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Behavioral and neurophysiological assessment of lateral line sensitivity in the mottled sculpin, *Cottus bairdi*

Sheryl Coombs and John Janssen

Parmly Hearing Institute and Biology Department, Loyola University of Chicago, Chicago, IL 60626, USA

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Summary. 1. The unconditioned feeding response of the mottled sculpin, *Cottus bairdi*, was used to measure threshold sensitivity of the lateral line system to a vibrating sphere as a function of stimulus position (i.e. sphere near head, trunk or tail) and vibration frequency. In addition, extracellular recording techniques were used to measure threshold sensitivity curves for posterior lateral line nerve fibers for the same stimulus position used for measuring trunk sensitivity in behavioral measurements.

2. For all stimulus positions, behaviorally-measured threshold sensitivity was relatively independent of vibration frequency from 10 to 100 Hz when defined in terms of water acceleration, rather than velocity or displacement. Best thresholds for stimuli placed 15 mm away from the head were around −75 dB re: 1 m/s^2, approximately 20 dB less than that for stimuli placed at the same distance near the tail. Trunk sensitivity was intermediate.

3. Physiologically-measured threshold sensitivity, in terms of acceleration, was also relatively independent of frequency from 10 to 100 Hz in most fibers. A smaller number of fibers showed a decline in acceleration sensitivity after 10–30 Hz, with the rate of decline being equivalent to equal velocity sensitivity. Best sensitivity of all fibers fell between −40 and −70 dB re: 1m/s^2.

4. These results indicate that (a) behavioral thresholds are based on acceleration-sensitive endorgans – most likely lateral line canal (rather than superficial) neuromasts, (b) behavioral performance can be accounted for on the basis of information from a single population of fibers, and (c) sensitivity varies along the fish's body in a manner that corresponds to the size and distribution of neuromasts.

Key words: Lateral line – Sensitivity – Tuning curves

Introduction

Although there have been a number of studies on the sensory capabilities of the lateral line system in surface feeding fish (e.g. Bleckmann 1988; Bleckmann et al. 1989) and amphibians (e.g. Elepfandt 1989; Görner and Mohr 1989), systematic and quantitative descriptions of subsurface detection abilities are fewer. This has largely been due to the lack of suitable devices for measuring subsurface water motions and a lack of a reliable behavioral indicator of detection in these fish. With the use of hot-film anemometry to measure subsurface vibrations (Coombs et al. 1989), we have determined the detection abilities of the lateral line in the mottled sculpin, *Cottus bairdi*, a benthic scorpaeniform fish that uses its lateral line system in detecting prey (Hoekstra and Janssen 1985, 1986). Blinded fish respond to stimulation with an unconditioned feeding response that consists of a movement of the fish's head towards the source and a bite (Hoekstra and Janssen 1985, 1986). This response appears to be mediated solely by the lateral line system as chemically inert stimuli elicit the response, whereas pharmacological (streptomycin or Ca^{2+} free water with Co^{2+} inhibitor) or mechanical blocking of the lateral line system results in a complete, but reversible loss of the response (Hoekstra and Janssen 1985, 1986).

The purposes of the experiments reported here were to determine (1) under what conditions the feeding response could be used as a reliable indicator of stimulus detection in measuring behavioral sensitivity, (2) how behavioral sensitivity varies as a function of frequency and stimulus position along the spatially distributed lateral line system and (3) how behavioral sensitivity is encoded by peripheral lateral line nerve fibers. Preliminary results from some of these experiments have been reported in Coombs and Janssen (1989a, b).

Methods

Experimental animals. Data were collected from mottled sculpin (*Cottus bairdi*) ranging from 70 to 130 mm (standard length). Animals were collected from Lake Michigan at depths ranging from 6 to 9 m by SCUBA divers. For behavioral experiments, fish were blinded by removing the eyes under anesthesia (MS-222) and housed in individual 5-gal tanks. For physiological experiments, fish were kept in 20-gal tanks housing between 3–5 fish each. Both behavioral and physiological animals were hand-fed with squid pieces delivered by forceps.

Stimulus generation and measurement. Stimulus generation and measurement were nearly identical for behavioral and physiological experiments. The dipolar stimulus consisted of a plastic sphere (6 mm in diameter) attached to the end of a 16-gauge, 120 mm long syringe needle. The syringe needle was mounted to a B & K minishaker (model 4810) that was sinusoidally activated. For behavioral experiments, electronic signals driving the minishaker were produced by an analog function generator, shaped by an electronic rise/fall switch (10 ms rise/fall times), attenuated, and amplified. For neurophysiological experiments, signals were digitally synthesized and converted to analog signals by a programmable function generator (Modular Instruments, Inc.) interfaced with an IBM-XT, low-pass filtered (400 Hz cut-off frequency) and then attenuated and amplified.

Water motion created by the sphere was measured directly with a displacement-sensitive hot-film anemometry system (TSI, Inc.), as described in Coombs et al. (1989). The sensing element of this system is electrically heated to a constant temperature and the output of the system is a measure of the electrical current required to maintain constant temperature and to counteract the cooling effects of water flowing past the sensor. Stimulus measurements were made in the same test tank where physiological experiments were made, but in the absence of the fish. The sensing element was positioned at the same distance from sphere center as the lateral surface of the fish and was oriented to measure the amplitude of water motion along the axis of sphere vibration. The signal was amplified and then measured with a wave analyzer through a 3 Hz bandwidth. Spectrum levels of ambient noise declined from -75 dB re: 1 m/s^2 at 10 Hz to at least -95 dB re: 1 m/s^2 at 40 Hz and above, where the noise floor was determined by the electrical noise of the measuring system. In addition, there were day-to-day variations in the amount of noise at 30 Hz, where the system showed considerable resonance (Coombs et al. 1989). Despite some evidence that the anemometer sensor may have been switching from a displacement to a velocity-sensitive device below 10–20 Hz (Coombs et al. 1989), thresholds reported here are based on the assumption that measurements were made with a displacement-sensitive device over the entire frequency range of interest. For data presentation, p-p displacement (d) levels measured directly with the anemometer were converted to p-p velocity (u) and acceleration (a) levels using the relationships $u = 2\pi fd$ and $a = 4\pi^2 f^2 d$, where f is the vibration frequency.

Behavioral procedures. Behavioral experiments were conducted in a glass tank, measuring 80 cm long, 47 cm wide and 36 cm high and filled with 8 cm of water. Fish rested on a glass plate separated from tank bottom by a plastic grating (egg crate). The glass plate could be slid over the grating to position the fish relative to the vibrating sphere (Fig. 1 A). Sphere center was 12 mm above the plate (corresponding to the average elevation of the lateral line system on the body of the fish) and the axis of vibration was perpendicular to the plate. One of several concentric circles drawn on the plate (as illustrated in Fig. 1 A) marked the actual, radial distances of 15 mm from the projection of sphere center onto the plate. A video camera directly below the tank afforded a ventral view of the fish, silhouetted against the concentric circles so that the fish could be accurately positioned (Fig. 1 A). A plastic cylinder (30 cm in diameter, 17 cm high) on top of the glass plate corraled the fish, which was then positioned with either the base of its tail (caudal peduncle), the middle of its trunk canal (at the junction between first and second dorsal fin), or the middle of its head (at the eye socket) tangential to the outer edge of the circle delineating 15 mm from sphere center. For any given condition, animals were randomly positioned with either the left or the right side tangential to the circle, thus making it difficult for animals to remember the sphere's position.

Once the fish was positioned, the experimenter initiated a computer-controlled sequence that determined a random intertrial interval (from 5 to 15 s) followed by a 5-s trial during which the sphere was vibrated. The sculpin's response was scored as either

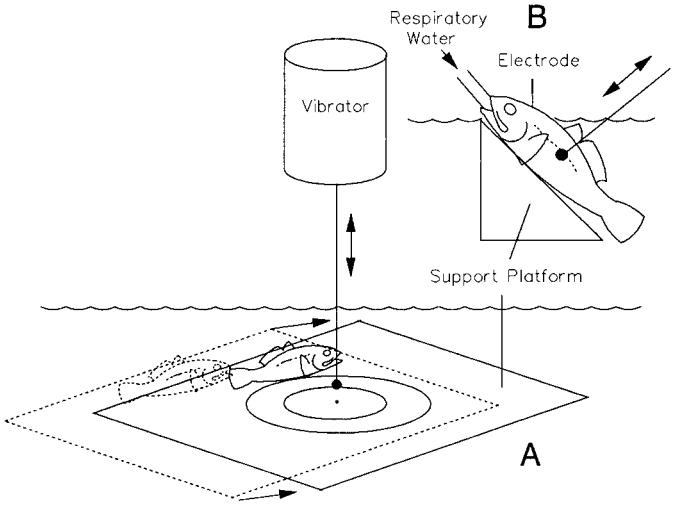

Fig. 1 A, B. Diagrams showing how fish are positioned next to a vibrating sphere for behavioral **A** and neurophysiological **B** measurements of lateral line sensitivity

a detection (movement towards or strike (bite) at the sphere) or non-detection (no movement or movement away) response. Detection responses were reinforced by feeding the fish with small pieces of squid. If the fish moved before the trial began, it was repositioned, and a new trial sequence was begun. To minimize repositioning and aborted trials, it was occasionally necessary to enhance the proclivity of sculpin to sit motionless by reinforcing progressively longer periods of inactivity with food rewards.

To measure the probability that detection responses occurred by chance, 30% of all trials, randomly scheduled, were presented without sphere vibration (blank trials). If feeding responses to blank trials occurred more than 20% of the time during any given experimental session, the feeding responses during that session were judged to be under poor stimulus control, and data from that session were excluded from final data analysis.

At the beginning of each new condition in threshold experiments, the amplitude of sphere vibration was usually set between 20–40 dB above threshold (determined a posteriori). Once preliminary threshold measurements were made, vibration amplitude was set between 10–20 dB above threshold at the beginning of each experimental session on the same condition. To measure threshold, an adaptive tracking technique was used in which the fish's response to a trial determined the amplitude of the subsequent trial, such that a detection response resulted in decreasing the amplitude of the next trial by 5 dB, and a non-detection response resulted in increasing the amplitude of the next trial by 5 dB. The signal level midway between that for consecutive yes and no responses was defined as a transition threshold. Approximately 3 to 7 of these transition thresholds (ranging over 5 to 15 stimulus presentations) were obtained for each experimental session, and 20 of these were obtained for each fish per experimental condition. Experimental sessions lasted approximately one hour per day and ran 4 days a week for each fish. Experimental conditions (stimulus position and vibration frequency) were randomized across individuals, but stimulus position remained the same for each animal until thresholds were obtained at all frequencies.

To assess the reliability of the feeding response as an indicator of detection, experiments were performed to examine the effects of rewards and satiation on the percentage of times that the feeding response was elicited by a suprathreshold stimulus. This response frequency was measured for fish that were (1) fed during experiments by rewarding correct responses, (2) fed after experiments in their home tank and (3) fed before experiments in their home tanks. For feeding experiments, the frequency of detection responses to a 20 Hz signal 40 dB above threshold was measured

over 10 stimulus presentations during an experimental session. Each fish was run for 15 consecutive sessions (over the course of approximately one month) and rewarded after each correct response. This was the only time these fish were fed, as was the case for animals in threshold experiments. For up to 15 consecutive sessions following this, the same fish were not rewarded during the experimental sessions, but were fed afterwards when returned to their home tank. Following this regimen, fish were again reinforced with food during experimental sessions until the detection rate returned to greater than 80% for several consecutive sessions. Fish were then fed in their home tanks immediately prior to experimental sessions and additionally rewarded during the experiments. For all conditions except the last, the amount of food given per day was approximately the same (around 550 mg).

Neurophysiological procedures. Methods for recording evoked activity from single fibers of the posterior lateral line nerve are very similar to those used previously for recording from eighth nerve fibers in goldfish (Fay 1978) and have been described by Coombs and Janssen (1989a) for the mottled sculpin. Fish were anesthetized by immersion in MS-222 (200 mg/l) and immobilized with intramuscular injections of Flaxedil (2 to 4 μg/g body weight). Fish were then transferred to the experimental tank ($27.5 \times 17.5 \times 10.5$ cm) where they were placed on a plate of plexiglass that formed a 45° angle with the water surface (Fig. 1B). The head was positioned above water and clamped onto a respiratory tube which delivered gravity-fed water through the mouth and over the gills. Respiratory flow, as measured with a flowmeter inserted between the water reservoir and the respiratory tube, was maintained at levels (between 0.01 and 0.05 l/min) determined to produce neither stimulatory nor hypoxic effects. The trunk of the fish, including the trunk lateral line system, was submerged. The vibrating sphere was placed at the same position (middle of the trunk canal at the junction between first and second dorsal fins, as shown in Fig. 1B), distance (15 mm away from the lateral body surface of the fish) and in the same vibration axis (perpendicular to the horizontal plane of the fish) at it was for behavioral experiments.

A hole was made in the top of the skull to expose the brain, and excess fatty tissue and fluids were aspirated away to reveal the posterior lateral line nerve root where it entered the brainstem. Micropipettes filled with 3 *M* KCl (tip impedance ranging from 10 to 50 MΩ) were placed on the nerve with a micromanipulator and advanced through the nerve with a motorized microdrive in 1 μm steps. The output of the microelectrode was amplified within a 300 to 3,000 Hz bandwidth, and single spikes were distinguished by a voltage-level discriminator which converted them into TTL pulses. Spike activity was both visually and acoustically monitored. Data acquisition components of a modular hardware system (Modular Instruments, Inc.) took in spontaneous spike activity in the form of interspike intervals and evoked activity in the form of elapsed times from stimulus onset to the occurrence of each spike. Data could be displayed on-line as interspike interval histograms for spontaneous activity or peristimulus time histograms for evoked activity. Data were also stored on disk for subsequent conversion into period histograms and extraction of average rate and phase-locking (synchronization coefficient) (Anderson 1973) measures of responsiveness.

Pulsed, low-pass (200 Hz cut-off frequency) noise signals were used to search for evoked activity from single fibers. Once a responsive fiber was located, spontaneous activity was recorded for 10 s to obtain a measure of spontaneous rate. The fiber was then stimulated with 10 repetitions of a 10-cycle sinusoidal burst (interburst interval = 500 ms) at a frequency and intensity that gave a robust response. Each sinusoidal burst was gated on at a 0° starting phase. Rise/fall times were determined by the waveform fine structure and thus varied with frequency from 1 ms at 250 Hz to 25 ms at 10 Hz. After obtaining a robust response from any given fiber, the stimulus level was then lowered by 10 dB and the bursts were repeated. This descending intensity series was continued until the fiber no longer responded and was followed by an ascending series which began at a signal level 5 dB below the lowest level used in the descending series. In this way, responses below and above threshold were obtained for signal levels 5 db apart and any long term changes in responsiveness could be seen by comparing results obtained from the descending series with those of the ascending series. This procedure was continued until data were collected in response to frequencies ranging from 10 to 500 Hz. In order to span as much of the frequency range as possible before a fiber was lost, testing began in the middle of the frequency range (typically between 30 and 100 Hz) and continued by alternating between lower and higher frequencies until the frequency range was exhausted. Since threshold measures of response based on evoked spike rate depended on spontaneous spike rate (see following paragraph), it was also necessary to make repeated measurements of spontaneous rate. These measurements were obtained between the descending and ascending intensity series at each frequency during a single stimulus presentation (i.e. 10 bursts) for which the stimulus was turned off. This method, rather than collecting data over a 10 s period, as was done in the beginning for each fiber, ensured that the time window for spontaneous rate measurements was the same as that for evoked rate measurements at any given frequency. If the fiber's response was still robust after collecting all of the frequency data, we investigated two factors which may have affected the fiber's frequency response. The possible effect of respiratory flow on spontaneous activity was investigated by monitoring spike rate and interspike interval distributions for successive 10 s periods after the flow rate was changed. Alternatively, the effect of stimulus position on tuning and sensitivity was investigated by moving the stimulus to a position 15 mm away from the base of the tail (one of the same positions used in behavioral experiments) and repeating tuning curve measurements.

Threshold levels of response were defined in two different ways for each fiber: as the signal level at which spike rate began to rise above spontaneous levels and at which the synchronization coefficient (1 being perfect synchronization and 0 being none) was 0.4. A value of 0.4 was chosen to keep the synchronization coefficient above that to be expected by chance (i.e. when no stimulus was present) and in a range where the value varied linearly with the degree to which spontaneous rates were modulated. Because synchronization functions are generally sigmoidal in shape, coefficients much above or below 0.5 are generally in a non-linear range of the function. Finally, since the synchronization coefficient cannot go above 0.5 when evoked rate does not exceed spontaneous rate, it was expected that a synchronization criterion of 0.4 would yield threshold measurements very close to those obtained with rate criteria. The strategy of using two quasi-independent threshold criteria was chosen to maximize valid threshold measurements at the high and low frequency end of the tuning curve. Because it is difficult to drive evoked spike rates above spontaneous levels at low stimulus frequencies, threshold measurements based on spike rate criteria are often unattainable at frequencies below 20–30 Hz. Similarly, synchronization criteria are poor at higher frequencies because fibers often respond with rapid adaptation. This tends to give an inflated estimate of phase locking, because a major proportion of spike activity is time-locked to the beginning of the stimulus. Synchronization coefficients are also not very useful in characterizing thresholds in fibers without any spontaneous activity.

Acceleration levels at threshold were plotted as a function of frequency to yield rate- and synchronization-based tuning curves for each fiber. In order to classify fibers according to the shape of their threshold curves, we also characterized fibers according to the slopes of their threshold functions (from 10 to 100 Hz) plotted on a log-log scale. Data at 30 Hz was excluded from this analysis because of resonance in the stimulus at this frequency (Coombs et al. 1989). Since sensitivity was expressed in terms of signal acceleration at threshold, a fiber having a threshold function with a slope of 0 dB/frequency doubling (octave) and thus, showing no change in sensitivity as a function of frequency, was defined as acceleration-sensitive. A fiber having a threshold function with a slope of 6.02 dB/octave (a slope which would be 0 dB/octave if

plotted in terms of signal velocity) was defined as velocity-sensitive. Threshold functions based on rate and synchronization criteria were evaluated separately for each fiber, and it was assumed that each threshold function would be associated with a fiber that was either acceleration or velocity sensitive. A linear regression was performed on each function, and the slope, error sums of squares, and error degrees of freedom were calculated. The error sums of squares and error degrees of freedom were summed over all threshold curves based on the same criterion to yield a single pooled error mean square for rate-based threshold curves and a single error mean square for synchronization-based threshold curves. This measure yields the best estimate of error variance. The standard error of the slope of each threshold curve was then calculated from the pooled error mean square using the standard formula (error variance/sums of squares $\log_2$ (frequency)). A 95% confidence interval was constructed for each expected slope. For acceleration, this interval was equal to $0.0 \pm t_{0.05} \times$ slope standard error and for velocity, $6.02 \pm t_{0.05} \times$ slope standard error. Because each threshold curve was defined by data at a different number of frequencies, the slope standard errors, and hence, confidence intervals varied between curves. However, only fibers for which there were threshold data for a minimum of 5 frequencies (over the range 10–100 Hz) were used. Threshold curves based on fewer frequencies were eliminated from this analysis because the standard error of the slope variance was too large, making it very difficult to classify fibers.

Behavioral results

Response rate and threshold detection as a function of feeding

Results from experiments in which the feeding regimen was varied were obtained from four animals (Fig. 2). When animals were rewarded during the experimental session, response rates to stimulus trials were generally greater than 80% and those to blank trails less than 20% (Fig. 2, panel A). Response rates fell outside of these limits for less than 10% of the total number of trials. When reinforcement was withheld, responses from all fish were slow to extinguish, usually taking 6 to 8 days before response rates dropped below 80% (Fig. 2, panel B). Reinstatement of reinforcement resulted in an almost immediate return to response rates greater than 80% (Fig. 2, panel C). Feeding fish until they refused to accept food reward during the experiment resulted in a dramatic and nearly immediate decline in response rate (Fig. 2, panel D). The various feeding regimens appeared to have no appreciable affect on false alarm rates, as shown by the bottom panel of Fig. 2.

We also measured 73 consecutive threshold detection levels (20 Hz signal positioned at the trunk) for one fish, the first 33 with stimulus-associated food and the next 40 without. The mean threshold for the first 33 determinations was less than 2 dB different from that obtained over the next 40 determinations and the standard deviations were 3.3 and 3.0 dB, respectively. These results showed that withdrawal of stimulus-associated food had no effects on threshold measurement within this time frame.

Response rate for blank trials

To examine the propensity of fish to respond in the absence of a stimulus, false alarm rates from all threshold experimental sessions (a total of 307 from 4 animals)

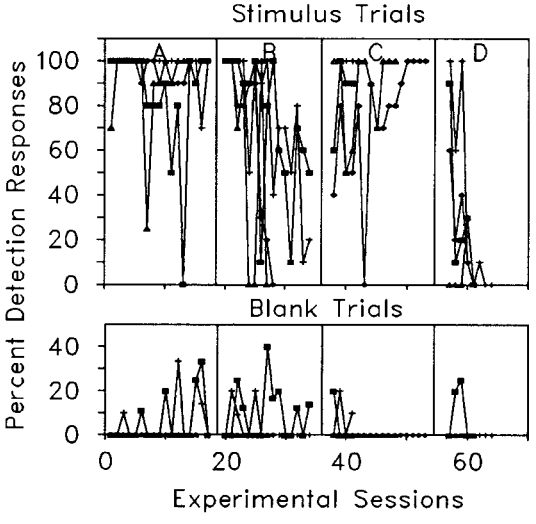

Fig. 2. Frequency of responding in the presence (upper panels) and absence (lower panels) of a suprathreshold stimulus for different feeding regimen. Panel A: fish fed during experiments following each correct response; Panel B: fish fed in their home tanks after each experimental session is over; Panel C: same as panel A; Panel D: fish fed to the point of satiation in home tanks before experiments are begun

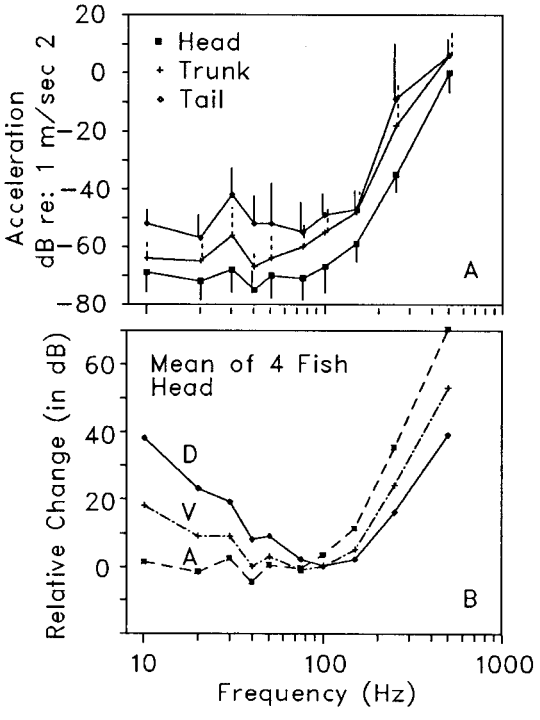

Fig. 3. A Sensitivity as a function of stimulus position and frequency. Each data point represents the mean of 80 threshold measurements from a total of 4 fish. Vertical lines: standard deviations from the mean. **B** Mean sensitivity to stimuli at the head expressed in terms of displacement (*D*), velocity (*V*) and acceleration (*A*). Best sensitivity was normalized to 0 dB which represents −178 dB re: 1 m/s, −123 dB re: 1 m/s and −70 dB re: 1 m/s, respectively

were pooled into a single frequency distribution. Feeding responses to blank trials during this experiment were relatively rare and occurred during only 25% of all experimental sessions. Of these, only 39% (10% of all experimental sessions) showed false alarm rates greater than 20%, the cut-off rate for rejecting threshold data. A session-by-session record of false alarm rates from feeding experiments (Fig. 2) shows similar results, with the majority of false alarm rates falling below 20%.

Sensitivity as a function of stimulus frequency and position

The mean of 80 threshold transitions (20 from each of 4 fish) (Fig. 3A) per condition is plotted as a function of stimulus position and frequency. The data are plotted as water acceleration, but the same data for the head position are plotted again as displacement and velocity (Fig. 3B). Acceleration thresholds varied little as a function of frequency in the range from 10 to 100 Hz for all stimulus positions. Acceleration thresholds were around -75 dB re: 1 m/s^2 for stimuli placed near the head. Acceleration thresholds for the tail were approximately 20 dB higher than head thresholds and trunk thresholds were intermediate. Spectrum levels of ambient noise were at least 20–25 dB below thresholds at the head for all frequencies except 10 and 30 Hz, where spectrum levels were -72 and -85 dB re: 1 m/s^2 respectively.

These results (Fig. 3) suggest that acceleration sensitivity varies with stimulus position but not with frequency over the range of 10 to 100 Hz[1]. For statistical analysis, data were analyzed as a three-factor analysis of variance (ANOVA), the factors being: fish, stimulus position, and frequency (from 10–500 Hz). The dependent variable was the mean threshold (as acceleration) for each fish at any given condition. Thus, in the data matrix, there was one cell for each fish × position × frequency combination. Data at 30 Hz were exluded from this and subsequent analyses due to a mechanical resonance in the stimulus at this frequency (Coombs et al. 1989). The analysis showed significant interposition variation (discussed later, $F=42.66$; 2, 93 df; $P<0.0001$) and interfish variation ($F=14.82$; 3, 93 df; $P<0.0001$) in sensitivity. Although this analysis also showed a significant inter-frequency variation ($F=138.0$; 8, 93 df, $P<0.0001$), a subsequent application of Tukey's test revealed that in general this variation did not occur over the frequency range from 10 to 100 Hz ($P>0.05$) (see Fig. 4 for details).

The above analysis indicates that, when sensitivity is expressed in terms of the acceleration of the signal, there is essentially no tuning over most of the bandwidth of detection (i.e. the threshold response function is flat in the approximate frequency range 10–100 Hz). When sensitivity is expressed in terms of signal velocity or displacement, the frequency response in that frequency range is not flat, as shown in Fig. 3B. A linear regression was performed on displacement-,velocity-, and acceleration-based sensitivity functions over the frequency range 10–100 Hz and the slopes are shown in Table 1 for different stimulus positions. This regression analysis shows that the slopes of velocity and displacement functions are significantly different from 0, but that of acceleration functions are not. Moreover, the slopes of velocity functions were not significantly different from the expected slope of -6.02 ($t=-0.37$, -1.40, 0.31, 4 df, $P>0.10$) for an acceleration detector. Similarly, the slopes of displacement functions were not significantly different from the expected slope of -12.04 ($t=-0.39$, -1.57, 0.38, 4 df, $P>0.10$) for an acceleration detector.

Because the 10 to 100 Hz portions of acceleration response functions are flat (and hence parallel), their elevations (i.e. threshold sensitivity) for the 3 positions can be compared in an analysis of covariance, with frequency (transformed to octaves) as the covariate and position and fish as fixed variables. This analysis again yields a slope not different from 0 and a significant interposition variation ($F=50.2$; 2, 64 df, $P<0.0001$). There is also significant inter-fish variation ($F=21.86$; 3, 64 df, $P<0.0001$). Subsequent comparisons between posi-

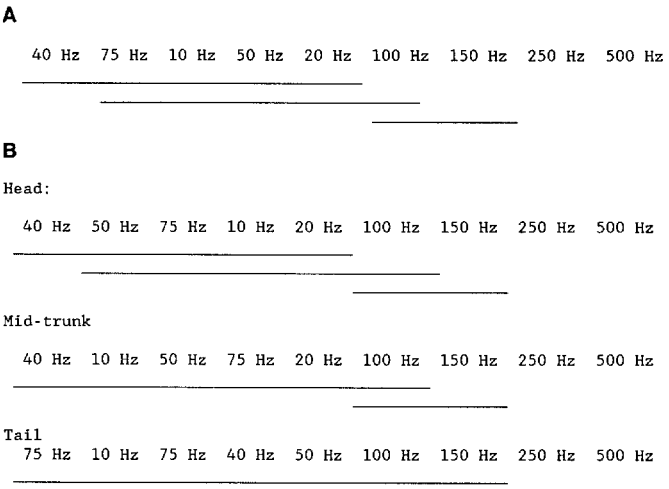

Fig. 4A, B. Ordering of frequencies (from left to right) according to mean sensitivity (from best to worst) for an analysis by Tukey's test across combined stimulus positions **A** and broken down by stimulus position **B**. Underlining indicates that mean sensitivity measures at these frequencies are not statistically distinguishable ($P>0.05$). Sensitivity measures at frequencies ordered in **A** between 40 and 20 Hz and also between 10 and 100 Hz are also not distinguishable at $P>0.2$. However, fish were significantly less sensitive at 250 Hz than at all lower frequencies (10–150 Hz) and at 500 Hz, less sensitive than at all lower frequencies (10–250 Hz) ($P<0.001$ in both cases). Sensitivity measures for all 3 positions in **B** over the frequency range 10–75 Hz were always indistinguishable at $P>0.2$

[1] It should be pointed out that the acceleration functions shown in Fig. 3 are slightly flatter in the frequency range 10–100 Hz than those published from preliminary results based on data from two animals (Coombs and Janssen 1989a). The major reason for this is that previously published figures were based on the assumption that the anemometer used in measuring signal levels was velocity-sensitive over the frequency range of interest, and not displacement-sensitive, as has beeen shown experimentally by Dubbelday (1986) for a different anemometer system and later confirmed in our own application (Coombs et al. 1989).

Table 1. Slopes of sensitivity functions based on linear regressions of sensitivity measures as a function of frequency (log base 2) measured behaviorally for the 3 positions. Sensitivity is expressed in terms of either acceleration, velocity, or displacement. The frequency range is 10 to 100 Hz and the response is flat (slope not statistically distinguishable from 0) only when measured in terms of acceleration of the signal. This applies to all 3 positions. t is the Students t statistic for the null hypothesis that the slope of the linear regression equals zero; P is the corresponding significance level

	Acceleration			Velocity			Displacement		
	Slope	t	P	Slope	t	P	Slope	t	P
Position									
Head	0.35	0.31	>0.5	−5.6	−5.0	<0.0001	−11.6	−11.6	<0.0001
Mid-trunk	1.9	1.41	>0.2	−4.1	−3.0	<0.04	−10.0	−7.7	<0.002
Tail	−0.58	−0.38	>0.5	−6.5	−4.2	<0.02	−12.5	−10.3	<0.002

tions using Tukey's test indicates that sensitivity at each position is significantly different from that of other positions with the head position most sensitive and the tail position least sensitive ($P<0.001$).

Neurophysiological results

Physiological status of neural preparations and spontaneous activity

Recording sessions lasted up to 12 h or until the animal died. Physiological status of the animal was evaluated by examining melanophore dispersion in the skin and blood flow in cranial vessels. Tuning curve data were collected from a total of 66 posterior lateral line fibers in 18 fish. Average spontaneous rates in these fibers ranged from 0 to 134 spikes/s with most fibers having spontaneous rates in the 30–40 spikes/s range (Fig. 5). Spontaneous activity from these fibers was of 3 types. 68% of all fibers showed bursting activity (multimodal interspike interval histogram). 14% were irregular (Poisson-like distribution of intervals) in their spontaneous activity and 18% were silent or nearly silent (<10 spikes/s). Intraburst intervals were almost always around 3 ms and interburst intervals ranged from 4 to 40 ms with some fibers showing preferred interburst intervals and others, random interburst intervals. Regular spontaneous activity (uni-modal interspike interval histogram) was not encountered in any posterior lateral line fibers – not even in those not responding to stimulation.

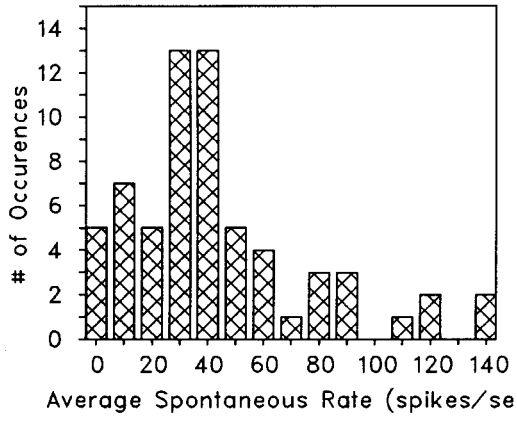

Fig. 5. Frequency distribution of average spontaneous rate in posterior lateral line fibers from which tuning curve data were collected

Threshold sensitivity curves

Although differences between threshold measurements based on phase-locking and spike rate criteria could be as large as 30 dB, differences generally clustered around 0 dB (Fig. 6). In general, there were no obvious trends for one criterion to produce higher or lower thresholds relative to measurements based on the other criterion, except possibly at 10 Hz, where synchronization-based thresholds tended to be slightly lower than rate-based thresholds. This difference probably exists because of the limited sample size and questionable validity of thresholds at 10 Hz using a rate criterion. Despite this potential difference, the two criteria usually resulted in tuning curves that were similar in overall sensitivity and shape.

Threshold sensitivity curves were generally of two types (Fig. 7). The input/output functions on which

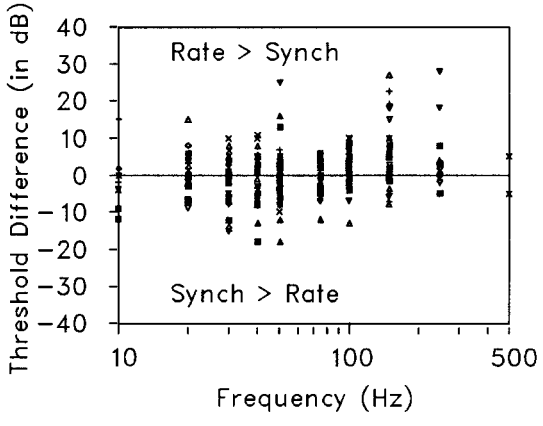

Fig. 6. Differences in rate- and synchronization-based threshold measurements as a function of vibration frequency. Data points falling below the zero line show the number of times that synchronization thresholds were lower than rate thresholds, whereas those above the line represent rate thresholds that indicated greater sensitivity than synchronization measurements

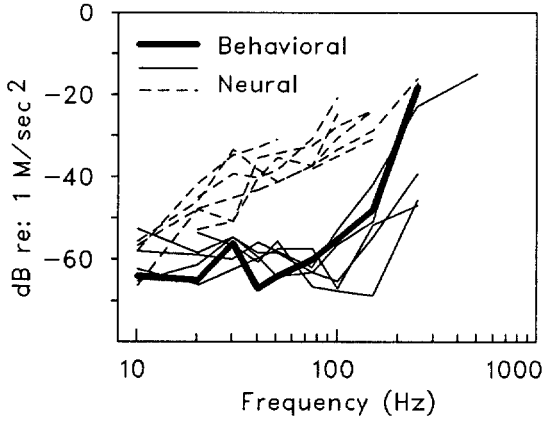

Fig. 7. Acceleration-sensitive fibers with tuning curves (solid lines) that are similar in overall shape to the behaviorally measured threshold curve (heavy solid line). Velocity-sensitive fibers with tuning curves (dashed lines) that are dissimilar in overall shape to the behaviorally measured threshold curve

these threshold curves are based are shown for one fiber of each type (Fig. 8). The majority of fibers responded with approximately equal acceleration sensitivity over the frequency range from 10 to 100 Hz (Fig. 7, solid lines). Best sensitivity in this range fell between −40 dB and −70 dB re: 1 m/s^2 and fibers tuned in this way were generally among the most sensitive of fibers encountered. For these fibers, sensitivity usually began to decline above 100 or 150 Hz. In contrast, a smaller number of fibers showed a decline in sensitivity after around 10–30 Hz (Fig. 7, dashed lines). These fibers responded with equal velocity sensitivity over most of their frequency range of response.

We also classified fibers according to the slopes of their threshold curves between 10 and 100 Hz in order to see if slopes would cluster around 0 and 6 dB/octave, the predicted slopes for equal acceleration and velocity sensitivity (see Methods 'Neurophysiological procedures' for details). The results of this classification procedure are consistent with a conclusion that both acceleration- and velocity-sensitive fibers mark the extremes of a narrowly defined continuum of slopes (Fig. 9). Using spike rate criteria, 17 of 27 fibers could be classified as either acceleration (Fig. 9, plus signs) or velocity (Fig. 9, open squares) sensitive (i.e. the fiber fit within the confidence interval for acceleration or velocity but not both). Forteen of 23 fibers could be similarly classified based on synchronization criteria. The remaining fibers could not be classified as either acceleration or velocity-sensitive for one of the following 3 reasons: (1) fibers fell within the confidence interval of both acceleration and velocity-sensitivity (filled triangles, Fig. 9; $n=7$ for rate criteria, $n=5$ for synchronization), (2) fibers fell within the confidence interval of neither (circled data points in Fig. 9; $n=3$ for both rate and synchronization

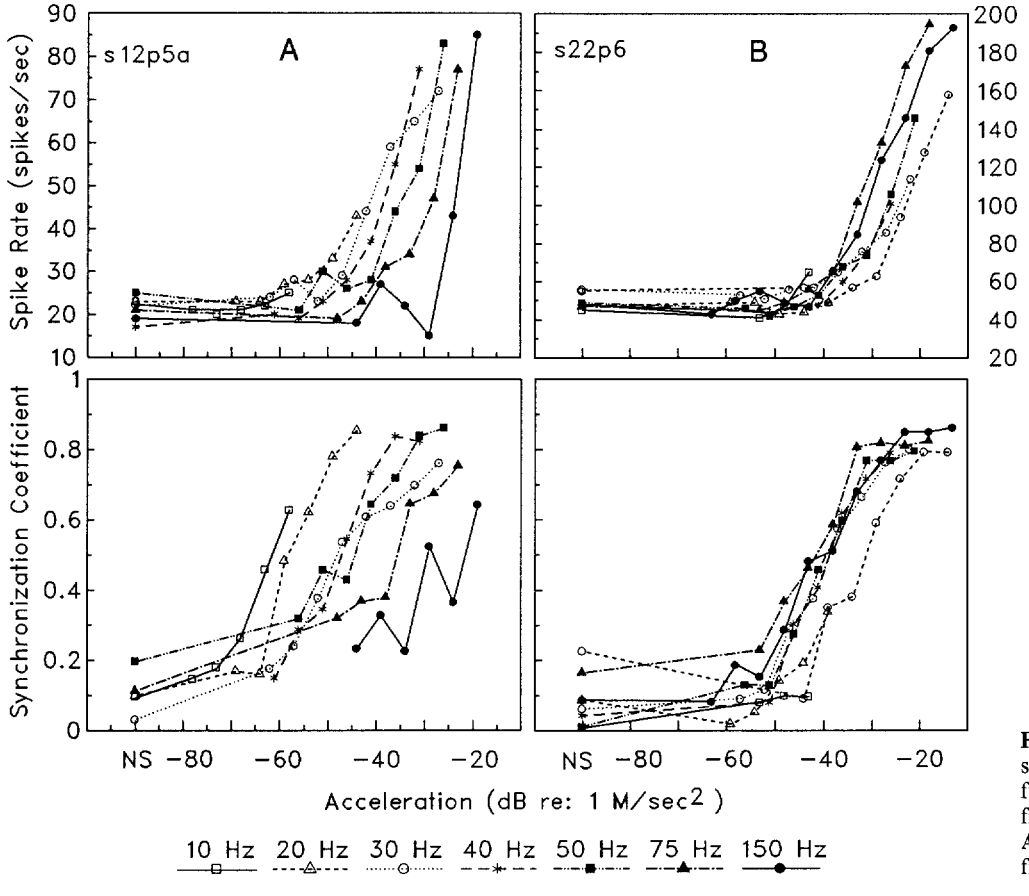

Fig. 8A, B. Rate- and synchronization-intensity functions at different vibration frequencies for a velocity-sensitive **A** and acceleration-sensitive **B** fiber

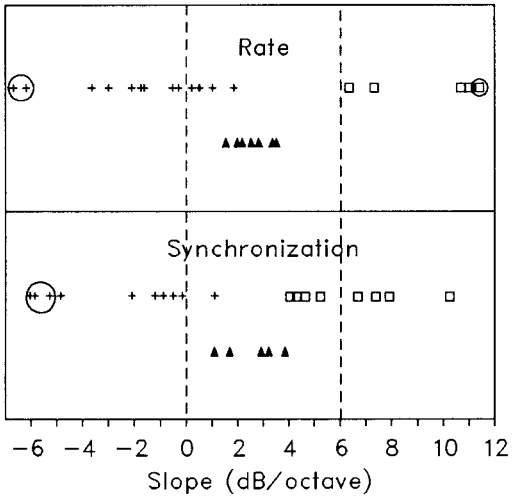

Fig. 9. Classification of fibers by tuning curve slopes. Each data point represents the slope characterizing the 10–100 Hz leg of tuning curves of fibers for which there were enough data for the analysis. Top panel represents classification on the basis of rate criteria, whereas the bottom panel represents classification by synchronization criteria. Plus signs not enclosed by circles represent slopes that were within the confidence intervals for indicating acceleration sensitivity (i.e. statistically indistinguishable from 0 dB/octave). Encircled plus signs represent slopes outside the confidence interval that were significantly less than 0 dB/octave. Open squares represent slopes falling with the confidence intervals for being velocity sensitive (6 dB/octave in acceleration units), whereas encircled squares show slopes that were significantly above 6 dB/octave. Slopes falling within the confidence intervals of both acceleration and velocity (i.e. between 0 and 6 dB/octave) are designated with filled triangles

criteria), and (3) fibers were classified as acceleration-sensitive using one criterion and velocity-sensitive using the other ($n=3$). Since it is expected that about 1.35 ($=0.05\times 27$) fibers (based on rate criteria) and 1.15 ($=0.05\times 23$) fibers (based on synchronization criteria) would fall outside both confidence intervals, the 3 outliers observed for each criterion is not unreasonably beyond expectation. Hence, there is little evidence that a 3rd category, such as displacement-sensitivity (expected slope of 12 dB), is needed to adequately describe the response characteristics of these fibers. Given that a number of fibers had slopes falling within the confidence intervals of both acceleration and velocity-sensitive slope populations, we cannot say for certain that intermediate response types did not exist. The fact that success-rates for classifying fibers as either acceleration- or velocity-sensitive were even greater for fibers having data for all 6 frequencies between 10 to 100 Hz, however, suggests to us that the sample size of frequencies tested, rather than the bimodal classification scheme, was the major limiting factor in this analysis. Results from tests in which respiratory flow levels (tested for 16 fibers) or stimulus positions (tested in 4 fibers) were changed revealed no substantial effects that could account for the tuning curve differences reported above.

Discussion

The feeding response as a reliable indicator of sensory detection

Because the prey-catching, orienting responses used as indicators of detection by the lateral line system in other animals (e.g. the clawed frog, *Xenopus laevis*, Elepfandt 1982, 1985) can be extremely variable, it is important to establish that the orienting response of the mottled sculpin is a reliable indicator of sensory detection under the conditions of our experiments. We examined two aspects of response reliability – the proclivity of experimental animals to respond when no stimulus was present and the consistency of the response to suprathreshold stimuli over time. The percentage of times that experimental fish showed feeding responses was generally equal to or greater than 80% in the presence of a suprathreshold stimulus and usually less than 20% in the absence of a stimulus (Fig. 2).

Although food reinforcement is not necessary to condition the orienting response of the mottled sculpin to a vibrating bead, as is apparently the case in a similar task with topminnow at some, but not all vibration frequencies (Bleckmann 1988), it is nonetheless possible to affect the rate at which this unconditioned response occurs by changing the regimen by which fish are fed. Withdrawal of stimulus-associated food resulted in a reduction of the rate at which mottled sculpin responded to a vibrating bead (Fig. 2, panel B). Similar reductions in response rate due to stimulus-associated food withdrawal have been reported for the topminnow (Bleckmann et al. 1981), but over what appears to be a much shorter time frame (one to two experimental sessions compared to 6–8 required for the sculpin). This relative resistance to extinction is also supported by limited results from one fish showing that up to 20 consecutive threshold measurements (taken over 6 experimental sessions) are unaffected by the withdrawal of food reward. Satiation also causes a reduction in response rate (Fig. 2, panel D) and together, these results are consistent with the conclusion reached by Elepfandt (1982) for *Xenopus* – namely, that reliance on the lateral line system for food is critical in maintaining reliable and consistent responses. Most importantly, these results show that under the feeding regimen used in the threshold experiments described here, the feeding response of the mottled sculpin is a reliable indicator of detection.

Effects of stimulus frequency and position on behavioral detection.

When expressed in terms of acceleration levels, behaviorally-measured thresholds were relatively independent of frequency over the range from 10 to 100 Hz, but best sensitivity in this frequency range was significantly affected by stimulus position. Stimuli near the head resulted in thresholds approximately 20 dB better than stimuli near the tail. These regional differences in sensitivity to a vibrating bead are similar to those reported

in experiments which measured the average reactive distance (Hoekstra and Janssen 1986) and the threshold distance (distance at which 50% were detected) (Coombs and Janssen 1989a) for the detection of live *Daphnia*. In both experiments, the distance at which *Daphnia* could be detected near the head was clearly greater than that for detection along the trunk and distances near the tail were essentially immeasurable because there were so few responses.

Regional differences in sensitivity appear to be correlated with the distribution density of canal neuromasts and size of canal neuromasts on the sculpin; neuromast density and size is largest on the head, intermediate on the trunk and smallest on the tail (Janssen et al. 1987). An alternative explanation for these regional differences in sensitivity is that only neuromasts on the head are responsible for signal detection and that positioning the stimulus further away from these detectors, as would be the case at the trunk and tail, essentially attenuates the signal. Since blockage of trunk neuromasts results in loss of responsiveness to trunk stimulation (Hoekstra and Janssen 1985), we think that this explanation is unlikely. Furthermore, since the signal from a dipolar source attenuates at the rate of 18 dB per distance doubling and the distance between the head and the stimulus is more than quadrupled when the stimulus is moved to the tail, one would predict a much greater decrement in sensitivity than the 20 dB measured.

Neural encoding of behavioral sensitivity

One of the major goals of these experiments was to determine the extent to which the response properties of single afferent fibers in the lateral line system of the mottled sculpin could account for behaviorally-measured detection abilities. When behavioral thresholds measured in response to trunk stimulation are compared to threshold sensitivity curves from single fibers innervating the trunk lateral line system, it is quite clear that there are single fibers as sensitive and as broadly tuned as the behavioral functions obtained under nearly identical stimulus conditions (Fig. 7). These fibers, characterized by an acceleration sensitivity that is independent of frequency out to at least 100 Hz, represent one of at least two basic tuning types encountered in the posterior lateral line nerve. A second type, for which acceleration sensitivity declines with increasing frequency, cannot account for behavioral detection on the basis of information from a single fiber. The almost perfect match between tuning curves of single peripheral fibers and the behaviorally-measured sensitivity curve has been reported for only a few other hair cell systems, including the lateral line system of the topminnow (Bleckmann and Topp 1981; Topp 1983) and the auditory (sacculus) system of the goldfish (Fay and Ream 1986). In general, however, behavioral detection is thought to involve an integration of information across many differently-tuned fibers that span the frequency range of behavioral detection (e.g. Kiang 1965), as is the case for nearly all vertebrate auditory systems. Despite this difference and the limited frequency range of detection, the overall sensitivity of the lateral line system, as measured physiologically and behaviorally for the mottled sculpin, rivals that of most vertebrate hair cell systems (Kroese and van Netten 1989), with best displacement sensitivity at 100 Hz around -179 dB re: 1 m (3×10^{-9} m).

Since there were no consistent differences in neural tuning or sensitivity based on the choice of threshold criteria, it is difficult to draw any firm conclusions about whether behavioral decisions were based on a spike rate or phase-locking code. However, given that 82% of all fibers for which tuning curve data were collected had spontaneous activity greater than 10 spikes/s and that the majority of fibers had spontaneous rates around 30–40 spikes/s (Fig. 5), it is difficult to imagine an effective rate code at the lower end of the frequency range, where it is difficult to drive spike rate above spontaneous levels. This, in addition to the fact that phase locking is robust over most of the frequency range of this system, suggests that temporal encoding of information is the more likely of the two candidate codes.

Although the placement of our recording electrode (i.e. at the cranial nerve root) did not allow us to identify which endorgans (superficial or canal neuromasts) were innervated by the fibers we recorded from, it is likely that fibers classified as velocity-sensitive originated from superficial neuromasts and that those defined as acceleration-sensitive originated from canal neuromasts. This tentative conclusion is based on both theoretical (Kalmijn 1988, 1989) and empirical (Münz 1985; Denton and Gray 1983, 1988; Gray 1984; Kroese and Schellart 1987) determinations of the response properties of these two types of peripheral endorgans in different fish species. If this assumption is correct, information encoded by canal organs is sufficient to account for behaviorally-measured sensitivity curves, whereas information from superficial neuromasts may not be used by the mottled sculpin in this detection task.

This conclusion is consistent with behavioral results from two other fish species for which feeding responses were used as behavioral indicators of stimulus detection. When behaviorally-determined displacement thresholds for the topminnow, *Aplocheilus lineatus* (Bleckmann 1980), stimulated with surface waves, and the ruff, *Gymnocephalus* (= *Acerina*) *cernua* (Kuiper 1967), stimulated with a magnet placed on a skin-covered canal on the head, are replotted as acceleration thresholds (see Coombs and Janssen 1989a), the picture of nearly equal acceleration sensitivity over the range 10–100 Hz is remarkably similar to behavioral results reported here. Since head canals on the ruff were stimulated directly, it is highly unlikely that the feeding responses used to measure acceleration sensitivity in this animal were evoked by stimulation of superficial neuromasts.

Other possible factors affecting neural tuning and behavioral sensitivity curves

An alternative explanation for fibers with different tuning, given that we did not know the origin of fibers

recorded from, is that fibers are tuned differently not because they originate from morphologically different endorgans, but because the endorgans they innervate are in different locations relative to the stimulus, normally fixed in position at the junction between the first and second dorsal fins. We attempted to move the stimulus along the trunk in order to find the approximate location of endorgans by locating the stimulus position which (a) caused the largest response or (b) caused the 180° phase shift predicted for a canal neuromast as the stimulus moves from one canal pore to the next on either side of the neuromast (Sand 1981; Münz 1985). However, this proved to be very difficult, probably because the vibrating sphere (due to its size and distance from the fish) did not create the kind of punctate stimulus necessary for such determinations. Although we did not systematically study the effects of stimulus position on tuning, we did measure these effects in 4 fibers and in no case was tuning altered in a way that could account for the classification of fibers into the two populations reported here.

There are several other methodological factors that could have affected the tuning curve data reported here. The first is the possibility that the water flow used to respirate the fish may have stimulated the lateral line and thus, interfered with threshold measurements. A second is that the anesthetic (MS-222) may have desensitized the laterel line system (Späth and Schweickert 1977). Although we cannot entirely rule out either of these two possibilities in all cases, the remarkable similarity between physiologically-measured threshold curves of acceleration-sensitive fibers and behaviorally-measured threshold curves (Fig. 7), for which these confounding factors did not exist, argues strongly against them in most cases. Additional arguments against anesthetic effects (Coombs and Janssen 1989) include the fact that the anesthetic was delivered for only a few (5–10) min at the beginning of each experiment at least an hour before recordings began and was washed out with a continuous flow of fresh water throughout the remainder of the experiment. Although fibers showed changes in spontaneous activity associated with changes in respiratory flow levels, the latency of these changes (1–5 min) is more consistent with changes due to hypoxia than to mechanosensory stimulus-evoked changes.

Finally, it is conceivable, given that rise/fall times were frequency-dependent, that energy splatter at the onset and offset of high frequency signals could have resulted in overestimates of sensitivity. However, several observations argue against this possibility as a significant determinant of overall tuning: (1) anemometer recordings of signal waveforms showed no evidence of onset or offset transients (see Fig. 11 from Coombs et al. 1989) (2) acceleration-sensitive fibers appear to be equally-sensitive from 10 to 100 Hz, despite changes in rise/fall times from 20 to 2.5 ms and moreover, are nearly identical in their tuning to behavioral sensitivity curves, obtained with signals shaped with 10-ms sinusoidal rise/fall times and (3) velocity-sensitive fibers show decreasing sensitivity with frequency – the opposite of what would be expected had increasing rise/fall times affected sensitivity.

It is conceivable that behavioral thresholds were masked at 10 Hz where spectrum levels of ambient noise were within a few dB of signal levels at threshold. The probable source of this ambient noise is substrate vibration, since the amount of noise reported here represents a significant reduction over that present before shock absorbers were used to dampen vibrations (Coombs et al. 1989). We feel that masking at 10 Hz is unlikely for two reasons. First, it is unlikely that whole tank vibration stimulates the lateral line, since movement of the fish and the surrounding water together would not provide an adequate stimulus (Kalmijn 1988, 1989). The second is that we see no evidence that threshold detection at 10 Hz is any worse than what might be expected, assuming that the system is responding to equal accelerations throughout most of its frequency range of detection. It would be expected that any elevation of the threshold at 10 Hz due to masking would be most apparent for thresholds measured at the head, since this is the most sensitive region of the body. However, thresholds at 10 Hz relative to those at 20–100 Hz are the same for all stimulus positions.

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