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Peripheral encoding of behaviorally relevant acoustic signals in a vocal fish: single tones

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Abstract The midshipman fish, *Porichthys notatus*, generates acoustic signals for intraspecific communication. Nesting males produce long-duration "hums" which attract gravid females and can be effectively mimicked by pure tones. In this study we examine the encoding of tonal signals by the midshipman peripheral auditory system. Single-unit recordings were made from afferents innervating the sacculus while presenting sounds via an underwater loudspeaker. Units were characterized by iso-intensity spike rate and vector strength of synchronization curves, as well as by peri-stimulus time histograms. Additionally, response-intensity curves and responses to long-duration (up to 10 s) stimuli were obtained. As has been seen in other teleosts, afferents had highly variable activity profiles. Excitatory frequencies ranged from 60 to over 300 Hz with most units responding best around 70 or 140 Hz. Thresholds at 90 Hz ranged from 95 to 145 dB re 1 μPa. Strong synchronization provided a robust temporal code of frequency, comparable to that described for goldfish. Spike rate showed varying degrees of adaptation but high rates were generally maintained even for 10-s stimuli. The midshipman peripheral auditory system is well suited to encoding conspecific communication signals, but nonetheless shares many response patterns with the auditory system of other teleosts.

Key words Hearing · Communication · Teleost fish · VIIIth nerve · Auditory

Abbreviations *BEF* best excitatory frequency · *PST* peri-stimulus time · *VS* vector strength

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Introduction

Although hearing capacities have been investigated in a number of fish species, relatively few studies in fish have examined the underlying neural coding of sound. Most of what is known is from the goldfish (reviews: Fay and Popper 1980; Popper and Coombs 1982; Hawkins 1993; Popper and Fay 1993), and includes considerable data on the encoding of acoustic stimuli by its peripheral auditory system (e.g., Fay 1978a, 1981, 1990). There have only been a small number of comparable single-unit physiological studies of the auditory periphery in other teleost species (Enger 1963; Buwalda and Van der Steen 1979; Fine 1981; Horner et al. 1981; Moeng and Popper 1984; Fay and Edds-Walton 1997a,b; Lu et al. 1998).

Goldfish are known as "hearing specialists," because they possess Weberian ossicles linking the swimbladder and ear. While this morphology provides enhanced sensitivity to acoustic pressure, it is not obvious what acoustic signals the goldfish auditory system has evolved to encode. Unlike many fish species, goldfish have no specialized sound production apparatus and no known acoustic communication signals. It is therefore of interest to extend investigation of sound encoding to species that use stereotyped acoustic signals for communication. An example of the latter is the midshipman fish, *Porichthys notatus*, a marine toadfish found along the Pacific coast of North America. The midshipman lacks specializations connecting the gas bladder to the inner ear, and is thus representative of teleosts with a more general mechanism of hearing ("nonspecialists").

Nesting male *P. notatus* use specialized swimbladder muscles to produce long-duration "hums." Aquarium observations (Brantley and Bass 1994) and playback experiments (Ibara et al. 1983; McKibben and Bass 1998) have shown that hum-like signals attract gravid females, and, to a lesser extent, males. *P. notatus* is nocturnally active and nests under rocks; thus, acoustic signals may be critical for locating mates. Males and

females both produce short, 50- to 200-ms grunts that are apparently directed toward conspecifics in agonistic contexts (Brantley and Bass 1994).

Midshipman hums are simple signals with only a few parameters likely to be of importance for recognition or preference. Although hums contain harmonics, single tones at the fundamental frequency (around 100 Hz) are sufficient to evoke phonotaxis. Playback experiments have demonstrated that the frequency, amplitude and duration of a tonal stimulus can affect its attractiveness to gravid female *P. notatus* (McKibben and Bass 1998). The goal of this study is to characterize the encoding of such behaviorally relevant, single-tone stimuli in midshipman auditory afferents. Toward this end, we undertook a single unit study of the VIIIth nerve branch innervating the sacculus. The sacculus is considered to be the major auditory end organ of the inner ear in most fish species (von Frisch 1938; Platt and Popper 1981; Popper and Coombs 1982; Popper and Fay 1993), and microphonic recordings support this role in the midshipman (Cohen and Winn 1967).

Materials and methods

Experimental animals

Fish were collected during the summer breeding season from Tomales Bay in Marin County, California. Prior to experimentation they were maintained in artificial seawater at 15 °C and fed live minnows. Responses were recorded from over 250 neurons in 23 male and 4 female fish ranging from 12.3 to 18.2 cm standard length and from 20 to 84 g in weight. All experimental procedures were approved by the Cornell University Animal Care and Use Committee.

Surgery and anesthesia

Before surgery, fish were fully anesthetized in either a solution of MS222 or 0.2% benzocaine. Taking advantage of their natural tolerance for emersion at low tide, fish were kept cool and moist but not respired during surgery. Midshipman have flat heads which allowed the overlying skin and muscle to be pulled back and a relatively large area of skull exposed. Using a scalpel and forceps, an opening was made extending approximately from the midline to the medial edge of the ear at the level of the VIIIth nerve (Fig. 1). Membranes over the brain and nerve were gently torn with fine forceps. The horizontal semicircular canal sometimes had to be pushed out of the way and in some preparations was severed without apparent effect on saccular afferent activity. A plastic cylinder (1 cm high) was fitted around the skull opening and sealed to the skull and surrounding skin using dental cement and histoacryl tissue glue. This allowed the fish to be lowered just under the water surface. The cranial cavity was filled with Fluorinert (3 M) to enhance clarity and oxygenation and prevent drying. For recording, fish were given intramuscular injections of pancuronium bromide for immobilization (0.5 mg kg^{-1}) and fentanyl (1 mg kg^{-1}) for analgesia.

Experimental setup

The experimental tank is shown in Fig. 2 and is similar to that used by Fay (1990). The loudspeaker (UW-30, University Sound, Buchanan, Mich., USA) rested in sand on the bottom of a 30-cm-diameter, 24-cm-high Nalgene tank. An outlet at the bottom of the tank connected to a variable-speed pump and a thermostatically controlled Peltier device, which recirculated artificial seawater (aquarium aged) through a respiration tube in the fish's mouth. Tank temperature was maintained at 15–17 °C during recording. The respiration tube fitted into a rigid Plexiglas head holder and lightly held the fish's bottom jaw against an extension under its head. The fish was further held in place with a peg affixed to its skull with dental cement. The fish's tail was supported by gauze hung across the tank. This arrangement allowed the fish to be lowered until the sacculus was 1–1.5 cm below the water surface and approximately 9 cm from the speaker face. The tank rested on 4 cm of open-cell foam; the tank, the stand for the head holder assembly, and the electrode manipulator stand all were positioned on a pneumatic vibration-isolation table inside an acoustic-isolation chamber (Industrial Acoustics, New York, N.Y., USA) with all recording and stimulus generation equipment located outside.

Recording

Recording began after the fish recovered from anesthesia and continued for several hours. Condition of the fish was monitored by observing blood flow in vessels near the ear. Single-unit recordings were made using 3 mol l^{-1} KCl-filled glass micropipettes with resistances of 20–40 MΩ. The electrode was visually positioned over the saccular nerve near its exit from the brain (Fig. 1), then lowered from outside the sound chamber using a hydraulic microdrive (Kopf). No search stimulus was used; however, even units with little or no background activity were easily detected by d.c. potential shifts or a few initial, presumably mechanically induced, action potentials. The electrode signal was pre-amplified (Getting 5 A), filtered (Krohn-Hite 3550) and amplified (Ithaco 255), then fed through an adjustable threshold discriminator (modified WPI model 121 window discriminator). Spike discrimination was visually monitored to insure single unit recordings and capture of all spike times. Spike times were recorded (10 μs buffer) with a custom-designed synthesis and recording program (Cassie, developed by Julian Vrieslander at Cornell University).

Most neurons could be held for several minutes to an hour. Occasionally neurons fired rapid streams of large spikes that were presumed to result from injury, and these neurons, as well as a few neurons with highly unstable background activity were disregarded during analysis. High and variable background activity rates made the collection of meaningful tuning-curve data problematic. For this reason, and because distinguishing between varying frequencies at a level above threshold is the relevant real-world task, iso-intensity data (the same pressure at all frequencies) were collected from each unit. When time allowed, stimuli at various sound levels or durations were presented.

Stimulus generation and calibration

Acoustic stimuli were generated with the Cassie software running on a Macintosh IIci with a 12-bit DA board (MacAdios). Stimuli were attenuated (Tucker Davis programmable attenuator), amplified (NAD stereo amplifier 3020 A), and played through the UW-30 underwater loudspeaker. Sound pressure measurements were made with a mini-hydrophone (Bruel and Kjaer 4130) both in the position normally occupied by the fish's head and, with the fish in place, next to the fish's head very close to the ear. For equalization across frequencies, relative measurements were made with a spectrum analyzer and calibrated by peak-to-peak voltage measurements on an oscilloscope trace. Synthesis levels were then adjusted within the software. The equalized response was flat within ±2 dB from 60 to 1000 Hz and was relatively insensitive to small changes in horizontal position.

Basic stimuli consisted of single tones 500 ms in duration with 50-ms rise and fall times, presented for five repetitions at a rate of one every 1.5 s. For the iso-intensity curves, sound pressure at the location of the fish's sacculus was set at 140 ± 3 dB for all frequencies. The order of frequency presentation was randomized,

though the range was usually divided beforehand in order to prioritize collection of data at frequencies below 150 Hz. For level and duration experiments, most stimuli were at 90 Hz. Just as with isointensity stimuli, this puts the emphasis on neural responses to a given signal as opposed to the inherent properties (such as absolute threshold) of a neuron. Stimuli for response-level experiments were presented in order of increasing amplitude. Long-duration 5-, 8-, and 10-s stimuli were presented for five repetitions at rates of one every 6, 13, and 15 s, respectively.

Analysis

Spike times were quantified using two measures: average spike rate and vector strength of synchronization (VS) which is a description of the temporal pattern of firing (i.e., phase locking). Spike rates were averaged over the 500-ms stimulus duration. For most units, background activity was measured over five repetitions of the stimulus interval with no stimulus present. For the few units for which data were lacking, spike rates were estimated based on the silent portion of the stimulus period for ineffective frequencies. VS of synchronization is a measure of the degree to which the spike train encodes stimulus periodicity, in this case, the tone frequency. VS is equivalent to the mean vector length for the circular distribution of spikes over the period of the stimulus, and was calculated according to Goldberg and Brown (1969), using 2-ms bins. VS varies from 0 for a uniform or random distribution to 1 if all spikes fall in the same bin. High VSs associated with very low spike rates were not significant ($P > 0.05$, Rayleigh test for randomness; Batschelet 1981) and were eliminated from the analysis. Peristimulus (PST) histograms were constructed with 10-ms bins, and interspike interval histograms with 1-ms bins. All sound levels are presented as decibels re 1 μPa. Further details of analysis are explained in the Results.

Results

Background activity

Saccular afferent background activity ranged continuously from 0 to over 120 spikes s^{-1} with a median spike rate of 26.4 spikes s^{-1} (Fig. 3A). In order to facilitate

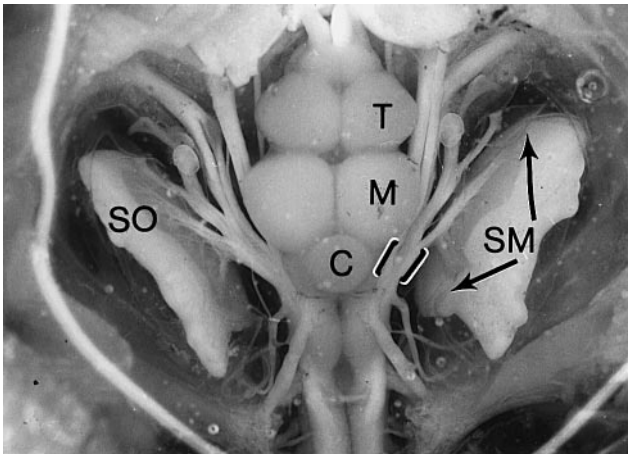

Fig. 1 Dorsal view of midshipman brain and ears. Sacculi have been deflected laterally to open up view of nerves. Brackets mark section of VIIIth nerve innervating sacculus where recordings were made. *C* cerebellum; *M* midbrain; *SM* saccular macula; *SO* saccular otolith; *T* telencephalon

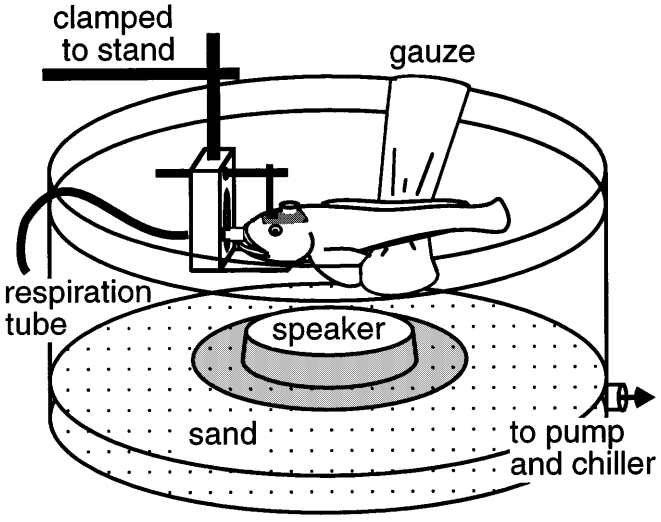

Fig. 2 Diagram of experimental tank showing speaker embedded in sand on tank bottom and fish attached to headholder

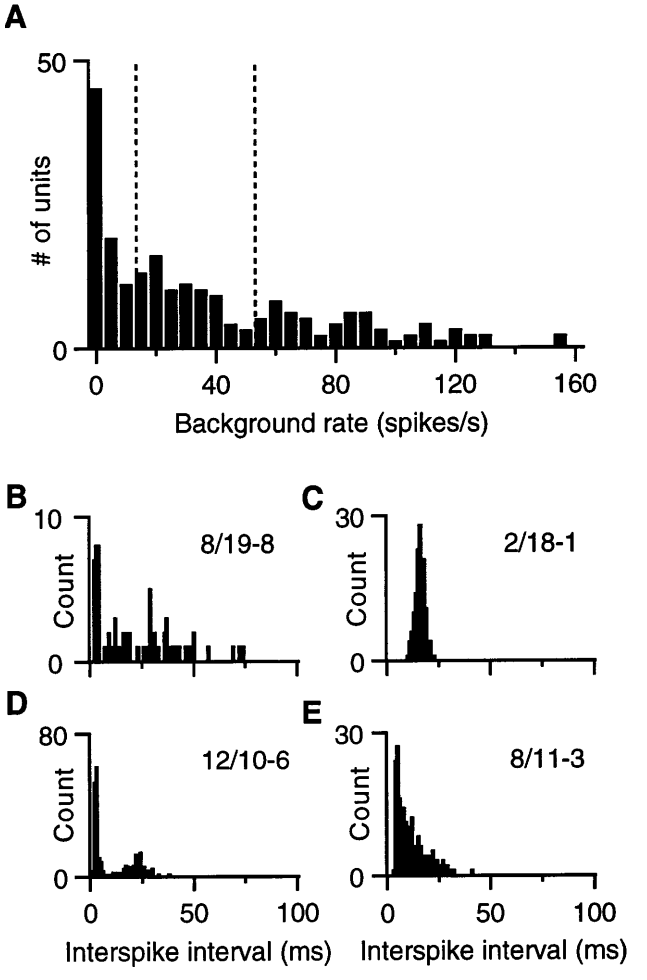

Fig. 3A–E Background activity. **A** Distribution of background spike rates for 213 midshipman saccular afferents. *Dashed lines* delineate low (< 15), moderate (15–50), and high (> 50) rate categories. **B–E** Interspike-interval histograms showing four patterns of background activity: **B** variable; **C** regular; **D** bursting; **E** irregular. Spike rates corresponding to each unit: **B** 6.4 ± 2.1; **C** 60 ± 0.6; **D** 92.8 ± 2.9; **E** 83.6 ± 4.3

descriptions, units are divided into low (<15 spikes s^{-1}) moderate (15–50 spikes s^{-1}) and high (>50 spikes s^{-1}) background rates (indicated with dashed lines in Fig. 3A). Four general patterns of spontaneous activity were seen, examples of which are illustrated with inter-spike interval histograms in Fig. 3B–E. Low spontaneous rate and some moderate spontaneous rate units had highly variable, scattered intervals (Fig. 3B). A few moderate to high spontaneous units had regular spike trains with intervals distributed around a central peak (Fig. 3C). The majority of high spontaneous and some moderate spontaneous rate units had bursting patterns of spontaneous activity (Fig. 3D). This is seen in inter-spike interval histograms as multiple peaks, representing short intra-burst intervals and longer inter-burst intervals. The remainder of the high spontaneous units had irregular spike intervals (Fig. 3E). Units with moderate spontaneous activity and very regular, large spikes which were not responsive to sound were also encountered, and were presumably vestibular in function (Highstein and Baker 1985).

Frequency encoding: iso-intensity curves

Figure 4 shows representative iso-intensity response data plotted both as average spike rate (open squares, left axes) and as VS to the stimulus frequency (filled circles, right axes). Spike rate plots, for 178 units with sufficient data, can be divided into four categories: "low-pass" (42.2%), "band-pass" (28.7%), complex or multi-peaked (24.1%), and suppressive (5%). "Low-pass" units (Fig. 4A,B) had their highest spike rates around 60 Hz (the lowest frequency presented) and a fall-off in response that could be smooth or have small peaks at one or more higher frequencies. Band-pass units (Fig. 4C,D) had their highest spike rates at intermediate frequencies (around 90–180 Hz), and complex units (Fig. 4E,F) had two or three distinct response peaks. Suppressive units (Fig. 4G) were certain high spontaneous units (12 of 32 units with background rates above 85 spikes s^{-1}) that showed a reduction in spike rate when driven at the lowest frequencies (<100–150 Hz). All four types of unit were found in the same preparation and multiple types in the same electrode pass. Low-pass, band-pass, and complex iso-intensity rate response types included units with a wide range of background activity, and there were no significant differences between background rates in these three groups (Mann-Whitney U test, P-values >0.1). Units stimulated at multiple intensities generally maintained the same iso-intensity categorization, although the relative sizes of spike rate peaks sometimes changed as responses to lower frequencies became saturated.

Iso-intensity synchronization curves were less variable than the spike rate plots, and tended to either fall monotonically from a low frequency peak (e.g., Fig. 4A,C,D–E) or remain flat over a considerable portion of the excitatory frequency range (e.g., Fig. 4B,F). For units with low to moderate spontaneous activity, synchronization often extended to higher frequencies than evoked spike rate increases (e.g., Fig. 4B,F).

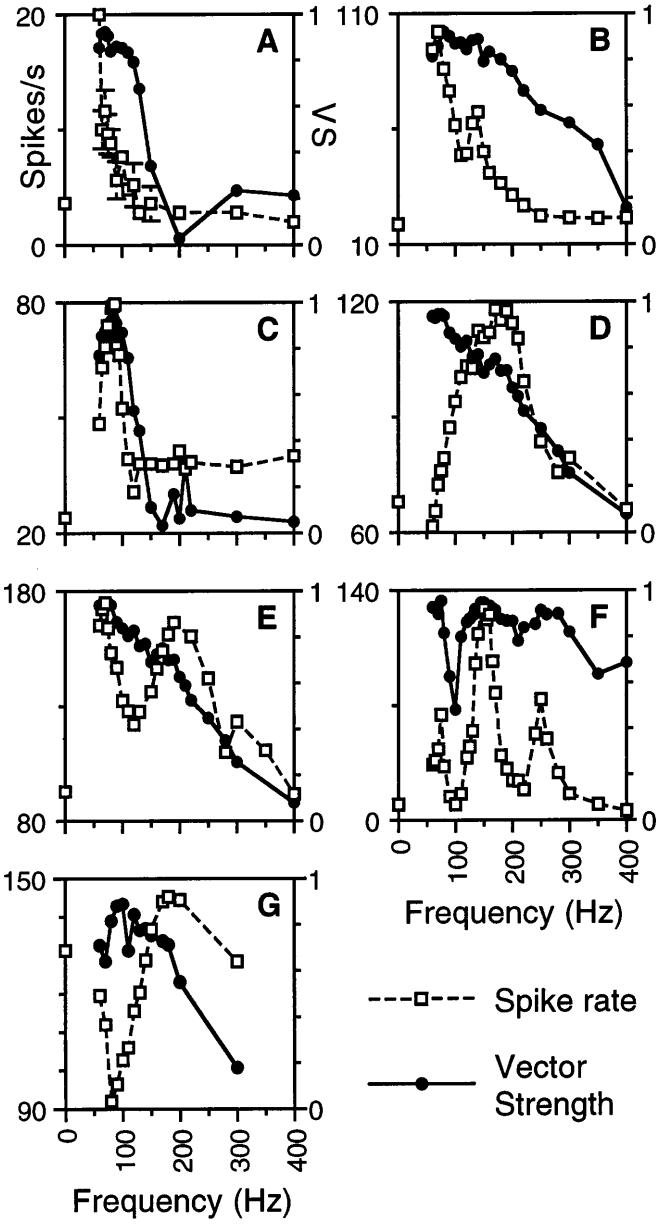

Fig. 4A–G Iso-intensity responses for seven units plotted both as spike rate (*open squares*, *left axes*) and as vector strength of synchronization (VS) to the stimulus frequency (*filled circles*, *right axes*). Standard errors for spike rates are plotted but for most points are too small to be visible. For each unit, background spike rate is plotted with an *open square on the left axis*. Groupings based on iso-intensity spike rate profiles: **A,B** Low-pass; **C,D** Band-pass; **E,F** Complex; **G** Suppressive

Variation in synchronization (i.e., phase locking) across frequencies can explain several features of the spike rate iso-intensity curves. Figure 5 shows iso-intensity data for four of the units in Fig. 4, this time plotted as spikes per stimulus cycle (average spike rate/frequency; filled circles). Average spike rate (open

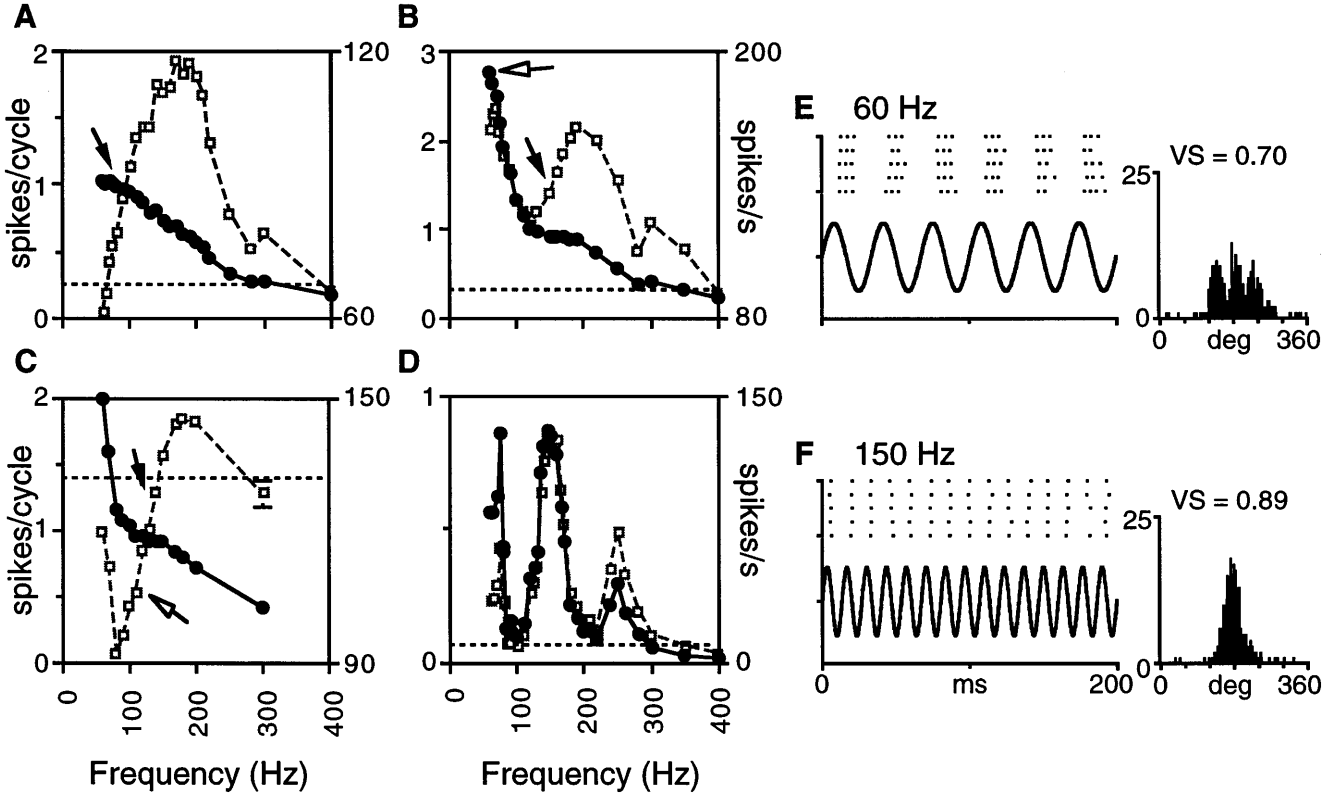

Fig. 5A–F Iso-intensity responses for four units, plotted as spikes per stimulus waveform cycle (*filled circles, left axes*). Average spike rate is also shown for comparison (*open squares, right axes*), and spontaneous rate for each unit is indicated with *dashed line*. **A,B,C** Three units (Fig. 4D,E,G respectively) which had very different iso-intensity rate curves but similar, monotonic, spikes-per-cycle curves. Over certain regions spike rate increased with frequency, due to phase-locked, approximately one-to-one firing (*filled arrows* in **A–C**). At low frequencies, elevated spike rates corresponded to multiple spikes per cycle (*open arrow* in **B**). Phase-locked, one-to-one firing to frequencies less than the background rate could reduce spike rates below background (*open arrow* in **C**). **D** Complex unit (Fig. 4F) which retained complexity when response was plotted as spikes per cycle. **E,F** Raster plots (200 ms of stimulus and response) and period histograms corresponding to *open* (**E**) and *filled* (**F**) *arrows* in **B**

squares) is also shown for comparison. Though in terms of spike rate the units in Fig. 5A–C were, respectively, band-pass, complex, and suppressive, all showed similar, nearly continuous, declines in spikes per cycle with frequency. Low-pass units (not shown) also had monotonic spikes per cycle curves, and the examples in Fig. 5 are representative of the variation seen.

At the lowest frequencies (60–90 Hz), cells often fired multiple spikes per cycle, boosting phase-locked spike rates (open arrow in Fig. 5B, raster and period histogram for same response in Fig. 5E). In these cases, multiple peaks were often seen in the period histogram, and the increased period histogram spread tended to decrease the calculated synchronization values. At low to intermediate frequencies (up to around 200 Hz), cycle-by-cycle firing could result in spike rate increasing with frequency (Fig. 5F and filled arrows in Fig. 5A–C). In some units with very high background rates, phase-locking to low-frequency stimuli resulted in depression of the firing rate from background levels (open arrow in Fig. 5C), a suppressive response. At relatively high frequencies (i.e., ≥300 Hz), spikes-per-cycle curves no longer represent phase-locked activity.

Not all complex iso-intensity rate responses were simplified when viewed as spikes per cycle. For example, in Fig. 5D the spikes per cycle and average spike rate curves both have distinct peaks and troughs; thus, the complexity probably represents true changes in responsiveness with frequency and not just the effects of phase locking on spike rate. Response intensity plots show that, while this unit responded strongly to 150 Hz, corresponding to a peak in its iso-intensity spike rate plot, it was virtually impossible to drive it at 90 Hz, an iso-intensity trough (see Fig. 8E,F).

Tuning across the fiber population

Peak iso-intensity responses are summarized for both spike rate and synchronization measures in Fig. 6. Since iso-intensity spike rate plots often had more than one major peak (e.g., Fig. 4E,F), best excitatory frequency (BEF) data are plotted both for the highest spike rate (primary peak) and the second highest peak (secondary peak), if present (81 of 175 units). The secondary peak could be higher or lower in frequency than the primary peak. Most units had their highest spike rates at or below 70 Hz, though there was a smaller peak in response around 140 Hz. A few units responded most strongly at higher frequencies, from 180 to 250 Hz.

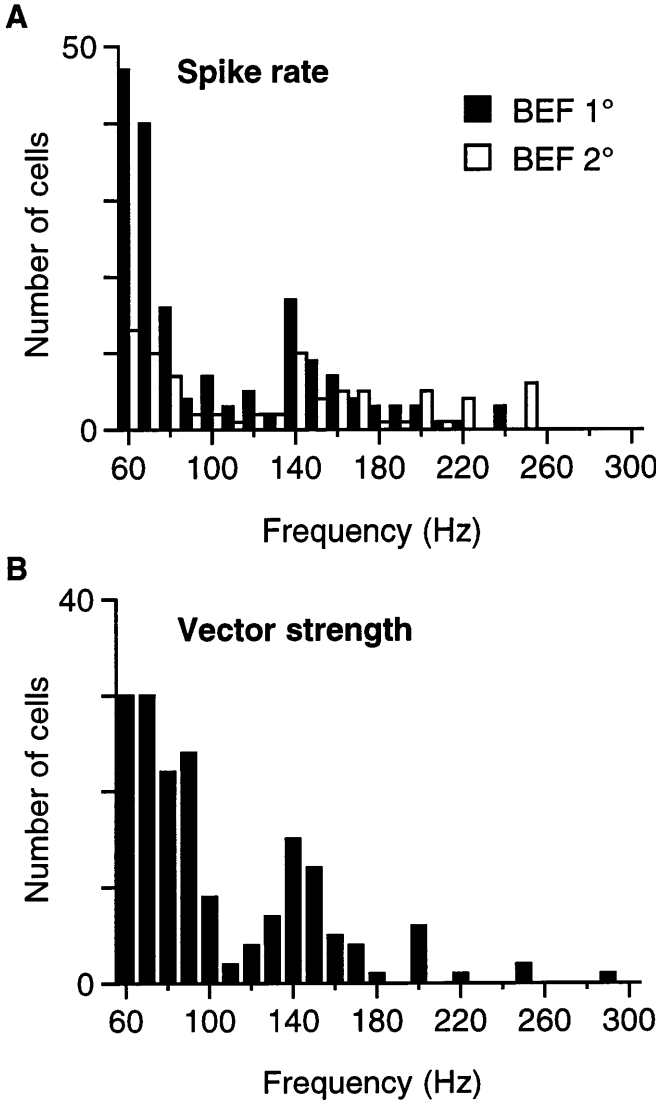

Fig. 6 **A** Tuning across fibers (n = 175) based on iso-intensity responses to 140-dB tones. Frequency values are lower limits of 10-Hz bins. **A** Distribution of frequencies with highest spike rates. For units with more than one prominent peak in the iso-intensity rate response, both the frequency with the highest spike rate, or best excitatory frequency (*BEF 1°*, *filled bars*), and the second highest peak (*BEF 2°*, *open bars*) are included. **B** Distribution of frequencies with highest VS

Figure 6B plots BEFs based on synchronization. As was the case for spike rate, the majority of best responses were to low frequencies (60–90 Hz), and there was a smaller peak around 140 Hz. Due to the flatness of many synchronization iso-intensity curves, this best-synchronization distribution is more a reflection of regions of strong response than of distinct response peaks. For nearly all units, best synchronization values indicated tight phase locking to 140-dB tones (mean maximum VS = 0.85 ± 0.13).

Figure 7A shows synchronization across frequency plotted in terms of population percentile values. Median VS declined from 0.79 at 80 Hz to 0.15 at 400 Hz, but the top 10–25% of units (90th and 75th percentiles) at a given frequency showed strong synchronization at frequencies as high as 300 Hz. An alternative to VS for quantifying the strength of firing periodicity is to measure the standard deviation (SD) of the period histogram spike distribution. In Fig. 7B the median and 90th percentile synchronization data have been converted to SDs in milliseconds (see legend to Fig. 7). SD serves as a measure of temporal jitter and thus can give a sense of the precision of temporal encoding of stimulus frequency. For units at or above the 90th percentile, SDs ranged from slightly over 1 ms at 60 Hz to approximately 0.4 ms at 200–400 Hz. Also plotted in Fig. 7B are median SDs for comparable data from low-frequency goldfish afferents from Fay (1978b) and goldfish behavioral frequency discrimination thresholds converted to milliseconds (Fay 1970, 1978b, 1988).

Level encoding

Rate-intensity plots were sigmoid shaped with dynamic ranges of 15–40 dB for excitatory frequencies between 60 and 360 Hz (for those units that reached saturation within the range of stimulus levels presented). Figure 8 shows rate-intensity curves and the corresponding synchronization curves at various frequencies for three representative units. These units were classified as band-pass (Fig. 8A,B; with slight low-pass "tail" at 60–70 Hz), "low-pass" (Fig. 8C,D) and complex (Fig. 8E,F; same unit as Fig. 5D). In units with spontaneous activity, synchronization typically increased at lower levels than affected spike rate (Fig. 8C,D). VS generally plateaued, showing little or no change with level at intensities where spike rate curves were steepest (Fig. 8B,D,F). Also, VS values tended to converge across frequencies at a maximum value as stimulus level was increased.

To illustrate the variation among afferents in encoding level, Fig. 9 presents response-level curves in response to a tone at a single behaviorally relevant frequency, 90 Hz. These representative units from two fish show a range of spike-rate thresholds, slopes, and dynamic ranges (Fig. 9A,C). The distribution of rate-intensity slopes (over the approximately linear portions of curves) for 65 units stimulated at 90 Hz is shown in Fig. 9E. Spike rate increased by a mean of 3.2 ± 2 spikes s^{-1} for every decibel increase in sound level. Threshold was approximated by visual inspection of the rate-intensity curves to determine the stimulus level at the base of the upward slope that evoked activity consistently above background. In this manner, threshold values could be determined for 60 units (Fig. 9F) and ranged from 96 to 150 dB re: 1 μPa, with a mean of 119 dB. VS curves were less variable and tended to differ more in threshold than in slope or saturation level (Fig. 9B,D).

PST histogram variety and duration encoding

The spike rate and VS measures presented up to this point have been averages over the entire stimulus dura-

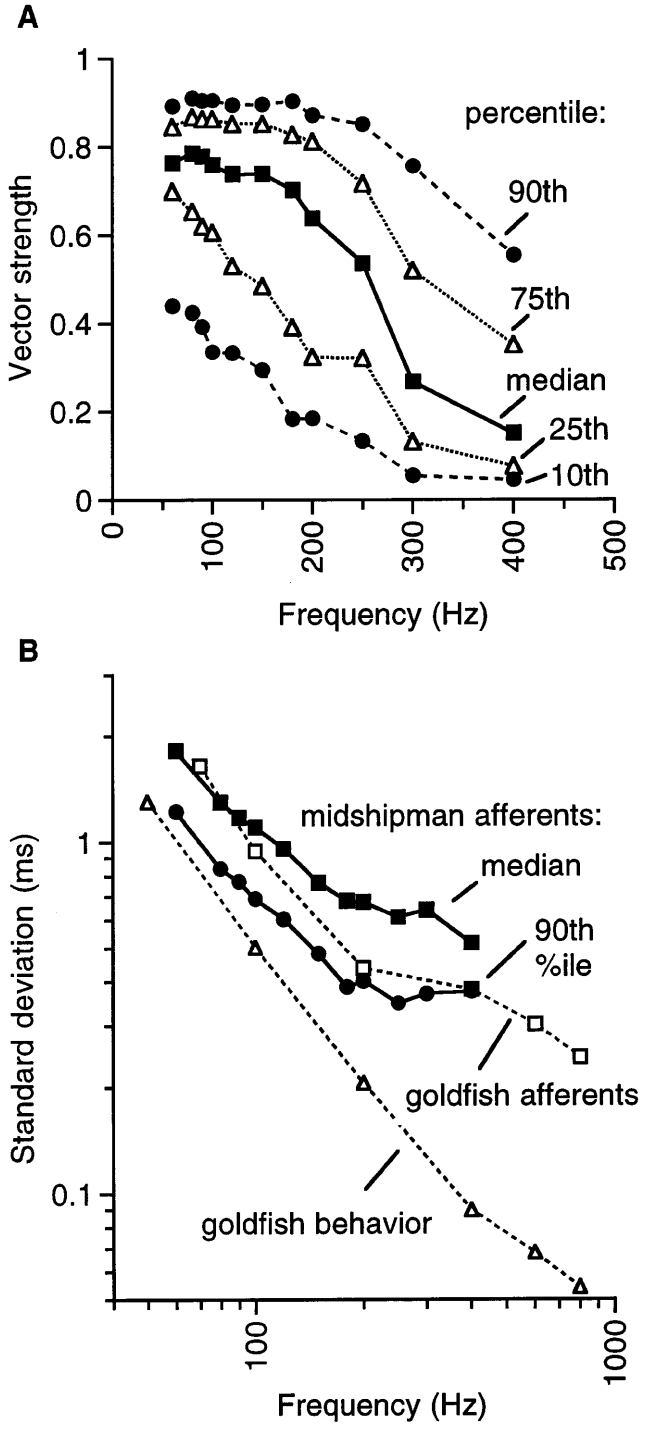

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Fig. 7 A Population percentile values for synchronization to a range of frequencies. Plot shows vector strength values for 133–180 units at each frequency in terms of the median (*filled squares*), 10th percentile (*filled circles*, *lower curve*), 25th percentile (*open triangles*, *lower curve*), 75th percentile (*open triangles, upper curve*) and 90th percentile (*filled circles*, *upper curve*). **B** Strength of firing periodicity quantified as standard deviation (in milliseconds) of period histogram spike distribution. Median (*filled squares*) and 90th percentile (*filled circles*) values for same midshipman data as in **A**. VSs were converted to mean angular deviation (s) in degrees ($s = 180°/\pi[2(1-VS)]^{1/2}$; Batschelet 1981) from which standard deviation in milliseconds was calculated according to stimulus period. For comparison, median data from goldfish afferents are also shown (*open squares*; Fay 1978). Median values were calculated from Fay's plot of individual standard deviations for 7–26 low-frequency fibers at each frequency. Goldfish behavioral frequency discrimination thresholds in milliseconds are also shown (*open triangles*; Fay 1970, 1988)

tion. The time-course of a unit's response is revealed by PST histograms. PST histograms showed response-pattern variation not only between units, but also within units at different stimulus frequencies and levels. Examples of PST histograms from low (Fig. 10A,B), moderate (Fig. 10C,D), and high (Fig. 10E,F) spontaneous rate units at three stimulus frequencies are shown in Fig. 10. Some low spontaneous units and all moderate and high spontaneous units sampled fired for the duration of 500-ms stimuli at all excitatory frequencies. For other low spontaneous units, firing patterns varied from phasic to tonic depending upon stimulus frequency and intensity. Sustained responses often had an initial response peak followed by varying degrees of adaptation. Suppression of spontaneous activity was commonly seen following the stimulus and could last as long as the 500-ms stimulus (e.g., Fig. 10B–E). Some high spontaneous rate units showed little change in firing rate when stimulated, and thus no apparent adaptation. PST histogram shape for other high spontaneous units was clearly affected by phase locking. The unit in Fig. 10F fired multiple phase-locked spikes per cycle in response to a 60-Hz stimulus resulting in a sustained increase in firing rate (left column). However, at 90 Hz (center column) it fired a single spike per cycle, reducing spike rate below background.

Responses to longer duration (5–10 s) stimuli were collected from a subset of units ($n = 14$) in order to examine encoding of hum-length signals. Patterns of response to long-duration tones at 90 Hz are summarized in Fig. 11. For each unit represented ($n = 10$), average spike rate was calculated for every 500-ms segment of the stimulus period. Spike rates were then normalized for each unit to the segment with the highest average rate (Fig. 11A). Firing was sustained for the duration of the stimulus, though most units showed continuing gradual adaptation over a period of seconds. Initial response bursts and rapid adaptation are obscured in this analysis. VSs calculated over the same segments are shown in Fig. 11B. Even though some units showed clear spike-rate adaptation, synchronization was consistently high for the entire stimulus period.

Discussion

The particularly interesting aspect of the midshipman system for studies of auditory encoding is this fish's known use and responsiveness to simple acoustic signals. Thus, though this paper is a characterization of basic auditory afferent response properties, the primary focus is on how particular behaviorally relevant signals are encoded in the auditory periphery.

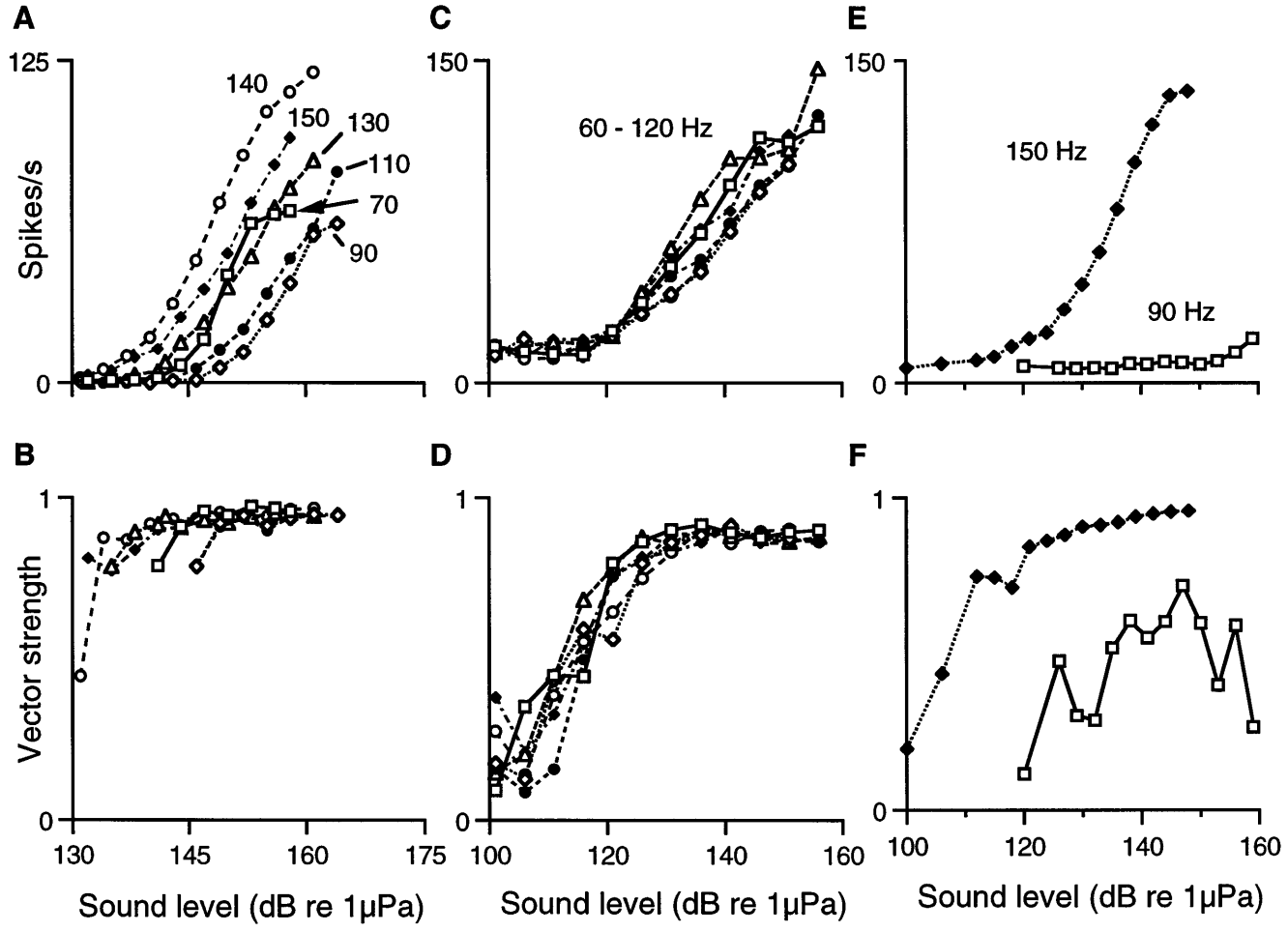

Fig. 8 Response-intensity curves for three units at various frequencies: **A**,**C**,**E** rate-intensity curves; **B**,**D**,**F** corresponding synchronization-intensity curves. Stimulus frequencies: **A**,**B** 70–150 Hz as indicated in **A**; **C**,**D** 60, 70, 80, 90, 100, 120 Hz; **E**,**F** (complex unit in Fig. 5D), 90 Hz (*open squares*) and 150 Hz (*filled diamonds*)

Appropriate stimulus

Any sound wave can be measured in terms of particle motion or pressure. Otolith organs are essentially acceleration detectors, responding to movement of the animal and to gravity. As acoustic sensors, they respond directly to displacement of the fish by particle motion in the medium (Hawkins 1993). However, indirect stimulation by acoustic pressure, via gas-filled bladders, also occurs. Stimulus level was recorded, equalized and presented here as sound pressure. Previous studies using hearing specialists such as goldfish (e.g., Fay and Ream 1986) and mormyrids (Crawford 1993) have utilized a similar experimental setup and made the assumption that pressure was the overriding relevant stimulus measure. The extent to which pressure stimulation via the swimbladder is important to fish with no specialized connections between the swimbladder or other gas bladders and the inner ear is less clear (Fay and Popper 1980; Popper and Coombs 1982). However, given that natural or artificial air bladders can affect hearing sensitivity and range without direct apposition to the ear (Chapman 1974; Jerkø et al. 1989), and that the midshipman swimbladder extends anteriorly into two points that lie just caudal to the ears, the midshipman is probably somewhat sensitive to the pressure component of sound. The relationship between pressure and particle motion in small tanks is complex (Parvulescu 1967; Fay and Popper 1980; Hawkins 1981), and quantification or equalization, when both aspects are probably relevant, is difficult.

The shape of a tuning curve or of a response to isointensity stimuli may vary depending upon the reference parameter. However, it is not necessary to know the physics of signal transduction at the end organ in order to gain insight into the encoding of sound waveforms and communication signals. The potentially most confounding difference between pressure and particle motion measurements in this study is the directionality of the latter. Measurements of pressure differences between points in the experimental tank confirmed that the primary axis of particle motion was vertical. The saccular otoliths and epithelia in the midshipman are oriented approximately vertically (Fig. 1), and it seems likely that this is a sensitive axis for direct stimulation of the midshipman ear. Studies in other teleost species have shown

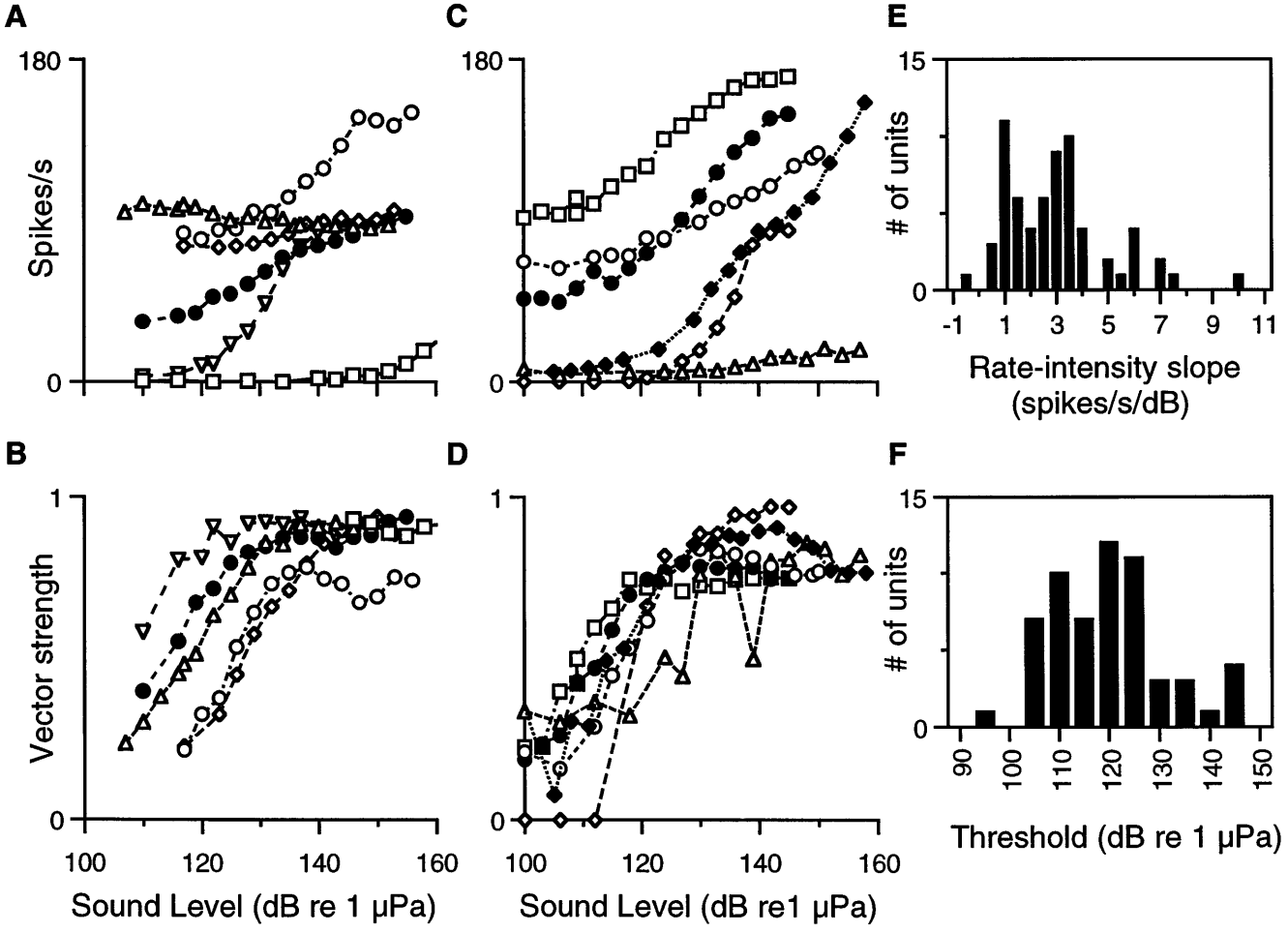

Fig. 9A–F Response-intensity curves at 90 Hz for six units each from two fish. **A**,**C** Rate-intensity curves. **B**,**D** Corresponding synchronization-intensity curves. **E** Distribution of 90-Hz rate-intensity slopes for 65 units. **F** Distribution of spike rate thresholds taken from 90-Hz rate-intensity curves ($n = 60$). Bin numbers are lower boundaries

that at least some of the neurons innervating vertically oriented otolith organs respond strongly to dorso-ventral acceleration (Fay 1984; Fay and Edds-Walton 1997a; Lu et al. 1998). However, considerable variation is seen in toadfish and other teleosts in hair cell orientation across the epithelial surface, and not all afferents are expected to be responsive to vertical acceleration (Platt and Popper 1981; Edds-Walton and Popper 1995; Fay and Edds-Walton 1997a). Direct acceleration of the fish using a shaker system would be a useful next step in characterizing midshipman auditory afferent responses (Fay and Popper 1980; Fay 1984; Fay and Edds-Walton 1997a,b).

Background activity

Midshipman saccular afferents showed considerable variation in rates and patterns of background activity. Though the possibility that some of this activity was driven by noise sources extrinsic to the animal cannot be definitively ruled out, similar spontaneous activity has been recorded in studies of other teleosts. Recordings from saccular afferents in sculpin (Enger 1963), goldfish (Fay 1978a; Fay and Ream 1986), cod (Horner, Hawkins and Fraser 1981), a marine catfish (Moeng and Popper 1984), oyster toadfish (Fay and Edds-Walton 1997a), and sleeper gobies (Lu et al. 1998) all showed spontaneous rates ranging continuously from zero up to around 200 spikes s^{-1}. Likewise, there seem to be common patterns of activity for spontaneously active afferents, with regular, irregular or random and bursting firing patterns identified in each of the four studies above. One difference in this study is that some units with regular spontaneous activity were responsive to sound, both increasing their spike rates and synchronizing to the stimulus. This inherent variability in afferent activity is the context in which afferent responses to specific signals occur.

Frequency encoding

Fish auditory systems have been presented as models for temporal encoding of frequency (Fay 1978a,b, 1986), because of the absence of an obvious place mechanism,

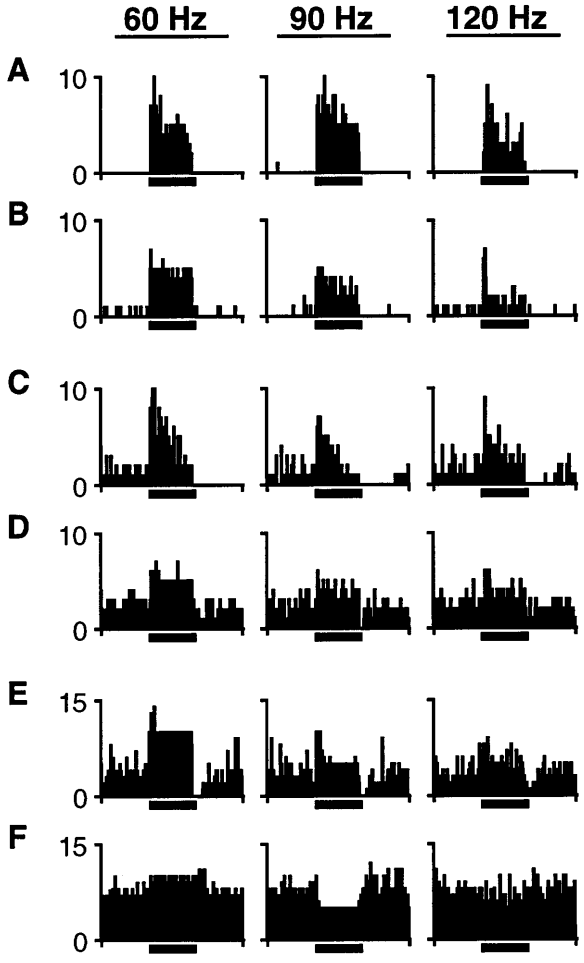

Fig. 10 Peri-stimulus time (PST) histograms for low (**A,B**), moderate (**C,D**), and high (**E,F**) spontaneous units at (from left to right) 60, 90, and 120 Hz. Stimulus period (500 ms) marked with *black bars*. Spike counts are for five repetitions of stimulus, 10-ms bins. Spontaneous rates (spikes per second): **A** 0.8 ± 0.2; **B** 4.4 ± 0.4; **C** 18.8 ± 1.7; **D** 34.8 ± 2.0; **E** 55.2 ± 2.4; **F** 131.2 ± 1.5

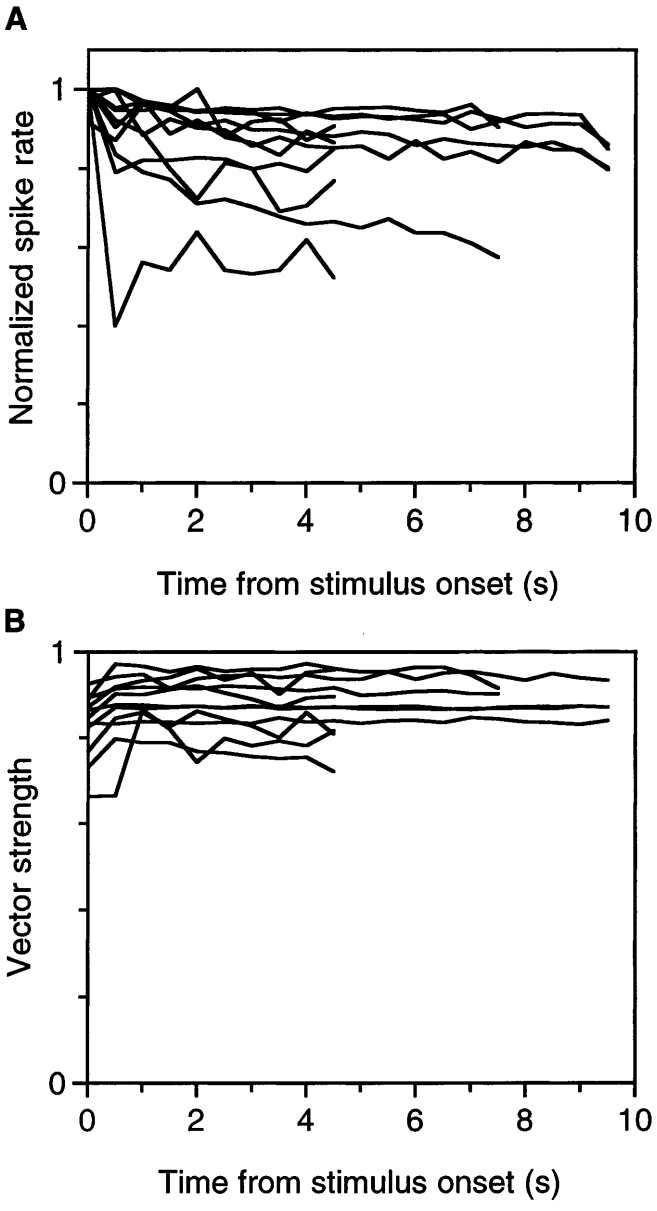

Fig. 11 Responses to long-duration (5, 8 or 10 s) 90-Hz stimuli by ten units. **A** Spike rate. Spike rates were averaged for each 500-ms segment of the response and normalized to each cell's maximum. Times plotted are beginnings of 500-ms segments. Extent of line indicates stimulus duration. **B** VS corresponding to the units and 500-ms segments in **A**

such as the mammalian cochlea, for frequency analysis. However, it is clear that saccular afferents vary in their frequency responses (e.g., Fay 1978a; Moeng and Popper 1984; Fay and Edds-Walton 1997b). This study reports frequency sensitivity in the midshipman based on iso-intensity data (all frequencies presented at the same pressure). Iso-intensity curves show responses to above threshold signals and are probably more behaviorally relevant than threshold-tuning curves (Fay 1978a; Capranica 1992). Also, the high levels of spontaneous activity and variety of PST histogram shapes seen make meaningful spike-rate tuning curves difficult to acquire for a large percentage of saccular neurons (Fay 1978a; Fay and Ream 1986).

Iso-intensity data are presented here in terms of both average spike rate and vector strength of synchronization to the sinusoidal period of the stimulus. Iso-intensity spike-rate curves varied from simple low-pass (down to 60 Hz), or band-pass, to more complex, multi-peaked responses. These groupings highlight some of the variation seen, but do not necessarily represent distinct classes of neurons. A high degree of synchronization accounts for some of the changes in spike rate with frequency, as does the frequency-dependent firing of multiple spikes per cycle. Indeed, much of the complexity in iso-intensity curves disappears when spike rates are plotted as spikes per stimulus cycle (Fig. 5).

Most units showed a continuous decline in spikes per cycle with increasing frequency; however, some responses remained complex. The higher peaks in complex responses tended to approximate harmonics of the

lowest-frequency peak. Such a response could enhance detection of harmonic signals such as a midshipman hum. However, before any functional conclusions could be drawn about complex iso-intensity responses, it would be necessary to rule out artifacts of the stimulus and recording set up. Simple and complex curves were found in the same preparations, and complex units often could not be effectively driven at response troughs, even with high stimulus levels. Thus, the complexity apparently does not reflect an effective imbalance in sound pressure. It is possible that complex responses result from the interaction of particular directional sensitivities and the acoustic particle motion in the experimental tank. If units are responding to particle motion, frequency-dependent variation in the major axis of motion could produce complex iso-intensity profiles even in the absence of spectral tuning. Fay and Edds-Walton (1997a) found toadfish saccular afferents to have directional nulls as well as preferred axes of stimulation. An accelerometer attached to the fish's head could help resolve this issue. The validity of complex tuning is supported by the observation of multiple-peaked frequency responses in *Opsanus tau*. In response to direct acceleration along a single axis, some saccular afferent filter functions had peaks around both 70 and 140 Hz (Fay and Edds-Walton 1997b).

BEFs for midshipman saccular fibers ranged from 60 Hz (the lowest frequency presented) to 250 Hz. The majority of units responded best to frequencies below 100 Hz, with a second cluster of BEFs around 140–180 Hz. The range of excitatory frequencies extended up to at least 400 Hz. However, by 300 Hz most units did not have spike rates significantly above background. Best synchronization values were also concentrated at the lowest frequencies, with a second group around 140 Hz. Plotting synchronization in terms of population percentiles showed that some units had vector strengths of 0.6 or above at 400 Hz, though most units did not synchronize well to tones above 250 Hz. This frequency range, from 60 Hz or less to around 400 Hz (Figs. 6, 7) is well suited for detecting midshipman communication signals, which have temperature-dependent fundamental frequencies that can range from 70 to 140 Hz (Brantley and Bass 1994; Bass et al. 1999). Harmonics, at least up to the third, should also be detectable. In playback experiments, gravid female midshipman approached tones from 80 to 140 Hz; and this is not necessarily the entire range of effective frequencies, especially on the lower end (McKibben and Bass 1998).

The afferent data presented here generally accord with the midshipman frequency-sensitivity curve based on saccular microphonics presented by Cohen and Winn (1967). They found the greatest pressure sensitivity at low frequencies, down to 30 Hz, and another dip in threshold around 150 Hz, before a steep increase up to 240 Hz. Threshold values are also comparable, remarkably so given the differences in stimulation and recording technique. By interpolation, their microphonic threshold for a 90-Hz tone would be approximately 115 dB re 1 μPa, within the range determined from rate-level curves in this study (Fig. 9F).

The midshipman hearing range is comparable to that found in other hearing nonspecialist fish. Most data consist of audiograms obtained through conditioning experiments, though there are some physiological data (reviews: Hawkins 1981; Popper and Fay 1993; also Fay and Edds-Walton 1997b). Again, the actual shape of a hearing-sensitivity curve may depend upon the stimulus parameter, pressure or a particle motion derivative, measured. Afferent filter functions, based on reverse correlation, in the toadfish, *O. tau*, showed broad tuning from less than 50 Hz to 250 Hz with some variation in high cut-off frequencies (Fay and Edds-Walton 1997b). These data were obtained by acceleration of the fish and are presented as such. The only study of toadfish saccular afferents using a sound-pressure stimulus showed greatest sensitivity (as low as 77 dB re 1 μPa) to tones at or below 90 Hz, and a continuous decrease in sensitivity at higher frequencies (Fine 1981). However, since the speaker was in air and the top of the fish's head above the water surface, these results are likely not directly comparable to those reported here. Previous conditioning experiments had indicated sound pressure thresholds for *O. tau* from around 100 dB (re 1 μPa) below 100 Hz to 140 dB at 500 Hz (Fish and Offutt 1972). All of these studies with *O. tau* indicate a general hearing range similar to that reported here for the midshipman. The goldfish has a hearing range that extends beyond 1000 Hz, but it also has low-frequency fibers with best frequencies at 200 Hz or below (Fay and Ream 1986). Fay and Ream (1986) suggest that low-frequency fibers are a general characteristic of fish auditory systems.

Nearly all midshipman auditory afferents synchronized strongly to a range of pure tones. Such synchronization could be the basis for a temporal frequency code. Fay (1978b) showed for goldfish that phase locking, at least by the afferents with the highest vector strengths, could account for psychophysical frequency difference detection limits. This conclusion was based on the reasoning that a discrimination threshold would occur when the frequency difference was approximately equal to the standard deviation in the afferent periodicity code. Goldfish behavioral frequency discrimination thresholds, along with median standard deviations at each frequency for low-frequency units, are compared with midshipman data in Fig. 7B. Values for midshipman afferents are virtually identical to the goldfish values below 100 Hz and are only slightly higher up to 400 Hz. At 200 Hz and above, however, goldfish performance would be enhanced by higher frequency fibers not included in this plot. The 90th percentile values for midshipman approach the goldfish behavioral thresholds at low frequencies, suggesting that midshipman should have frequency discrimination abilities similar to goldfish below approximately 100 Hz. The limits of frequency discrimination have not been studied in midshipman, though two-choice behavioral tests

(McKibben and Bass 1998) showed selective phonotaxis when tones from 80 to 100 Hz differed by 10 Hz, approximately a 1.4- to 0.9-ms difference in period.

Level encoding

Rate-intensity plots for midshipman auditory afferents are generally sigmoid shaped but vary considerably in thresholds and slopes. Though individual fibers may code level with rate only over 15–40 dB, the population can encode a range of at least 60 dB. Rate-intensity slopes are likely to influence psychophysical level sensitivity (Fay 1985; Winter and Palmer 1991; Coombs and Fay 1993), though it is not obvious how spike rates across the afferent population would be processed. Midshipman afferent rate-intensity slopes for 90-Hz tone stimuli ranged from <1 to 10 spikes s^{-1} dB^{-1}. In phonotaxis experiments, midshipman discriminated between 90-Hz tones that differed by 3 dB (McKibben and Bass 1998). That translates to an average spike rate difference of 9.6 spikes s^{-1} for the approximately linear portions of the rate-intensity curves reported here.

Psychophysical tests have shown goldfish tonal increment detection thresholds of 1.3 dB (Fay 1985) and thresholds for amplitude modulation of tones as low as 0.2–3.7 dB, depending on stimulus level (Hall et al. 1981). Data for pulsed-tone discrimination are comparable at around 1.4–2.5 dB (Fay 1989). For any tonal stimulus, a subpopulation of neurons will be most strongly driven, and those where the stimulus falls in the steepest portion of their rate-intensity responses should provide the best information about stimulus level. Fay (1985) reported steep goldfish afferent rate-intensity slopes of up to 20 spikes s^{-1} dB^{-1} in response to pulsed noise. Given the differences in stimuli, it remains possible that midshipman have similar level encoding and behavioral sensitivity to goldfish. Spike rate changes for the midshipman data were constrained by phase locking to the low-frequency stimulus, and 90 Hz was not the BEF for most of the units tested. Stimulus duration can also affect average spike rate values, due to high spike rates at stimulus onset and adaptation or suppression as the stimulus continues (Fay 1985; Yates 1987; Fay and Ream 1992). This study presented relatively long-duration, 500-ms stimuli, since the major communication signals of interest are also long duration. Spike rates calculated for stimulus onset would be higher, and rate-intensity slopes would probably be steeper (Fay 1985).

Synchronization seems secondary to spike rate in encoding level. Units with background activity do show synchronization at lower levels than drive increases in spike rate, and this could increase dynamic range. (Synchronization may be more important than spike rate for encoding level by lateral line afferents, especially at low levels (Coombs and Fay 1993).) However, synchronization saturates relatively quickly with level, and units with zero or very low spontaneous activity have essentially flat synchronization-intensity curves. This stability of synchronization with level may be important for frequency encoding.

Duration, PST histogram shape, and adaptation

Midshipman hums are unusually long-duration signals, typically for periods of seconds to minutes. In contrast, the grunts associated with agonistic contexts are only 50–200 ms long (Brantley and Bass 1994; Bass et al. 1999). In playback experiments, gravid female midshipman readily approached speakers playing tones as short as 1–2 s, but trains of grunts or of 100- or 500-ms tone pulses did not elicit phonotaxis (McKibben and Bass 1998). Given that signal duration is clearly important to midshipman behavior, we examined the time-course of auditory afferent activity in response to 500-ms and longer duration tones.

Teleost auditory afferents are notable for their variety of response patterns, as seen in PST histogram shape, and midshipman were no exception. Individual responses varied in onset peaks, rates of adaptation, suppression of background activity, and off-suppression, which was sometimes followed by a rebound (Fay 1978a, 1986, 1990; Horner et al. 1981; Fay and Ream 1986; Coombs and Fay 1987). Response time-course often varied with stimulus frequency and level. Such PST histogram variety has also been seen in amphibians (Megela 1984), but not in mammals until the level of the cochlear nucleus (review: Pickles 1988). The coding significance is not known (Coombs and Fay 1987). In goldfish, PST histograms evidencing both excitation and suppression were found in low-pass and untuned fibers (Fay and Ream 1986), further suggesting commonality between low-frequency fibers in hearing specialists such as the goldfish, and nonspecialists such as the midshipman.

In midshipman, entirely phasic excitatory responses were mostly confined to high-frequency stimuli that elicited a burst of spikes at stimulus onset, followed by suppression. Rarely, phasic responses to low frequencies were seen in units with very low spontaneous activity. This is similar to what was seen in sleeper gobies (Lu et al. 1998); and adaptation was most prominent in low- or non-spontaneous fibers in goldfish (Fay 1978a) and cod (Horner et al. 1981).

Most midshipman afferents responded for the duration of 500-ms stimuli. Longer-duration stimuli, up to 10 s, also elicited sustained firing, with most units showing only minor slow adaptation (Fig. 11). Overall, midshipman afferents encode behaviorally significant signal durations by the duration of their sustained firing. Onset peaks and offset suppression may further indicate signal beginning and end. Even in cases with marked adaptation, synchronization to the stimulus tone remained high. Thus, information about the frequency of long-duration stimuli is consistently encoded by spike periodicity.

Summary and conclusions

Midshipman hums are simple signals that can be effectively mimicked by pure tones at their fundamental frequencies. Thus, the tonal stimuli presented in this study are not only amenable to analysis of auditory encoding but are also of behavioral relevance. Midshipman saccular afferents resemble those of other teleosts in having considerable variation in background activity, PST histogram shape, and rate-intensity curves. The frequency range of afferent responses, from 60 Hz or less to at least 300 Hz, is comparable to that of other nonspecialists and to the low-frequency fibers of hearing specialists such as the goldfish. This range is well suited to encoding midshipman acoustic signals. Synchronization to the stimulus waveform was generally high and resistant to changes in level or stimulus duration; thus, frequency is robustly encoded in the temporal pattern of spikes. Accordingly, midshipman frequency discrimination performance seems likely to be as good as that of goldfish at low frequencies. Iso-intensity spike rate profiles often reflected phase locking to the stimulus rather than simply changes in responsiveness across frequencies. Though variable degrees of adaptation were seen, units generally fired for the stimulus duration, making spike rate a reliable measure of stimulus level even for long-duration sounds. This study has revealed a general similarity over the midshipman's hearing range between the peripheral auditory processing of midshipman and that of other teleosts, including the less vocal but better-studied goldfish. Though the midshipman peripheral auditory system may not be uniquely adapted for acoustic communication, it unambiguously encodes acoustic parameters known to be critical for behavioral signal recognition and preference. Knowing how simple signals are encoded in such a vocal fish establishes a basis for understanding the extraction of behaviorally meaningful information from sound.

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