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Physiological evidence for binaural directional computations in the brainstem of the oyster toadfish, *Opsanus tau* (L.)

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SUMMARY

Comparisons of left and right auditory input are required for sound source localization in most terrestrial vertebrates. Previous physiological and neuroanatomical studies have indicated that binaural convergence is present in the ascending auditory system of the toadfish. In this study, we introduce a new technique, otolith tipping, to reversibly alter directional auditory input to the central nervous system of a fish. The normal directional response pattern (DRP) was recorded extracellularly for auditory cells in the first-order descending octaval nucleus (DON) or the midbrain torus semicircularis (TS) using particle motion stimuli in the horizontal and mid-sagittal planes. The same stimuli were used during tipping of the saccular otolith to evaluate changes in the DRPs. Post-tipping DRPs were generated and compared with the pre-tipping DRPs to ensure that the data had been collected consistently from the same unit. In the DON, ipsilateral or contralateral tipping most often eliminated spike activity, but changes in spike rate (±) and DRP shape were also documented. In the TS, tipping most often caused a change in spike rate (±) and altered the shape or best axis of the DRP. The data indicate that there are complex interactions of excitatory and inhibitory inputs in the DON and TS resulting from the convergence of binaural inputs. As in anurans, but unlike other terrestrial vertebrates, binaural processing associated with encoding the direction of a sound source begins in the first-order auditory nucleus of this teleost.

Key words: auditory, descending octaval nucleus, torus semicircularis, directional hearing.

INTRODUCTION

There is a general consensus that fishes are unlikely to localize sounds using the same central neural computations as terrestrial vertebrates (Rogers and Zeddies, 2008). Sound localization by terrestrial vertebrates requires binaural comparisons (for reviews, see Brown and May, 2005; Christensen-Dalsgaard, 2005; Trahiotis et al., 2005), beginning with parallel neural pathways that encode small acoustic time of arrival differences and sound level differences. In water, the speed of sound is about five times faster than in air, making interaural time differences five times shorter underwater than in air for a vertebrate of similar head size. In addition, the fish's ears are relatively close together physically, and the skull is surrounded by tissue that is approximately the same density as water. Therefore, acoustic cues for interaural time differences (ITD) and interaural level differences (ILD) are expected to be very small or non-existent, and the binaural processing mechanisms typical of terrestrial vertebrates would not be sufficient to detect those minute differences. However, there are multiple lines of evidence indicating that directional hearing in fishes involves binaural processing and that interaural comparisons could be present in the directional hearing pathway of fishes (reviewed by Fay, 2005).

Sound has both a kinetic, particle motion component, and a pressure component. In fishes, the axis of acoustic particle motion is encoded by the variously oriented sensory hair cells on the otolithic endorgans of the ear (Sand, 1974; Hawkins and Horner, 1981; Fay, 1984; Fay and Edds-Walton, 1997a; Fay and Edds-Walton, 1997b; Edds-Walton et al., 1999; Lu at al., 1998). Each endorgan has a sensory epithelium and calcareous otolith, which together function like an inertial accelerometer (de Vries, 1950). The hair cells on the epithelium are anatomically specialized neurons with mechanically activated surface structures that are physiologically polarized with a 'best axis' (Flock, 1964). Particle motion along the best axis maximally excites the hair cell. Particle motion perpendicular to that axis causes a null in neural activity, and excitation at other angles causes a graded response that is proportional to a cosine function of the angle of particle motion with respect to the hair cell's best axis. In most fishes, auditory hair cells are activated by particle motion between about 50 and 1000 Hz. There are three otolithic endorgans in teleost fishes that may have auditory response characteristics (saccule, lagena and utricle). Research on a variety of fish species indicates that the saccule is the greatest contributor to auditory processing in most fishes (Fay, 2005).

Sand (Sand, 1974) recorded microphonic potentials from left and right ears of a perch (*Perca fluviatilis*, Linnaeus) in response to oscillations of the fish in the horizontal plane and showed that the two saccules responded with slightly different directional response patterns. Sand hypothesized that interaural comparisons would be sufficient to determine sound source direction in the horizontal plane. Schuijf (Schuijf, 1975) provided early behavioral evidence that two ears were necessary for cod (*Gadus morhua*, Linnaeus) to discriminate between two different sound source locations. Using the same species, Horner and colleagues (Horner et al., 1980) provided physiological evidence that binaural auditory processing sites were present through unilateral and bilateral electrical blockage of input from the saccule, which resulted in reduction or elimination of auditory responses in the medulla and the midbrain.

Our lab (Edds-Walton et al., 1999) has shown that auditory afferents from the saccule of the toadfish (*Opsanus tau*, Linnaeus) contact multiple hair cells that have similar or identical best

directions. The direction of stimulation is transmitted *via* primary afferents in cranial nerve VIII to the descending octaval nucleus (DON) in the medulla (Edds-Walton and Fay, 1998; Edds-Walton and Fay, 2008). Both the left and right DONs project to the midbrain torus semicircularis (TS) where directional information is retained (Wubbels and Schellart, 1998a; Wubbels and Schellart, 1998b; Wubbels et al., 1995; Ma and Fay, 2002; Edds-Walton and Fay, 2003) and enhanced directionality (directional sharpening) is common (Edds-Walton and Fay, 2005a). Thus, encoding the axis of particle motion for audio frequencies is one of the consistent functions of the ascending auditory pathway in the toadfish. We have hypothesized that binaural computations could account for the wide range of best axes and directional sharpening documented in the torus semicircularis of toadfish (Edds-Walton and Fay, 2003).

Anatomical tract-tracing studies consistently show that the auditory midbrain receives input from first-order and second-order auditory nuclei in the left and right auditory medulla in a variety of teleost fishes (reviewed by McCormick, 1999). In addition, Edds-Walton (Edds-Walton, 1998) demonstrated a commissural tract carrying axons between the DONs in toadfish. Therefore, both the DON and TS have the neural basis for interaural comparisons, but binaural response characteristics indicative of directional auditory processing have not been confirmed anywhere along the ascending auditory pathway of a fish.

In this paper we will describe further physiological evidence that convergence of left and right directional inputs occurs in the DON and TS of the toadfish. Using a new, readily reversible technique, we show that manipulating the contralateral saccular otolith can change the responsiveness of a cell in the DON or in the TS. These data indicate that binaural mechanisms play a role in the neural representation of direction in the brainstem of the toadfish.

MATERIALS AND METHODS

Anatomy

The anatomical terminology used herein corresponds to that used in our previous publications, which is consistent with the detailed description of the toadfish medulla (Highstein et al., 1992), but also includes the divisions of the DON described in *O. beta* (Bass et al., 2001). The DON lies within the octaval column, between the entrance of cranial nerves VIII and X. Near the anterior ramus of VIII, the ventro-lateral DON transitions into the tangential octaval nucleus and the dorso-lateral DON transitions into the magnocellular octaval nucleus. The dorso-medial division appears near VIII and transitions into the dorsal secondary octaval nucleus medially. In order to limit recordings to the DON, recording sites were restricted rostro-caudally to the area between the posterior ramus of VIII and IX. The presence of a large blood sinus prevented access to the dorso-medial division of the DON.

A preliminary investigation of auditory sites in the DON was conducted using neurobiotin injections following characterization of an auditory unit. Those studies were conducted prior to commencement of the present experiments involving otolith manipulations (described below). Neurobiotin was injected into the medulla *via* positive current (1600–2000 nA) from the recording electrode for 20–30 min. The surgical opening was sealed and the fish placed in aerated seawater for label transport to occur over 8–12 h. The toadfish then was anesthetized and perfused through the ventricle with buffered toadfish saline followed by perfusion with fixative (4% paraformaldehyde in 0.1 mol l^{-1} PBS). The brain was excised and postfixed for 1 h, rinsed and refrigerated in PBS overnight. The brain was cryoprotected by infusion of 40% sucrose over 24 h, embedded in Tissue-Tek OCT, and sectioned (50 μm). The neurobiotin was visualized in floating sections using a standard ABC-DAB reaction (Elite kit, Invitrogen, Carlsbad, CA, USA) with metal intensification (modified from Hancock, 1982). The sections were stored in buffer until mounted onto gelatin-coated slides, dehydrated through alcohols, cleared in Citrisolv (Fisherbrand, Pittsburgh, PA, USA) and cover-slipped for examination on an Olympus BX50 with drawing tube and digital camera (DP12 camera system: Olympus, Center Valley, PA, USA).

Our previous anatomical and physiological work in the DON and the TS allowed us to visually define appropriate recording sites (Edds-Walton, 1998; Edds-Walton et al., 1999; Edds-Walton and Fay, 1998; Edds-Walton and Fay, 2003; Edds-Walton and Fay, 2005a; Edds-Walton and Fay, 2005b; Edds-Walton and Fay, 2008). Small neurobiotin injections (1600–1900 nA, 10–15 min) were used to confirm the recording site in some fish (N=15). For those cases, physiological recording ceased after the injection, and the fish was prepared for histological examination of the tissue (see above). Details of injection methods and illustrations have been published previously (Edds-Walton and Fay, 2003; Edds-Walton and Fay, 2008). Recording sites were not labeled in the midbrain during this study because the location and physiology of auditory cells in the TS (nucleus centralis) have been investigated in detail in a previous study (Edds-Walton and Fay, 2005a; Edds-Walton and Fay, 2005b). During the search for auditory units in the midbrain, the recording electrode traverses areas with predictable neural activity or lack thereof (e.g. the ventricle). The auditory TS lies immediately below the ventricle, and no other auditory sites could be encountered.

Electrophysiology and stimuli

Pulled, broken glass electrodes (5–20 MΩ) were used for extracellular recordings of individual units in the DON and TS (Sutter Instruments, Novato, CA, USA). Electrodes were filled with 3 mol l^{-1} NaCl or 2 mol l^{-1} NaCl with 4% neurobiotin (Invitrogen) for labeling recording sites, as described above.

All electrophysiology was conducted in the particle motion stimulus system (shaker dish) designed by R.R.F. and used for all previous hearing research on toadfish. Briefly, the fish was positioned in a custom-designed head-holder attached to the edge of the dish. The dish contained local seawater up to the level of the opening in the skull. A 50 ml syringe was used to remove and replace water in the dish as needed to maintain appropriate temperature and oxygenation. Particle motion was produced in the horizontal plane by two pairs of mini-shakers (Bruel and Kjaer, Odense, Denmark) positioned back–front and left–right of the fish, and in the mid-sagittal plane by the front–back paired mini-shakers and a vertical shaker (Bruel and Kjaer). Dish movement caused the fish to experience particle motion directly. A diagram and detailed description of the apparatus were provided in our earlier publication (Fay and Edds-Walton, 1997a). Three orthogonally positioned accelerometers (PCB Piezotronics, Depew, NY, USA) mounted on the outer surface of the dish monitored movement and were used to calibrate all directional and frequency stimuli prior to each experiment to ensure consistency over the 4 years of data collection.

The stimuli (500 ms; 20 ms rise and fall times) were presented in preprogrammed order at the designated level (dB re: 1 nm) with eight repetitions. Spike times were recorded with 0.1 ms resolution (Tucker-Davis Technologies, Gainesville, FL, USA), and the spike rate data were used to plot iso-level directional response patterns (DRPs) and iso-level frequency response curves. The DRPs were used to evaluate the best axis (most excitatory) in azimuth and in elevation prior to, during and following experimental manipulation of the otolith whenever possible (see below). Phase locking (how

consistently the cell produces spikes at a particular phase of the sinusoidal stimulus) and the phase angle of the response were also recorded. Background activity was recorded in the absence of deliberate stimulation.

The response of cells to particle motion was evaluated at 30 deg. intervals in the horizontal and mid-sagittal planes at 100 Hz, an appropriate frequency for the broadly tuned auditory units in toadfish based on previous experimentation (Fay and Edds-Walton, 1997b; Edds-Walton and Fay, 2008). In addition, the frequency response was evaluated at the same frequencies used in our previous studies of the DON and TS (50, 65, 84, 100, 141, 185, 244, 303 Hz) with a stimulus angle of 30 deg. azimuth to the left (0 deg. elevation) or 30 deg. elevation (0 deg. azimuth). The frequency response functions were used during these experiments to help confirm that the data obtained were from the manipulated cell, and not the activity of an adjacent cell. Stimuli were presented at 5 dB increments in a sufficient range of levels to evaluate the cell's sensitivity and generate data for the DRP.

Surgery and otolith manipulation

The care and use of toadfish were approved by the Animal Care and Use Committees at the Marine Biological Laboratory and Loyola University of Chicago. For all surgical procedures the gills of the fish were washed with buffered seawater containing MS222 (3-aminobenzoic acid methane-sulfonate salt, 1:1000, pH 7.4; Sigma-Aldrich, St Louis, MO, USA) until opercular movements ceased. Fish were paralyzed with an intramuscular injection of pancuronium bromide (0.05 mg kg^{-1}; Sigma-Aldrich) and placed in a Plexiglas enclosure with aerated sea water. Lidocaine (Henry Schein, Melville, NY, USA) was applied to the skin, a rectangular incision was made dorsally, and the skin reflected. The muscle was removed and the skull was opened to expose the medulla or midbrain. Intracranial fluids were replaced with a fluorocarbon plasma substitute (FC-77, 3M Corp. Minneapolis, MN, USA) to provide a clearer view. The bone of the midline suture was removed and the triangular dura was peeled caudally to permit access to both sides of the brain, taking great care to avoid a major dorsal vessel that descends from the cranium and divides repeatedly to supply the midbrain.

Additional surgery was necessary to access the DON. After the brainstem had been exposed, the pia along the left medulla was removed gently with a glass probe to expose the dorso-lateral surface of the medulla between cranial nerves VIII and IX, to access the main body of the DON. Although the DON extends medially near VIII and caudally beyond IX, these areas are difficult to access, and they were not the target of this project.

The saccule is located within the translucent membranous labyrinth of the ear and is bathed in endolymph. The large, calcareous otolith lies adjacent to the brainstem, where it is easily observed without surgical modification of the area (Fig. 1A). The saccular epithelium lies within a small central depression on the medial surface of the otolith [see figure 1 in Edds-Walton et al. (Edds-Walton et al., 1999)] and is not visible without additional surgery. Therefore, we could not evaluate the condition of the saccular epithelium directly, but inferred its condition based on the consistency of data acquisition.

After the fish had been secured in the shaker dish, two or three 3-D micromanipulators were positioned on opposite sides of the fish. One micromanipulator held the recording electrode and ground. The second (and third) micromanipulator held the hand-made tipper: a borosilicate micropipette or synthetic electrode filler (World Precision Instruments, Sarasota, FL, USA) attached to a 1 ml syringe. The tipper was positioned just above the dorsal edge of the

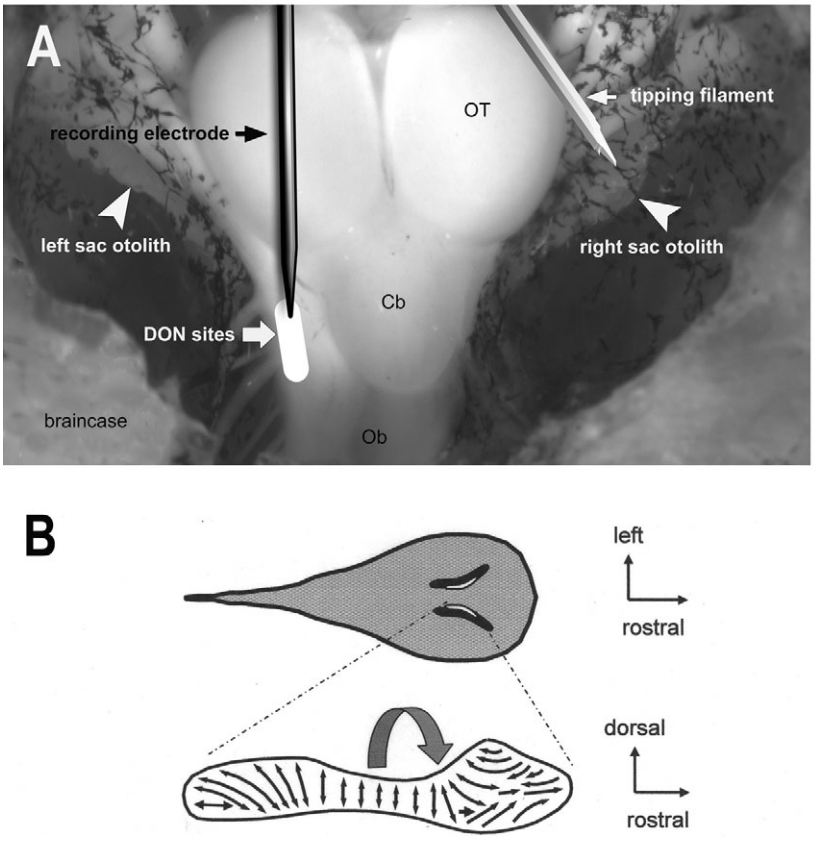

Fig. 1. (A) Dorsal view of toadfish brainstem illustrating recording and otolith tipping methods. The recording electrode surveyed the approximate area of the descending octaval nucleus (DON) shown as a white oval. The tipping probe and recording electrode are drawn to indicate approximate locations during experimentation. The tipping probe was used to tilt the curved edge of the saccular otolith 20–30 deg. laterally from normal orientation with respect to the midline of the fish (contralateral tip illustrated). (B) Cartoon of dorsal view of toadfish to illustrate location of paired saccules (not drawn to scale). The black structures represent the otoliths, and white structures medially represent the sensory epithelia. The right epithelium is enlarged to illustrate the hair cell orientation pattern (Edds-Walton and Popper, 1995). Arrowheads point to the best direction for hair cells in that region; note that well-defined, opposition zones (orthogonal hair cells) are not present on the toadfish saccule. The large curved arrow indicates the direction of rotation during contralateral tipping. Abbreviations: Cb, cerebellum; Ob, obex; OT, optic tectum of midbrain; sac, saccule.

right (contralateral) and/or left (ipsilateral) saccular otolith (Fig. 1A). The saccule was chosen for manipulation for several reasons. First, we have characterized the directional auditory responses from the saccule in this species and know that it projects heavily to the auditory region of the DON. In addition, experiments by Schuijf and Siemelink (Schuijf and Siemelink, 1974) revealed that surgical elimination of input from one saccule (and possibly the lagena) impaired the ability of a cod to orient to a sound source at different locations in azimuth, in the presence of intact utricles. The toadfish ear is organized similarly to that of the cod, and there is no physiological evidence to date that either the utricle or the lagena contribute to directional hearing in either species.

All stimuli were presented prior to tipping the otolith to obtain a normal, pre-tipping DRP and frequency response at two to three different levels. Then gentle pressure was applied to the dorsal edge of the saccular otolith to tip the saccule away from the brain and alter the normal orientation of the sensory epithelium without damaging the labyrinth (Fig. 1A,B). Care was taken to ensure that the saccular nerve was not strained, which could alter normal neural activity. In general, the vertical orientation of the otolith edge was altered by 20–30 deg. All of the stimuli were presented again to obtain DRP and frequency response at two to three levels during tipping. Lastly, the tipper was retracted slowly by reversing the micromanipulator. In most cases, a 5–10 min recovery period was permitted prior to the post-tipping repetition of the stimuli, again at two to three levels. In all cases, post-tipping data were collected to confirm that tipping had not permanently altered the response of the unit under study. If the unit did not respond post-tipping, the unit was considered lost, and the data were eliminated from further consideration.

The experimental procedures were identical for recordings in the DON and in the TS. The contralateral otolith was tipped in the majority of the experiments reported here. In a few fish, the skull was large enough to permit the use of two tippers, one that targeted the ipsilateral saccular otolith and a second that targeted the contralateral otolith while the recording electrode was placed in the DON. However, the probe was applied sequentially, not simultaneously, to each side, due to space limitations. Sequential tipping was usually done only if contralateral tipping was unsuccessful. There was never sufficient space to allow simultaneous ipsilateral and contralateral tippers with the recording electrode located in the midbrain.

Tipping was employed as an easily reversible method of temporarily altering the orientation of the saccule, although it did not produce predictable results with every application of the tipper. In preliminary experiments, we showed that tipping the ipsilateral saccular otolith caused consistent reductions in multi-unit activity in the DON (Fig. 2). However, in subsequent recordings from single units, tipping the ipsilateral otolith did not always produce a change in the DON cell's activity (e.g. Fig. 3). There are two potential explanations for the lack of an effect: (1) the DON cell did not receive auditory input from the ipsilateral saccule or (2) the saccular inputs to that particular DON cell were not affected by tipping because all regions of the saccular epithelium are not affected equally by tipping. Of these two possibilities, we believe the second is the most likely reason for a lack of change in the DRP during ipsilateral tipping. Based on the ipsilateral otolith tipping experiments, the lack of a change in the DRP of a DON cell during contralateral otolith tipping cannot be interpreted as evidence that the cell does not receive contralateral input. Similarly, tipping did not always affect the responsiveness of TS cells (Fig. 3), but altered responses were obtainable with this method. We will focus on the cases in which we were able to obtain a reversible change in the spike rate or shape of the DRP of a unit in the DON or the TS during tipping of the contralateral saccular otolith. To be included in this data set, we required complete directional data at two stimulus levels with consistent DRPs.

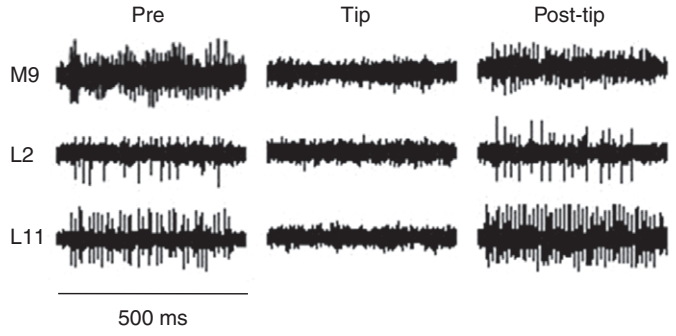

Fig. 2. Multi-unit activity illustrated prior to tipping the ipsilateral saccular otolith (Pre), during tipping (Tip) and after otolith returned to its original orientation (Post-tip) at three different recording sites in the DON of two fish (M, L).

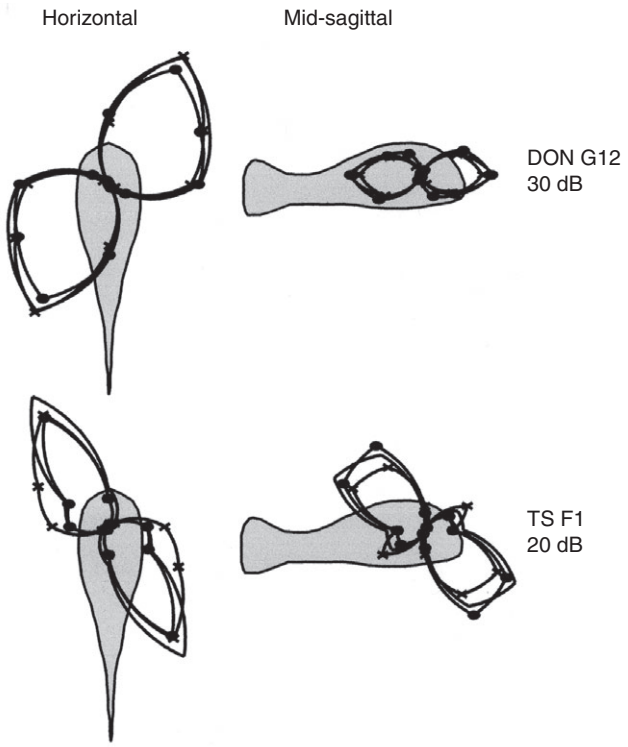

Fig. 3. Examples of negative results from tipping the saccular otolith. Tipping of the ipsilateral saccular otolith resulted in no change in the directional response pattern (DRP) of unit G14 in the DON to stimuli in the horizontal or mid-sagittal planes; tipping of the contralateral saccular otolith did not cause a change in the DRPs of unit F1 in the torus semicircularis (TS). Stimulus level shown in dB re: 1 nm. Note that each unit was held for consistent data acquisition throughout the experiment and that the slight variation in response magnitude at each direction is consistent with normal variation in the absence of manipulation. Filled circle on curve for pre-tip data; no symbol for data during tipping; x used for post-tip data.

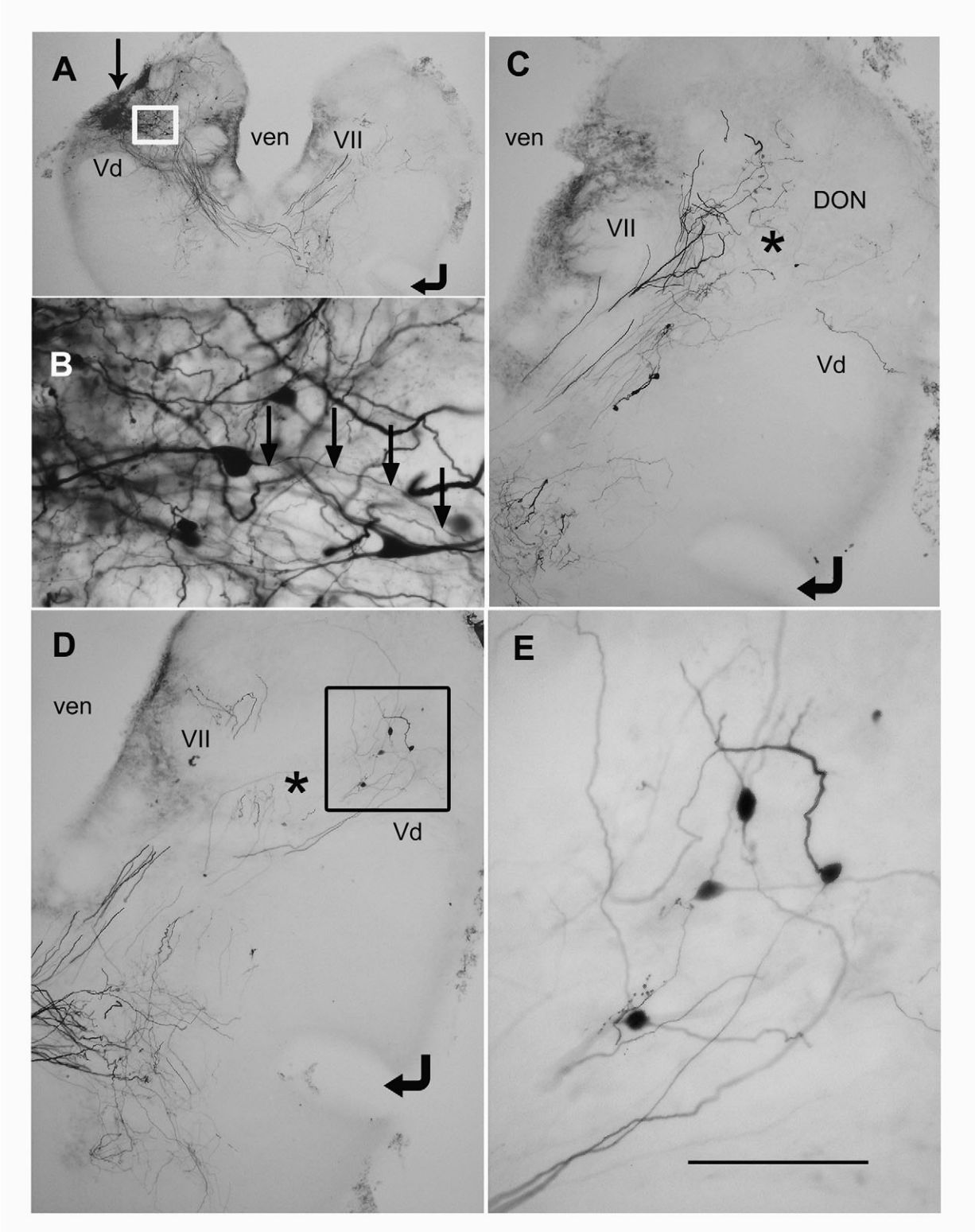

Fig. 4. Anatomical evidence of a commissural auditory pathway connecting the DONs. (A) Coronal section with large neurobiotin injection at physiologically characterized auditory site (vertical arrow) in left dorsal DON. White box indicates area enlarged in B (right-angle arrow indicates contralateral cut in brain prior to sectioning). (B) Projection cell in DON among other filled somata; axon (arrows) entered the commissural tract. (C) Contralateral site in the same fish, 100 μm caudal of A, with terminal fields in the right dorsal DON (asterisk) from projection cells in the left dorsal DON. (D) Contralateral site in the same fish, 50 μm rostral of A with terminal fields (asterisk) from left dorsal DON projections and retrogradely filled somata of projection cells in the right dorsal DON (black box, enlarged in E). (E) Retrogradely filled projection cells from D. Scale bar, 100 μm in B and E; 400 μm in C and D; 1 mm in A. Vd, descending tract of trigeminal; ven ventricle; and VII, sensory facial nerve.

RESULTS

Anatomical confirmation

The larger neurobiotin injections (20–30 min) at confirmed auditory sites produced clear evidence that a subset of dorsal DON cells provides input to the contralateral dorsal DON (*N*=5 cases). Neurobiotin-filled axons were common crossing the midline, and terminal fields were widespread in the contralateral DON. In addition, retrogradely filled cells were present in the contralateral DON (Fig. 4A–D). Therefore, these data extend previous anatomical work (Edds-Walton, 1998) that identified a commissural connection between the left and right DONs and support the hypothesis that binaural auditory cells are likely to be present in the DON of toadfish.

The locations of cranial nerves VIII and IX are dependable landmarks for the rostro-caudal extent of the DON. Neurobiotin injections were limited to maximize use of each fish, as fish had to be killed immediately after injection to prevent label transport from the injection site. Neurobiotin was injected only when a recording site was at the extremes of the desired recording area, e.g. particularly rostral, medial or deep (*N*=15). Three data sets were eliminated due to the location of the injection in an adjacent nucleus. No injections were placed in the midbrain. Our previous work in the TS provided sufficient experience and confidence of location when recording auditory activity.

Responses to tipping

For brevity, the simple term tipping will be used to indicate that the saccular otolith was tipped, and either ipsi (ipsilateral) or contra (contralateral) will indicate which saccular otolith was tipped with respect to the recording site. The presence of binaural input was evaluated in 70 left DON auditory units, and 30 left and one right TS auditory unit. Of the experiments in the DON, 21 were ipsi tipping, 42 were contra tipping, and seven were sequential tipping of the contra and ipsi saccular otoliths. The TS tipping experiments consisted of two ipsi, 29 contra and a single sequential tipping.

The ipsilateral tipping experiments conducted while recording in the DON validated the repeatability of the technique and confirmed that a cell could be held throughout the acquisition of the directional and frequency data before, during and following tipping. As described in Materials and methods, the limitations of the tipping technique were revealed when tipping the ipsilateral saccular otolith failed to cause a change in the DRP of a DON unit. These data were interpreted as indicating that tipping may not always affect the region of the saccular epithelium that provided input to the unit from which we were recording, as saccular input is present throughout the dorsal DON (Edds-Walton et al., 1999). Similarly, we concluded that lack of a change in the directional response of a cell during contralateral tipping could not be interpreted as a lack of contralateral input, as data resulting from unsuccessful or inadequate tipping could not be distinguished from data indicating a lack of binaural input. Therefore, we considered only cases in which changes in the DRP were observed during contra tipping to assess the potential role of contralateral input to the DON.

Bilateral auditory input to the TS has been established by a previous physiological study in the toadfish (Edds-Walton and Fay, 2005b). Therefore, either ipsilateral or contralateral tipping could provide insight into the potential role(s) of binaural convergence for directional processing. As noted above, most of the tipping experiments in the TS were conducted with the contralateral otolith (29/31) due to space limitations in the braincase. As in the DON, a lack of change in the DRP of an auditory unit in the TS could not be interpreted with regard to our research question.

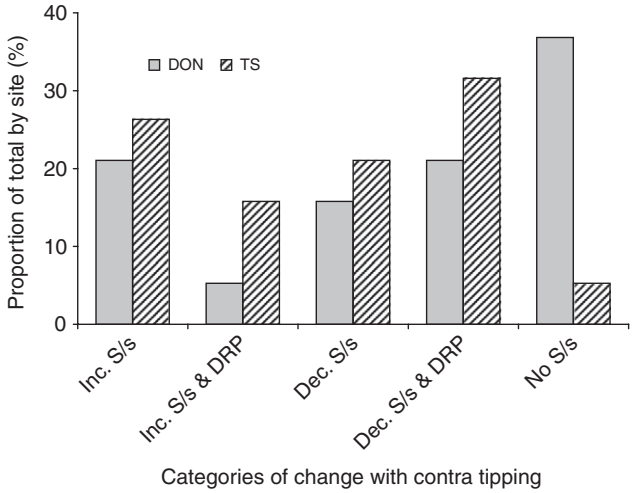

Fig. 5. Categories of responses to contralateral tipping of the saccular otolith in the DON (shaded) and the TS (hatched) of the toadfish. Data are graphed as a proportion for each site (i.e. total of 100% for DON, total of 100% for TS). Note that all units exhibited a change in spike rate: data are divided to illustrate the proportion of units that exhibited only spike rate changes and those that exhibited spike rate changes along with shifts in the best axis or altered shape of the DRP. No spikes category (No S s^{-1}) indicates units that lost spike activity during tipping, but resumed spike activity following removal of the tipper; S s^{-1}, spikes s^{-1}; Inc., increase; Dec., decrease. DON: *N*=38; TS: *N*=23.

We designated categories of alterations in the DRP based on the differences we observed during data analyses: changes in overall spike rate (increase or decrease) without a shift in the shape or best direction of the DRP *versus* shifts in the DRP shape or best direction, which were always accompanied by a change in the spike rate (increase or decrease). The final category of changes recorded during tipping was a complete loss of activity (0 spikes). For all cases in the latter category, the post-tip DRP and frequency response confirmed that the loss of activity was due to tipping and not to loss of contact with the DON or TS cell. Units with inconsistent DRPs across different stimulus levels were not included in the data set reported here. All of the TS and DON units included had DRPs with best directions consistent with our earlier work on directional auditory processing.

Of the 36 auditory units evaluated in the DON, seven DON cells exhibited changes that were inconsistent, and nine DON cells exhibited no change in the DRP during contralateral tipping. Of the 23 auditory units evaluated in the TS, three TS cells exhibited changes that were inconsistent at different stimulus levels, and only one cell exhibited no change in the DRP during contralateral tipping.

The most common result of tipping was a change in spike rate without a shift in the shape or best direction of the DRP (47% of TS cells; 37% of DON cells). In both the TS and the DON, an increase in spike rate was slightly more common than a decrease in spike rate (Fig. 5). Total loss of activity (no spikes during tipping, with recovery post-tipping) was more common in the DON (37%) than in the TS (5%; Fig. 5). Shifts in the shape of the DRP or the best axis (in one or both planes) were always accompanied by an increase or decrease in spike rate in both the TS and the DON. At both sites, shifts in the DRP were most often accompanied by a decrease in spike rate (Fig. 5). Overall, alterations in the shape of the DRP or best axis were more common in the TS (48% of cases) than in the DON (26%).

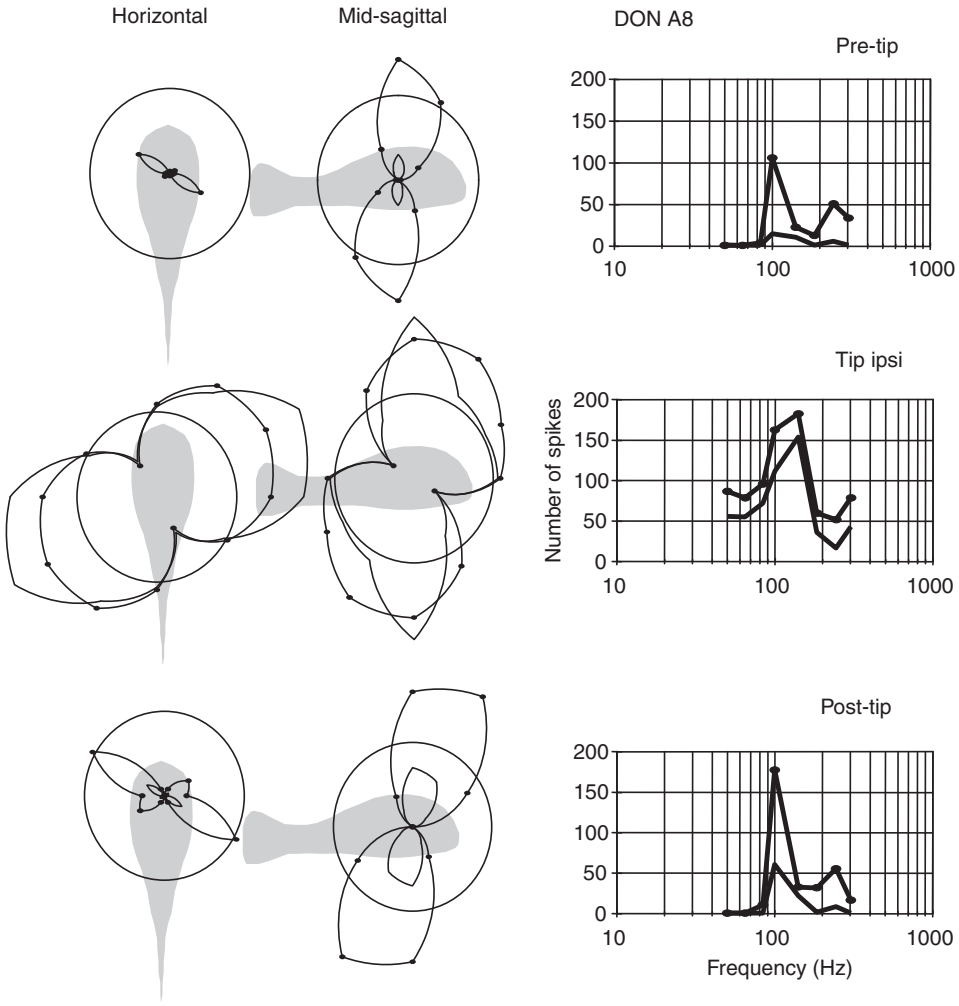

Fig. 6. Example of DRP changes with tipping of the ipsilateral saccular otolith for a DON unit. DRPs for both stimulus planes and frequency responses are shown pre-tip, during tip and 5 min post-tip. Two different stimulus levels are plotted for each DRP graph (20 and 25 dB re: 1 nm, higher level has symbols); spike rate indicated by the circle (100 spikes s^{-1}). Interpolated best axes (average of two levels) pre-tip: –30 deg. (left) azimuth, 85 deg. elevation; tipped: 35 deg. (right) azimuth, 62 deg. elevation; post-tip: –30 deg. (left) azimuth, 80 deg. elevation.

DRPs in the DON

Ipsi tipping could produce dramatic results in the DON. As illustrated in Fig. 6, the best axis and DRP shape were altered substantially in both planes for unit A8. The best axis in the horizontal plane was rotated 90 deg., and the responsiveness increased at angles adjacent to the best axis in elevation (loss of sharpening), particularly at the higher of the two stimulus levels shown. When the tipping probe was backed away from the otolith (post-tip), the DRP in both planes became very similar to the pre-tipping DRP. Although the shape of the post-tip DRP is not identical to the original for A8, the slight variation is consistent with a near complete return to the pre-tipping state. The frequency response for DON A8 shifted during tipping, revealing a higher best frequency (141 Hz *versus* 100 Hz), but the post-tip frequency responses were very similar to the pre-tip responses.

Contra tipping often caused an overall change in responsiveness with little or no shift in the shape of the DRP. Three DON units that exhibited rate changes are presented in Fig. 7. G4 exhibited an increase in spike activity (tipping data shown with symbols). L14 exhibited a decrease in responsiveness and a slight shift in the DRP (tipping data shown with symbols). Among DON cells, the most common result of contralateral tipping was complete loss of activity (Fig. 5). The pre-tip (larger DRP) and post-tip data (smaller DRP) are presented for cell D5 (Fig. 7) because there were no spikes produced during tipping. The smaller post-tip DRP and frequency response indicate that the unit had not recovered pre-tip activity, but the data indicate that the unit had not been lost.

The phase of the sinusoid at which a DON unit phase locked was examined pre-tip *versus* during contra tipping to determine whether there was a change in phase locking when other changes were noted. There was no consistent evidence that phase locking was affected by a change in the contralateral input. Small, inconsistent changes were observed, but there were no trends indicative of a mechanically induced shift in phase response.

DRPs in the TS

A dramatic effect of contra tipping is shown for TS unit H1 in Fig. 8. During tipping the response increased in the horizontal plane, and rotation of the best axis is present in both planes compared with the pre-tip data. The shift in the DRP is nearly 30 deg. in each plane, and there is an interesting loss of sharpening shown at the higher level in the mid-sagittal plane. Withdrawal of the tipping probe resulted in a return to the original best axis in both planes. The frequency response data are difficult to compare due to the low spike count. The stimulus axis consistently used for the frequency stimuli was near the null for that cell; recovery was not complete, and the spike rate was too low for a valid frequency response post-tip at the lower of the two stimulus levels.

The assortment of responses to tipping obtained in the midbrain are illustrated in Fig. 9 (tipping data shown with symbols). TS unit P6 exhibited a rotation of the best axis in both planes during contra

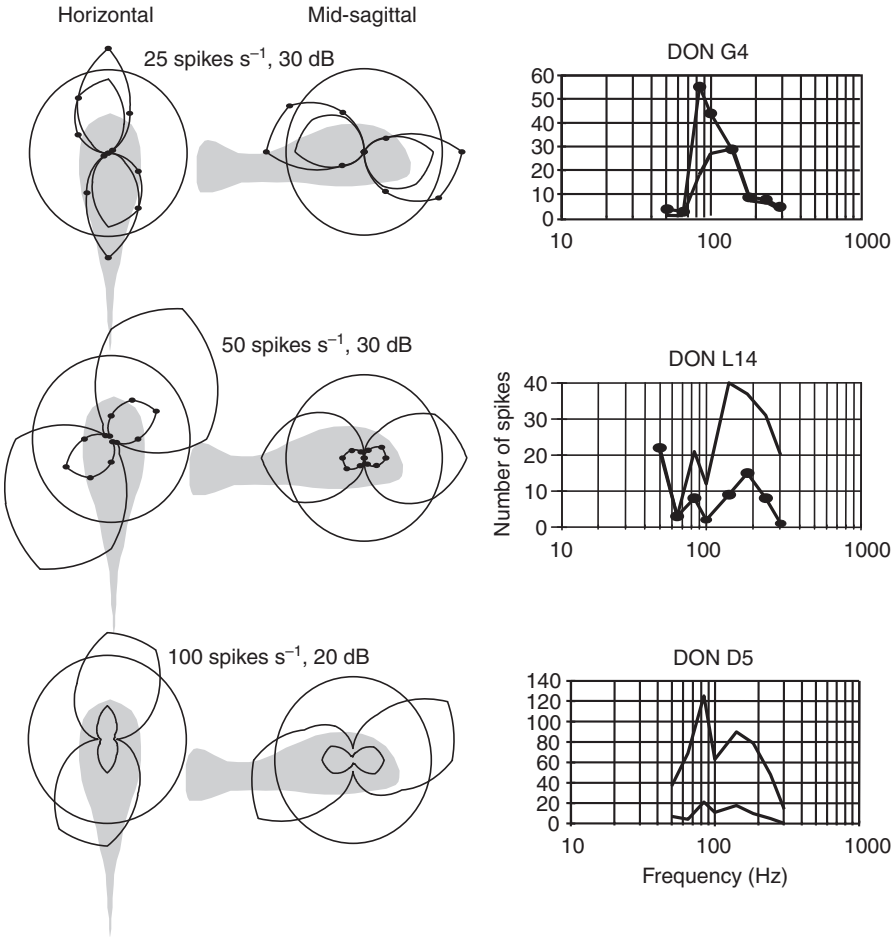

Fig. 7. Examples of different spike rate changes in DON units due to tipping contralateral saccular otolith. Symbols identify data obtained during tipping, except for D5. G4: increase in spike rate; L14: decrease in spike rate. D5: all activity was lost during tipping; spike activity was lower post-tipping (smaller curves) than pre-tipping (larger curves) due to incomplete recovery. Frequency responses shown on the right. S s^{-1}, spikes s^{-1}, indicated by the circular scale; stimulus levels in dB re: 1 nm.

tipping, with a substantial decline in responsiveness in the mid-sagittal plane. The frequency response exhibited a shift as well. TS unit F8 showed an increase in responsiveness when the ipsi otolith was tipped. Note that F8 was nearly omnidirectional in the horizontal plane prior to tipping, and the 90 deg. best axis in the mid-sagittal plane went to 0 deg. elevation (coinciding with the front–back axis in the horizontal plane). TS unit M5 exhibited no change in the best axis for the DRPs in either plane with contra tipping, but an increase in spike rate primarily in the mid-sagittal plane. Note that the minor secondary axis of response in the horizontal plane is present pre-tip and during tipping. The frequency response also shifted during tipping as though the input that responded well to 140 Hz had been removed. TS unit M1 exhibited an overall reduction in responsiveness (DRPs and the frequency response) with contra tipping. Although we generally saw the same types of changes in TS DRPs during tipping as seen in the DON, there was one major difference. Contra tipping resulted in a complete elimination of spike activity in only one TS cell. Lastly, phase studies were not conducted with the TS cells as they do not exhibit phase locking.

DISCUSSION

The experiments reported here illustrate that saccular otolith tipping could eliminate saccular input completely or could cause a change in the response characteristics of DON or TS cells. In some cases, spike rates were altered (increased or decreased) without a change in the directional responses. In other cases, the best axis or the shape of the DRP shifted along with spike rate changes. The shape of the DRP is an important consideration because sharpened DRPs must be the result of central computations, given the more rounded DRPs (cosine functions) characteristic of primary afferents from the saccule (Fay and Edds-Walton, 1997a; Edds-Walton and Fay, 2005a). These experiments reveal some clues as to the nature of directional computations that occur due to binaural convergence in the DON and the TS.

Otolith tipping resulted in four responses in DON cells, presented here in the order of their likelihood: (1) loss of activity, (2) increase in spike rate without DRP shift, (3) decrease in spike rate without DRP shift, and (4) a shift in the DRP shape/best axis. Given that we documented both increases and decreases in spike rate (with or without shifts in the DRP) during contralateral tipping, contralateral projections must include both inhibitory and excitatory inputs. The prevalence of loss of activity in the DON during contralateral tipping indicates that contralateral excitation is required for at least a subset of DON cells to reach threshold, further suggesting that the relative activity of the left and right ears (Fig. 1A) may be compared within the DON. Sand (Sand, 1974) illustrated that the left and right ears of the perch respond with different directional response functions when stimulated by particle motion in the horizontal plane, and he suggested that the combination of right and left saccule would provide complementary directional information with regard to azimuth of a sound source. The binaural data from the toadfish DON are consistent with that hypothesis. The toadfish data are also consistent with the results of the study by Horner and coleagues (Horner et al., 1980) in cod. They recorded reductions in the activity of auditory cells in the medulla (no site was labeled) with ipsilateral or contralateral blocks of the eighth nerve (Horner et al., 1980). In

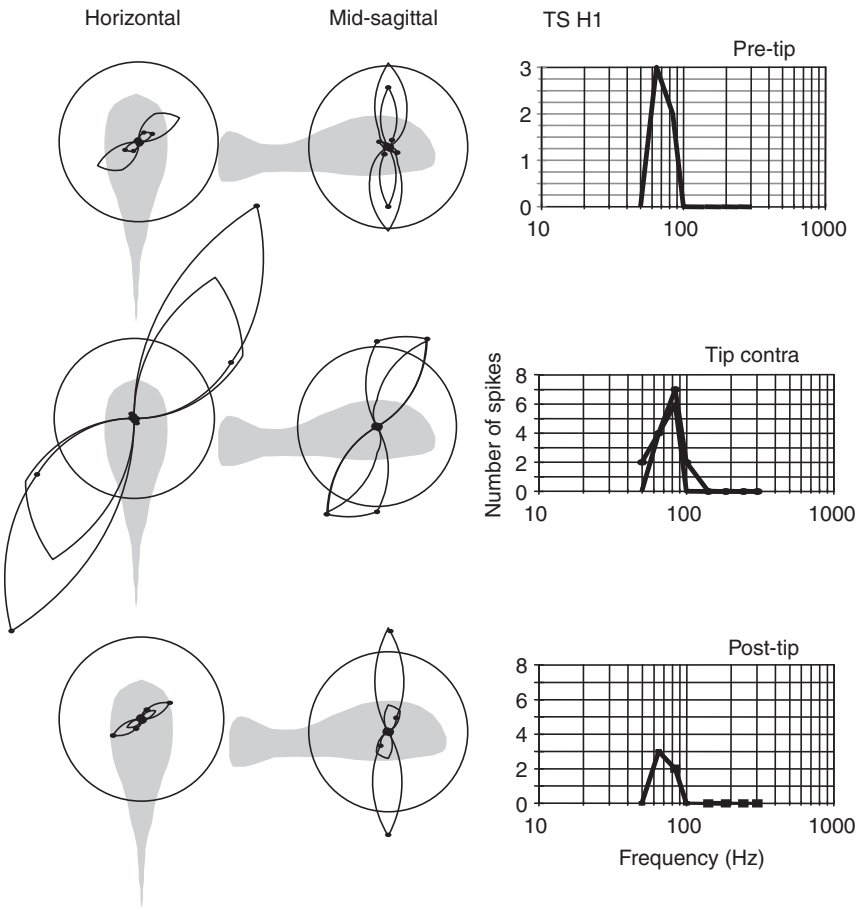

Fig. 8. Experimental data set for cell H1 in the TS. DRP and frequency response are shown pre-tip, during tip and 5 min post-tip of the contralateral saccular otolith; higher stimulus level indicated by symbols. The higher stimulus level had a slightly smaller DRP in both planes pre-tip. Increased spike rates and a shift in the best axis in the DRP occurred in both planes during tip. Interpolated best axes (average of two levels): pre-tip azimuth 60 deg., elevation 90 deg.; tipped azimuth 37 deg., elevation 65 deg.; post-tip azimuth 55 deg., elevation 85 deg. Stimulus levels: 20 and 25 dB re: 1 nm; circular scale for DRP is 20 spikes s^{-1}. Frequency responses are minimal because the standard stimulus axis was near the null for this unit.

addition, they were able to inhibit activity completely with a bilateral block. Although we did not attempt bilateral tipping due to space limitations for the tippers and the recording electrode in the skull, we expect that the same result might be obtained with DON cells using the tipping method.

Edds-Walton presented anatomical evidence for a homotopic commissural tract linking the dorsal auditory division of the DONs of toadfish (Edds-Walton, 1998). The ventral vestibular division of the DON (Highstein et al., 1992; Mensinger et al., 1997) also contributes homotopic projections to the commissural tract, leading to speculation that the commissural tract may be part of a common mode rejection circuit, as has been described in the electrosensory medulla of skates (New and Bodznick, 1990; Bodznick et al., 1999). Rejection of redundant bilateral information (or self-generated noise from opercular movements) may be a component of the DON commissural tract, but comparison of left and right auditory inputs by toadfish has an obvious role in directional hearing as well (Fay, 2005).

Contralateral tipping altered the DRP of an auditory cell more often in the TS than in the DON; however, auditory cells in the midbrain rarely lost activity during tipping. Both findings are important for modeling the directional circuit in teleosts. Spike rates among cells in the TS increased or decreased during tipping, indicating that inhibition or excitation can be associated with contralateral inputs. The persistence of some activity during tipping (and lack of complete loss of activity) suggest that there may be greater convergence of excitatory inputs in the TS than in the DON, which is consistent with the convergence of binaural inputs from DON and secondary octaval populations to the TS in toadfish, which has been demonstrated anatomically (Edds-Walton and Fay, 2005b). Finally, shifts in the best axis for the DRP in one or both planes (Figs 8 and 9) and, in some cases, a broadening of the directional response (i.e. loss of sharpening; Fig. 8) further support a role for binaural convergence in directional computations.

Encoding the axis of particle motion of a sound source is clearly a major driving force for the organization of the auditory pathway in toadfish and probably other teleost fishes. Evidence from taxonomically diverse species confirm that auditory hair cells are organized on auditory endorgan(s) along a variety of axes, providing wide-ranging directional sensitivity. Species with adaptations to respond to the pressure component of sound (e.g. air-filled structures connected mechanically to the ear) are scattered throughout the many families of fishes (Braun and Grande, 2008), but the endorgans in the ear of the pressure-sensitive species retain particle motion sensitivity as well. Although pressure reception is potentially important for phase comparisons that could clarify source direction (reviewed by Fay, 2005), our work addresses the directional vector inherent in particle motion that is encoded by the ears of all jawed fishes, including the more ancient lineages (sturgeons) (Meyer et al., 2005). The neural circuitry and computations associated with the detection of particle motion in primitive fishes served as the template for directional circuitry in modern fishes and, possibly, some terrestrial vertebrates (Carr and Edds-Walton, 2008).

The terrestrial tetrapod ear responds to the pressure component of sound in air, and the auditory response from the two ears is compared in the central nervous system, ultimately providing

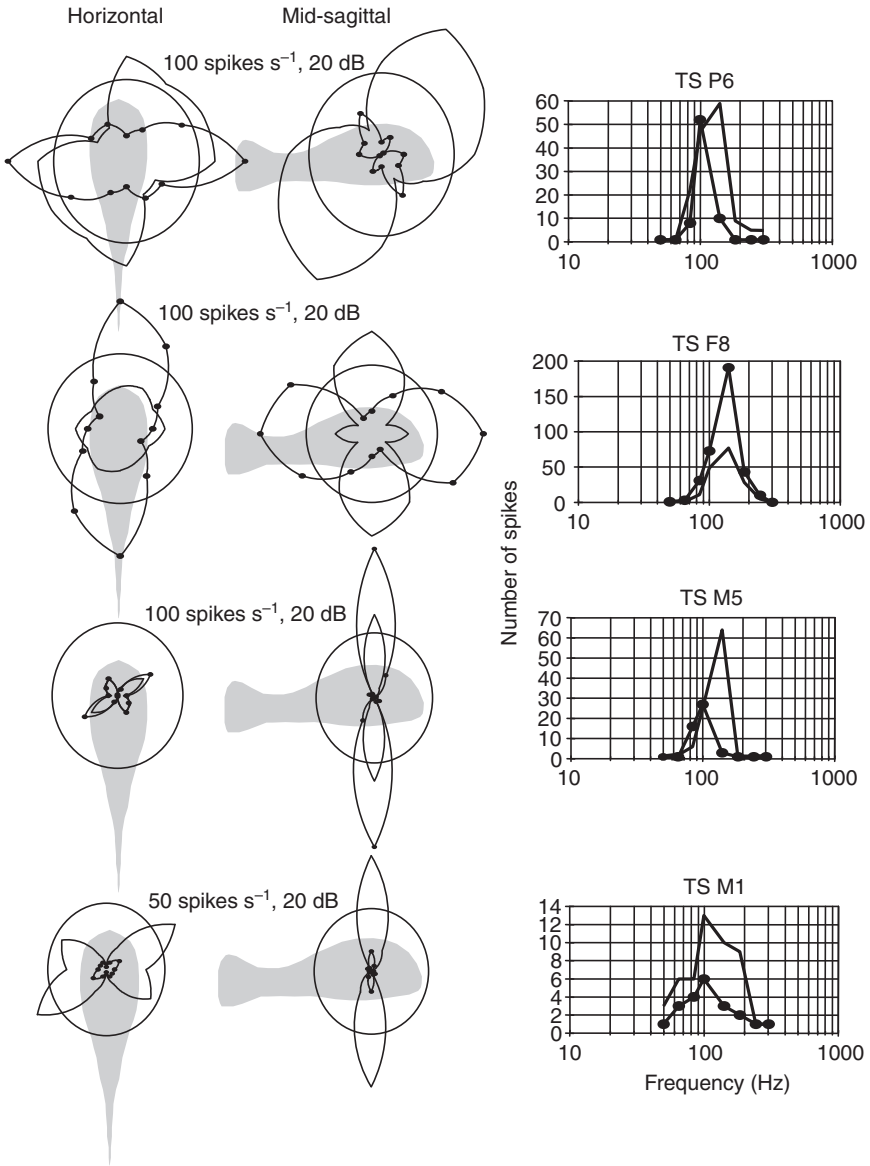

Fig. 9. Responses of four units in the TS to tipping. Circular symbols identify tipping data. P6: altered DRP and decreased spike rate in mid-sagittal plane, altered best axis in horizontal plane. F8: DRP altered in both planes, increased sharpening in horizontal plane and rotation of DRP in mid-sagittal plane. M5: change in spike rate in vertical plane only, no shift in best axis. M1: major reduction in spike rate in both planes, little or no change in DRP. Frequency response shown on the right. Spikes s^{-1}, indicated by circular scale; stimulus levels in dB re: 1 nm.

interaural data to 'compute' the location of the sound source [e.g. amphibians (Feng and Shofner, 1981; Christensen-Dalsgaard, 2005); birds (Klump, 2000); mammals (Brown and May, 2005)]. The first site of binaural comparisons along the auditory pathway for most terrestrial vertebrates is a second-order nucleus in the medulla (e.g. the superior olive) (reviewed by Carr and Edds-Walton, 2008). Frogs provide an interesting variation, however. The auditory endorgans of the anuran ear project ipsilaterally to the dorsal medullary nucleus (DMN), which projects bilaterally to a secondary auditory nucleus (called the superior olive). There also is a commissural tract connecting the two DMNs (Feng, 1986). Feng and Capranica (Feng and Capranica, 1976) provided early evidence of binaural cells in the DMN and suggested that contralateral input could be either excitatory or inhibitory. A study by Christensen-Dalsgaard and Kanneworf (Christensen-Dalsgaard and Kanneworf, 2005) revealed directional sharpening in the DMN, which they suggested was the result of binaural interactions.

Our data indicate that despite major differences in the auditory periphery, the auditory medulla in toadfish and frogs may be organized similarly with regard to binaural sites, although there are probably differences in the details of the computations that occur there. Anurans have a tonotopic organization to the DMN, and there is good evidence that both ILD and ITD are reflected in the response properties of some DMN cells (for reviews, see Feng and Schellart, 1999; Christensen-Dalsgaard, 2005). There are no comparable physiological data from a teleost fish.

To date, no well-defined vector map of auditory space has been found in toadfish (Edds-Walton and Fay, 1998). For example, vertical tracks in the dorsal DON sometimes yield cells with similar characteristics, but not consistently (P.L.E.-W. and R.R.F., unpublished data). Furthermore, no study has been able to reveal a division of the auditory circuit with response characteristics relevant to the resolution of the 180 deg. confusion inherent in the particle motion component of sound (Fay, 2005; Wubbels and Schellart, 1998b). Spatial patterns of activity, as described for directional mechanoreceptor maps in crickets (Jacobs, 1995) is another organizational framework worthy of consideration for the DON and TS in fishes.

Taken together, the data from the dorsal DON and the auditory TS indicate that there are inhibitory and excitatory inputs with

bilateral origins that are combined in unpredictable ways, resulting in a variety of directional responses. Principles proposed by Huggins and Licklider [page 299 (Huggins and Licklider, 1951)] seem especially applicable here:

> "The principle of sloppy workmanship states that it is dangerous to postulate a neural structure that is precisely arranged in detail…One of the basic facts of neurophysiology is that the nervous system works despite a considerable amount of misarrangement of detail…The principle of diversity states that the nervous system often hedges: Instead of presenting a single transform of the peripheral stimulation to the higher centers, the auditory tract may present a number of transforms…The principle of diversity suggests that a simple description of the auditory process may not be possible because the process may not be simple."

The variety of results that we obtained suggests that the circuit is not as simple as originally modeled (Fay and Edds-Walton, 1999; Edds-Walton and Fay, 2003).

LIST OF ABBREVIATIONS

DMN	dorsal medullary nucleus (anurans)
DON	descending octaval nucleus (medulla of fish)
DRP	directional response pattern
ILD	interaural level difference
ITD	interaural time difference
TS	torus semicircularis (midbrain)

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