factor of 1.7 at force optima, comparable to that reported previously after chemoreceptor activation (Watson and Hessinger, 1989a) or after stimulation of hair bundles with vibrations at key frequencies (Watson et al., 1998). Thus, contact with the tentacles at the appropriate forces (0.33 and 1.10 mN) is alone sufficient to stimulate maximal discharge.

Effects of NANA and/or nearby vibrations on force-dependent discharge

Upon activating chemoreceptors for N-acetylated sugars, maximal discharge of nematocysts occurs across a broader, expanded range of forces such that the response curve is no longer bimodal. Nevertheless, discharge decreases to baseline at 1.50 mN. Likewise, in seawater alone and in the presence of

nearby vibrations at 55 Hz (SW55), discharge is enhanced across the range of forces tested such that the response curve featured a broad maximum. In this case, however, the decrease in discharge at relatively large forces is less pronounced than in the NANA curve described above. Accordingly, although the minimum occurred at 1.50 mN, the enhancement in discharge observed at maxima was only by a factor of 1.2 above the minimum. It is reasonable to question whether maximal discharge was achieved. In SW55, impact with test probes at 1.50 mN triggers levels of discharge significantly larger than observed for the other treatments tested at 1.50 mN. On the other hand, levels of discharge at 0.33 mN in SW55, taken as a representative maximum, are indistinguishable from maxima at 0.33 mN obtained using other stimuli. Thus, in SW55, whereas discharge at the minimum is elevated, discharge at the maxima is typical.

We emphasize that hair bundles in anemones are tunable. In seawater alone, key frequencies, those that stimulate maximal discharge, include 55 Hz. In the presence of NANA, key frequencies shift downward to include 30 Hz (Watson et al., 1998). Thus, it is not surprising that in the combined presence of NANA and nearby vibrations at 30 Hz, a broad maximum is observed with discharge decreasing to baseline at 1.50 mN.

Apparently, in our previous studies (e.g. Watson and Hessinger, 1989a; Watson and Hudson, 1994; Watson et al., 1998), in which the force of stimulation was not precisely controlled, tentacles likely were most commonly contacted with forces other than those that stimulate maximal discharge. Thus, in seawater alone, mean levels of discharge were modest. In the presence of additional stimuli such as NANA or nearby vibrations at key frequencies, levels of discharge changed. In those studies, had tentacles consistently been contacted at a force of 0.33 mN, levels of discharge would have remained the same despite the presence or absence of NANA and/or nearby vibrations at key frequencies.

A model for the regulation of nematocyst discharge

In seawater alone, anemones discharge nematocysts maximally into objects that strike the tentacle with forces of 0.33 and 1.10 mN. We speculate that such forces overlap those produced by ideally sized prey. Perhaps the maximum observed at 1.10 mN overlaps the force produced when the body of a planktonic crustacean swims into direct contact with the tentacle and the maximum observed at 0.33 mN overlaps the force produced by a swimming appendage contacting the tentacle. Obviously, experiments are necessary to test this idea. In this regard, we must consider how it could be possible for the prey to arrive at the tentacle without having notified the anemone to its arrival beforehand by virtue of chemoreceptor and vibration-sensitive mechanoreceptors. Perhaps prey swimming upstream to contact the anemone would meet these criteria because the odorants (mucins) would not diffuse very far from the prey organism in the direction of the tentacle.

In the event that anemone chemoreceptors for N-acetylated sugars are activated, the force response curve broadens such that maximal discharge occurs over a wider range of relatively small forces and then decreasing to baseline at 1.50 mN. Because N-acetylated sugars are common constituents of glycoproteins and glycolipids, activated chemoreceptors for N-acetylated sugars announce the arrival of potential prey. Upon receiving such information, the anemone system accepts a wider range of smaller forces as stimulatory, perhaps because of the possibility

that approaching prey may strike the tentacle with glancing blows of either the body or swimming appendages. Nevertheless, the anemone system still rejects contact by forces that are likely to be produced by prey that are too large (those striking the tentacle at forces of 1.50 mN) because such forces trigger only baseline discharge. In the event that nearby vibrations are detected in the presence of N-acetylated sugars, the response varies according to the frequency of vibration. In the combined presence of NANA and vibrations at 30 Hz, discharge is maximal at smaller forces and then decreases to baseline at 1.50 mN. This result is consistent with the detection of nearby, calmly swimming prey. Thus, upon receiving such information, the anemone system accepts a wider range of smaller forces as stimulatory (as described above) but still functions to reject large forces that may accompany contact by larger prey.

Prey selection according to the size of the prey is known to occur for jellyfish that prey upon the same type of prey over a range of sizes (Sullivan et al., 1997). Thus, there is precedence for the idea that cnidarians select prey according to their size.

Signal processing in anemones

We were surprised to see a bimodal response curve in the combined presence of NANA and nearby vibrations at 55 Hz (NANA55). In this case, the response curve resembled that obtained in seawater alone (i.e. the absence of NANA) and in the absence of nearby vibrations at a key frequency. Indeed, the peaks obtained at 0.33 and 1.10 mN, are comparable to those observed in seawater alone and in the absence of nearby vibrations. Nevertheless, the curves are not identical to each other. Although 55 Hz is not a key frequency in the presence of NANA, it is intriguing to see that the anemone system seems to largely ignore NANA in the presence of nearby vibrations at 55 Hz (a non-key frequency in NANA). With these data, we are getting a glimpse of how anemones integrate sensory input. It appears that sensory input is processed according to a hierarchy in which vibrations are considered to be more important than are chemical stimuli. In this case, vibrations detected at 55 Hz, the 'wrong frequency' cancel out the 'right odorant' (NANA) that otherwise alone signifies proximity of potential prey. In response to such conflicting information, it appears that the anemone system attempts to return to the default response such that only the optimal forces in seawater alone (0.33 and 1.10 mN) stimulate maximal discharge. It certainly will be interesting to further investigate combinations of stimuli to determine how anemones integrate sensory input using their 'simple' nervous system.

Comparative force-dependent regulation of nematocyst discharge

Finally, we compare the responsiveness of anemones to that reported for *Hydra*. In *Hydra*, maximal discharge of desmonene nematocysts occurs at 19 mN (Scappaticci et al., 2010). In the present study, maximal discharge of microbasic p-mastigophore nematocysts occurs in response much smaller forces (0.33 and 1.10 mN, respectively) for test probes moved into contact with intact animals in seawater alone. Either the anemone mechanoreceptor system is at least an order of magnitude more sensitive than that for *Hydra*, or differences in the experimental design contributed to such observed differences. In the study on *Hydra*, discharge was tested after test probes contacted single cnidocils of nematocytes located on excised tentacles. In this

study, test probes were moved into contact with whole tentacles (i.e. such that they contacted hundreds of cells) of intact anemones. At this point, we cannot rule out the possibility that differences in experimental design contributed to the apparent differences of the anemone and *Hydra* mechanoreceptor systems. It would be interesting to compare the mechanoreceptor systems under more comparable experimental conditions.

Competing Interests

The authors declare that there are no competing interests.

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