Frequency Response Properties of Lateral Line Superficial Neuromasts in a Vocal Fish, With Evidence for Acoustic Sensitivity

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Weeg, Matthew S., and Andrew H. Bass. Frequency response properties of lateral line superficial neuromasts in a vocal fish, with evidence for acoustic sensitivity. J Neurophysiol 88: 1252–1262, 2002; 10.1152/jn.00077.2002. The mechanosensory lateral line of fish is a hair cell based sensory system that detects water motion using canal and superficial neuromasts. The trunk lateral line of the plainfin midshipman fish, Porichthys notatus, only has superficial neuromasts. The posterior lateral line nerve (PLLn) therefore innervates trunk superficial neuromasts exclusively and provides the opportunity to investigate the physiological responses of these receptors without the confounding influence of canal organs. We recorded single-unit activity from PLLn primary afferents in response to a vibrating sphere stimulus calibrated to produce an equal velocity across frequencies. Threshold tuning, isovelocity, and input/output curves were constructed using spike rate and vector strength, a measure of phase locking of spike times to the stimulus waveform. All units responded maximally to frequencies of 20–50 Hz. Units were classified as low-pass, band-pass, broadly tuned, or complex based on the shapes of tuning and isovelocity curves between 20 and 100 Hz. A 100 Hz stimulus caused an increase in spike rate in almost 50%, and significant synchronization in >80%, of all units. Midshipman vocalizations contain significant energy at and below 100 Hz, so these results demonstrate that the midshipman peripheral lateral line system can encode these acoustic signals. These results provide the first direct demonstration that units innervating superficial neuromasts in a teleost fish have heterogeneous frequency response properties, including an upper range of sensitivity that overlaps spectral peaks of behaviorally relevant acoustic stimuli.

INTRODUCTION

The mechanosensory lateral line system of fish is used to detect water motion relative to the surface of the animal and mediates such varied behaviors as prey detection, predator avoidance, hydrodynamic imaging, rheotaxis, schooling, and courtship communication (Coombs and Montgomery 1999). The lateral line system has two receptor types: superficial neuromasts, which lie on the surface of the skin, and canal neuromasts, which are found in subdermal canals that open to the external environment via a series of pores. Physiological investigations of the anterior and posterior lateral line nerves, which innervate neuromasts on the head and trunk respectively, have identified two classes of primary afferents based on their physiological characteristics in response to a small vibrating sphere. One class of fibers is sensitive to acceleration and responds to frequencies ranging from 30 to 200 Hz, while a second class is sensitive to velocity and responds best to frequencies <30 Hz (Coombs and Janssen 1990; Kroese and Schellart 1992). Although theoretical studies suggest that acceleration-sensitive, high-pass units innervate canal neuromasts and velocity-sensitive, low-pass units innervate superficial neuromasts (Denton and Gray 1983; Kalmijn 1988, 1989), there remains to be a direct demonstration of this functional dichotomy in fish (but see Kroese et al. 1978 for frogs).

In contrast to most teleost fish with both head and trunk canal neuromasts, the plainfin midshipman, Porichthys notatus, lacks a trunk canal and instead has several rows of superficial neuromasts (Greene 1899). The posterior lateral line nerve (PLLn) therefore innervates superficial neuromasts exclusively and offers a unique physiological advantage to examine the responses of this class of lateral line receptors to a dipole stimulus. The first goal of this study, then, was to examine the frequency response properties of midshipman superficial neuromasts and test the hypothesis that they can be modeled as velocity detectors.

The organizational pattern of the midshipman trunk lateral line also afforded us the opportunity to examine the heterogeneity of superficial neuromast responses without the presence of canal neuromasts confounding our interpretations. Indeed, most studies have focused on the differences between superficial and canal neuromasts with little emphasis placed on the range of responses within a single class of endorgan (although see Coombs and Montgomery 1994). Kroese and van Netten (1989) suggested that structural features could affect neuromast response properties; thus variation in the physical characteristics of hair cells could produce a range of frequency responses within a single class of neuromast. Therefore a second major goal of this study was a detailed examination of the variability of response properties for a receptor population composed only of superficial neuromasts.

The third major goal of this study was to examine peripheral lateral line responses in the context of behaviorally relevant vocalizations. Underwater sound sources produce a near field component of particle motion that, given the appropriate frequency composition, is theoretically detectable by the lateral line when the fish is close to the source (Coombs and Montgomery 1999; Denton and Gray 1983; Harris and van Bergeijk 1962; Kalmijn 1988, 1989). Midshipman produce vocaliza-
tions with fundamental frequencies near or < 100 Hz (Bass et al. 1999; Brantley and Bass 1994). While peripheral and central auditory neurons encode the frequencies of these vocalizations (Bodnár and Bass 1997, 1999, 2001a,b; McKibben and Bass 1999, 2001b), it is unknown what role, if any, the lateral line system might play in the detection and processing of these sounds. These vocalizations are within the sensitivity range of the lateral line of other teleosts (Coombs and Montgomery 1999), and presumably produce a considerable near-field system might play in the detection and processing of these sounds. These vocalizations are within the sensitivity range of the lateral line system might play in the detection and processing of these sounds. These vocalizations are within the sensitivity range of the lateral line system might play in the detection and processing of these sounds. These vocalizations are within the sensitivity range of the lateral line system might play in the detection and processing of these sounds.

Portions of this work have been reported in abstract form (Weeg and Bass 2000b, 2001).

METHODS

Sixteen adult type I male plainfin midshipman fish, ranging in body size from 24.6 to 82.9 g (standard length, 13.2–18.4 cm) were included in this study. Midshipman were collected from nest sites in Tomales Bay, CA, and maintained in artificial seawater tanks at 16°C on a regular diet of perch minnows and goldfish. All experiments were conducted under the guidelines of the Cornell University Institutional Animal Care and Use Committee and the National Institutes of Health.

Prior to surgery, fish were anesthetized by immersion in 0.2% ethyl p-amino benzoate (Sigma, St. Louis, MO) in seawater from their home aquaria until gilling movements ceased. Animals were then placed in a recessed dish filled with cold seawater. This allowed the trunk of the animal to remain submerged during surgery, leaving only the head exposed to air. Apart from the surgical area, all exposed surfaces were covered with moist tissue. The PLLn was exposed dorsally, just distal to the point at which it enters the brain stem. Excess fluid was blotted from the cranial cavity and replaced with an inert fluid (Fluorinert, 3M, St. Paul, MN). Following surgery, fish were transferred to a 32 cm diam tank filled with 16°C artificial seawater and clamped by the head onto a small plastic platform. The tank was on a vibration isolation table inside an acoustic isolation chamber (Industrial Acoustics, New York, NY). A small plastic tube inserted into the mouth of the animal provided a constant stream of recirculated seawater across the gills. Fish were given intramuscular injections of pancuronium bromide (0.5 mg/kg body wt, Astra Pharmaceutica Products, Westborough, MA) for immobilization and fentanyl (1.0 mg/kg body wt, Sigma) for analgesia. Condition of the animal was monitored by watching blood flow in the dorsal vasculature of the brain.

Hydrodynamic stimuli were delivered via a small plastic sphere (12 mm diam) attached by a metal rod to a minishaker (Brüel and Kjær). Stimuli were synthesized using a customized signal-generation/data-acquisition software package (CASSIE, designed by Julian Vrieslander, Cornell University), attenuated (PA4, Tucker-Davis Technologies, Gainesville, FL) and amplified (NAD 3220PE, NAD Electronics, Boston, MA) before being delivered through the minishaker. The minishaker was placed in a large metal clamp inserted into the chuck of a drill press such that the sphere’s axis of motion was in the horizontal plane, parallel to the long axis of the fish. The sphere was positioned at the midpoint of both the rostrocaudal and dorsoventral planes of the fish, 15–25 mm from the body surface. The drill press was isolated from the vibration isolation table on which the preparation was positioned to minimize transfer of substrate vibrations.

Movement of the sphere was equalized to produce a constant maximum source velocity across the range of stimulation frequencies (20–200 Hz). Peak-to-peak displacement of the sphere was measured under a dissecting microscope (resolution, 15 μm) and adjusted to produce a constant velocity across frequencies according to the flow field equations for a dipolar sound source (Kalmijn 1988). To ensure that velocity measured at the source was an adequate predictor of particle velocity in the flow field (i.e., that the experimental tank did not greatly distort flow fields), we measured the sound pressure at various points around the source using a mini-hydrophone (4130, Brüel and Kjær) whose output was fed into a spectrum analyzer (SR780, Stanford Research Systems, Sunnyvale, CA). Measured pressure at these points was compared with predicted pressure, which was calculated using flow field equations for a dipolar sound source in an unbounded medium (Kalmijn 1988). Measured and predicted pressure showed similar gradient patterns, so we feel confident that equalizing for velocity at the source translated into equalized particle velocity in the stimulus flow field.

Single-unit extracellular potentials were recorded from the posterior lateral line nerve using glass microelectrodes filled with 4M NaCl. Electrodes were visually placed on the surface of the nerve and advanced using an hydraulic microdrive (Kopf Instruments, Tujunga, CA). Signals were amplified, filtered through 300-5000 Hz (A-M Systems 1700, A-M Systems, Everett, WA) and digitized onto a Macintosh Centris running CASSIE. Visually identified single units were discriminated using a pattern matching algorithm within CASSIE.

On isolation of a single unit showing a robust response to a pulsed 40 Hz sound stimulus, the approximate location of the receptive neuramost was identified by monitoring spike rate in response to a water jet directed against the side of the fish. After localization of the receptive neuramost, the following stimulus protocol was initiated. Stimuli consisted of 10 repetitions of a 700 ms tone burst at 1 s intervals. Threshold was determined for each unit and a random series of 20 to 100 Hz tones in 10 Hz steps was presented in an ascending series of 6 dB increments from threshold level to a level 30 dB above threshold. The stimulus level was then decreased by 3 dB, and a descending series of 6 dB increments was presented. At each stimulus level, spontaneous activity was measured by recording 10 s repetitions in which no signal was sent to the minishaker. If the unit was still viable, data at additional stimulus levels, as well as at frequencies ±200 Hz were collected.

Neural responses were measured in terms of average evoked spike rate and vector strength of synchronization (VS). Spike rate was measured for each repetition and averaged across the 10 repetitions for each stimulus. Threshold was determined as the level at which evoked spike rates were >2 SDs above spontaneous levels. VS is a measure of phase locking of the neural response to the stimulus waveform (Goldberg and Brown 1969). Typically, all repetitions of a given stimulus are collapsed into a single cycle’s worth of time, and a single VS value (R) is measured. The Rayleigh statistic (Z) is then calculated using this VS and the total number of spikes (n) occurring throughout all stimulus repetitions (Z = R^2/n) (Batschelet 1981). We measured VS for each repetition individually, using the number of spikes in each repetition to calculate Z. Threshold was arbitrarily defined as the stimulus level at which Z > 3 (which corresponds to P < 0.05) for at least 50% of the repetitions. We chose this criterion because it corresponded to a robust response that was well below saturation, and proved to be more conservative than traditional methods of calculating the Rayleigh statistic.

Phase of the neural response was calculated from the period histograms and expressed relative to the displacement of the sphere. Sphere displacement was measured using a dissecting microscope and a strobe that could be adjusted to match the stimulus frequency. The timing of the strobe that corresponded to the maximum displacement of the sphere was recorded by the data-acquisition software, and the difference between the peak in the stimulus waveform from the computer and the peak displacement of the sphere was measured. This procedure was done at each stimulus frequency to account for fre-
frequency dependent delays between the signal from the computer and the output of the sphere.

RESULTS

The data reported come from 81 single units that were held long enough to obtain nearly complete data sets consisting of threshold tuning curves and isovelocity curves. Some units were lost before complete data sets could be obtained, which accounts for the differences in sample sizes.

Spontaneous activity

The distribution of mean spontaneous rates ranged from 1.8 to 104.8 spikes/s (Fig. 1A). Interspike interval (ISI) histograms of spontaneous activity revealed four patterns of resting discharge activity (Fig. 1, B–E). Bursting units (71.6%; Fig. 1B) were most commonly seen, with ISI distributions characterized by a sharp peak at low ISIs, representing ISIs within bursts, followed by a smaller secondary peak at higher ISIs, representing interburst intervals. Irregular units (12.3%; Fig. 1C) showed a peak at low ISIs followed by a broad tail out to higher ISIs. Regular units (3.7%; Fig. 1D) were characterized by a normal distribution of ISIs, while variable units (12.3%; Fig. 1E) had a broad distribution of ISIs that lacked a robust peak. Although variable ISI distributions were only seen in cells with low spontaneous activity (1.8–18.2 spikes/s), spontaneous rates for bursting (26.4–104.8 spikes/s), irregular (19.5–81.2 spikes/s), and regular (35.9–55.5 spikes/s) ISI distributions were overlapping.

Spike rate sensitivity

All units showed nonadapting, phase-locked responses throughout the duration of the stimulus (Fig. 2). At lower stimulus frequencies, most units fired multiple spikes per cycle of the stimulus waveform as can be seen in the multiple peaks of the period histograms in Fig. 2, A (in response to a 20 Hz stimulus) and B (40 Hz stimulus). At higher frequencies, units typically fired one or fewer spikes per cycle (Fig. 2C, 100 Hz stimulus). Peristimulus time (PST) histograms showed that units responded with sustained firing throughout the duration of the stimulus, although many units fired a burst of spikes at stimulus onset followed by a tonic response (e.g., Fig. 2C). We did not examine this onset response systematically, but it tended to occur more often at frequencies above 50 Hz than below, and at high stimulus amplitudes.

The phase of the neural response relative to the phase of the vibrating sphere was extremely variable both between units at a given frequency and between frequencies within a given unit (Fig. 3). For a given unit, response phase shifted as frequency increased by an average of $+62.5° \pm 16.1°/10$ Hz (mean ±
Best sensitivity was defined as the lowest stimulus level that VS thresholds were usually lower than spike rate thresholds. Spike rate curves were often sharper than VS curves, whereas measures resulted in similarly shaped tuning curves. However, with the location of the receptive neuromast on the fish, consistent phase at any given frequency. Phase of response was not correlated with the location of the receptive neuromast on the fish.

Threshold tuning curves

Threshold tuning curves were constructed based on both spike rate ($n = 60$) and VS ($n = 80$) criteria (Fig. 4). Both measures resulted in similarly shaped tuning curves. However, spike rate curves were often sharper than VS curves, whereas VS thresholds were usually lower than spike rate thresholds. Best sensitivity was defined as the lowest stimulus level that elicited a threshold response. Characteristic frequency (CF), defined as the frequency at best sensitivity, ranged from 20 to 50 Hz for both VS and spike rate (Fig. 4A). To provide an objective measure of tuning curve shape, we categorized tuning curves based on whether bandwidth between 20 and 100 Hz could be measured at 12 dB above best sensitivity. Units for which a 12 dB bandwidth could be measured were classified as band-pass. Units for which a 12 dB bandwidth could not be measured were classified as either low-pass or broadly tuned, depending on the unit’s sensitivity to 100 Hz stimuli. Some units exhibited large notches in the tuning curve and were classified as complex. Using these criteria, 66.7% of the units based on spike rate and 56.3% of the units based on VS were categorized as low-pass (Fig. 4B), 23.5% (spike rate) and 13.8% (VS) were categorized as band-pass (Fig. 4C), 5.0% (spike rate) and 21.3% (VS) were categorized as broadly tuned (Fig. 4D), and 5.0% (spike rate) and 8.8% (VS) were categorized as complex (Fig. 4E). There was no systematic relationship between tuning curve category and location of the receptive neuromast.

Of 59 units for which both spike rate and VS tuning curves were constructed, 62.7% ($n = 36$) of the units were categorized the same for both measures, while 37.3% ($n = 23$) of the units were categorized differently. Of those units that were categorized differently, the shapes of spike rate and VS tuning curves were generally fairly similar, and the differences in categorization were largely due to spike rate tuning curves being sharper than VS curves (Fig. 4F). Both the low- and high-frequency slopes of spike rate tuning curves were steeper than those of VS tuning curves (low: $P = 0.0061$; high: $P = 0.0035$, Mann-Whitney U test). This resulted in an increased probability that the low, high, or both ends of the spike rate tuning curves would be $>12$ dB above best sensitivity, while those of the VS tuning curves were more likely to be $<12$ dB above best sensitivity (Fig. 4F).

Low- and high-frequency slopes were measured for low-pass, band-pass, and broadly tuned tuning curves. Low-frequency slopes were measured between 20 Hz and characteristic frequency, and high-frequency slopes were measured between characteristic frequency and 100 Hz or, if the unit was not responsive to 100 Hz, the highest frequency that elicited a threshold response. Both low- and high-frequency slopes were highly variable and widely distributed (Table 1). Low-pass and broadly tuned units had shallower low frequency slopes than band-pass units, and broadly tuned units had shallower high-frequency slopes than low-pass and band-pass units. Tuning curve slopes were independent of neuromast location.

Isovelocity curves

Spike rate ($n = 71$) and VS ($n = 80$) isovelocity curves were constructed at all stimulus levels above best sensitivity, but only curves at 12 dB above best sensitivity (rate: $n = 55$; VS: $n = 78$) were used to determine best excitable frequency and response categories (Fig. 5). Best excitable frequency, defined as the frequency that elicited the greatest response, ranged from 20 to 50 Hz for spike rate and 20 to 80 Hz for VS (Fig. 5A). We again recognized four categories based on the shapes of isovelocity curves and whether the response at 20 and 100 Hz fell $<$70% of the response at best excitable frequency. Using spike rate as a measure, isovelocity curves were categorized as either low-pass (43.6%; Fig. 5, B and D) or band-pass (56.4%; Fig. 5, C, E, and F). Using VS as a measure, isovelocity curves were categorized as low-pass (44.9%; Fig. 5, B and F), band-pass (23.1%; Fig. 5C), broadly tuned (17.9%; Fig. 5D), or complex (14.1%; Fig. 5E). As with threshold tuning curves, there was no relationship between isovelocity curve category and the location of the receptive neuromast.

The shapes of isovelocity curves varied slightly with stimulus intensity. Spike rate isovelocity curves generally became more sharply tuned as stimulus intensity was increased, while VS curves became more broadly tuned. Best excitable frequency remained constant as stimulus intensity increased for 40.8% of spike rate curves and 22.5% of VS curves. The remaining units showed either a consistent increase (spike rate, 22.5%; VS, 21.3%), consistent decrease (spike rate, 8.5%; VS, 6.3%), or random fluctuations (spike rate, 28.2%; VS, 50.0%) in best excitable frequency as stimulus intensity increased. The maximum variation was 20 Hz for all spike rate curves and 88.8% of VS curves. Variation ranged from 30 to 80 Hz for the remaining VS curves, although this extreme variation was related to either very high stimulus levels or low spike rates, which tended to inflate VS values.

Of 52 units for which both spike rate and VS isovelocity curves were constructed, 44.2% were categorized the same for both measures while 55.8% were categorized differently. This high percentage of units that were categorized differently was largely due to the fact that none of the spike rate curves were categorized as broadly tuned or complex. Of the 29 units that were categorized differently, 21 had broadly tuned or complex VS isovelocity curves (Fig. 5, D and E). The eight remaining units were all classified as band-pass by spike rate criteria and low-pass by VS criteria (Fig. 5F). Like threshold tuning curves, these were generally similar in overall shape, although
The low-frequency slopes of the spike rate curves were steeper than those of the VS curves.

Vector strength is sensitive to the number of spikes occurring during each cycle of the stimulus waveform. Multiple spikes per cycle broaden the distribution of spike times, thus lowering VS values. Therefore low VS values could arise from the neuron not encoding the stimulus or from the neuron firing more than one spike per cycle. This distinction becomes par-

TABLE 1. Mean tuning curve slopes from primary afferents in the posterior lateral line nerve

<table>
<thead>
<tr>
<th></th>
<th>Low-Frequency Rate Slope</th>
<th>Low-Frequency VS Slope</th>
<th>High-Frequency Rate Slope</th>
<th>High-Frequency VS Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low pass</td>
<td>-6.0 ± 4.3</td>
<td>-3.6 ± 5.5</td>
<td>13.9 ± 6.5</td>
<td>12.3 ± 4.1</td>
</tr>
<tr>
<td>Band pass</td>
<td>-16.6 ± 9.6</td>
<td>-17.9 ± 8.1</td>
<td>18.5 ± 4.9</td>
<td>18.2 ± 9.1</td>
</tr>
<tr>
<td>Broad</td>
<td>-8.3 ± 3.3</td>
<td>-5.4 ± 4.2</td>
<td>7.1 ± 2.3</td>
<td>4.6 ± 2.8</td>
</tr>
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</table>

Values are means ± SD. VS, vector strength.
particularly important when considering low-pass versus band-pass units in which the differences between filter shapes arose primarily from band-pass units having low VS values at 20 Hz (Fig. 5). Visual inspection of raster plots suggested that band-pass units did not encode a 20 Hz stimulus as well as low-pass units (Fig. 6, A and D). This was reflected in both PST (Fig. 6, B and E) and period (Fig. 6, C and F) histograms. In general, low-pass units fired multiple spikes per cycle within a limited range of the stimulus waveform, whereas band-pass units fired spikes throughout the entire waveform. In addition, there was not a significant difference between the number of spikes per cycle at 20 Hz for low-pass and band-pass units ($P = 0.1066$, Mann-Whitney $U$ test). Thus the difference in 20 Hz VS between low- and band-pass units appeared to be a function of the degree of phase locking rather than band-pass units firing more spikes per cycle than low-pass units.

**Input/output curves**

Input/output (I/O) curves were constructed for stimulus levels up to at least 18 dB above best sensitivity for 79 units (rate $n = 43$; VS $n = 78$). I/O functions showed the greatest differences between spike rate and vector strength measures. Of 43 units, 53.5% did not show an increase in spike rate to a
100 Hz tone as stimulus intensity was increased (Fig. 7A), while 46.5% did (Fig. 7C). In contrast, only 17.9% of units did not show a significant increase in VS (Fig. 7B), while 82.1% of units did significantly synchronize to a 100 Hz tone as stimulus level was increased (Fig. 7D). The magnitude of the response was also more robust for VS than for spike rate. While spike rate for most units could be driven just above threshold levels at 100 Hz, maximum VS at 100 Hz was often as high as that at 20 and 40 Hz.

**DISCUSSION**

The PLLn of the plainfin midshipman innervates only superficial neuromasts, which provided us the unique opportunity to investigate the response properties of this class of lateral line receptors in a teleost fish. Specifically, we tested the hypothesis that primary afferents innervating superficial neuromasts respond as velocity detectors, examined the variability in superficial neuromast responses, and asked whether the midshipman lateral line system is capable of encoding behaviorally relevant vocalizations. We have shown that the midshipman lateral line system shares several basic features with that of other teleosts, including best responses to frequencies at or <50 Hz, and sustained responses that usually consisted of an increase in spike rate and synchronization of spike times to the stimulus waveform. However, based on phase data and the slopes of threshold tuning curves, we conclude that the responses of primary afferents innervating midshipman superficial neuromasts do not match the predicted responses of a particle velocity detector. Midshipman superficial neuromasts also exhibit a large degree of variation in their frequency response properties, which has not been previously documented. This includes sensitivity to frequencies >100 Hz, suggesting that these receptors are capable of detecting species specific vocalizations.

**Spontaneous activity**

Patterns of spontaneous activity were similar to those described for other teleosts (Coombs and Janssen 1990; Kroese and Schellart 1992; Montgomery et al. 1988; Munz 1985; Tricas and Highstein 1991; Wubbels et al. 1990). Unlike previous studies, we did not encounter silent units. However, variable units typically had low spontaneous activity and may be analogous to these silent units. There was no relationship between spontaneous activity and the shape of threshold tuning and isovelcity curves. Because the location of the sphere was fixed and distance between the sphere and receptive neuromast varied between units, we were unable to compare sensitivity, which is often correlated with spontaneous rate (see Popper and Fay 1999), between units.

**Spike rate vs. vector strength**

Midshipman superficial neuromasts exhibited sustained, nonadapting responses to a vibrating sphere that consisted of an increase in spike rate, synchronization of spike times to the stimulus waveform, or, more commonly, a combination of the two. These types of responses are a common feature of lateral line primary afferents (Coombs and Janssen 1990; Coombs et al. 1998; Mogdans and Bleckmann 1999; Mohr and Bleckmann 1998; Montgomery et al. 1988; Munz 1985; Wubbels et al. 1990).
The sustained response provides information about stimulus duration, while the degree of synchronization and magnitude of the spike rate increase encode stimulus amplitude. Synchronization also provides a temporal code of frequency and thus a mechanism for frequency analysis at the periphery.

We analyzed the responses of superficial neuromasts in terms of spike rate and VS, as both measures likely play an important role in encoding sensory information. Vector strength usually started increasing at stimulus levels lower than those that caused an increase in spike rate, and saturated at lower stimulus levels than spike rate. For most units, we did not present stimulus amplitudes large enough to cause saturated spike rate responses, so we were unable to compare dynamic ranges of spike rate versus VS. However, the combination of the two measures clearly increased the overall dynamic range of the system, increasing the amplitude range over which the neuromast was responsive. Moreover, at stimulus levels that caused VS saturation, an increase in spike rate caused an increase in the overall depth of modulation of neural activity. Similar interplay between spike rate and VS is seen in the midshipman VIIIth nerve (McKibben and Bass 1999). Thus although we have presented data in terms of spike rate and VS as separate measures, neural encoding probably employs a combination of the two to reconstruct the features of the stimulus waveform.

### Velocity detection

Superficial neuromasts are modeled as particle velocity detectors (Kalmijn 1988, 1989), which produces a set of predictions regarding their response to a vibrating sphere. A velocity detector is expected to respond at a 90° phase lead relative to the displacement maximum of the source. We found that the response phase of midshipman superficial neuromasts at any given frequency was highly variable across units. This contrasts with previous studies showing that the response phase of superficial neuromasts is stable across units, suggesting either velocity (Kroese and Schellart 1992; Kroese et al. 1978) or acceleration (Kroese et al. 1980; Munz et al. 1984) sensitivity. The difference in results is unclear, although Kroese and Schellart (1992) and Kroese et al. (1978) showed 90° phase leads only at low stimulus amplitudes within the linear response range of the system and at frequencies <10 Hz. The variability in response phase may have arisen from filtering mechanisms (i.e., synaptic delays and neural propagation delays) that would introduce a phase shift dependent on neuromast location. However, delay-induced phase shifts should occur in all species, yet did not obscure the phase response in trout, which are larger than midshipman and have receptors distributed over an even greater area (Kroese and Schellart 1992). Phase shifts therefore cannot solely explain the variability in our study. Regardless, it is doubtful that neural response phase would prove useful to...
midshipman in this situation, given the large variation in response phase across a spatially distributed array of receptors.

Along with the 90° phase lead, velocity detectors are predicted to respond to a constant velocity stimulus with a slope of 0 dB/octave. While some low-pass units had low-frequency threshold tuning curve slopes close to this theoretical value, there was a wide range of variation across units. As with phase measures, previous studies have measured the low-frequency slopes of gain curves calculated using linear frequency analysis and described units whose frequency responses matched those of either a theoretical velocity detector (Kroese and Schellart 1992; Kroese et al. 1978) or a theoretical acceleration detector (Munz 1985; Munz et al. 1984). The responses of lateral line afferents in this study were clearly nonlinear, and thus linear frequency analysis was inappropriate. However, Coombs and Janssen (1990) did report low-frequency slopes close to theoretical velocity detector values using analyses similar to ours, so it is unclear whether the wide range of low-frequency tuning curve slopes reported here resulted exclusively from nonlinearities in the neural response.

These results suggest that, under the present experimental conditions, midshipman superficial neuromasts do not follow the predicted responses of velocity detectors. This is not to say that these neuromasts do not respond in proportion to velocity at the receptor level, but this information is obscured at the level of the primary afferent. In addition to presenting stimulus amplitudes beyond the linear range of the system, a key methodological difference between this and previous studies was the placement of the stimulus relative to the receptive neuromasts. Most demonstrations of velocity sensitivity of the lateral line have been done either in vivo with the vibrating sphere located in the same position relative to each receptive neuromast (Kroese and Schellart 1992; Kroese et al. 1978) or in vitro using isolated patches of skin containing superficial neuromasts (Harris and Milne 1966; Kroese et al. 1978; Strelloff and Honrubia 1978). Our study was done in vivo, but the stimulus was not in the same position relative to the receptive neuromast for each unit because the sphere’s position was fixed. Variability in the distance between the stimulus and the receptor may have caused the variability in phase and low frequency slope of the response. It is possible that under experimental conditions like those described in Kroese and Schellart (1992), midshipman superficial neuromasts would respond in a velocity sensitive manner. Our methodology, however, allows us to consider a more realistic situation in which an array of lateral line organs is presented with a stimulus at some point in space rather than with a stimulus that is equidistant from each receptor. Moreover, it is likely that natural stimuli will be of sufficient amplitudes to drive the lateral line system beyond the linear response range. Thus in a more naturalistic context, the responses of superficial neuromasts might be too complex to be modeled solely in proportion to velocity.

Frequency sensitivity

While characteristic frequency and best excitable frequency of midshipman superficial neuromasts fell within a relatively narrow frequency range, there was considerable variation in the shapes of threshold tuning curves and isovelocity curves. Comparisons with previous studies are difficult because most have reported physiological responses in terms of displacement or acceleration rather than velocity, as we have done in this study. The shapes of frequency response functions are dependent on the stimulus parameter to which data are referred (Kalmijn 1988, 1989), and translating frequency response curves between different components of the stimulus is not appropriate if the responses are not linear (see Montgomery et al. 1988). Low-pass units were similar in shape to velocity-sensitive units in the antarctic fish, Trematomus bernacchii (Coombs and Montgomery 1994; Montgomery and Coombs 1992), while band-pass units were more similar in shape to acceleration sensitive units reported in these studies (see Fig. 7 in Coombs and Montgomery 1994). Although the low-frequency parts of the curves are similar, antarctic fish have much steeper high-frequency slopes than midshipman. This might be attributed to the much colder temperatures inhabited by Trematomus.

Complex units were characterized by a deep notch in either the threshold tuning or isovelocity curves. In most units, this notch occurred at 70 Hz, and the width of the notch was variable. It is unclear whether this notch was biological in nature or an artifact of our experimental setup. The fact that the notch usually occurred at 70 Hz suggests that it may have resulted from nonuniformities in the stimulus field at 70 Hz. However, the majority of units did not contain a notch, implying that such nonuniformities only existed at particular points in space around the stimulus. We were unable to map out the velocity field around the stimulus, so we cannot determine if this was the case.

Broadly tuned units were particularly interesting in that they exhibited an almost flat response across a wider frequency range than has been reported for superficial neuromasts in other species. These responses extended into the frequency range typically thought of as the domain of canal neuromasts (Coombs and Montgomery 1999). Coombs and Montgomery (1992) hypothesized that the high-frequency slope of canal neuromasts is determined by hair cell and primary afferent membrane time constants, which steepen the slope relative to predicted responses. Higher-order filtering mechanisms may also function in superficial neuromasts and might account for the broad tuning of some units. In fact, the loss of a trunk canal may have favored adaptations to increase the frequency range of superficial neuromasts to compensate for what would otherwise result in a loss in functionality. It would be interesting to examine the response properties of superficial neuromasts in other teleosts that lack a trunk canal to see whether this is a general phenomenon.

The frequency response properties of midshipman superficial neuromasts show a higher degree of variation than has been previously reported for this class of lateral line receptors (see Coombs and Montgomery 1994). Midshipman superficial neuromasts have two dermal papillae on either side of the cupula (Greene 1899). Within a given line of neuromasts, the papillae are oriented along the same axis, although the size and overall structure is variable both between and within lines. These papillae may form a rudimentary canal (see also Coombs et al. 1988) with some of the same filtering effects of intact canals (Denton and Gray 1988) and could account for the broadening of tuning exhibited by some primary afferents. Alternatively, the response variation might arise from physical variation in hair cells between neuromasts. Kroese and van Netten (1989) suggested that the frequency responses of a
neuromast are influenced by the mechanical properties, length, and number of stereocilia. Superficial neuromasts can have multiple developmental origins (Coombs et al. 1988), which could give rise to such structural variability. Although all midshipman trunk neuromasts are superficial, the developmental origins of individual organs are unknown. Ultrastructural and developmental studies will be required to determine the degree of variability within midshipman neuromasts and whether this might account for variability in the physiological responses of the system.

For ease of analysis and presentation, we used specific criteria to classify units into four categories, although this categorization was not always distinct. Therefore although we operationally defined distinct categories, responses may fall along a continuum of filter shapes. Nevertheless, these responses are clearly highly variable and extend the functional range of these receptors beyond the low-pass detectors that superficial neuromasts are typically described as.

**Sound detection**

The role of the lateral line system in sound detection has long been debated (see Sand 1981 for review). The acoustic field around an underwater sound source consists of a particle motion dominated near field and a pressure wave dominated far field (Kalmijn 1988, 1989). Because the effective stimulus to the lateral line system is movement of the surrounding water relative to the fish, near field particle motion is the only sound component capable of directly stimulating the lateral line (Harries and van Bergeijk 1962). Further, the steep gradients of particle motion confine direct stimulation to the innermost region of the near field, within a few body lengths from the source (Denton and Gray 1983, 1988; Kalmijn 1988, 1989). It is therefore likely that acoustic stimulation of the lateral line will occur in close proximity to the source.

Male midshipman produce loud (~125 dB re: 1 μPa) acoustic signals with fundamental frequencies at or <100 Hz, well within the response range we have shown for superficial neuromasts. The near field dominates the sound field up to a distance of λ/2π from the source, which is on the order of 2–3 m for a midshipman vocalization with a fundamental frequency of 100 Hz (Bass and Clark 2002). During the breeding season, nocturnally active males produce advertisement “hums” to attract females to their nests and agonistic “grunts” to fend off conspecific males during territorial defense (Bass et al. 1999; Brantley and Bass 1994; McKibben and Bass 1998, 2001a). These interactions often occur within several body lengths and place the recipient within the inner regions of the near field of the vocalizing fish, where stimulation of the lateral line system would occur.

The lateral line system might also be stimulated indirectly via the swim bladder. As an air-filled sac, the swim bladder oscillates when placed within a pressure wave. This oscillation re-radiates the sound, creating an indirect near field signal. Displacement thresholds of the roach (*Rutilus rutilus*) lateral line are below the particle displacements of re-radiated pressure waves, suggesting that indirect stimuli are detectable by the lateral line system in these animals (Sand 1981). Thus it is reasonable to predict that the midshipman lateral line could indirectly detect acoustic signals at distances beyond the normal range of direct lateral line stimulation.

This study clearly shows that midshipman superficial neuromasts are capable of encoding frequencies within the range of natural vocalizations. The question remains then, what additional information might the lateral line provide that is not available via the inner ear? It is unknown what a female midshipman bases her decision on when choosing a mate, but vocalizations likely provide information regarding male quality, especially in a nocturnal habitat where visual cues are limited. While the auditory system is clearly used to guide a female to a humming male’s nest (Brantley and Bass 1994; McKibben and Bass 1998, 2001a), the final mating decision may take place within the inner regions of the near field using input from the lateral line and auditory systems. Similarly, the lateral line system may be used to assess the size of a vocalizing rival male during close agonistic interactions. The behavioral data, together with the current study’s physiological results and our previous demonstration of anatomical overlap between the ascending lateral line and auditory pathways (Bass et al. 2000; Weeg and Bass 2000a), suggest an interaction between the lateral line and auditory systems that may be common to all aquatic anamniotes, especially those that vocalize.

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