Morphodynamic Hair Bundles Arising From Sensory Cell/Supporting Cell Complexes Frequency-Tune Nematocyst Discharge in Sea Anemones

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ABSTRACT

Discharge of nematocysts from cnidocytes occurs in response to appropriate chemical and mechanical stimulation. In sea anemone tentacles, activating chemoreceptors for N-acetylated sugars shifts maximal discharge into vibrating targets to low frequencies corresponding to prey movements and induces hair bundles to elongate by approximately 1–2 µm. Until now, only indirect, correlative evidence linked these two events. Using cytochalasin D, we provide evidence that bundle elongation is necessary for the frequency shift. Moreover, we find that only bundles associated with sensory cell/supporting cell complexes elongate with chemosensitization, and not bundles associated with cnidocyte/supporting cell complexes as was previously thought. Cytochemical labeling of sensory cells, purported to be bipolar neurons connected to the nerve net, suggests that sensory cells may interconnect with each other and with cnidocytes. Taken together, these findings are incompatible with the classical view that cnidocytes are independent effectors of nematocyst discharge and, furthermore, implicate the involvement of morphodynamic neurons in fine-tuning vibration-dependent discharge of nematocysts into swimming prey.

The classic explanation for regulation of nematocyst discharge in sea anemones is the independent effector hypothesis, proposed by Parker ('16). According to this hypothesis, cnidocytes (cells containing and deploying nematocysts and other cnidae) function both as receptor and effector cells in regulating discharge of nematocysts and do not require intervention by the nervous system. Subsequent to Parker's hypothesis, the nervous system has been implicated in superimposing mainly an inhibitory effect on modulating discharge but with cnidocytes still in direct control over regulating discharge in response to specific chemical and mechanical cues presented by prey (for review, see Mariscal, '74). Recent discoveries that discharge into vibrating and nonvibrating targets is modulated by exogenous N-acetylated sugars and that chemoreceptors for the sugars are located on the surface of supporting cells adjacent to cnidocytes have prompted another revision of the hypothesis (Thorington and Hessinger, '88; Watson and Hessinger, '87, '89a). This revision suggests that cnidocyte/supporting cell complexes (CSCCs) function as independent effector units regulating nematocyst discharge. With respect to vibrating targets, exposing animals to optimal levels of N-acetylated sugars shifts maximal discharge to lower frequencies matching prey movements and induces hair bundles on tentacles to elongate by approximately 1–2 µm. A model has been proposed suggesting that the hair bundles are vibration-sensitive mechanoreceptors that, upon sugar-induced elongation, tune to frequencies matching those produced by swimming prey (Watson and Hessinger, '89b, '91). Thus, the combination of chemoreception of sugars and the mechanoreception of vibrations would predispose cnidocytes to trigger discharge upon contact between the tentacle and the prey.

Until now, only indirect evidence linked the elongation of bundles to the downward frequency shift in nematocyst discharge. Cytochemical studies using phalloidin indicate that bundles elongate by polymerization of actin within the individual stereocilia comprising the bundles (Watson et al., '92). Using cytochalasin D, at concentrations thought to specifically block actin polymerization (Cooper, '87), we now find that bundle elongation is necessary for the sugar-induced frequency shift in discharge.

Two types of cell complexes in the epidermis of anemone tentacles form hair bundles that protrude into the seawater (Peteya, '75; Mariscal et al., '78; Watson and Hessinger, '89a). One type is the CSCC
in which a single cnidocyte contributes a single kinocilium to the center of the bundle and adjacent supporting cells each contribute many stereocilia to the periphery of the bundle. The other type is the sensory cell/supporting cell complex (SNSC) in which a single sensory cell contributes a single kinocilium and 5–10 large diameter stereocilia to the center of the bundle and adjacent supporting cells each contribute many small diameter stereocilia to the periphery. The sensory cells of anemone tentacles are purported to be bipolar neurons whose axons connect to the underlying nerve net (Peteya, '75; van Marle, '90).

Previous studies reporting changes in bundle lengths with chemosensitization involved measurements of mixed populations including both CSCC and SNSC bundles (Watson and Hessinger, '91). This was due to the difficulty in distinguishing the bundle types using light microscopy without the aid of computer enhancement. The primary, tunable, vibration-sensitive mechanoreceptors were presumed to be the CSCC bundles because CSCCs are highly abundant and house the nematocysts, and SNSCs were thought to be extremely rare based on TEM observations. Using computer-enhanced light microscopy, CSCC and SNSC bundles can be readily distinguished. We now report that only SNSC hair bundles elongate when specimens are exposed to N-acetylated sugars, whereas CSCC hair bundles remain unchanged.

In intact tentacles, SNSCs and basal processes of all epidermal cells are difficult to resolve with light microscopy due to the high abundance of bi-refringent nematocysts within cnidocytes. In this study, we use two methods to visualize sensory cells and their interconnections: DiI labeling via crystal loading of intact, fixed tentacles and silver impregnation of tentacles that have been depleted of nematocysts. We see possible interconnections between multiple sensory cells and between sensory cells and cnidocytes.

MATERIALS AND METHODS

Reagents

Paraformaldehyde was obtained from Ted Pella, Inc., Redding, CA, and (3), 1,1'-dioctadecyl-3,3',3''-tetramethylindocarbocyanine perchlorate (DiIC18) was obtained from Molecular Probes, Eugene, OR. N-acetylenuraminic acid (NANA), type VI, cytochalasin D (CD), glutaraldehyde, and chemicals not otherwise specified were obtained from Sigma Chemical Co., St. Louis, MO. Potassium seawater (K'SW) was prepared in deionized water as follows: NaCl (382 mM), KCl (50 mM), MgCl2-6H2O (23.9 mM), MgSO4-7H2O (25.5 mM), CaCl2 (12.2 mM), and NaHCO3 to adjust pH to 8.3.

Animal maintenance

Monoclonal specimens of the sea anemone Haliphanella luciae were kept in Pyrex dishes containing natural seawater at 32°C, held at 22–24°C, and maintained on a 12:12 photoperiod. The anemones were fed brine shrimp nauplii (San Francisco Bay) twice weekly. Experiments involving assaying bundle length and nematocyst discharge were performed approximately 72 h after feeding.

Measurements of hair bundles

Whole specimens were anesthetized for 2 h in K'SW. For the experiments comparing CSCC bundles to SNSC bundles, the anesthetized animals were incubated for 5 min either in fresh K'SW (seawater controls), or K'SW containing 10^-7 M N-acetylenuraminic acid (NANA treatment). For the cytochalasin experiments, the anesthetized animals were incubated in K'SW containing specified concentrations of CD either for 20 min (seawater + CD treatment), or for 15 min and subsequently incubated in K'SW containing specified concentrations of CD with 10^-7 M NANA for 5 min (NANA + CD treatment). After treatment, whole specimens were fixed in K'SW containing 2.5% glutaraldehyde and 1% paraformaldehyde for 1 h and then rinsed twice in K'SW for 15 min each. Fixed tentacles were excised and prepared as wet mounts on glass coverslips. Bundles were viewed with video-enhanced DIC optics (DPlanApo100UV objective lens, n.a. = 1.30; BH3-2 APlanat Achromat DIC condenser, n.a. = 1.4 modified to fit an IMT2 Olympus Inverted Microscope; 100 watt illumination using a scrambler, Technical Video, Inc.; analog video enhancement, Colorado Video Model 605 video contrast enhancer; digital enhancement, Hamamatsu Argus 10 processor). Images were captured, contrast enhanced, and bundles analyzed using Image1 software and hardware. Lengths of bundles were determined by measuring individual bundles (n = 25) from tip to base, parallel to the long axis of the bundle. The tip was defined as the apical terminus of the longest stereocilium of the bundle and the base as the point of intersection of the bundle with the tentacle surface. CSCC bundles were identified by the presence of a microbasic p-mastigophore nematocyst directly beneath the bundle. SNSC bundles were identified by the absence of a nematocyst or other cnida directly be-
neath the bundle and by the presence of large diameter (0.2–0.5 μm) stereocilia rising above small diameter stereocilia (0.05–0.2 μm) at the tip of the bundle. Prior to data collection, images were coded and scrambled by computer so that all of the bundle measurements were performed blind with respect to treatment.

Dil labeling

Whole animals were anesthetized and fixed as described above. Fixed specimens were rinsed in K'SW for several days, then most of the K'SW was removed, allowing only a thin film of K'SW to cover the specimens. Dil crystals were sprinkled onto tentacles and allowed to remain for 24 h while specimens were kept in the dark. Labeled specimens were rinsed several times in K'SW for 15 min each. Tentacles were excised and prepared as wet mounts on glass slides. Tentacles were viewed with a Nikon Optiphot upright microscope at 600x final magnification (10x oculars, objective lens Plan-Apo60/1.40) and labeled cell types identified by the presence of large diameter stereocilia arising from the apices of the cells. Cnidocytes were identified by conspicuous nematocysts or other cnidae within the cells. Areas of interest were then viewed with confocal microscopy (Biorad-600). Images of optical serial sections were captured by computer (COMOS software) and subsequently processed using IPPLUS software (Media Cybernetics).

Silver impregnation

Whole animals were anesthetized as above and then depleted of nematocysts and other cnidae by exposure to a hypotonic solution, prepared by diluting K'SW 1:1 with distilled water, for 1 h and then returned to K'SW for a 1 h recovery. These specimens were then fixed for 30 min as described above and rinsed in distilled water. Fixed specimens were stained using a modification of Porter's and Davenport's Golgi staining method (Davenport, '60) that involved incubating specimens in a solution of 0.5% AgNO₃ and 2.5% glutaraldehyde in distilled water for 4 d in the dark, rinsing 2 h in distilled water, and subsequently incubating in 2.5% K' (CrO₄)₂ in distilled water for 4 d. Tentacles were excised, prepared as wet mounts, and viewed with video-enhanced DIC optics as described above. Images were captured, enhanced, and analyzed using Image1.

Dose responses and frequency responses of nematocyst discharge

Dose responses and frequency responses of nematocyst discharge were performed as described elsewhere (Watson and Hessinger, '89a, b, '91). Briefly, animals were removed from the mass culture, placed in small Petri dishes filled with natural seawater (NSW), and allowed 3–4 h to recover. The NSW was then replaced either with fresh NSW (seawater controls) or NSW with 10⁻⁷ M NANA added (NANA treatment), in which animals were incubated for 5 min. For the cytochalasin experiments, the NSW was replaced with NSW containing specified concentrations of CD, in which animals were incubated either for 20 min (seawater + CD treatment), or for 15 min followed by 5 min in NSW containing specified concentrations of CD with 10⁻⁷ M NANA added (NANA + CD treatment). Tentacles were then touched with gelatin-coated test probes made to vibrate at specific frequencies. For the frequency responses, probes were made to vibrate at 5 Hz intervals over a range of 5 to 100 Hz. For the dose responses to CD, probes were made to vibrate either at 55 Hz (seawater + CD treatment), or at 5 Hz (NANA + CD treatment). Probes were fixed in 2.5% glutaraldehyde in NSW for 1 min and then prepared as wet mounts. The microbasic p-mastigophore nematocysts discharged into the gelatin were counted in a single field of view (10x oculars, 40x objective, and phase-contrast optics). The field of view subjectively evaluated as having the greatest number of nematocysts on the probe was scored. Four replicate probes were used for each experimental condition, one probe for each of four anemones, and data were averaged from two separate experiments.

RESULTS

SNSC bundles elongate with NANA treatment but not CSCC bundles

Anemone hair bundles appear as cone-shaped protrusions from the tentacle surface (Fig. 1). CSCC bundles are composed of small diameter stereocilia (0.05–0.20 μm) surrounding a kinocilium (Mariscal et al., '78). Directly below a CSCC bundle is a conspicuous nematocyst (Fig. 1A). SNSC bundles are composed of an outer circle of small diameter stereocilia surrounding an inner circle of 5–10 longer, large diameter stereocilia (0.20–0.50 μm) and a central kinocilium (Peteya, '75). Directly below the SNSC bundle, a nematocyst is conspicuously absent (Fig. 1B). In seawater controls, SNSC
Fig. 1. Video-enhanced photomicrographs (DIC) portraying different types of hair bundles protruding from epidermal surfaces of sea anemone tentacles. Tentacles were excised from whole animals that had been anesthetized in K'SW for 2 h, fixed in a K'SW solution containing 2.5% glutaraldehyde and 1% paraformaldehyde for 1 h, and rinsed twice in K'SW for 15 min each. A: A hair bundle extends into the seawater from a cnidocyte/supporting cell complex, CSCC, and is composed of smaller diameter stereocilia (s) surrounding a central kinocilium (k). Directly beneath the bundle is a conspicuous microbasic p-mastigophore nematocyst (n). B: A hair bundle extends into the seawater from a sensory cell/supporting cell complex, SNSC, and is composed of small diameter stereocilia (s) at the periphery and longer, large diameter stereocilia (l) surrounding a central kinocilium (k). Directly beneath the bundle, a nematocyst or other type of cnida is conspicuously absent, however, cnidocytes (c) containing cnidae can be seen elsewhere within the epidermis. Scale bar = 5 μm.

Bundles ranged in length from approximately 6 to 10 μm. CSCC bundles were more homogeneous in length, ranging from approximately 4 to 6 μm. With NANA treatment, SNSC bundles elongated by an average of 1.6 μm and 1.9 μm in two replicate experiments (Fig. 2A,B). In contrast, CSCC bundles did not elongate but showed average decreases of 0.02 μm and 0.03 μm in replicate experiments (Fig. 2A,B). All bundle length measurements were performed blind. Statistical analyses comparing means in seawater to NANA treatment indicated significant differences for SNSC bundle lengths (Student's t-test, P < 0.00001 in each case) but insignificant differences for CSCC bundle lengths (P > 0.85 in each case).

**Visualization of sensory cells and interconnections between cells**

DiI crystal loading of tentacles resulted in intense labeling of many entire sensory cells including bundles, cell bodies, and basal processes. In some regions of the tentacles, patches of sensory cells were labeled and at other areas solitary sensory cells were labeled. Using confocal microscopy, it was possible to optically section the labeled sensory cells. Computer reconstructions of optical sections (Figs. 3,4) revealed that the kinocilium and large diameter stereocilia protruded from a ring-shaped cell apex. The elongate cell body was oriented at a sharp angle relative to the bundle and appeared to wind through the epidermis, gradually tapering toward the base. Radiating from a central point at the base of the sensory cell were 3–6 narrow processes, although it was not clear whether these structures were basal processes of sensory cells or processes of other neurons of the underlying nerve net. Within the patches of sensory cells, the basal processes of two or more cells often appeared to intersect (Fig. 3). Occasionally, processes extending from the bases of solitary sen-
Fig. 2. Bar graphs of mean lengths (± SEM) of CSCC and SNSC hair bundles in seawater controls and with NANA treatment. With NANA treatment, SNSC bundles significantly elongated in two replicate experiments (A and B, respectively; Student’s t-test, \(P < 0.00001\) in each case), while CSCC bundles showed insignificant changes in length in replicate experiments (A and B, respectively; Student’s t-test, \(P > 0.85\) in each case). All bundle length measurements were performed blind with respect to treatment. Tentacles were excised from whole animals that had been processed as described in Figure 1, except for NANA treatment, whereby anesthetized specimens were incubated in K’s SW containing 10- M N-acetylneuraminic acid for 5 min prior to fixation.

Sensory cells traversed relatively long distances, up to several hundred microns, running parallel to the long axis of the tentacle.

Less frequently, plasma membranes of cnidocytes labeled with DiI (Fig. 4). The nematocysts did not label but appeared as black silhouettes in contrast to the brightly labeled plasma membranes, thus permitting cnidocytes to be easily identified. On occasion, patches of labeled cnidocytes appeared to converge basally. Sometimes basal processes of cnidocytes and sensory cells appeared to meet at a common locus (Fig. 4).

Silver impregnation of cnida-depleted tentacles resulted in labeling of a continuous, regular reticulum of processes located deep within the epidermis (Fig. 5). Occasionally, the processes formed radial arrays that converged near a circular object. Measurements were made to compare the structures observed with silver impregnation to the structures labeled with DiI. The average diameter of the silver-stained circular objects, 2.32 \(\mu\)m, was in close agreement with the average diameter of DiI-labeled bases of sensory cells, 2.29 \(\mu\)m. The mean diameters of processes were also similar, 0.72 \(\mu\)m with silver impregnation and 0.63 \(\mu\)m with DiI label. Furthermore, the angles between processes at junctions were comparable, with average angles of 103.8° and 103.2° in silver-stained specimens and DiI-labeled specimens, respectively.

**Discharge responses in seawater and with NANA treatment to “key” frequencies are differentially sensitive to CD**

The effects of various doses of CD on nematocyst discharge were tested at an optimal frequency with NANA treatment, 5 Hz, and at an optimal frequency in seawater alone, 55 Hz (Fig. 6). Both responses were unaffected by CD at concentrations of \(10^{-9}\) M or lower. At \(10^{-8}\) M CD, the NANA response was reduced to 61% while the seawater response was unaffected. At \(3 \times 10^{-8}\) M CD, the NANA response was reduced to 50% while the seawater response was reduced to 87%. At \(10^{-7}\) M CD, both responses were reduced to approximately 40%, comparable to baseline levels, i.e., the levels of discharge that normally occur with contact regardless of treatment.

**Frequency responses of nematocyst discharge are altered by CD**

To more precisely describe the effects of CD on frequency-dependent discharge in seawater and with NANA treatment, frequency–response curves were generated in the presence of CD at four concentrations: \(10^{-4}\), \(3 \times 10^{-4}\), \(10^{-3}\), and \(10^{-2}\) M (Figs. 7, 8). For each curve, peaks were determined statistically by Newman–Keuls analysis (\(P < 0.05\)).

In seawater controls, peaks of discharge were detected at 30, 50–55, 65, and 75 Hz (Fig. 7A). In the presence of \(10^{-6}\) M CD, statistically significant peaks of discharge were detected at 50–55, 65, and 75 Hz, but not at 30 Hz (Fig. 7B). In \(3 \times 10^{-6}\) M CD, peaks of discharge were detected at 50–65 and 75 Hz, but also at 85 and 95 Hz (Fig. 7C). In \(10^{-5}\) M CD, statistically significant peaks of discharge occurred over a range of frequencies from 65–100 Hz (Fig. 7D).

With NANA treatment, peaks of discharge were detected at 5, 15–20, 30, and 40 Hz (Fig. 8A). In the combined presence of NANA and \(10^{-9}\) M CD, peaks of discharge occurred at 5, 15, 30, and 40 Hz, but not at 20 Hz (Fig. 8A). In NANA and \(10^{-6}\) M CD, statistically significant peaks of discharge...
were detected at 10, 20, 30, 45–55, 65, and 75 Hz (Fig. 8B). In NANA and 3 x 10^{-9} M CD, peaks of discharge occurred at 50–55, 65, 75, 85–90, and 100 Hz (Fig. 8C). In NANA and 10^{-8} M CD, peaks of discharge were detected at 55 Hz and over a range of frequencies from 70–100 Hz (Fig. 8D).

**Bundle elongation with NANA treatment is inhibited by CD**

The effects of CD on SNSC bundle lengths were tested both with and without NANA treatment. Mean bundle lengths were subjected to one-way ANOVA followed by post hoc least significant difference (LSD). To facilitate comparisons between experiments performed on different days, means were normalized by subtracting the mean bundle length of seawater controls from the mean bundle lengths for each treatment within a single experiment. Thus, data are presented as the change in mean bundle length (+ or −) compared to a seawater control (0 value) with statistically significant differences indicated (Fig. 9).

In the absence of CD or in 10^{-9} M CD, bundles significantly elongated by approximately 2 μm with NANA treatment compared to seawater controls ($P < 2.0 + 10^{-4}$ in each case). In 10^{-8} M CD with
Fig. 4. Computer-generated overlay image from optical serial sections of a DiI-labeled sensory cell and cnidocyte within an anemone tentacle visualized with confocal microscopy. The entire sensory cell is labeled, including large stereocilia (l) arising from a ring-shaped cell apex (a) and the cell body (b) that appears to wind through the epidermis tapering basally. Whereas the plasma membrane of the cnidocyte (c) is labeled, the nematocyst within the cell is not labeled. The bases of the two cells appear to meet at a common locus (i) on one of the four basal processes radiating outward. Cells were "crystal loaded" with DiI as described in Figure 3. Scale bar = 12 μm.

NANA treatment, bundles elongated by only 0.3 μm, while in seawater and 10^{-8} M CD, bundles shortened by 0.1 μm, both of which were statistically indistinguishable from seawater controls (P > 0.5). Likewise, in 3 × 10^{-3} M CD with or without NANA treatment, bundles were comparable in length to seawater controls (P > 0.7). In 10^{-6} M CD with or without NANA treatment, bundles significantly shortened by approximately -1.3 μm (P < 0.01).

The insignificant differences found between the mean bundle lengths for seawater controls and for specimens in 10^{-8} M CD with NANA treatment (Fig. 9) were surprising since differences were detected in the frequency responses between specimens incubated in seawater (Fig. 7A) and in 10^{-8} M CD and NANA (Fig. 8B). To determine if the effects of this concentration of CD on frequency responses could be explained by differences in the distributions of the bundle length data that were not detectable by comparing means, median values were used as indicators of the distributions of the data sets. To facilitate comparisons between experiments performed on different days, data sets were normalized by subtracting the mean bundle length of seawater controls from each bundle length within a single experiment. Data sets are presented as normalized distributions with median values indicated (Fig. 10). At most doses of CD, median values paralleled means. The median values for all seawater treatments in CD, regardless of CD concentration, were comparable to the median for the seawater controls, with the exception of the highest concentration of CD tested (10^{-6} M). In 10^{-6} M CD, medians with and without NANA treatment were substantially lower than seawater controls by approximately -1.3 μm. The median values with NANA treatment in the absence and presence of 10^{-6} M CD were approximately 2 μm higher than seawater control medians. In 3 × 10^{-8} M CD, the median with NANA treatment was approximately 0.2 μm higher than controls. However in 10^{-8} M CD, the median with NANA treatment was approximately 0.8 μm higher than the median for seawater controls, an increase of approximately 0.5 μm above the change in mean bundle length from

Fig. 5. DIC video-enhanced photomicrograph of modified Golgi impregnation of cnida-depleted tentacles. A continuous, regular reticulum of processes (p) is labeled deep within the epidermis, occasionally forming radial arrays (r) that converge near a circular object (o). Measurements were made comparing these structures to sensory cell structures labeled with DiI (e.g., in Figs. 3 and 4; see Results). Anesthetized animals were osmotically stressed to promote extrusion of cnidae, fixed for 30 min as described in Figure 1, and rinsed in distilled water. Fixed specimens were incubated in a solution of 0.5% AgNO3 and 2.5% glutaraldehyde in distilled water, and subsequently incubated in 2.5% K2Cr2O7 in distilled water for 4 d. Scale bar = 5 μm.
SENSORY CELL BUNDLES TUNE NEMATOCYST DISCHARGE

Fig. 6. Dose responses of nematocyst discharge into vibrating test probes to cytochalasin D (CD). Discharge levels in seawater are depicted by open circles, with the first point indicating discharge in absence of CD. Discharge levels in NANA treatment are depicted by closed circles, with the first point indicating discharge in the absence both of added NANA and CD and the second point indicating discharge in the presence of added NANA but without CD. For seawater treatments, anemones were incubated in natural seawater containing specified concentrations of CD for 20 min then touched with gelatin-coated test probes made to vibrate at 55 Hz. For NANA treatments, anemones were incubated in natural seawater containing specified concentrations of CD for 15 min and subsequently for 5 min with the addition of $10^{-7}$ M NANA, then touched with test probes made to vibrate at 5 Hz. Microbasic p-mastigophore nematocysts discharged into four test probes were counted for each dose of CD tested. Data are presented as the mean number of nematocysts counted ± SEM for two replicate experiments.

the same experiment. Thus, the median for NANA treatment in $10^{-8}$ M CD was approximately at the halfway point of elongation (1 μm) occurring with NANA treatment in the absence of CD (2 μm).

DISCUSSION

Until now, the tunable, vibration-sensitive mechanoreceptors of anemone tentacles were presumed to be hair bundles arising from cnidocyte/supporting cell complexes (Watson and Hessinger, '89a, '91). This study shows that only bundles arising from sensory cell/supporting cell complexes elongate in response to chemodetection of sugars, and not bundles arising from cnidocyte/supporting cell complexes. Sensory cells of anemone tentacles are purported to be bipolar neurons whose axons connect to the underlying nerve net (Peteya, '75; van Marle, '90). However, little is known about the function of these sensory cells or whether sensory cells interact with each other or with other cell types. In this study, two methods (DiI and silver impregnation) are used to visualize sensory cells and their possible interconnections to each other and to other cells. Combining information from the different labeling methods, we conclude that sensory cells are likely to be interconnected to each other and to cnidocytes. Although it is unclear whether the connections are direct between these cells or whether the connections are indirect via interneurons of the nerve net, it appears that pathways exist whereby sensory cells could communicate with each other and with cnidocytes.

This is the first study to provide evidence that sugar-induced bundle elongation is required for tuning nematocyst discharge to low frequencies. While this idea was suggested previously based on dose responses to NANA of bundle elongation and frequency-dependent discharge (Watson and Hessinger, '91), direct evidence was not provided that established the interdependency of these two events. The effects of CD on frequency responses of discharge and on bundle lengths in seawater and with NANA treatment suggest that a direct relationship exists between bundle lengths and frequencies to which maximal discharge of nematocysts occurs.

Exposure to $10^{-6}$ M CD causes bundles to shorten such that they are comparable in length without and with NANA treatment, but shorter than seawater controls (Fig. 9). These data suggest that at $10^{-6}$ M, CD is interfering with the maintenance of bundles at normal resting lengths while also inhibiting sugar-induced elongation. However, this dose of CD does not render the bundles insensitive to vibrations, as peaks of discharge are detected at frequencies higher than is normally detected even in seawater controls (compare Figs. 7A,D and 8D). Thus, we find a relationship between abnormally short SNSC bundles and discharge of nematocysts from cnidocytes at abnormally high frequencies.

Frequency responses of specimens in $3 \times 10^{-6}$ M CD with or without NANA treatment are more or less comparable to each other and to seawater controls, each exhibiting statistically significant peaks of discharge at 50–55, 65, and 75 Hz (compare Figs. 7C, 8C, and 7A), along with several additional peaks at higher frequencies in the presence of CD (Figs. 7C, 8C). While these additional peaks suggest that at this concentration CD may be interfering with the maintenance of resting length of at least some bundles, we could not detect, by our
methods, a significant shortening of bundles induced by $3 \times 10^{-8}$ M CD as compared to seawater controls (Figs. 9, 10). It is clear, however, that exposure to $3 \times 10^{-8}$ M CD blocks the elongation of bundles and drastically alters the frequency responses of discharge normally induced by NANA treatment (Figs. 8A,C, 9, 10).

Decreasing the CD concentration to $10^{-6}$ M virtually restores vibration-dependent discharge to normal for specimens exposed to this concentration of CD without NANA, as statistically significant peaks of discharge are detected at essentially the same frequencies as for seawater controls (Fig. 7A,B). However, with NANA treatment, $10^{-8}$ M CD generates peaks of discharge at frequencies typical of seawater controls (30, 50–55, 65, and 75 Hz) in addition to peaks at 10, 20, 30, and 45 Hz (Fig. 8B), frequencies slightly higher than typical for NANA-treated specimens in the absence of CD (Fig. 8A). Hence, at this concentration, CD may partially inhibit bundle elongation induced by NANA without significantly interfering with the maintenance of resting bundle length. Our morphological data for specimens exposed to $10^{-8}$ M CD with NANA treatment agree with this interpretation if we use the median bundle length, and not the mean bundle length, to characterize the population of bundles on the tentacle. In $10^{-8}$ M CD with NANA treatment, the median bundle length was $0.8 \mu m$ longer than seawater controls (Fig. 10). In the absence of CD, NANA treatment caused the median bundle length to increase by $2.0 \mu m$ as compared to seawater controls (Fig. 10). Comparisons of bundle length based on means failed to detect such a trend in the data. Thus, the median may be a more sensitive parameter than the mean to predict responsiveness of the bundles.

Finally, exposure to $10^{-9}$ M CD neither affects
Fig. 8. Vibration-dependent discharge of nematocysts in anemones treated with NANA and CD. Tentacles of intact sea anemones were touched with vibrating test probes after 15 min in seawater containing CD followed by 5 min in seawater containing CD and 10^{-7} M NANA. Microbasic p-mastigophore nematocysts discharged into four test probes were counted for each frequency and dose of CD tested. Data are presented as the mean number of nematocysts counted ± SEM for two replicate experiments. A: Closed circles depict data for control specimens (exposed only to NANA). Open circles depict data for specimens exposed to 10^{-4} M CD. B: Data are shown for the ability of NANA to induce bundles to elongate (Figs. 9, 10) nor to tune vibration-dependent nematocyst discharge to 5, 15, 30, and 40 Hz (Fig. 8A).

At submicromolar concentrations, CD is thought to specifically inhibit actin polymerization, while higher concentrations (>1 μM) of CD are required to disrupt pre-existing cytoskeletal structures, such as stress fibers, or to interfere with sliding of actin filaments, such as in smooth muscle contraction (Cooper, '87; Adler et al., '83). Studies using phalloidin to label F-actin and DNaseI to label G-actin indicate that upon chemosensitization with NANA, the F-actin content of bundles increases while the G-actin content of apices of sensory cells decreases (Watson et al., '92; G.M. Watson, unpublished). These observations suggest that NANA-induced elongation of bundles involves polymerization of actin within the individual stereocilia comprising the bundles. It is conceivable that the intermediate doses of CD (10^{-8} and 3 × 10^{-8} M) inhibit bundle elongation by blocking actin polymerization without significantly affecting resting bundle length.

Taken together, the results of this study provide evidence that contradicts the classical view that cnidocytes are independent effectors that do not employ nervous intervention to regulate discharge of nematocysts. We conclude that upon chemo-detection of N-acetylated sugars, tuning the response of nematocyst discharge to movements of
swimming prey requires elongation of vibration-sensitive mechanoreceptors arising from sensory cell/supporting cell complexes. Because SNSCs do not house the nematocysts, they must, either directly or indirectly, communicate with cnidocytes to regulate discharge of nematocysts.

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**LITERATURE CITED**


