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RESEARCH ARTICLE

Potential role of the anterior lateral line in sound localization in toadfish (*Opsanus tau*)

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ABSTRACT

Male oyster toadfish (*Opsanus tau*) acoustically attract females to nesting sites using a boatwhistle call. The rapid speed of sound underwater combined with the close proximity of the otolithic organs makes inner ear interaural time differences an unlikely mechanism to localize sound. To determine the role that the mechanosensory lateral line may play in sound localization, microwire electrodes were bilaterally implanted into the anterior lateral line nerve to record neural responses to vibrational stimuli. Highest spike rates and strongest phase-locking occurred at distances close to the fish and decreased as the stimulus was moved further from the fish. Bilateral anterior lateral line neuromasts displayed differential directional sensitivity to incoming vibrational stimuli, which suggests the potential for the lateral line to be used for sound localization in the near field. The present study also demonstrates that the spatially separated neuromasts of the toadfish may provide sufficient time delays between sensory organs for determining sound localization cues. Multimodal sensory input processing through both the inner ear (far field) and lateral line (near field) may allow for effective sound localization in fish.

KEY WORDS: Fish, Mechanosensation, Sensory system

INTRODUCTION

Batrachoid fishes (*Opsanus* spp. and *Porichthys* spp.) have been used as biological models for investigating muscle physiology (Elemans et al., 2014; Rome and Klimov, 2000), excretory function (Barimo and Walsh, 2006), vestibular physiology (Rabbitt et al., 1995) and hearing (Edds-Walton and Fay, 2008; Sisneros, 2009). However, sound generation and reception is an integral part of their natural history. Both male and female oyster toadfish produce broadband grunts by means of rapid contraction of sonic muscles surrounding the swim bladder and sexually mature male toadfish produce a vocalization, termed a boatwhistle, which is used to acoustically attract females to nesting sites (Gray and Winn, 1961; Maruska and Mensinger, 2009). Although the production and reception of sound has resulted in investigations on both sonic muscle and auditory physiology (Edds-Walton et al., 2013; Mensinger, 2014; Nelson et al., 2013), the physiological mechanisms by which female fish locate males remains largely unknown. Recent studies have indicated that directional cues may be available through particle motion elicited by their vocalizations (Zeddies et al., 2010, 2012).

The saccule is considered the primary auditory endorgan in fishes (Popper and Fay, 1993, 2011). However, both the saccule and utricle are sensitive to linear accelerations, acoustic particle motion, display directional sensitivity, and function predominantly as low frequency (60–1000 Hz) detectors (Boyle et al., 2001; Fay, 1984; Lu et al., 2004; Maruska and Mensinger, 2015). The mechanism by which otoliths contribute to sound localization remains unclear. While terrestrial vertebrates use interaural time delays (ITDs) to localize sound in azimuth (Schnupp and Carr, 2009), the small distances between otolith pairs and the speed that sound travels underwater (1475 m s^{-1} at 20°C in seawater) (Urich, 1983) makes the use of time differences challenging for teleosts. For example, the maximum ITD for fish with an inter-otolith distance of 3 cm would be less than 20 µs, which is at or near the threshold ITD of the most sensitive terrestrial vertebrates (Grothe et al., 2010).

Fishes also possess a hair cell-based mechanosensory lateral line that functions in schooling behavior (Partridge and Pitcher, 1980), rheotaxis (Montgomery et al., 1997), hydrodynamic imaging (Weissert and von Campenhausen, 1981), social interactions (Butler and Maruska, 2016) and predator–prey interactions (Montgomery et al., 1995). The functional unit of the lateral line is the neuromast, which contains the same sensory hair cells as the inner ear. The lateral line is a near-field particle displacement detector that potentially contributes to acoustic sensitivity and sound localization. Although the role of the lateral line in sound detection has long been debated (Braun et al., 2002), several studies have suggested that a fish's mechanosensory lateral line may play a role in sound localization (Higgs and Radford, 2013; Mirjany et al., 2011; Radford and Mensinger, 2014; Weeg and Bass, 2002). Particle motion speed, combined with interneuromast distance, afferent nerve length and conduction velocities, may be sufficient for aquatic sound localization using time of arrival differences at neuromasts.

Sound localization in fishes has recently been reviewed (Sisneros and Rogers, 2016) and there is compelling evidence for midshipman fish to locate distant sound sources using particle motion. The lateral line's ability to detect near field particle motion suggests that it could function in shorter-distance sound localization. As male toadfish can be found in shallow water and soft bottoms, which can quickly attenuate sound, final mate selection may hinge on accurate sound localization in the near field. The present study investigates whether the anterior lateral line can potentially use differential input to contralateral neuromasts and/or time delays for determining sound source location.

MATERIALS AND METHODS

Animal husbandry

Adult toadfish, *Opsanus tau* (Linnaeus 1766), with standard length of 26±3.8 cm (mean±s.e.m.) were collected near the Marine

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Biological Laboratory in Woods Hole, Massachusetts. Four males and two female fish were used in the study. They were kept in flow-through seawater tanks at temperatures of 18–22°C from mid-May to mid-August 2015 and were fed *ad libitum*. All animal care and experimental procedures conformed to institutional animal care protocols.

Electrode construction

To record neural activity, microwire electrodes were implanted into the left and right anterior lateral line nerves. Microwire electrodes consisting of a twin pair of insulated 20 µm diameter, 10% platinum/iridium wire (Sigmund Cohn) were custom fabricated for each implant. Each microwire was fixed to silver-plated copper multistrand wire (25 µm diameter, New England Wire) with conductive silver paint. The multistranded wire was attached to silver wire (320 µm diameter) that terminated in a multipin underwater connector. The anterior portions of the microwires were threaded through a 1 cm length of polymide tubing (180 µm outer diameter) to maintain the recording sites in proximity. Any exposed wire/connectors were encased in medical devise adhesive (Loctite 3341; Henkel Loctite Corp., Rocky Hill, CT, USA) and cured with ultraviolet light. Implanted electrodes ranged in impedance between 0.5 and 1.5 MΩ (FHC impedance meter).

Dorsal craniotomy

Toadfish were anesthetized by immersion in 0.005% tricaine (MS-222; 3-aminobenzoic acid ethyl ester) in seawater and paralyzed with an intramuscular injection of 0.01% pancuronium bromide (600 µm kg^{-1}). The fish was then placed in a custom-designed, Plexiglas stereotactic tank on a vibration isolation table. An incision was made through the dorsal musculature overlying the sagittal crest and the muscle was retracted. Two small holes were made in the cranium on both sides of the sagittal crest and posterior to the transverse crest to expose the right and left anterior lateral line nerves.

Electrode implantation

Each pair of microwire electrodes were implanted into the dorsal branch of the right or left anterior lateral line nerves proximal to their exit from the braincase. Extracellular potentials were differentially amplified (Dagan, Minneapolis, MN, USA) and monitored on a portable computer using LabChart 7 software on a Powerlab 4SP. After an afferent fiber was located, fish were left undisturbed for 30 min to ensure recording stability. Cyanoacrylate gel was used to attach the electrodes to the skull and to seal the craniotomy. The muscle was returned to its original position and the incision triple sutured (fascia, muscle, and epidermis) for a waterproof seal. The electrode connector was mated to a flexible 1.5 m tether that terminated in the head stage of the differential amplifier. A small brush was run over the fish's head to determine the approximate locations (±0.5 cm) of the neuromasts. The fish was allowed at least 1 h to recover from surgery prior to testing.

Experimental design

Fish were moved to a Plexiglas experimental tank (50 cm×85 cm), located on a vibration isolation table, with a water depth of 8–10 cm. Toadfish were free to move, but remained relatively inactive throughout experimental trials. A solid plastic sphere (15 mm diameter) was attached to a mini-shaker (Bruël and Kjaer, model 4810) by a 15 cm metal shaft and suspended vertically midway in the water column. An externally triggered function generator (Tektronix FG 501A; Beaverton, OR) was used to drive the mini-shaker at 60 Hz. Additional frequencies, such as 120 Hz and 180 Hz, which were more relevant to boatwhistle vocalizations were not tested because of difficulties in maintaining both electrodes for prolonged periods of time. The toadfish remained in position while the sphere was moved in a semicircle (−90 deg directly left, 0 deg in front, +90 deg directly right) using the midline of the premaxilla as the reference point. The vibrational stimulus was tested in 30 deg increments relative to the fish to test for directional sensitivity in anterior lateral line afferent fibers. Additionally, at each 30 deg position, seven distances (0.5–8.9 cm) were tested. Each stimulus trial was 25 s in duration with two 5 s stimulus periods bracketed by 5 s of spontaneous activity. Multi-unit recordings were amplified, filtered using a high pass setting of 100 Hz, and recorded onto a computer using Powerlab.

Particle acceleration measurement

Particle acceleration was determined at the head of the toadfish for each of the stimulus locations using a calibrated waterproofed triaxial accelerometer (model W356A12/NC; PCB Piezotronics, Depew, NY, USA; sensitivity: x=10.47 mV ms^{-2}, y=10.35 mV ms^{-2} and z=10.29 mV ms^{-2}). The accelerometer was made neutrally buoyant in water by using syntactic foam (Zeddies et al., 2012). The accelerometer was connected to a sensor signal conditioner (model 482C; PCB Piezotronics), which then connected to PowerLab. Using LabChart software (version 8), root mean square (RMS) values when the stimuli was both absent and present were recorded. RMS values for each axis were then calibrated to the sensitivity of the accelerometer. Calibrated values were then used to determine the magnitude of particle acceleration and converted to dB scale following the equation:

$$20 \times \log_{10}\left(\sqrt{x^2 + y^2 + z^2}\right). \quad (1)$$

To determine time of arrival differences, the sphere was placed 2.5 cm to the left of the toadfish operculum and the accelerometer was placed 2.5 cm right of the toadfish operculum at 5 cm water depth. The time of arrival of the stimulus to the accelerometer (distance 15.5 cm) was determined for 10 stimulus presentations trials with and without a toadfish. The toadfish measured 10.5 cm across at the operculum and the top of its head was approximately 5 cm below the water surface

Data analysis

Waveform analysis was performed on the data, using Spike2 software (Cambridge Electronic Design Ltd, version 7), to discriminate individual units in the extracellular recording. The spontaneous spike rate before and after the presentation of the vibrational stimulus was recorded for each neuron. Neural responses to the 60 Hz vibrational stimulus were quantified for evoked spike rate and vector strength. Spike rates were expressed as the maximum evoked spike rate minus the mean resting rate for each neuron. To determine whether the anterior lateral line responses were phase-locked, phase histograms were generated for each unit. The coefficient of synchronization (R) was calculated from the phase histograms to represent phase-locking strength (Goldberg and Brown, 1969). However, R is likely to be misinterpreted when the sample size (N) is small. To correct this issue, the Rayleigh statistic (Z) was used as a combined measure of the number of discharges and strength of phase locking (Lu and Fay, 1993). Z is defined as $N \times R^2$, where N is the total number of spikes (Batschelet, 1981) and represents the response magnitude of the anterior lateral line

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afferents. An afferent was significantly phase-locked if $Z>6.91$ ($P>0.001$). To describe the strength of phase locking of the afferents, a previously published criterion (Lu and Fay, 1993) was applied to distinguish strongly phase-locked afferents ($R\geq0.5$) from weakly phase-locked afferents ($R<0.5$). All phase-locking analysis was done in MATLAB using the CircStat toolbox. To determine directional sensitivity, R and Z were calculated for each isolated afferent at each 30 deg increment. Subsequently, polar plots of R were constructed to characterize an afferent's directionality, with maximum R values indicating the direction of best sensitivity for that particular afferent.

All other statistics were performed with SigmaStat. Values presented are means±s.e.m.

RESULTS

During stimulus, particle acceleration levels at the anterior-most point of the toadfish head, ranged from −3 dB re. 1 m s^{-2} when the sphere was at 0.5 cm and declined to approximately −35 dB re. 1 m s^{-2} at the most distant points (8.9 cm), but remained above ambient levels [−50 dB re. 1 m s^{-2}] at all stimulus points (Fig. 1).

Bilateral chronic electrodes were successfully implanted into the anterior lateral line of six fish and a total of 24 units (12 left side; 12 right side) were recorded and fully characterized to the experimental parameters. All units were spontaneous active afferent fibers with an average spontaneous spike rate of 14.9±0.3 spikes s^{-1} (range 7.4±0.3 to 28.9±0.6 spikes s^{-1}) (Fig. 2). All

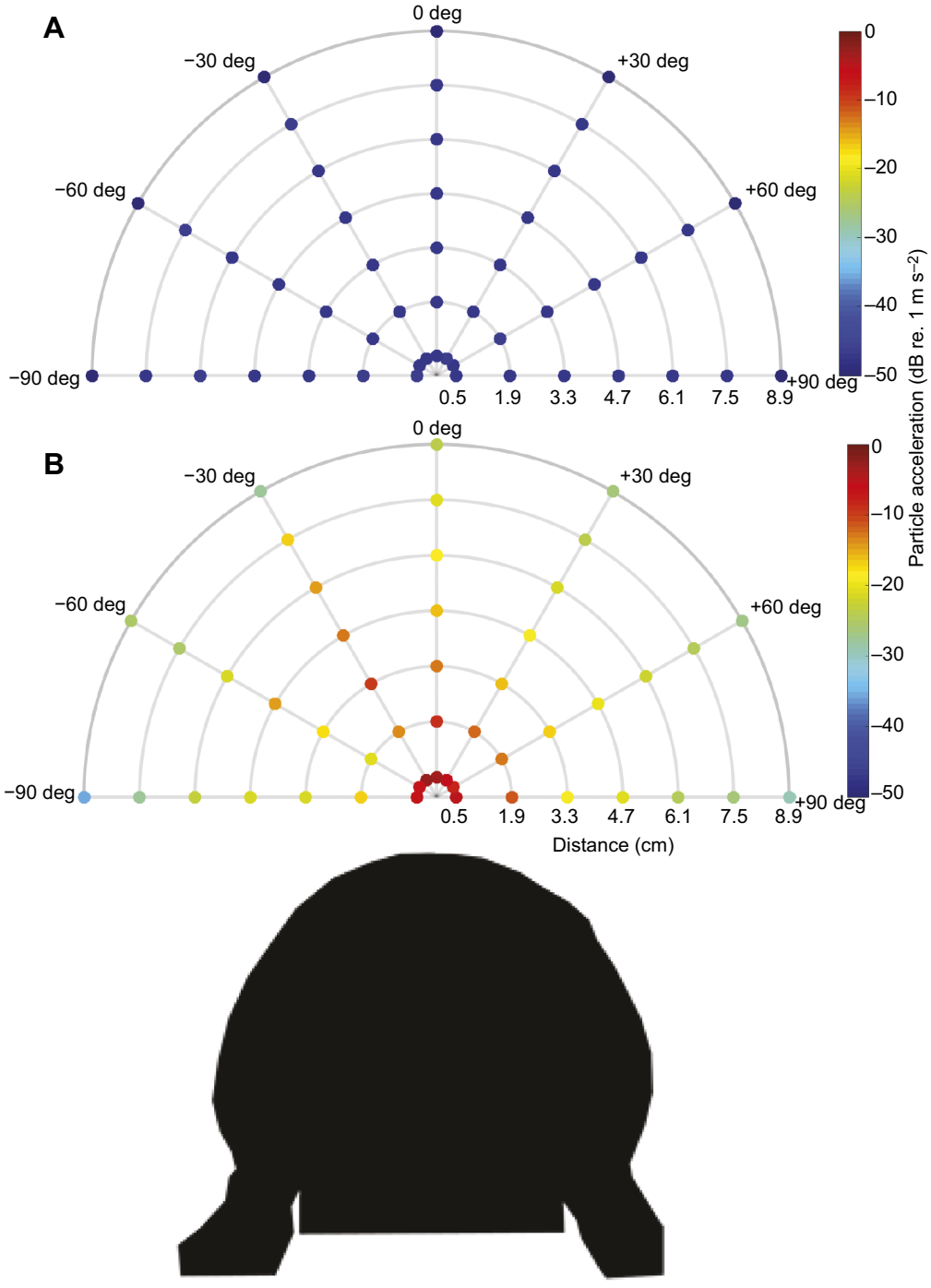

Fig. 1. Particle acceleration in the oyster toadfish (*Opsanus tau*). Particle acceleration was measured at a range of stimulus points from –90 deg to +90 deg and distances up to 8.9 cm. Distance (cm) is shown on bottom axis. The ambient (A) and experimental (B) particle acceleration (dB re. 1 m s^{-2}) recorded at the anterior point of the fish's head is shown for each stimulus location. The outline of a toadfish head (~10 cm wide) shows its position relative to the stimuli locations.

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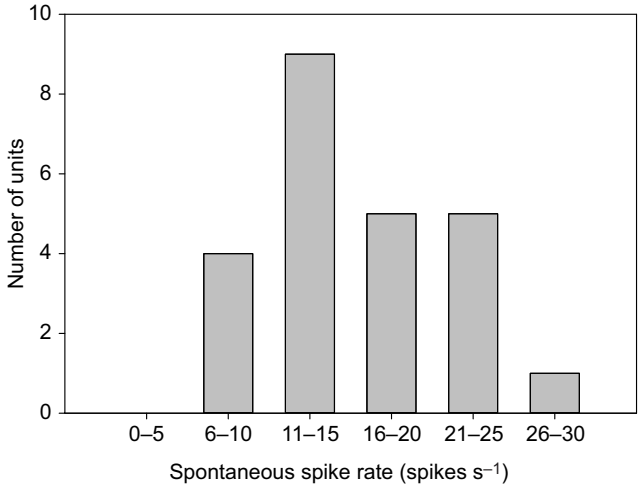

Fig. 2. Spontaneous mean neural firing activity of characterized toadfish afferent fibers. The spontaneous mean neural firing rates of the characterized afferent fibers (*N*=24) was determined prior to stimulation and the number of units within each 5 spike s^{-1} interval plotted.

fibers were tonic and showed continuous firing during stimulus presentation.

Twenty of the 24 fibers showed strong (*R*>0.5) and/or significant (*Z*>6.91) phase-locking to the vibrating sphere for at least one of the angles. Fig. 3 shows the neural activity recorded from two bilateral implants before, during and after the vibrational stimulus. The probe is 90 deg to the right of the toadfish and the ipsilateral fiber (left) shows strong phase-locking, whereas in this case, the contralateral fiber (right) does not appear to respond to the stimulus.

However, many neuromast responded to stimuli from both the ipsilateral and contralateral side. Fig. 4 plots the number of neuromasts in three different fish that showed significant phase-locking to stimuli at each of the sphere locations. The yellow segments indicate at least one contralateral fiber was also phase-locked with the ipsilateral fibers while red segments show all four fibers showing significantly phase-locking to the sphere at its indicated location. For example, in Fig. 4A, three (two ipsilateral and one contralateral) to four fibers (two ipsilateral and two contralateral) reacted when the stimulus was at 60 deg to the right and 0.5–6.1 cm away from fish. In Fig. 4B, all neuromasts responded when the sphere was at 90 deg left and 0.5–1.9 cm away, and 90 deg right and 3.3–4.7 cm distant. Relatively low sensitivity to stimuli directly in front (0 deg) was consistent among fish.

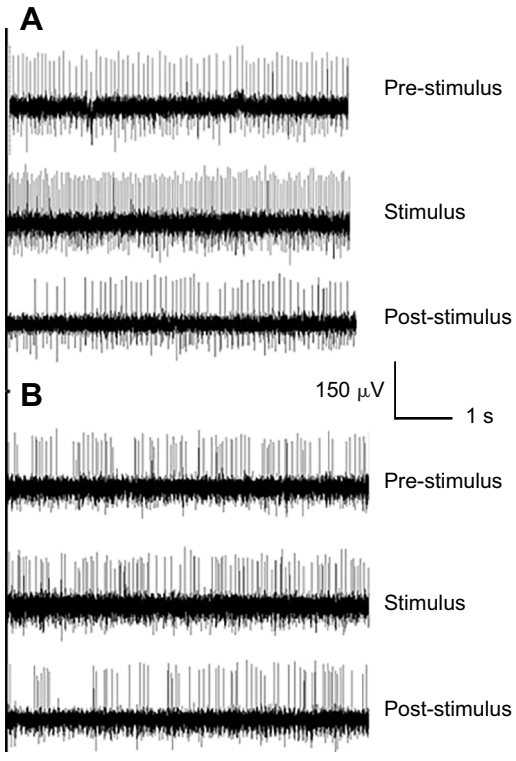

Fig. 3. Neural activity from bilateral electrode implant. Neural activity from two bilateral implanted electrodes in the anterior lateral line nerve of the toadfish. Each trace shows a 5 s neural recording from the lateral line nerves before, during and after a 60 Hz stimulus that was presented to the right side of the toadfish. (A) Modulated recordings from a neuromast on the ipsilateral (right) side. (B) Modulated recordings from a contralateral neuromast not responding to the stimulus.

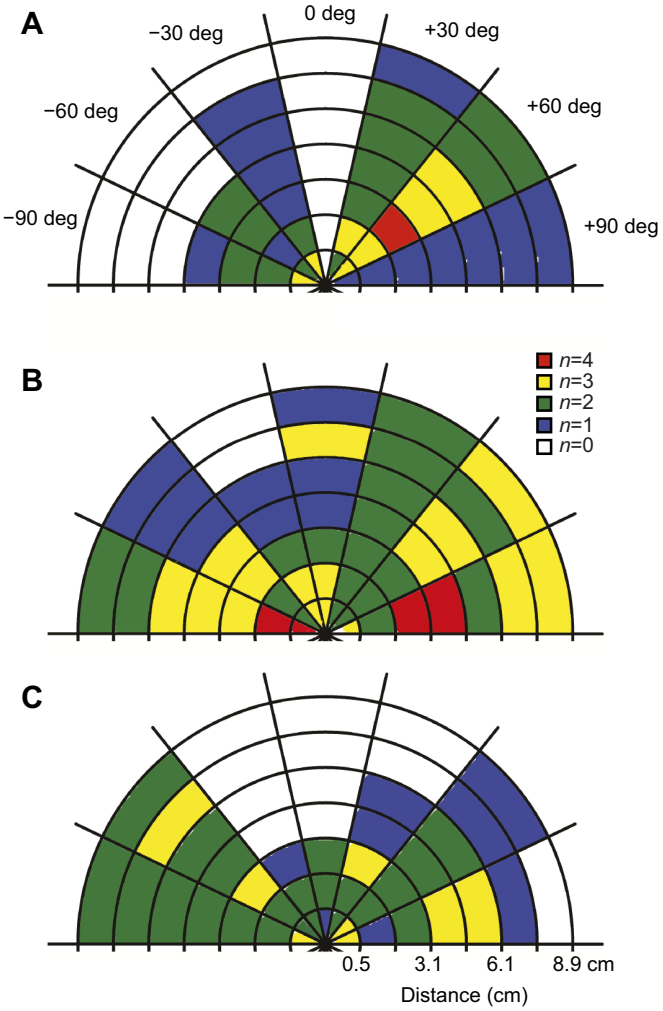

Fig. 4. Phase-locking plots. Polar plots showing the number of neuromasts (max. *n*=4) in three different fish (A–C) that displayed significant phase locking (*Z*>6.91) during a 60 Hz stimulus presentation. Two units were recorded from both the left and right lateral line nerve in each fish. The stimulus was a vibrating sphere that presented at seven distances (0.5 to 8.9 cm) and seven angles (−90 deg to +90 deg) in 30 deg increments. Segment colors indicate how many nerves exhibited significant phase-locking at each of the vibrating sphere locations (see key). Both ipsilateral and contralateral fibers were phase locking to the stimulus at the location with yellow (*N*=3) and red (*N*=4) segments.

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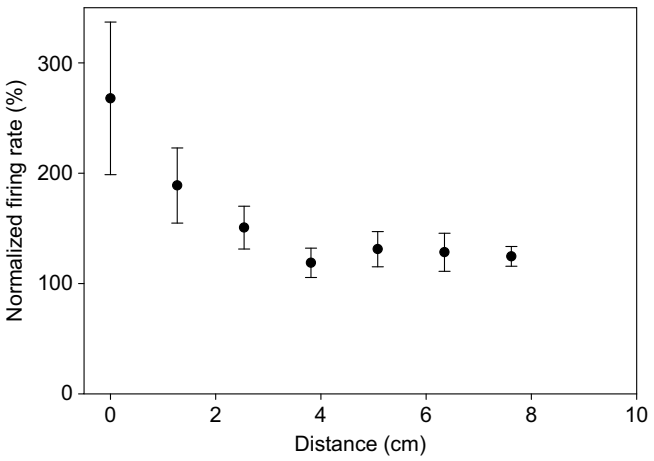

Fig. 5. The effect of distance on the evoked spike rate. Each data point represents mean normalized firing rate [(evoked/spontaneous)×100] of left neuromasts (*N*=12) during presentation of the stimulus directly in front of the fish (0 deg) versus the distance from the probe in cm. Values are means±s.e.m.

The vibrating probe had a relatively short range with a decreasing effectiveness as the probe was moved away from the neuromast (Fig. 5) corresponding with the decrease in particle acceleration observed in Fig. 1.

The approximate neuromast positions for 11 of the 12 implants were mapped on the toadfish head (Fig. 6). The left electrodes were consistently implanted in afferent fibers innervating neuromasts on the left side of the head, caudal to the eye and rostral to the operculum on the lateral side of the head. The right electrodes recorded from nerves slightly more rostral and closer to the posterior margin of eye with two additional locations suborbital and another rostral to the eye near the midline.

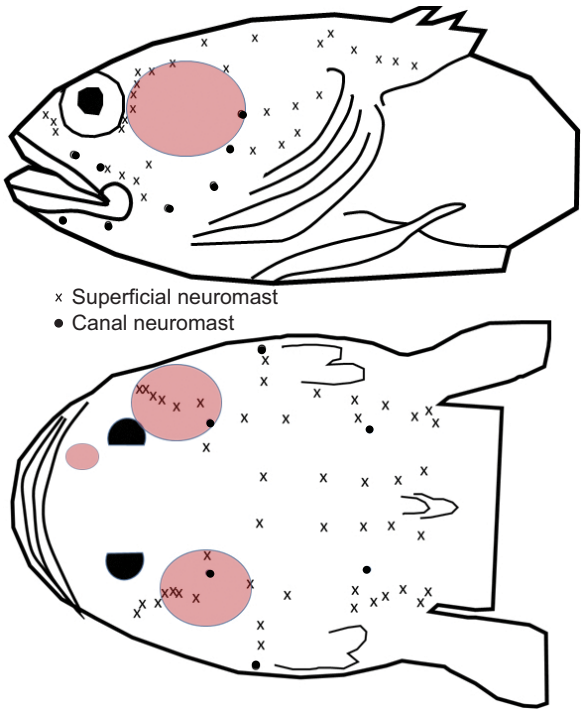

Fig. 6. Location of recorded neuromasts innervated by the anterior lateral line nerve. Outline of a lateral (top) and dorsal view (bottom) of the toadfish head with the location of superficial and canal neuromasts indicated. The figure is based on an illustration by Clapp (1898), which was scanned and traced. The schematic shows only the neuromast locations that were clearly visible on the original figure and should not be considered inclusive of every neuromast location. Eleven of twelve neuromast locations were localized for the six fish and the approximate area encompassed by these records are outlined by the pink circles.

Most lateral line neurons (83.3%) displayed directional sensitivity to the stimulus using either vector strength or spike rate as the measured criterion. Of these, the majority (33%) showed best sensitivity to the probe positioned at 60 deg to the left of the midline followed by (28%) reacting strongest to the probe positioned directly in front of the fish (0 deg) with the probe 90 deg to the right eliciting the next largest response (22%). Three general classes of fibers were encountered with sharply tuned fibers showing strong phase-locking to only one location, intermediate fibers that displayed phase-locking to two locations and broadly tuned fibers that exhibited strong phase-locking to all or most sphere locations (Fig. 7). Both ipsilateral (left) and contralateral (right) nerves phase-locked to the stimulus, with the left nerve firing ∼1.5 ms ahead of the right nerve (Fig. 8).

The speed of particle acceleration generated by the sphere was determined by placing a toadfish between the vibrating sphere and accelerometer and determining the time of arrival of the particle motion at the accelerometer. The particle motion was detected 3.77±0.31 ms after stimulus onset at the 15.5 cm distance between the sphere and accelerometer, resulting in an average velocity of 4.1 cm ms^{-1}. In three fish, phase-locking was observed in both left and right neuromasts. The distance between the neuromasts ranged from 6.6 to 7.9 cm (7.1± 0.6 cm; mean±s.e.m.) and the phase-locking time delays ranged from 1.26 to 2.04 ms (1.60±0.40 ms). Extrapolating the 3.77 ms delay from the 15.5 cm distance to the 7.1 cm interneuromast distance, the time of arrival delay is estimated to be ∼1.72 ms, which is within 10% of the mean (1.6 ms) recorded delay.

DISCUSSION

The objective of this study was to determine the effects of the particle motion generated by a vibrating sphere on contralaterally implanted lateral line neurons to determine if response characteristics during vibration stimulus could provide sufficient information that toadfish could use for source localization. This study differs from previous investigations on the lateral line as it successfully simultaneously recorded from bilaterally neuromasts, in awake, non-restrained fish that were completely submerged and were presented with stimuli from both the ipsilateral and contralateral sides. Earlier work on directional sensitivity was restricted to recording from a single neuromast and the stimulus was presented on the same side as the recording (Coombs and Conley, 1997; Montgomery and Coombs, 1998). Other studies that suggested time of arrival differences of water surface vibrations in feeding were sufficient to determine the location of the source in surface feeding fish were based on behavioral studies (Bleckmann et al., 1989). In the present study, response variations between individual neurons on different sides of the fish, combined with time of arrival differences, suggested that differential input can be integrated for source localization.

Previous experiments in the toadfish had a high percentage of success implanting a single chronic electrode; however, the bilateral implants were more challenging as the initial implant restricted the space available to maneuver the second electrode, and manipulations often caused the signal to be lost from the first electrode. Additional time was needed to secure both electrodes, suture the skin, wait for anesthesia recovery (neural activity returns to normal 90 min after anesthesia withdrawal in toadfish; Palmer and Mensinger, 2004), and fully characterize the different angles

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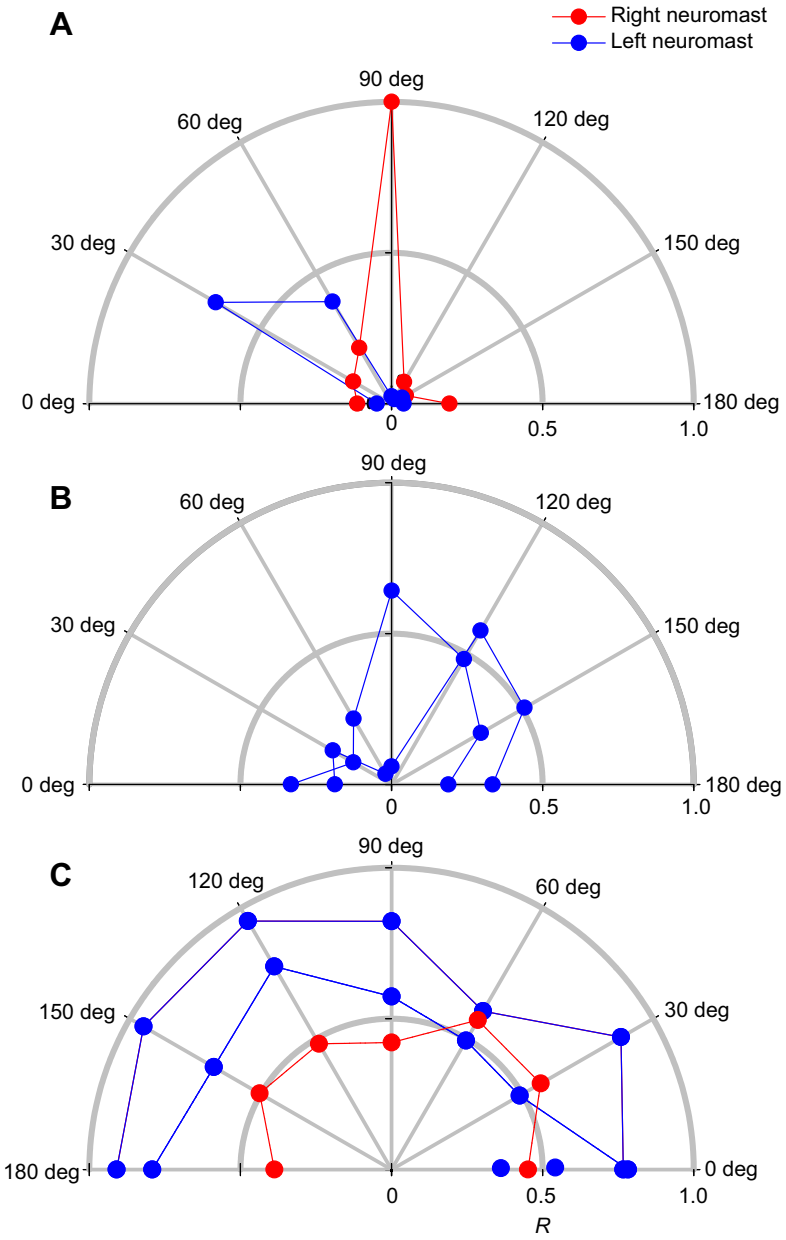

Fig. 7. Polar plots of neuromast tuning curves. Polar plots constructed using vector strength analysis to determine phase-locking responses of individual afferent fibers from both the left and right anterior lateral line nerves. Each point represents the *R* value at each angle (0–180 deg) at which the stimulus was presented. *R* values greater than 0.5 represent strong phase-locked afferent fibers. Connecting lines are used for illustrative purposes only. Left side (blue) and right side (red) neuromasts are indicated. (A) Sharply tuned units (one angle >0.5). (B) Intermediate units (two angles >0.5). (C) Broadly tuned (4 or more angles >0.5). In several fish, both the right and left neuromasts phase-locked to the stimulus when the sphere was at single location.

and distances. The extended time and manipulations often contributed to the loss of the signal from one of the electrodes. These factors resulted in a modest number of bilaterally implanted fish (N=6) and limited the tests to a single stimulus frequency; however, multiple units were characterized in each implant, which allowed comparison of 24 individual nerves.

All teleost fishes possess three inner ear end organs (the saccule, utricle and lagena) that contain functionally similar hair cells with functional overlap in both auditory and vestibular modalities, but their respective contributions to sound localization remain largely unclear (Sisneros and Rogers, 2016). However, underwater sound consists of both a pressure and a particle-motion component. The sound wave moves relatively fast, while the particles oscillate around their original position with a relatively small particle velocity. Sound pressure will pass relatively unimpeded through soft animal tissue; however, the path of particle motion is not quite direct and could be deflected by the toadfish head or body, resulting in a block or delay in the stimulus to the contralateral neuromasts. Thus, fishes could potentially use both sound pressure and particle motion to detect and locate stimuli.

The question remains regarding the differential contribution of the lateral line and inner ear to sound source localization. Both systems are hair cell-based receptors that show directional sensitivity to pure tones and toadfish vocalizations (Maruska and Mensinger, 2015; Mensinger, 2014; Radford and Mensinger, 2014). For successful toadfish mating, females need to localize male calls to deposit their eggs in nests (Gray and Winn, 1961). The swimbladder in toadfish may allow pressure detection by the inner ear as suggested for sound source localization in the midshipman which would allow long distance detection. However, near-field particle displacement has been identified as the determining factor in mate choice (Bhandiwad and Sisneros, 2016; McKibben and Bass, 1998). Therefore, components of the inner ear and lateral line may contribute in some degree to fish sound localization.

Reproductive female oyster toadfish (*O. tau*) and plainfin midshipman (*Porichthys notatus*) will exhibit phonotaxis to the

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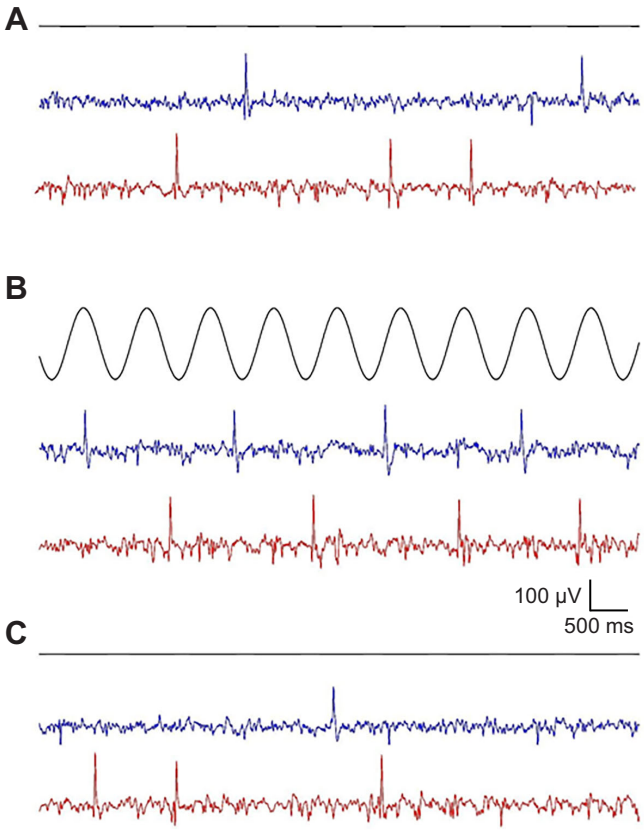

Fig. 8. Time delays between afferent fibers of the anterior lateral line nerve. Toadfish with bilaterally implanted electrodes in the right and left anterior lateral line nerve were presented with a 60 Hz vibrational stimulus. Strong phase-locking (R>0.5) was found simultaneously between the ipsilateral (left, blue) and contralateral (right, red) anterior lateral line during a 60 Hz vibrational stimulus with the probe positioned to the left of the fish. (A) Neural activity from two afferent fibers exhibiting no phase-locking before vibrational stimulus presentation. (B) Neural activity from simultaneously phase-locked afferents. (C) Neural activity displaying no phase-locking trend after the presentation of the vibrational stimulus.

playback of the male's advertisement signals (McKibben and Bass, 2001a,b, 1998; Winn, 1972). The fundamental frequency of the tonal portion of the toadfish boatwhistle is temperature dependent and in the Cape Cod area ranges from ∼150 to 200 Hz. Underwater, the wavelengths for these frequencies range from ∼11.5 to 7.5 m, respectively. Thus, particle motion can provide fish with acoustic cues at large distances from sound sources and offer directional information on sound source localization. Recent experiments demonstrated that plainfin midshipman locate sound sources using particle motion (Zeddies et al., 2010, 2012). However, the relative contribution of the swim bladder and lateral line to sound localization, remained unclear. Subsequent studies revealed that gravid midshipman with partially deflated swimbladders continue to show positive phonotaxis, which indicates that pressure reception may be required for sound source localization. However, in the same studies, partial ablation of the lateral line decreased the phonotaxis response, suggesting that the lateral line system is probably not required for sound source localization, but it may be important for fine-tuning the approach to the sound source (Coffin et al., 2014).

The variation in phase-locking strength, directionality and time of arrival differences between afferent fibers in the toadfish, suggested a potential mechanism for stimulus source detection. Both the left and right anterior lateral line nerve exhibited units with strongly phase-locked directional properties, suggesting differential phase-locking as a mechanism for stimulus detection. In many cases, the lateral line on the same side of the sphere responded to the stimulus while contralateral neuromasts were not affected. Whether this was due to the stimulus being blocked by the intervening tissue, the contralateral neuromasts being out of range of the stimulus or oriented off axis to the stimulus, is unknown. However, it clearly demonstrates stimulus location can result in differential input to the right and left lateral line that could be used for source localization. Although sensory deprivation experiments suggested time of arrival cues at different neuromasts in comparison to directional tuning of individual neuromasts for source localization in fishes (Bleckmann et al., 1989), units that are either narrowly tuned (i.e. strongly phased-locked to one source location) or have sensitivity restricted, for example, to the contralateral or ipsilateral, could provide additional locational information.

Superficial neuromasts are located on the surface of the skin and canal neuromasts are located in subdermal canals. Superficial neuromasts have a smaller frequency sensitivity range, responding best to lower frequencies, compared to canal neuromasts (Montgomery et al., 2000, 1995; Radford and Mensinger, 2014; Voigt et al., 2000), so canal neuromasts may play a larger role in increasing hearing sensitivity and sound localization (Higgs and Radford, 2013). However, there are ∼140 neuromasts arranged in three dimensions around the toadfish head with the majority being superficial neuromasts (Clapp, 1898). Thus, closely spaced and even adjacent neuromasts may differ in their axis of directional sensitivity and will react differentially to stimuli from the same location. The superficial neuromasts are visible macroscopically as each is flanked by two finger-like projections or flaps. The functional significance of these protuberances remains unknown; however, it has been speculated that they protect neuromasts from sediment deposition in silty estuarine habitat. Regardless of their function, their presence allows the directional sensitivity of each neuromast to be determined because the hair cell sensitivity axis lies perpendicular to the projections. The area mapped in Fig. 5 has ∼20 superficial neuromasts and several canal pores all arranged in slightly different directions and/or angles (Clapp, 1898), which undoubtedly contributed to the variation in the directional sensitivities observed.

Terrestrial animals use interaural time delays to locate sound sources in the horizontal plane. However, the close proximity of inner ear otoliths and the speed of underwater sound compared with the air makes this difficult for fishes (Sisneros and Rogers, 2016). Lateral line neuromasts potentially have greater separation distance than otoliths (i.e. anterior versus posterior lateral line), and could provide alternative mechanisms for sound source localization. For example, in adult toadfish, neuromasts of the anterior lateral line can be separated by over 10 cm, while anterior and posterior lateral line neuromasts can be over 25 cm apart. If a 25 cm standard length fish is presented with sound directly in front of it, the rostral neuromast will detect the sound 16 µs prior to the posterior lateral line neuromasts at the base of the tail. Factoring in toadfish cranial nerve diameters (1–12 µm; Mensinger and Highstein, 1999), conduction speeds of myelinated nerves of these diameters (10–50 m s^{-1}) and afferent lengths to second order neurons (up to 5 cm length for anterior and 20 cm for posterior lateral line primary afferents; A.F.M., unpublished results), delays in response to sound pressure stimulus from anterior and posterior lateral line neuromasts to second-order neurons in the central nervous system would range from ∼400 µs to 2 ms. These time delays between posterior and anterior lateral line neuromasts are within the time frame used by other vertebrates for sound localization (Grothe et al., 2010).

Thus, even closely spaced neuromasts may be able to use time of arrival difference as observed in surface feeding fish (Bleckmann et al., 1989). Stimulus (particle motion) speed was determined by calculating the time of arrival from the sphere to an accelerometer 15.5 cm away. Particle motion was detected 3.77±0.31 ms after stimulus, resulting in an average velocity of 4.1 cm ms^{-1}. In three fish, phase-locking was observed in both left and right neuromast pairs. The distance between the pairs ranged from 6.6 to 7.9 cm (7.1±0.6 cm) and the phase-locking time delays ranged from 1.26 to 2.04 ms (1.60±0.40 ms). Extrapolation of the calculated 3.77 cm ms^{-1} to the 7.1 cm interneuromast distance, resulted in a time of arrival difference of 1.87 ms (in contrast, the pressure wave of sound would have been expected to travel this distance in 47 µs). The time of arrival difference calculations did not factor head curvature, possible delays due to particle motion impact with tissue or that particle motion speeds will decrease with distance from the source. Therefore, the phase-locking time delays were within the range of time of arrival differences for particle motion between neuromasts.

In this study, bilateral anterior lateral line neuromasts displayed differential directional sensitivity to incoming vibrational stimuli, which suggests the potential for the lateral line to be used for sound localization in the near field. Based on our calculations of particle accelerations, inter-neuromast distances, and time delays of bilaterally recorded anterior lateral line nerves, our study also provides evidence that that the anterior and posterior lateral line system of the toadfish may provide sufficient time delays between sensory organs for determining sound localization cues.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.F.M., C.A.R.; Methodology: A.F.M., E.A.C., C.A.R.; Validation: A.F.M., C.A.R.; Formal analysis: E.A.C., C.A.R.; Investigation: A.F.M., E.A.C., C.A.R.; Resources: A.F.M.; Data curation: A.F.M.; Writing - original draft: E.A.C., C.A.R.; Writing - review & editing: A.F.M., E.A.C., C.A.R.; Visualization: C.A.R.; Supervision: A.F.M., C.A.R.; Project administration: A.F.M.; Funding acquisition: A.F.M., C.A.R.

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