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# Frequency and Amplitude Tuning of Nematocyst Discharge by Proline

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*ABSTRACT* In sea anemone tentacles, discharge of nematocysts into vibrating targets is regulated by hair bundles. N-acetylated sugars are known to induce hair bundles to elongate while tuning nematocyst discharge to low frequencies and small amplitudes corresponding to movements produced by calmly swimming prey. Proline is known to induce hair bundles to shorten while tuning nematocyst discharge to higher frequencies. In this report, we provide evidence suggesting that proline linearly tunes nematocyst discharge. Nematocyst discharge is tuned by increases of $10^{-18}$ M proline above background levels ranging from $10^{-12}$ M to $10^{-8}$ M proline, but only after anemones adapt to the background levels of proline. Anemones adapt more rapidly to $10^{-12}$ M proline than to $10^{-10}$ M, followed by $10^{-8}$ M proline. L-proline and D-proline comparably tune nematocyst discharge. Proline tunes discharge to higher frequencies and/or to larger amplitudes at the same lower frequencies produced by prey. We propose that N-acetylated sugars tune nematocyst discharge to the movements of calmly swimming prey. After the prey is wounded by nematocysts, it releases proline into the seawater. This proline tunes nematocyst discharge to higher frequencies and/or larger amplitudes at low frequencies corresponding to movements produced by struggling, wounded prey. Thus, the greatest numbers of nematocysts may be discharged into calmly swimming, fresh prey and into vigorously struggling, wounded prey. 

Sea anemones capture prey by using nematocysts and other cnidae, intracellular capsules containing tubules that rapidly and forcibly evert (Skaer and Picken, '65; Holstein and Tardent, '84). Everting tubules either penetrate the prey to inject potent toxins, adhere to the surface of the prey, or entangle its appendages, depending on the type of cnida (Mariscal, '74). In anemone tentacles, discharge of nematocysts is regulated, at least in part, by effector/receptor complexes consisting of cnidocytes and supporting cells surrounding them. Supporting cell chemoreceptors for N-acetylated sugars or for certain amino compounds, including proline, modulate discharge from cnidocytes into non-vibrating and vibrating targets (Thorington and Hessinger, '88a,b; Watson and Hessinger, '87, '89a,b, '91a,b).

For non-vibrating targets, activated chemoreceptors for N-acetylated sugars or for proline predispose cnidocytes to discharge nematocysts in the event of contact between the target and tentacle. Increasing the ligand concentration increases discharge to a maximum and then decreases discharge to seawater control levels (Thorington and Hessinger, '88a,b; Watson and Hessinger, '87, '89b).

For vibrating targets, chemoreceptors for N-acetylated sugars act antagonistically to those for proline. Discharge is frequency-specific so that discharge at tuned frequencies is 2–6 times greater than at other frequencies. Activating chemoreceptors for N-acetylated sugars tunes hair bundles regulating discharge to lower frequencies than seawater controls. These low frequencies correspond to those produced by swimming prey. Whereas proline alone has no effect on frequency-specific nematocyst discharge, activating chemoreceptors for proline after pretreatment with N-acetylated sugars tunes hair bundles to frequency maxima higher than seawater controls (Watson and Hessinger, '89a, '91b, and submitted).

Tuning involves changing the length of hair bundles such that exposure to N-acetylated sugars induces bundles to elongate by approximately 15%, whereas subsequent exposure to proline induces bundles to shorten by 30%. Such changes in bundle length are accompanied by increases and decreases, respectively, in the F-actin content of the stereocilia comprising hair bundles (Watson et al., '92). Receptor-induced changes in bundle length may be modulated by receptor-dependent changes in intracellular levels of cyclic-AMP (Watson and Hessinger, '92; Thibodeaux and Watson, '92).

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As with hair bundles from vertebrate systems, frequency responsiveness correlates to length such that longer bundles respond to lower frequencies (Frishkopf and DeRosier, '83; Holton and Hudspeth, '83; Watson and Hessinger, '91a). Hair bundles of sea anemones are extremely sensitive to proline-induced tuning with a threshold at $10^{-18}$ M proline. Furthermore, anemones can detect increases in proline of $10^{-18}$ M against a background concentration of $10^{-8}$ M proline (Watson and Hessinger, '91b, and submitted). In this report, we examine for the first time, amplitude-tuning of nematocyst discharge by proline. In addition, we examine further frequency-tuning of nematocyst discharge by proline and adaptation to proline.

## MATERIALS AND METHODS

### *Materials*

N-acetylneuraminic acid (NANA), type VI, L-proline (lot 88F-0023), D-proline (lot 70H-0675), and reagents not otherwise specified were obtained from Sigma Chem. Co. (St. Louis, MO).

### *Animal maintenance*

Monoclonal specimens of the sea anemone, *Haliplanella luciae,* were cultured in Pyrex dishes containing natural seawater, but tested in artificial seawater (ASW; Instant Ocean). Animals were maintained on a 12:12 photoperiod, cleaned daily, and fed brine shrimp nauplii (San Francisco Bay) twice weekly. Experiments were performed approximately 72 h after feeding to maximize nematocyst discharge (Thorington and Hessinger, '88b).

### *Assay for testing nematocyst discharge*

Animals transferred from mass culture to 35 mm diameter plastic Petri dishes filled with ASW were allowed 3 to 4 h recovery, at which point ASW was replaced with $10^{-7}$ M NANA in ASW. After 5 min, the medium was replaced with $10^{-7}$ M NANA and L-proline at specified concentrations. After an additional 10 min, vibrating test probes were moved into contact with anemone tentacles.

Test probes consisted of 2-cm segments of 0.14 mm-diameter, nylon fishing line (Stren Brand, 2-lb test, DuPont) coated at one end with 30% (w/v) gelatin to a thickness of approximately 200 μm (Watson and Hessinger, '89a,b). The uncoated ends of vibrating test probes were inserted into glass capillary tubes (ID = 0.27 mm) made to oscillate using a galvanometer driven and controlled by a function generator set to the sine-wave function as described previously (Watson and Hessinger, '89a, '91a, '92). This apparatus permits independent adjustments of frequency (0–75 Hz) and amplitude (amplitudes of ± 50–350 μm were possible over this range of frequencies). For experiments testing effects of proline on frequency-tuning, a fixed amplitude of ± 65 μm was employed. For experiments testing effects of proline on amplitude-tuning, specific fixed frequencies were employed while amplitude was varied in increments of 50 μm, ranging from ± 50 to 350 μm.

After contacting the tentacles, the gelatin-coated ends of the probes were fixed in 2.5% glutaraldehyde in ASW for 1 min. Probes were prepared as wet-mounts and microbasic p-mastigophore nematocysts discharged into the gelatin coating were counted in a single field of view at 400× magnification (0.16 $mm^2$) of an inverted microscope using phase-contrast optics. The field of view subjectively evaluated as having the greatest number of nematocysts on the probe was scored. On a given day, 4 replicate probes were used for each experimental condition, 1 probe for each of 4 anemones. Data are presented as the mean of 2 daily means ± the standard error.

In some cases, contour plots were generated from large data sets derived from several separate experiments. To minimize effects of daily fluctuations in baseline levels of discharge, data were normalized for each experiment by subtracting baseline levels of discharge. Baseline values for frequencies below 55 Hz consisted of discharge levels from ASW controls because ASW controls exhibit low levels of discharge at frequencies below 55 Hz. At frequencies of 55 Hz or higher, discharge levels from specimens treated in $10^{-7}$ M NANA constituted baseline values because NANA-treated specimens exhibit low levels of discharge at these frequencies. Contour plots depicted the mean number of nematocysts counted above baseline levels.

### Proline isomers

To determine if the receptor for proline discerns optical isomers of proline, dose-responses to L-proline and D-proline were compared. Specimens were placed in ASW for several hours, then exposed to $10^{-7}$ M NANA prepared in ASW for 5 min, followed by $10^{-7}$ M NANA and a specified concentration of either L-proline or D-proline in ASW for 10 min. At this point, discharge was tested at 10 Hz. Previous research using this treatment regime had shown that L-proline generates a single peak of discharge at 10 Hz coinciding with a decrease in discharge at 5 Hz (Watson and Hessinger, '91b).

### Time course for adaptation to proline

To determine the time course of adaptation to proline, specimens were transferred from natural seawater to reagent grade ASW (r-ASW; consisting of NaCl [423 mM], KCl [9 mM], $MgCl_2$ [23 mM], $MgSO_4$ [26 mM], $CaCl_2$ [12 mM] adjusted to pH 8.3 with sodium bicarbonate) where they remained for several hrs before being exposed to solution 1 (r-ASW containing $10^{-12}$ M to $10^{-8}$ M L-proline). Anemones were incubated in solution 1 from 15 min to 135 min. Following such exposure, anemones were incubated for 5 min in $10^{-7}$ M NANA (prepared in solution 1), followed by 10 min in $10^{-7}$ M NANA and $10^{-18}$ M L-proline (added to solution 1). Discharge was tested at 10 Hz.

## RESULTS

### *Frequency tuning by proline*

In ASW, major peaks of discharge appeared at 50–55, 65, and 75 Hz (Fig. 1; Watson and Hessinger, '89a, '91a). Exposing anemones to ASW containing $10^{-7}$ M NANA shifted maximal discharge to the lower frequencies of 5, 15, 30, and 40 Hz. In the continued presence of NANA and concentrations of proline up to $10^{-19}$ M, maximal discharge remained at 5, 15, 30, and 40 Hz. In $10^{-7}$ M NANA and proline in excess of $10^{-19}$ M, discharge shifted to progressively higher frequencies as proline concentrations were increased (Fig. 1). Generally speaking, proline-induced peaks at higher frequencies (55 Hz and above) were broader than at lower frequencies (below 55 Hz) (Fig. 1).

We tested a linear model for frequency-tuning of nematocyst discharge after mapping the coordinates of peaks from Figure 1. From 4 to 8 points appearing to align were selected for each line. Linearity was confirmed for 10 different lines with a minimum linear-regression coefficient of 0.85 (labelled a–j in Fig. 2; Table 1). The regression lines suggested divergence occurs during tuning such that discharge at a given frequency in NANA may be tuned by proline to different frequencies (e.g., compare lines a–c in Fig. 2). Other lines suggested convergence also occurs during tuning (e.g., compare lines c, d, g, in Fig. 2). Lines were weighted according to the den-

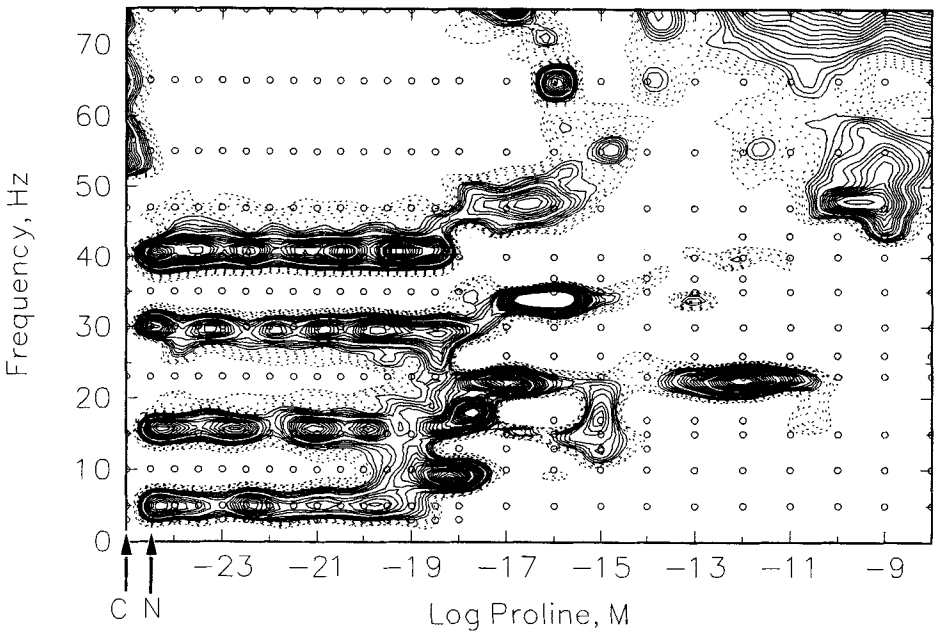


Fig. 1. Frequency-tuning of nematocyst discharge. In this contour plot, the frequency of vibrating test probes (Y-axis) is plotted versus proline concentration (X-axis) and the mean number of nematocysts discharged into the probes (Z-axis rising out of the plane of paper). A fixed amplitude of ± 65 μm was used. Sea anemones were tested after 10 min in ASW alone ("C" for control on X-axis), in $10^{-7}$ M NANA alone (N), or after 5 min in $10^{-7}$ M NANA followed by 10 min in $10^{-7}$ M NANA and L-proline at the concentration indicated. Each data point (open circle) represents the mean of two replicate experiments, each based on 4 test probes. For each test probe, microbasic p-mastigophore nematocysts were counted in a single field of view of an inverted microscope using phase contrast optics (10 × oculars and 40 × objective). Data were normalized by subtracting baseline levels from experimental levels of discharge. A minimum increase of 12 nematocysts above baseline levels constituted the threshold for contours to appear on the graph (outermost dots). In this graph, this occurred at the $20^{th}$ of 50 total contours. For an internal reference of peak height, the $30^{th}$ and $40^{th}$ contours appear as white bands. Each contour line represents an increment of approximately 1 nematocyst.

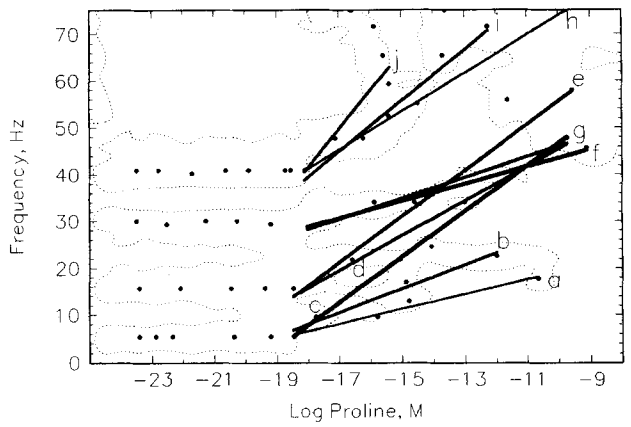


Fig. 2. Linear model for proline tuning of frequency-dependent nematocyst discharge to higher frequencies. Linear regression lines labelled a–j are plotted for data points (closed circles) corresponding to peaks of discharge in Figure 1. The thickness of each line represents the relative weight of the line as determined by the mean peak height and the density of data points along the line. Values for data points shared between lines were shared equally. Attributes of these lines appear in Table 1.

sity of points and relative magnitude of data points. Values for points shared between lines were divided equally. The outcome of this adjustment, represented graphically by the thickness of the regression lines (Fig. 2), suggested that, from 5 Hz, more discharge was tuned along line c, followed by lines b and a (Fig. 2; Table 1). From 15 Hz, slightly more discharge was tuned along line e than d (Fig. 2; Table 1). More discharge occurred from 30 Hz along line f than g (Fig. 2; Table 1). Finally, from 40 Hz, nematocyst discharge was comparably tuned along lines i and j, followed by h (Fig. 2; Table 1). Combining these weighted estimates of tuning, one hundred-fold increases in the concentration of exogenous proline tuned discharge to higher frequencies by a mean of 7.2 Hz, ranging from 3.3 to 18 Hz. Proline tuned discharge to higher frequencies over a total range of between 13 to 43 Hz (Table 1).

Whereas at lower frequencies, increasing proline consistently tuned discharge to higher frequencies, data from Figure 1 suggested that proline may also tune discharge from 75 Hz to lower frequencies (55 and 47 Hz) (Fig. 3). This trend was linear with regression coefficients at 0.85 or above (lines k,l in Fig. 3; Table 1). In these two cases, one-hundred fold increases in proline levels apparently tuned discharge to lower frequencies by a weighted mean of 16.9 Hz extending over a range of between 20 to 27 Hz (Table 1).

*TABLE 1. Attributes of regression lines from Figures 2 and 3*[1]

| Line | Relative weight | Slope Hz/100 [pr] | Range Hz | $r^2$ | Prob. F |
|---|---|---|---|---|---|
| a | 1.00 | 3.3 | 13 | 0.96 | 0.01 |
| b | 1.69 | 5.0 | 18 | 0.96 | 0.01 |
| c | 2.25 | 10.0 | 43 | 0.99 | $7 \times 10^{-8}$ |
| d | 1.66 | 7.4 | 33 | 0.94 | $2 \times 10^{-4}$ |
| e | 1.86 | 10.0 | 43 | 0.99 | $1 \times 10^{-6}$ |
| f | 2.34 | 4.0 | 14 | 0.85 | $7 \times 10^{-4}$ |
| g | 1.69 | 4.7 | 16 | 0.94 | $2 \times 10^{-4}$ |
| h | 1.17 | 8.2 | 35 | 0.99 | $3 \times 10^{-5}$ |
| i | 1.67 | 11.0 | 32 | 0.95 | $5 \times 10^{-4}$ |
| j | 1.65 | 18.0 | 26 | 0.88 | 0.04 |
| k | 2.02 | −22.5 | 20 | 0.85 | 0.02 |
| l | 1.26 | − 8.0 | 27 | 0.99 | $2 \times 10^{-4}$ |

[1]The relative weight of each line was adjusted according to the intensity of the points used and the density of points used to form a given regression line. The slope is represented as the change in frequency (Hz) for each 100-fold change in proline concentration. The total range (Hz) for each line, the linear regression coefficient ($r^2$) and probability value for F also are shown.

### *Amplitude tuning by proline*

At the lower frequencies tested (23 Hz and below), peaks of discharge in NANA occurred only at a low amplitude of ± 50–75 μm (Fig. 4). Increasing the level of proline resulted in the appearance of additional peaks of discharge at a higher amplitude of ± 200 μm. These additional peaks appeared first at 5 Hz, followed by 10, and by 15 Hz at $10^{-14}$ M, $10^{-13}$ M, and $10^{-12}$ M proline, respectively (Fig. 4). In addition, at 15 Hz, a peak of discharge at $10^{-16}$ M proline formed a double peak covering ± 100–125 μm (Fig. 4C). Increasing proline levels induced this double peak to disappear at 15 Hz by $10^{-14}$ M and then to appear as separate peaks at 23 Hz at $10^{-12}$ M proline (Fig. 4C,D).

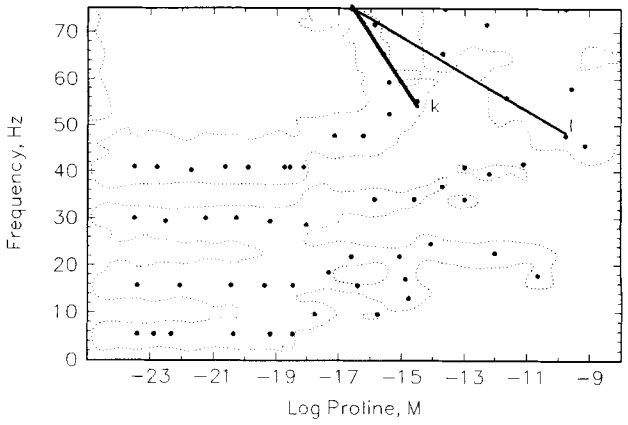


Fig. 3. Linear model for proline tuning of frequency-dependent nematocyst discharge to lower frequencies. Regression lines k and l exhibit a negative slope. The lines were otherwise plotted as described for Figure 2.

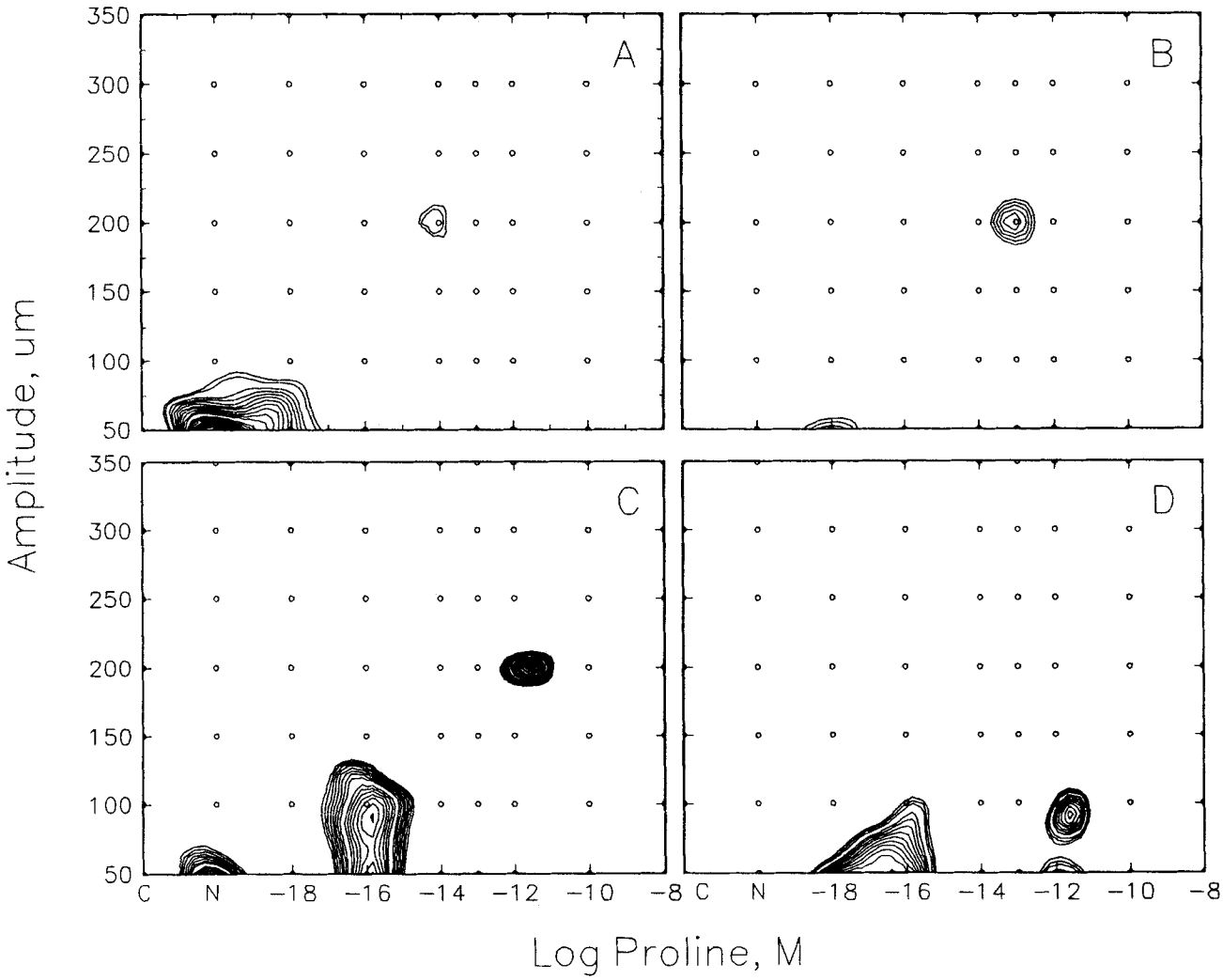


Fig. 4. Amplitude-tuning of nematocyst discharge at lower frequencies. In this contour plot the ± amplitude of vibrating test probes in μm (Y-axis) is plotted versus the proline concentration (X-axis) and the mean number of nematocysts discharged into the probes (Z-axis). A fixed frequency of **(A)** 5 Hz, **(B)** 10 Hz, **(C)** 15 Hz, or **(D)** 23 Hz was used. Sea anemones were tested after 10 min in ASW alone ("C" for control), in $10^{-7}$ M NANA alone (N), or after 5 min in $10^{-7}$ M NANA followed by 10 min in $10^{-7}$ M NANA and L-proline at the concentration indicated. Each data point (open circle) represents the mean of two replicate experiments, each based on 4 test probes. Nematocysts were counted as in Figure 1. Data were normalized by subtracting baseline levels (ASW controls) from experimental levels of discharge. A minimum increase of 11 nematocysts above baseline levels constituted the threshold for contours to appear on the graph. Each contour line represents an increment of approximately 1 nematocyst. For an internal reference of peak height, the $30^{th}$ and $40^{th}$ contours appear as white bands (valid for comparisons within each panel but not between panels).

At the higher frequencies tested (55 Hz and above), responsiveness in ASW spanned a larger range of amplitudes than observed at the lower frequencies, where peaks occurred only at low amplitudes near ± 50 μm. For example, in ASW the peak of discharge at 55 Hz extended from ± 50–150 μm (Fig. 5A). At 65 Hz, the ASW peak ranged from ± 50–325 μm (Fig. 5B). At 75 Hz, the ASW peak spanned the entire range tested from ± 50–350 μm (Fig. 5C). At each of these higher frequencies, exposure to NANA abolished peaks of discharge. Exposure to proline, after pretreatment in NANA, caused the peaks of nematocyst discharge to reappear first at the lower amplitudes at low levels of proline, then at higher amplitudes with increases in proline (Fig. 5). Furthermore, peaks reappeared first at 55 Hz, followed by 65 Hz and 75 Hz (Fig. 5). Tests for linearity failed to demonstrate significant linear trends in the data for 55, 65, or 75 Hz.

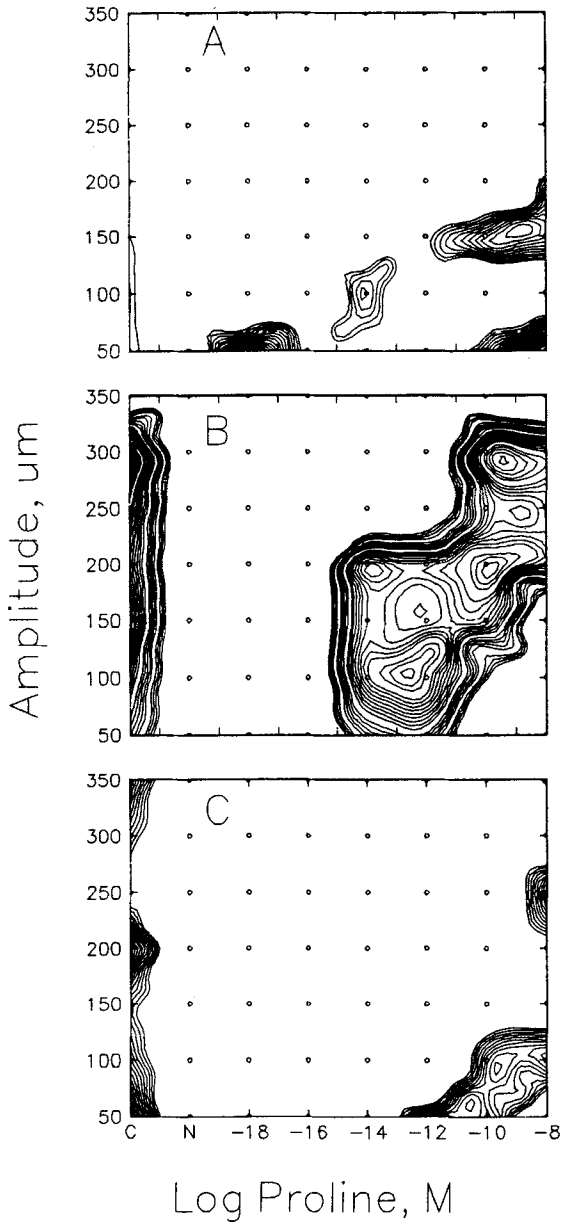


Fig. 5. Amplitude-tuning of nematocyst discharge at higher frequencies. In this contour plot, the ± amplitude of vibrating test probes in μm (Y-axis) is plotted versus the proline concentration (X-axis) and the mean number of nematocysts discharged into the probes (Z-axis). A fixed frequency of (**A**) 55 Hz, (**B**) 65 Hz, or (**C**) 75 Hz was used. Sea anemones were tested after 10 min in ASW alone ("C" for control), in $10^{-7}$ M NANA alone (N), or after 5 min in $10^{-7}$ M NANA followed by 10 min in $10^{-7}$ M NANA and L-proline at the concentration indicated. Data were collected, tabulated, and presented as described for Figure 4 except that baseline levels were based on mean discharge levels from NANA treated specimens. A minimum increase of 11 nematocysts above baseline levels constituted the threshold for contours to appear on the graph (except in A where the minimum was lowered to 9 nematocysts to show ASW discharge, unusually low in these experiments). Each contour line represents an increment of approximately 1 nematocyst.

### *Proline isomers*

Anemones were placed in ASW for several hours before they were exposed to $10^{-7}$ M NANA prepared in ASW for 5 min followed by 10 min to $10^{-7}$ M NANA and a specified concentration of D-proline or L-proline in ASW. At this point, discharge was tested at 10 Hz. This frequency was selected because L-proline tunes discharge from 5 Hz to 10 Hz (Fig. 1). Using this treatment regime, L-proline generated a peak of discharge at $10^{-18}$ M (Fig. 6). Similarly, D-proline generated a peak of discharge at $10^{-18}$ M (Fig. 6).

### *Adaptation to proline*

Previously, it was found that anemones adapt to background levels ranging from $10^{-12}$ M to $10^{-8}$ M proline (Watson and Hessinger, '91b, and submitted). This conclusion was based on results of experiments in which increases of $10^{-18}$ M proline consistently generated a peak of discharge at 10 Hz regardless of which background level of proline was used. However, in that study anemones were allowed several hours exposure to the background level of proline before they were tested. Consequently, the time course of adaptation was not determined.

Anemones were placed in reagent-grade ASW (r-ASW) for several hours before they were incubated in specified background levels of proline ranging from $10^{-12}$ M to $10^{-8}$ M proline (prepared in r-ASW). At 15 min intervals ranging from 15–135 min, groups of these anemones were then exposed 5 min to $10^{-7}$ M NANA followed by 10 min to $10^{-7}$ M NANA and $10^{-18}$ M proline (added to the appropriate background level of proline in r-ASW). Time course experiments indicated that 30 min incubation in background levels of proline ranging from $10^{-12}$ M to $10^{-8}$ M proline was insufficient for anemones to respond to increases of $10^{-18}$ M proline by tuning discharge to 10 Hz (Fig. 7). Following a more prolonged incubation in the background levels of proline, however, nematocyst discharge increased at 10 Hz (Fig. 7). Estimates of the time required to half-maximally tune nematocyst discharge to 10 Hz increased by increments of approximately 7.5 min for each 100-fold increase in concentration from 40 min for $10^{-12}$ M proline to 60 min for $10^{-8}$ M proline (Fig. 7).

## DISCUSSION

### *Sensitivity of anemones to proline*

The tuning response to proline of vibration-sensitive nematocyst discharge exhibits a thresh-

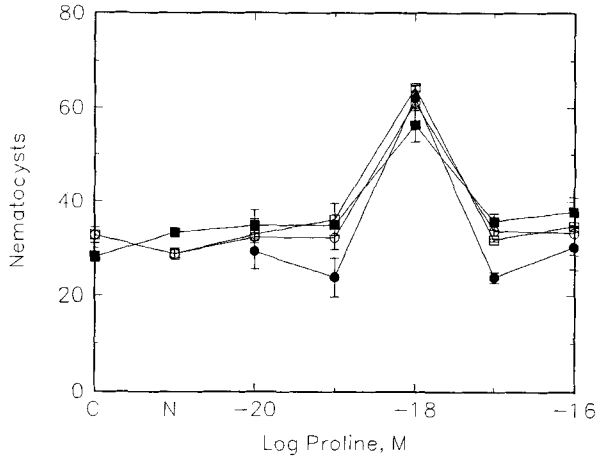


Fig. 6. Frequency-tuning of nematocyst discharge by optical isomers of proline. Specimens were placed in ASW for several hours, then exposed to $10^{-7}$ M NANA prepared in ASW for 5 min, followed by 10 min to $10^{-7}$ M NANA in ASW and a specified concentration of either L-proline (open squares for 1 replicate experiment, closed squares for the other replicate experiment) or D-proline (open circles for 1 replicate experiment, closed circles for the other replicate experiment). Discharge was tested at 10 Hz. Nematocysts were counted as in Figure 1. Each data point represents the mean number of nematocysts based on the mean of two replicate experiments, each based on 4 test probes (± SEM). Thus, a total of 4 replicate experiments are shown for D-proline and for L-proline, respectively.

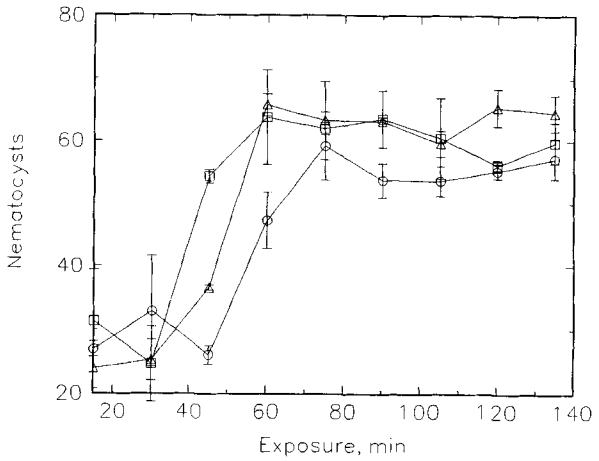


Fig. 7. Time course of adaptation to proline. Specimens were transferred from natural seawater to reagent grade ASW (r-ASW) where they remained for several hrs before being exposed to r-ASW containing L-proline at $10^{-12}$ M (squares), $10^{-10}$ M (triangles), or $10^{-8}$ M (circles). Anemones were incubated in this solution from 15 min to 135 min as indicated on the X-axis. Following such exposure, anemones were incubated for 5 min in $10^{-7}$ M NANA (added to this solution), followed by 10 min in $10^{-7}$ M NANA and $10^{-18}$ M L-proline (added to this solution). At this point, discharge was tested at 10 Hz. Nematocysts were counted on test probes as described for Figure 1. Each data point represents the mean number of nematocysts based on the mean of two replicate experiments, each based on 4 test probes (± SEM).

old at $10^{-18}$ M both for L-proline and D-proline (Fig. 6), suggesting that the receptor cannot readily discriminate between optical isomers of proline. Previously, it was shown that tuning of nematocyst discharge occurs at $10^{-18}$ M proline even against background concentrations as high as $10^{-8}$ M proline (Watson and Hessinger, '91b, and submitted). In this report, it was shown that adaptation to background concentrations of proline is an absolute prerequisite for anemones to respond to increases in proline of $10^{-18}$ M (Fig. 7). Furthermore, the time required for the completion of adaptation increases as background concentrations of proline are increased, suggesting that adaptation is a dynamic process.

### *Chemoreceptor tuning of hair bundles*

Chemoreceptors for N-acetylated sugars and for proline act antagonistically to modulate responsiveness of anemone hair bundles to specific frequencies (Watson and Hessinger, '91a,b, and submitted) and to specific amplitudes (Figs. 1–5). Whereas activated receptors for N-acetylated sugars tune hair bundles to lower frequencies and low amplitudes, activated receptors for proline tune hair bundles to higher frequencies and/or to larger amplitudes. We tested a linear model for frequency-tuning because, in many instances, peaks of discharge appeared to align linearly in a frequency versus log proline plot (Fig. 1). While our analyses of data for frequency-tuning of nematocyst discharge proved to be consistent with that possibility, we did not investigate other possible interpretations of the data which may be equally valid. According to the linear model, frequency-tuning does not appear to be the same for each of the bundles on the tentacle since some of the bundles tuned to a given frequency by NANA were tuned by proline to different frequencies (Fig. 2). Furthermore, the slope of the tuning function is larger at some frequencies than at others (Fig. 2; Table 1).

Vertebrate hair bundles are sensitive detectors of vibrations. At one extreme, hair bundles are sufficiently sensitive to detect thermal noise (Denk and Webb, '92). At the other extreme, large amplitude stimuli damage or destroy hair bundles (Tilney et al., '82; Pickles et al., '87). Within the range of nondestructive amplitudes, increasing the amplitude of vibrating stimuli increases, in a sigmoidal fashion, the flux of ions (Fettiplace, '92). The transduction current apparently flows through spring-gated ion channels located at the tips of stereocilia that open as the bundle oscillates in response to stimu-

lation (Hudspeth, '82, '83; Corey and Hudspeth, '83; Pickles et al., '84; Huang and Corey, '90). At lower frequencies, anemone hair bundles demonstrate amplitude selectivity (Fig. 4). Assuming that anemone hair bundles are similar to vertebrate hair bundles, it seems unlikely that even low amplitude vibrating stimuli fail to generate a transduction current. Thus, selectivity to amplitude may occur at the level of signal processing by the cell. Whereas low amplitude vibrations may generate a subthreshold ion flux, vibrations at optimal amplitudes may generate a larger ion flux capable of inducing downstream signalling events. Vibrations at excessive amplitudes may generate an even larger ion flux that inhibits signalling. The magnitude of the ion flux could be coupled to signal processing in a variety of ways. For example, calcium ions are likely to be a component of the transduction current. Incoming $Ca^{2+}$ could modulate calcium channels involved in downstream signalling events such that it activates them at low levels of calcium and then inactivates them at higher calcium levels. Comparable regulatory mechanisms have been proposed for other cell systems (Lechleiter and Clapham, '92). However, no such system is yet known to occur in anemones.

For anemone hair bundles, the situation is further complicated because at least some bundles on the tentacle exhibit amplitude-tuning. At frequencies of 5-15 Hz, a shift in amplitude responsiveness occurs at $10^{-14}$ M to $10^{-12}$ M proline from $\pm 50$ to $\pm 200$ μm (Fig. 4). In addition, a broadening of responsiveness to include amplitudes from $\pm 50$ to $\pm 100$ μm was detected at 15 Hz at $10^{-16}$ M proline (Fig. 4C) which persisted in subsequent frequency-tuning to 23 Hz at $10^{-12}$ M proline (Fig. 4D). At the higher frequencies of 55 Hz and above, progressive amplitude tuning occurs as a function of proline concentration such that optimum amplitude increases as the proline concentration is increased (Fig. 5). These data suggest that low-frequency bundles can incur stable, stepwise changes in selectivity to larger amplitudes while high-frequency bundles are subject to progressive shifts in selectivity to larger amplitudes. Such amplitude tuning could occur by means of receptor-dependent changes to the signal processing pathway (e.g., phosphorylation events). In this case, the activated proline receptors may induce modifications to the signal processing pathway that partially inhibit it so that a larger transduction current is required to initiate downstream signalling events. Thus, the bundles would become tuned to larger amplitude vibrations. On the other hand, amplitude tuning could occur by means of receptor-dependent alterations to the morphology of the bundle such that bundles stiffen. The stiffened bundles would be less sensitive to deflection by stimulation at a given amplitude, in effect decreasing the transduction current at that amplitude. Thus, larger amplitude vibrations would be required to generate transduction currents capable of initiating downstream signalling events. We further suggest that these two possible mechanisms for amplitude tuning are not necessarily mutually exclusive.

### *Biological significance of tuning by proline*

Conjugated N-acetylated sugars contained in mucins are likely to be at higher levels in the vicinity of prey. As the prey approaches the anemone tentacle, local levels of conjugated N-acetylated sugars increase to activate chemoreceptors on the tentacles. Activated receptors for N-acetylated sugars tune hair bundles on the tentacle to lower frequencies matching those produced by suitable prey (Watson and Hessinger, '89a). At this point, hair bundles are tuned to low amplitude movements ($\pm 50$ μm) corresponding to those produced by relatively small, calmly swimming organisms. Hence, prospective prey may be discriminated and selected according to type (frequency) and size (amplitude). Immediately after a calmly swimming, suitable prey contacts a tentacle, massive nematocyst discharge is triggered. Wound sites resulting from discharged penetrant nematocysts release proline from hemolymph (Gilles, '79) into the seawater. This process would be enhanced by any struggling movements produced by the prey. The wounded prey attempts to escape, producing higher frequency and/or larger amplitude movements. Activated chemoreceptors for proline on tentacles tune hair bundles to respond to higher frequency and/or larger amplitude ($\pm 100$–$200$ μm) movements at the same, low frequencies produced by the calmly swimming prey. Thus, additional, massive nematocyst discharge is triggered into the vigorously struggling prey. As the prey succumbs to injected nematocyst toxins, it produces lower amplitude movements (i.e., $<100$ μm) that no longer elicit high levels of nematocyst discharge. Hence, levels of nematocyst discharge would be highest into calmly swimming, fresh prey and into vigorously struggling, wounded prey, and then would decrease as the prey succumbs to nematocyst venoms.

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