What the Toadfish Ear Tells the Toadfish Brain About Sound

Peggy L. Edds-Walton

Abstract Of the three, paired otolithic endorgans in the ear of teleost fishes, the saccule is the one most often demonstrated to have a major role in encoding frequencies of biologically relevant sounds. The toadfish saccule also encodes sound level and sound source direction in the phase-locked activity conveyed via auditory afferents to nuclei of the ipsilateral octaval column in the medulla. Although paired auditory receptors are present in teleost fishes, binaural processes were believed to be unimportant due to the speed of sound in water and the acoustic transparency of the tissues in water. In contrast, there are behavioral and anatomical data that support binaural processing in fishes. Studies in the toadfish combined anatomical tract-tracing and physiological recordings from identified sites along the ascending auditory pathway to document response characteristics at each level. Binaural computations in the medulla and midbrain sharpen the directional information provided by the saccule. Furthermore, physiological studies in the central nervous system indicated that encoding frequency, sound level, temporal pattern, and sound source direction are important components of what the toadfish ear tells the toadfish brain about sound.

Keywords Descending octaval nucleus • Hearing • Saccule • Torus semicircularis

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1 Introduction

When considering the vast work on fishes conducted by Arthur Popper and Richard Fay, the obvious theme is a better understanding of how (and what) fish hear. However, each took a different approach. Much of Popper's fish research was driven by an interest in the organization and functions of the sensory hair cells, what one might call a "bottom-up approach." Much of Fay's research used psychophysical methods to investigate and define the limits of the sense of hearing in fishes, using a comparative approach and methods previously used for other vertebrates, particularly mammals, which one might call a "top-down approach."

Popper revealed an unexpected diversity of orientations for sensory hair cells (e.g., Fig. 1a, b) on the otolithic endorgans of fishes (e.g., Popper 1977). Of the three otolithic endorgans that may be involved in hearing (the lagena, the saccule, and the utricle), it was the saccule that showed the greatest variety among species that he
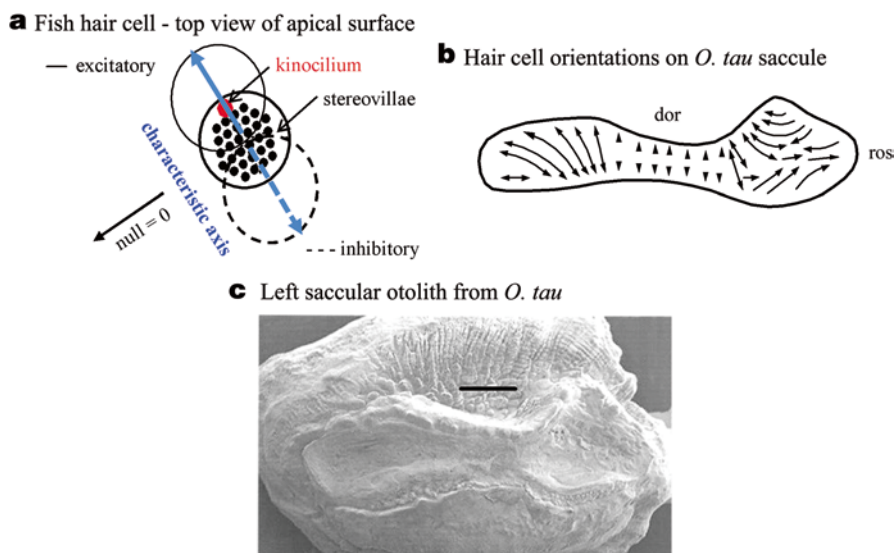

Fig. 1 Sensory hair cells on the toadfish saccule. (**a**) Apical view of morphological and physiological polarity of hair cell. The hair cell has a cosine response function, shown as a polar plot. The hair cell is excited by particle motion that bends the stereovillae toward the kinocilium (*solid blue arrow*), which results in excitation of the primary afferent. Hair cell activity is inhibited when the apical structures move in the opposite direction (*dashed blue arrow*) and there is a null (*thick black arrow*) along an axis perpendicular to the characteristic axis (*blue line*). (**b**) Hair cell orientations for regions on the epithelium are illustrated by an *arrow* indicating the excitatory direction [like *solid blue arrow* in (**a**)]. All orientations are opposed by a 180° counterpart, but the point of orientation reversal varies in the regions where the *arrows* are connected. In the middle of the saccule, the opposition line is generally along the center of the epithelium. (**c**) Scanning electron micrograph of the saccular otolith illustrating the location of the smaller epithelium along the sculptured otolith surface. A gelatinous layer connects the epithelium to the otolith (not shown). Note the curvature along the edges of the saccular depression. Scale bar = 1 mm (modified from Edds-Walton et al. 1999)

and others examined. Popper (1981) proposed a broad classification system for saccular hair cell orientation "patterns," and Coombs and Popper (1979) suggested a functional hypothesis for some of the diversity, based on their studies of Hawaiian squirrelfish species with different audiograms and different hair cell orientation patterns. Although that hypothesis has not proven to be a valid generalization for all fishes, those studies led to major questions about the functional significance of the variations observed among the saccules of teleosts (Popper and Coombs 1982). Work by Flock (1971) and Hudspeth and Corey (1977) on the physiological polarity of vertebrate hair cells provided the basis for a variety of hypotheses on the potential role that hair cell orientations might play in encoding the direction of a sound source (e.g., Moulton and Dixon 1967; Schuijf 1975, 1976; Saidel and Popper 1983; Schellart and deMunck 1987; Rogers and Zeddies 2008). Documented variations in the apical structures (a single kinocilium and a stair-step array of stereovillae) on individual hair cells also stimulated research on frequency response and the potential for regional response differences along the saccular epithelium (e.g., Furukawa and Ishii 1967; Sugihara and Furukawa 1989).

Around that time, Fay (1984) conducted a landmark study that introduced the field of fish bioacoustics to his three-dimensional particle motion stimulus system, which allowed him to collect the data that proved all three otolithic endorgans of the goldfish (*Carassius auratus*) had overlapping frequency responses. In addition, each endorgan encoded the axis of particle motion as predicted, based on the orientation of the endorgan and the orientations of the hair cells on each sensory epithelium. Fay went on to study many aspects of the sense of hearing in goldfish by classical conditioning (respiration or heart rate) and various psychophysical procedures (see Fay 1988 for details), first obtaining a response to a stimulus the fish could easily detect and then determining whether the fish could detect another stimulus with a difference in frequency content, intensity, or temporal parameters, or a stimulus in the presence of various types of "noise." The reader is referred to Fay (2014), wherein he summarized his contributions to our understanding of the sense of hearing in goldfish, especially as it compares to the sense of hearing in humans. It is important to note here that the goldfish is a nonvocal teleost fish that nonetheless has specializations of the auditory pathway (Weberian ossicles) that provide increased sensitivity to higher frequencies (e.g., 500 Hz to about 5000 Hz) than the majority of fish species investigated to date, which lack similar specializations and hear only lower frequencies (e.g., below 500 Hz; Popper and Fay 1999). The importance of Fay's work with goldfish was in revealing what the goldfish "knows" about the sounds (or noise) around it, including the concept of "auditory scene" analyses (introduced by Bregman 1990) or the "soundscape," which Fay has often addressed in his papers and presentations (Fay 2009, 2014).

As a beginning graduate student in the Popper lab, I was struck by the volume of information known about auditory processing in the non-vocal goldfish and the relative lack of information about auditory processing in *vocal* fish, particularly with regard to sound source localization. The oyster toadfish, *Opsanus tau* (Linnaeus 1766) was my first marine research subject as an undergraduate working in the lab of the famous "shark lady," Eugenie Clark, where I studied their shelter-seeking

behavior and social interactions. I also had become familiar with their vocal behavior (Tavolga 1958, 1964). The locally available species was an obvious choice in which to address questions about how a fish locates a vocalizing conspecific. Arthur Popper approved, and thus began more than 20 years of research on auditory processing in oyster toadfish.

This review of research completed during collaborations with Arthur Popper and Richard Fay not only summarizes some of the important contributions to understanding what toadfish (and other teleosts) hear but also reflects their influence on the questions asked, the hypotheses generated, and the interpretation of the data obtained. None of this research would have taken place without them.

The focus of my research has been on how the toadfish ear and central nervous system encode and process sounds of biological significance. Sound consists of the alternating compression and rarefaction of the medium through which sound travels, producing both a pressure wave and particle motion. Although some fish can detect the pressure component of sound through an indirect mechanism (involving an internal, gas-filled structure), all fish ears respond directly to the particle motion component (Popper and Fay 2011). Lu (2011) provides an excellent introduction to auditory processing in fishes in general and Radford et al. (2012) provide an experimental comparison of particle motion and pressure stimulation among three teleost species that differ in the presence or absence of an association between the ear and the gas bladder. The physiological research that we conducted on toadfish has investigated only how the toadfish ear responds to particle motion and how the vector of particle motion is encoded in the central nervous system as the basis for determining the location of a sound source.

As in other vertebrates, the ear in fishes has three orthogonal semi-circular canals and three otolithic endorgans that provide information about the position and movement of the head (orientation with respect to gravity, linear acceleration, angular acceleration). The reader is referred to Straka and Baker (2011) for a general introduction to the vestibular (or positional) sense, which is fairly consistent across species and is applicable to what the toadfish ear tells the toadfish brain with regard to position. Unlike other vertebrates, one or more of the three otolithic endorgans in fishes encode the particle motion component of sound. The otolithic endorgan acts as an inertial accelerometer: the sensory hair cells are stimulated by the shearing motion caused by relative motion of the sensory epithelium with respect to the much denser, calcareous otolith. The sensory hair cells are oriented in various directions on the endorgan, resulting in response characteristics across the endorgan that could be used to compute the direction of the sound source in 3-dimensional space (see Sect. 3.1). The focus of this paper will be what we have learned about what the toadfish ear tells the toadfish brain about the particle motion component of sound.

The behavioral repertoire of oyster toadfish includes the establishment of reproductive territories around a nest site constructed by males, sound production in both agonistic and reproductive contexts, and behavioral responses to conspecific sounds by both sexes (Gray and Winn 1961; Winn 1972; Fish 1972). Behavioral observations confirm that multiple reproductive males do not occupy the same nest sites, and that females are attracted to nest sites with vocal males (Gray and Winn 1961;

Fish 1972). Detailed observations of the natural behavior of the Lusitanian toadfish, a member of the same family (Batrachoididae) with similar reproductive behaviors, revealed that males avoid sites where a vocalizing male is present (Vasconcelos et al. 2010). Therefore, we have good indications that both sexes "listen" to conspecific vocalizations. In general, the agonistic sounds produced by both sexes are broadband pulses rapidly produced in brief bursts (<150 ms) that sound like a "grunt" (Winn 1972). The reproductive advertisement call is a sustained pulsing (up to 500 ms) called a "boatwhistle" with a harmonic structure that is a function of the pulse repetition rate (Watkins 1967). The harmonic structure of the boatwhistle varies somewhat among individuals and populations (Fine 1978; Edds-Walton et al. 2002); however, behavioral studies by Winn (1967) revealed that toadfish respond equally well to pure-tone sounds around 100–200 Hz. Therefore, the majority of our research on auditory processing in toadfish has used tonal stimuli rather than pulsed sounds. There is no doubt that the physiological data collected in our lab reflect normal auditory processing that functions during natural behavior.

2 Overview of Methods

2.1 Oyster Toadfish

The majority of the fish used for these studies were obtained from the waters around Woods Hole, MA or Cape Cod, MA by the Marine Resources Center (MRC) at the Marine Biological Laboratory (MBL). The fish were maintained in large cement tanks with flow-through local seawater at ambient temperatures in the MRC until removed to be research subjects. Toadfish chosen for experimentation were placed in plastic tubs with flow-through, filtered and chilled local seawater (usually 16–18° C) for at least two weeks. Fish were fed twice weekly with small fish or clam chunks obtained from the MRC. Fish were not fed for one week prior to use to ensure complete digestion of food to prevent regurgitation and fouling of the gills during anesthesia and to reduce fouling of the water during the experiments.

When local toadfish availability was limited, individuals were obtained from New Jersey waters by the MRC. Although believed to be the same species, the New Jersey toadfish were used only for anatomical experiments because their physiology appeared to differ from the local toadfish (e.g., they required more anesthesia and metabolized the anesthesia more quickly, possibly due to the warmer temperatures of their home waters). Therefore, we never used New Jersey toadfish for experiments in which we obtained auditory threshold data. In addition, Massachusetts toadfish were sometimes retained over the winter in the MRC for use in the spring months before local toadfish were being caught. Those "overwintered fish" were used one summer due to a total lack of appropriately sized local fish. The physiological data (e.g., hearing thresholds) from the overwintered fish differed significantly from the data obtained from freshly caught, local fish (unpublished data); thus, only anatomical (e.g., recording site) and tract-tracing data from overwintered toadfish

were included in data sets. The care and use of the toadfish during experimentation was approved by the IACUC at the MBL and at Loyola University Chicago, Parmly Hearing Institute.

2.2 Tract-Tracing

The tract-tracing studies included a variety of methods and labels, including DiI (1,1 dio-octadecyl 3,3,3,3 tetramethyl-indocarbocyanine-perchlorate, Sigma), biotinylated dextran amines (rhodamine, fluoroscein, cascade blue; 3000 and 10,000 MW, Molecular Probes), and neurobiotin (Molecular Probes). Details of the neuranatomical methods may be found in Edds-Walton (1998a, b), Edds-Walton et al. (1999), and Edds-Walton and Fay (2005a).

2.3 Physiology

All physiological studies were conducted using stimuli produced by the "shaker system" designed by Fay and described in detail in Fay and Edds-Walton (1997a, b). This unique stimulus system provides a particle-motion dominated sound field that is well controlled and predictable. The shaker system consists of a vibration-isolated, open cylinder attached to a single vertical shaker (beneath the cylinder) and paired mini-shakers (front–back and side–side stimulation). The shaker system is programmed (and calibrated daily) to provide particle motion stimulation at specified frequencies (50–300 Hz) along designated axes in the horizontal and midsagittal planes (0°, 30°, 60°, 90°, 120°, 150°). Particle motion is a vector quantity with direction, frequency, and magnitude, and it is the component of sound to which all fish ears respond (Fay 2005; Popper and Fay 2011; Radford et al. 2012). Simply, each stimulus (500 ms duration, 20 ms rise, fall; repeated 8 times) consisted of controlled movement along a single axis in a single plane (see Fig. 3 in Edds-Walton and Fay 2008) to simulate particle motion produced by a sinusoid at a single frequency and designated level (dB re: 1 nm). Displacement was measured by three orthogonally positioned accelerometers mounted on the cylinder. Stimulation with the mini-shakers did not permit determination of the excitatory direction along the designated axis, so all directional response pattern (DRP) illustrations consist of the best axis without regard to the excitatory versus inhibitory segment. In other words, although the DRPs for cells look like the cosine function of a single hair cell (Fig. 1a), the actual excitatory direction is not known, only the best axis (see Fay and Edds-Walton 1997a for a detailed explanation of the DRPs).

The fish was positioned in a custom head-holder within the cylindrical dish containing chilled seawater at a depth sufficient to submerge the gills. The dorsal surface of the fish was covered with a paper towel in contact with the water surface so that the entire body was kept moist, but the surgical area was free of water. An

injection of pancuronium bromide (0.1–0.4 mg in toadfish saline, dependent on fish size) in the tail musculature prevented swimming movements of the tail, but the opercula moved and aerated the gills normally. Opercular movements did not interfere with the recording of auditory afferent activity, but could modulate the activity of lateral line cells in the medulla or bimodal cells (lateral line and auditory responses) in the midbrain. Water temperature and oxygen levels were maintained by replacing half of the water in the cylinder at 2 h intervals.

The shaker system produced movement of the entire dish in nanometer amounts, simulating particle motion stimulation at biologically relevant levels. The attachment of the head-holder to the dish ensured that the fish moved with the dish, and no water movement was induced around the fish by the stimuli. Thus, there was no hydrodynamic flow around the fish and the lateral line system was not stimulated unless we produced water movement around the fish using a glass pipette (to test for lateral line responsiveness, see Edds-Walton and Fay 2005a).

The experiments that will be summarized in this chapter included physiological recordings from (1) primary afferents as they exited the saccule, cells in (2) the descending octaval nucleus (DON) and (3) the magnocellular octaval nucleus (MON) in the medulla, and cells in (4) the auditory nucleus centralis (and the lateral line nucleus ventrolateralis (NVL)) in the torus semicircularis of the midbrain (Fig. 2b, c). Although the surgical approach varied somewhat for each recording site, the surgical procedures included the same initial steps. The dorsal skin and musculature were removed and the dorsal braincase was scraped with dental tools until thin enough to remove without damaging the underlying tissues or blood supply. The required region of the otic capsule or the brain was exposed carefully and fluids around the ear and brain were replaced by a clear, inert fluorocarbon (FC-77). Pulled glass electrodes were mounted on a 3D micromanipulator and lowered into a branch of the VIIIth cranial nerve, an octaval nucleus of the medulla, or the midbrain (based on surface landmarks and confirmed by neurobiotin injections at recording sites). For more details about the methods, please see the original papers (e.g., Edds-Walton et al. 1999; Edds-Walton and Fay 2005b; Edds-Walton and Fay 2008).

3 Anatomy and Physiology of the Toadfish Auditory System

As noted earlier, the insights into auditory processing by oyster toadfish were obtained over more than 20 years. The work that is summarized here is organized anatomically from the periphery to the medulla to the midbrain. The peripheral investigations were limited to the saccule, whose inputs to auditory processing sites in the medulla had been confirmed anatomically by Highstein et al. (1992) and Edds-Walton (1998a, b). Systematic investigations of auditory responsiveness were not conducted on the utricle or the relatively small lagena due to technical difficulties in reaching their afferents consistently without altering their normal orientations in the otic capsule or encountering afferents from the semicircular canal cristae that are also associated with those branches of VIII (see Fig. 3 in Edds-Walton
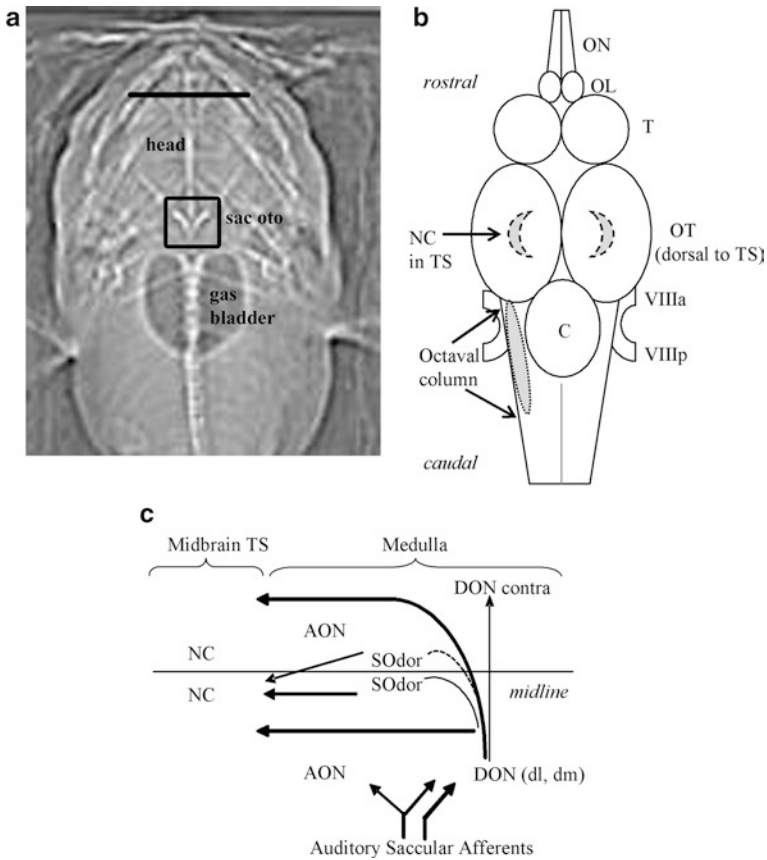
Fig. 2 Auditory structures and sites described in text. (**a**) Topogram of toadfish, revealing saccular otoliths (sac oto in the otic capsule, enclosed in box). The bi-lobed gas bladder is visible caudal to the otoliths; scale bar is 3.5 cm. (**b**) Cartoon of *Opsanus tau* brain: dorsal view showing general location of auditory regions. The octaval column consists of the five nuclei receiving input from VIII, which lie deep to the surface of the medulla, indicated by the grey oval surrounded by the *broken line*. The auditory nucleus in the midbrain (nucleus centralis, *dashed outline*) is beneath the ventricle in the midbrain (below the optic tectum). Drawing not to scale. (**c**) Auditory circuit in toadfish defined by anatomical and physiological studies. Confirmed auditory afferents from the saccule project to AON and dorsal DON, with some bifurcating fibers that project to both octaval nuclei. Auditory projections from DON to SOdor and from SOdor to the auditory midbrain (NC) were obtained anatomically, without physiological confirmation that SOdor has auditory response characteristics. *Dashed line* indicates limited evidence for projection from DON to contralateral SOdor. Abbreviations: *AON* anterior octaval nucleus, *C* cerebellum, *CC* crista cerebellaris, *contra* contralateral, *dl* dorsolateral, *dm* dorsomedial, *DON* descending octaval nucleus, *NC* nucleus centralis, *OL* olfactory lobe, *ON* olfactory nerve, *OT* optic tectum, *SOdor* dorsal secondary octaval nucleus, *T* telencephalon (forebrain), *TS* torus semicircularis (midbrain), *VIIIa* anterior branch of acoustic cranial nerve, *VIIIp* posterior branch of acoustic cranial nerve (topogram by D. Ketten, CSI Lab, Woods Hole Oceanographic Institution)

1998a). Therefore, while we have learned much about the contribution of the saccule to auditory processing, we do not know all that the ear tells the brain about sound (see Sect. 5). In each of the studies described here, auditory processing was evaluated in sexually mature toadfish of both sexes that were obtained during their spring and early summer breeding season.

3.1 The Ear of the Toadfish

Each of the otolithic endorgans consists of the sensory epithelium, a calcareous otolith, and a gelatinous substance that mechanically links the epithelium to the otolith in a fluid-filled sac within the otic capsule. The saccule is the largest of the otolithic endorgans in oyster toadfish. The saccular otolith is a dense, highly sculptured structure that is curved in both the vertical and horizontal planes (Figs. 1c and 2a). The sensory epithelium on which the hair cells are located is smaller in area than the otolith, but both are intricately associated such that the greater relative motion of the epithelium (e.g., from particle motion in a sound field) causes deflection of the apical structures on the hair cells.

As shown in Fig. 1, maximum excitation occurs along a central axis, toward the single kinocilium. The hair cell response is inherently directional with a single maximum excitatory direction (solid blue line in Fig. 1a), decreasing responsiveness along other axes and a null perpendicular to the best axis (modeled as a cosine function of relative activity versus angle of stimulation; Fig. 1a). The hair cell orientations on the sensory epithelium dictate the directional responsiveness of the endorgan. In toadfish, the saccule is oriented in the dorsal–ventral plane, with a sweeping hair cell orientation on both the rostral and caudal saccule, and a region of vertically oriented hair cells in the middle (Fig. 1b). Thus the hair cells on the saccule can respond to a particle motion stimulus at any angle in the vertical plane; however, the responsiveness in the horizontal plane is restricted by the angle of the endorgan with respect to the midline of the fish and the orientation of the endorgan on the curved otolith (Fig. 1c).

Like all components of the ear, the saccule is a paired endorgan. The saccular otoliths are heavily calcified and are obvious on X-rays, lying on either side of the midline (Fig. 2a) in the otic capsule. The rostral saccular otoliths lie at approximately ±35° with respect to the midline of the fish, and the caudal end is curved to lie adjacent to the midline in the otic capsule. This angled orientation is important because the left and the right saccules "point" into different regions of acoustic space, and their directional responses will be different, though complimentary. Non-parallel orientation of the saccules is also seen in other fishes that are not closely related to the toadfish (e.g., perch, Sand 1974; cod, Dale 1976; trout, Schellart and Buwalda 1990; sleeper goby, Lu and Xu 2002). The functional significance of the non-parallel orientation may lie in the location-dependent binaural contributions for encoding the direction of a sound source (see Enger et al. 1973; Schuijf and Buwalda 1975; Schellart and deMunck 1987; Schellart and Buwalda 1990).

3.2   Circuits: What Goes Where

The "acoustic nerve," cranial VIII, includes afferents from the sensory epithelia in the endorgans of the ear: the three canal cristae and the otolithic endorgans (the lagena, saccule, and utricle). Efferent axons (from the paired efferent nuclei in the midline of the medulla) are also found in VIII as they travel to their peripheral projection sites. Afferent axons from the peripheral endorgans send their input to the octaval column in the medulla (Fig. 2b, c). The octaval column has five nuclei in toadfish (and all teleosts): the anterior octaval nucleus (AON), the MON, the DON, the tangential octaval nucleus (TON), and the posterior octaval nucleus (PON), as reported in detail by Highstein et al. (1992).

3.2.1   Saccular Inputs

Labeling of the entire saccular nerve bundle revealed projections to four of the five octaval nuclei in the medulla, in order of greatest to fewest projections: DON, AON, MON, and minimal input to TON. Distinct neuron bundles from the rostral, middle, and caudal saccule were labeled individually and in pairs to evaluate the organization of input from hair cells with different orientations (compare regions in Fig. 1b) in the two nuclei believed to be involved in auditory processing, the DON (dorsal division, which includes both medial and lateral subdivisions) and AON (Edds-Walton 1998a, b). The hypotheses tested included (1) a topographic organization with afferent input in DON and/or AON reflecting the rostral-caudal organization of the saccule; (2) vector organization, representing hair cell orientations, and (3) extensive overlap of inputs consistent with analyses of the pattern of activity across the entire saccule.

The regional labeling of saccular afferents as they exited the sensory epithelium was designed to provide comparable projection data from (1) the afferents of the vertically oriented hair cells (90°, dorsal and ventral with regard to the fish) in the middle of the saccule, (2) from the rostral sweeping hair cell orientations (0–90° in the vertical plane), and (3) from the caudal sweeping orientations (also 0–90° in the vertical plane; Fig. 1b). The regional analyses revealed that there is great overlap in the primary afferent projections that represent different hair cell orientations (but see Fig. 6 in Edds-Walton 1998a for parallel input from the middle saccule). In other words, there was no evidence for a simple topographic or vector map in either the elongate DON or the much smaller AON. The data indicate that primary afferents from different hair cell orientations on the saccule converge in the lateral to medial axis *and* in the rostral to caudal axis of the dorsal region of the DON (= dDON, above the descending tract of cranial V), as well as throughout the medial AON. From the viewpoint of determining the direction of a sound source, the computations in the DON are likely to consist of "weighting" of multiple inputs that converge onto the dendrites of the principal cells (although some axosomatic endings were seen in dDON; Edds-Walton 1998a).

More specific projection data were obtained from physiologically characterized saccular afferents that were injected with neurobiotin (see Physiology below). Auditory afferents sometimes bifurcated and sent a process rostrally to AON and caudally to the dorsal subdivision of DON, indicating that the same auditory input can go to both nuclei (Edds-Walton et al. 1999). However, more afferents went exclusively to the dDON, and the input had a distinctive lateral to medial organization. Individual primary afferent fibers projected along the length (rostral to caudal) of the DON, with medial projections bearing bouton-like endings at multiple sites along the length of DON (Edds-Walton et al. 1999). These anatomical data indicate that there is redundancy in the input from the saccule that occurs along the length of the dDON, which may reflect a computational axis for directional analyses. Additional studies have shown that there are topographic commissural connections between the left and right DONs that would permit binaural computations (Edds-Walton 1998b; and see Physiology below).

Lastly, examination of the afferent branches from the saccular epithelium during the labeling study reported by Edds-Walton (1998a) revealed interesting variations in afferent organization that may provide clues to the location(s) of hair cells with response characteristics consistent with vestibular functions (e.g., tilt perception) on the saccule. In all toadfish, a small but distinct bundle of afferents (distinguished from efferents by the presence of somata in the periphery) exited the rostral tip of the saccule and merged with the anterior and horizontal canal cristae and utricular inputs, rather than with the rostral bundle of saccular afferents. In some toadfish, a small bundle of afferents exited vertically from the caudal saccule and joined VIII with afferents from the lagena and posterior canal crista (see Fig. 2A in Edds-Walton 1998a). These anatomical data suggest that if hair cells with vestibular response characteristics are present on the saccule, their distribution may be limited to the most rostral and most caudal sites along the saccular epithelium.

3.2.2 The DON

The organization of the DON is interesting in that the pattern of inputs across species indicates that DON has both vestibular and auditory roles (McCormick 2011). In toadfish, as in other teleosts, the dorsal regions receive inputs primarily from the otolithic endorgans (Fig. 3a); the ventro-lateral region of DON receives inputs primarily from the semicircular canal cristae (Highstein et al. 1992). Utricular afferents project to dorsolateral sites in DON (Highstein et al. 1992; unpublished data) where they may overlap with saccular inputs, but there is also substantial input from the utricle to the ventrolateral region (Highstein et al. 1992). The distribution of utricular inputs in DON is consistent with both auditory and vestibular (orientation) roles for that endorgan.

As noted previously, Fay (1984) provided physiological evidence that utricular and lagenar afferents in goldfish responded to 140 Hz with directional response characteristics consistent with the orientation of each endorgan and its hair cell orientation pattern, providing evidence that both endorgans could contribute to
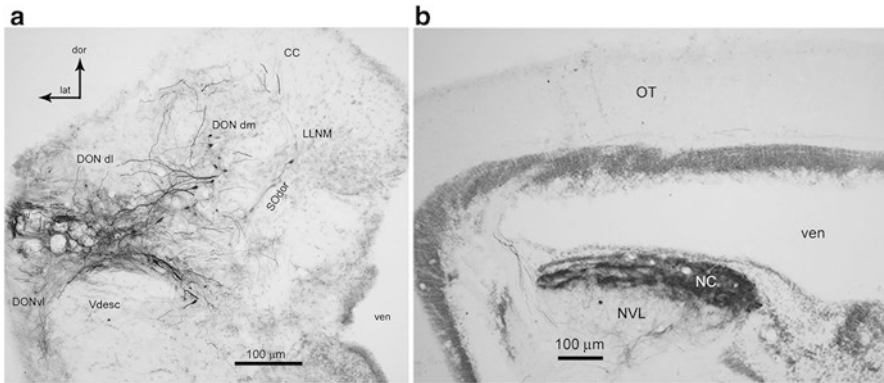
Fig. 3 Auditory processing regions in (**a**) the descending octaval nucleus in the medulla and (**b**) nucleus centralis of the midbrain torus semicircularis following injections of neurobiotin at physiologically confirmed auditory sites. Note the lack of auditory cells in ventrolateral DON (*vl*), which receives input from semicircular canal cristae. Some efferent fibers pass through DONvl. A subset of cells in the dorsolateral (*dl*) and/or dorsomedial (*dm*) region of DON project to the secondary octaval dorsal nucleus (*SOdor*); both dl and dm project to NC. The midbrain torus semicircularis has a dorsal auditory region (NC) and an underlying lateral line processing area where bimodal cells are also located (nucleus ventrolateralis, NVL). Other abbreviations: *dor* dorsal, *lat* lateral, *LLNM* lateral line nucleus medialis, *OT* optic tectum, *Vdesc* descending tract of cranial V, *ven* ventricle (**a** was modified from Edds-Walton et al. 2010)

directional computations along with the saccule. McCormick and Wallace (2012) conducted an elegant study in goldfish in which they were able to show that all three otolithic endorgans send projections to identified auditory projection cells in DON. Although the saccule contributed the most input to the auditory cells in the goldfish DON, most often with bouton-like endings on the somata, McCormick and Wallace found evidence that the utricle or lagena also provided input to some of those auditory projection cells (see Table 2, McCormick and Wallace 2012). The anatomy indicates convergence of the otolithic endorgan inputs in the dorsal regions of the DON, but the physiological response properties of the DON projection cells receiving those inputs are unknown. Given that the goldfish has an otophysic connection that mechanically links movement of the gas bladder to the fluids in the ear, resulting in enhanced reception of the pressure component of sound, input from the endorgan encoding the indirect particle motion from the gas bladder (presumed to be the saccule in goldfish) may be parceled in some way for phase comparisons with input from an endorgan receiving only direct particle motion.

If the organization of dDON in goldfish includes a division for phase comparisons, projections from otolithic afferent input to the dDON in a fish lacking an otophysic connection may reveal a different organization of inputs. Tomchik and Lu (2005) examined the central projections of afferents from all three otolithic endorgans in the "non-otophysic" sleeper goby, in which, like the goldfish, the saccule, utricle, and lagena have overlapping frequency responses and distinct directionality. They found inter-digitating projection sites for the three otolithic endorgans in the

octaval nuclei, including dDON. Therefore, convergence of otolithic inputs in the dDON may be common among fishes, including those with different peripheral anatomy.

3.2.3 Medulla to Midbrain

Injections of neurobiotin into physiologically characterized auditory sites in the midbrain nucleus centralis (NC in Figs. 2b, c and 3b) revealed the sites in the medulla that are components of the ascending auditory pathway. Inputs to the auditory midbrain originate primarily in the contralateral DON, with smaller contributions from the ipsilateral DON and secondary octaval (SO) nuclei in the medulla, which may receive input from both the ipsilateral and contralateral DONs (Edds-Walton and Fay 2005a) (Figs. 2c and 3b).

The function of AON in the auditory circuit of toadfish remains to be revealed. Cells in AON receive substantial saccular input (Edds-Walton et al. 1999) and respond to auditory frequencies (Edds-Walton, unpublished data), but the connectivity of AON is not known. A small number (<5) of retrogradely filled cells were present in AON in some, but not all, investigations of inputs to nucleus centralis (Edds-Walton 1998a; Edds-Walton and Fay 2005a). The scarcity of these fills suggests either that AON contributes little to the ascending auditory pathway or that AON contributes indirectly, via intermediate nuclei, such as the secondary octaval nuclei. For the second scenario, the retrograde fills in AON would have been due to trans-synaptic retrograde spread of the low molecular weight label (3000 mw dextran amine) from the dorsal SO nucleus (SOdor) to AON during incubation. Thus far, those two possibilities have not been investigated. Injections of label into AON (without involving other medullary nuclei or tracts) for anterograde transport that would reveal the target(s) of AON projection cells have not been successful. In addition, evaluating the origins of inputs to the SO nuclei is challenging due to the location and small size of the nuclei, which make discrete labeling of only those nuclei extremely difficult. However, it is critical to characterize the role that each may have in binaural processing of sound.

Although the MON and TON do not send projections to the auditory processing regions of the midbrain, the possibility remains that saccular projections to MON/TON reflect inputs for orientation and reflex responses to sound (see Physiology below). The utricle also provides input to these two nuclei, along with substantial inputs from the semi-circular canal cristae (Highstein et al. 1992). Extracellular recording followed by injection of neurobiotin at the recording site revealed that a subset of MON cells (in M2, M3 subnuclei designated by Highstein et al. 1992) respond to particle motion stimuli (mostly ≤100 Hz) and others (in M3) respond to both particle motion and lateral line stimuli (water motion), consistent with lateral line input to M3 (Highstein et al. 1992). The auditory and bimodal cells responded best to sounds in the horizontal plane, potentially reflecting inputs from the rostral or caudal saccule (Fig. 1b) or from the horizontally oriented utricle (Edds-Walton et al. 2013). In addition, ipsilateral label injections into auditory sites in MON also

filled contralateral somata in MON, indicating that there is a commissural tract that provides the potential for integration of auditory (and lateral line) inputs from the left and right sides of the fish (Edds-Walton et al. 2013). These data are consistent with other studies of circuits that regulate body position in space (e.g., pitch and roll), which also could include rapid responses to sound (discussed in Straka and Baker 2011).

Although this review focuses on the directional hearing circuit, it is worth noting that auditory input is combined with lateral line input in the midbrain of oyster toadfish. Application of label to regions in the midbrain torus semicircularis (TS) revealed sites where lateral line and auditory inputs converged (Edds-Walton and Fay 2005b) and retrogradely filled projection cells were present in both DON and the lateral line nucleus medialis. Consistent with the anatomy, physiological studies confirmed bimodal cells in the TS that responded well to auditory particle motion around 100 Hz, and to lateral line stimuli (water movement) ipsilaterally, or contralaterally, or on both sides of the fish (Fay and Edds-Walton 2001; Edds-Walton and Fay 2005a). An additional finding was the presence of cells for which lateral line stimulation inhibited the spiking activity during auditory stimulation (Edds-Walton and Fay 2005a). Therefore, there are potentially interesting interactions between these two sensory systems in toadfish, as well as other fishes (Braun and Sand 2014). The lateral line may be involved in orientation during the "final approach" within centimeters of a sound source, rather than sound source localization from a distance. A carefully conducted behavioral study with the closely related midshipman fish (*Porichthys notatus*, Batrachoididae) revealed that the lateral line is not required for sound source localization by free-swimming females approaching a speaker that projected male reproductive calls (Coffin et al. 2014).

3.3   Physiology: What the Ear Hears

Fay (1984) showed that all three otolithic endorgans in the goldfish responded to particle motion at 140 Hz and each had directional responses that reflect the hair cell orientations of that endorgan. Lu et al. (1998, 2003, 2004) also showed that all three otolithic endorgans in the sleeper goby (*Dormitator latifrons*) can respond to similar frequencies, however, the sound levels required to stimulate each endorgan varied to the extent that all three are unlikely to be stimulated simultaneously.

Fay and Edds-Walton have focused on understanding auditory processing of the saccule in the oyster toadfish, although an auditory role for the utricle or the lagena have not been ruled out, nor have our physiological studies ruled out a potential role for the saccule in orientation or equilibrium common for the vestibular systems of other vertebrates. The potential for each otolithic endorgan to respond to low frequency linear acceleration associated with "vestibular" function and frequencies in the auditory range, the "mixed function" hypothesis (Platt and Popper 1981; Popper and Fay 1993), remains an intriguing possibility.

In 2011, Vasconcelos worked with another member of the Batrachoididae, the Lusitanian toadfish (*Halobatrachus didactylus*), and used the shaker system to com-

pare the relative sensitivity of the saccule and the utricle to particle motion at auditory frequencies. In addition, Vasconcelos removed the otolith from the endorgan (unilaterally or bilaterally) to assess the role of the saccule and the utricle in body posture and movement following recovery from the surgery. The results of those behavioral experiments indicated that the utricle functions both as an auditory and vestibular endorgan in the Lusitanian toadfish (Vasconcelos et al. 2012). Although the auditory responsiveness for the saccule was consistent with previous work on auditory processing in this species, the results of the postural experiments were less clear, and additional research is being done. Therefore, although likely that the toadfish brain receives both auditory and vestibular information from the utricle and the saccule, more work is required to delineate the specific contributions of each to orientation, auditory processing, and sound source localization.

The third otolithic endorgan, the lagena, is the smallest of the otolithic endorgans in toadfishes, difficult to access in vivo, and its association with the posterior semicircular canal indicates a vestibular role. In goldfishes, the lagena is nearly equivalent in area to the saccule (Edds-Walton and Popper 2000), lies directly adjacent to the saccule (Platt 1977), and the nerve bundle from the lagena joins the saccular bundle prior to joining the other components of VIII, all of which are consistent with a similar sensory role for the saccule and lagena in that species (for physiological comparisons, see Coombs et al. 2010; Dailey and Braun 2011). Therefore, clarification of the role of the lagena in toadfishes would be of interest, but it was not included in any of the studies by Fay and Edds-Walton.

As described above (Sect. 3.2.1), saccular afferents often occur in bundles that reflect their site of origin on the epithelium: rostral, middle, caudal. Fay and Edds-Walton (1997a, b; Edds-Walton et al. 1999) evaluated afferents from those regions of the saccule and concluded that (1) in general, primary afferents are broadly tuned with most afferents responding best to the 100 Hz stimulus; (2) there is no evidence for a frequency map along the rostral-caudal axis of the saccule; (3) the majority of afferents are directional in that their responses reflect responses from a single hair cell orientation (producing a cosine response almost identical to that of a single hair cell; Fig. 1a); (4) the saccule provides information about the axis of stimulation for all angles in the vertical plane (0–90°), consistent with the hair cell orientation pattern described by Edds-Walton and Popper (1995); in the horizontal plane, responses are consistent with the orientation of the saccule in the otic capsule (Fig. 2a).

The variations in the saccular data also provided interesting insights into the auditory system. The best response directions (best stimulus axis) among saccular afferents that were filled with neurobiotin (to identify the location of their dendritic arbors on the saccular epithelium) did not always coincide with the predicted best direction based on the hair cell orientation drawing. Edds-Walton et al. (1999) provided evidence that the epithelium does not lie flat against the otolith, and the unexpected best directions (in particular, low elevations of cells along the edge of the epithelium) are likely to be due to curvature of the epithelium where it lies along the sculptured otolith (see Fig. 1c). Fay and Edds-Walton (1997a) also noted that some afferents (about 20 %) are nearly omnidirectional, lacking a null in the directional response plot. They hypothesized that those afferents contacted hair cells with two different orientations, based on a simple model (see Fig. 11 in Fay and Edds-Walton

1997a), and that those afferents could function as simple sound detectors. Alternatively, those cells may reflect a step in the maturation of the synaptic connections on the sensory epithelium. New hair cells and new connections develop as the endorgan grows throughout the life of the fish; some of those connections may be temporary, particularly if correlated activity is favored for maintaining synaptic contacts between an afferent and multiple hair cells.

Thresholds among saccular afferents varied between 300 and 0.1 nm rms, similar to saccular afferents of the goldfish (Fay and Ream 1986; Fay and Edds-Walton 2000). These data were important because they showed that the otophysic connection (mechanically connecting the ear and gas bladder) in goldfish does not provide substantial improvement in sensitivity to particle motion at lower frequencies (below 200 Hz) when compared to the toadfish, which lacks the otophysic connection. The most sensitive afferents (sensitivity is the inverse of threshold) rival mammalian cochlear afferents. Also, similarly to mammalian cochlear afferents, toadfish afferents with low spontaneous (or background) activity tended to have higher thresholds than those with higher spontaneous activity, though there was a continuum (not a dichotomy) of responses in all three locations investigated along the saccule (Fig. 5 in Fay and Edds-Walton 1997a).

Additionally, a subset of afferents located all along the saccule, with 0–2 spikes/s spontaneous activity, exhibited consistent level-dependent phase shifts with increasing stimulus levels (mean slope ± s.e.m.: 3.7°/dB ± 0.16°). Although the phase shift may seem inconsequential, for a 5dB difference in stimulus level, there could be a 20 deg shift in the occurrence of a phase-locked spike (Fig. 4). For a biologically relevant frequency such as 100 Hz, a 5 dB difference would translate to a 55 ms temporal difference in the spiking activity of an afferent that exhibits phase-advance (Fig. 4d) versus a phase-locked afferent that does not exhibit phase-advance (Fig. 4b). If we look at an octaval cell that receives input from both of the above cells, the phase-advanced afferent representing the louder sound would provide its "information" earlier to a cell that receives both inputs. Taken together, the directional response data and phase-advance data provide evidence that computations of interaural level differences are possible, and a binaural comparison of the activity from the nonparallel saccules could contribute to sound source localization. These results led to the investigation of response characteristics within the medullary nucleus of the octaval column (Fig. 2b) that receives the majority of input from the saccule: the dorsal division of the descending octaval nucleus (dDON).

3.4 Physiology: What the Brain "Knows"

A key question to address is what the brain does with the information from the ear. Our studies of the dDON in the medulla and its midbrain target, nucleus centralis (NC), revealed that the auditory circuits perform various computations that "improve" the information about frequency, temporal pattern, and the axis of particle motion of a sound source from the information provided by the auditory endorgans of the ear. At this point, we cannot state which endorgans of the ear contribute
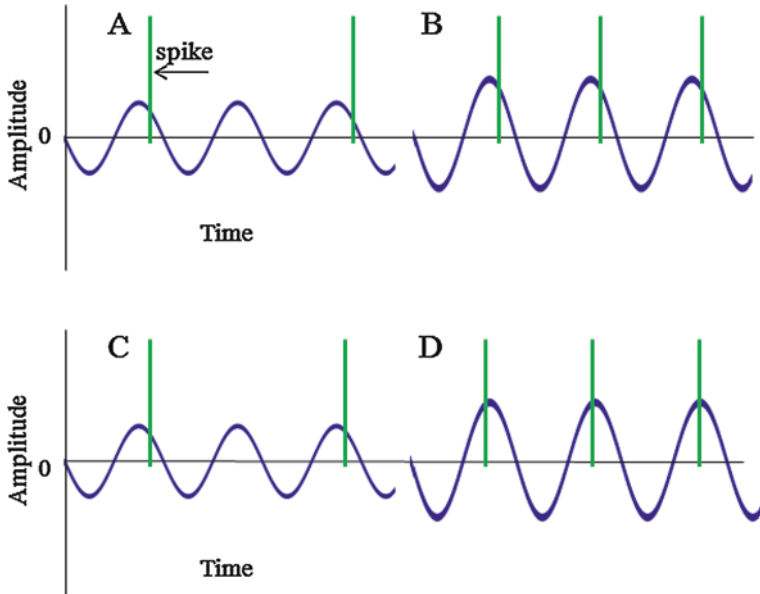
Fig. 4 Phase-locking in auditory afferents. (**a**) Spikes (*green vertical lines*) produced by a phase-locked afferent consistently occur at the same phase of a sinusoidal stimulus (*blue line*). At lower sound levels, the spikes do not occur during every cycle. At sound levels well above threshold for the same afferent, a spike will be produced for every cycle of the stimulus at the same phase, as shown in (**b**). (**c**) Spikes from an afferent that exhibits a level-dependent phase shift also occur at a particular phase of the sinusoid, but as sound levels increase, a spike consistently occurs earlier in the sinusoid, as shown in (**d**). The advance in the phase response results in earlier spike times (compare **d** to **b**) that could be a mechanism for sound level comparisons

all of the information that the brain uses. As noted previously, we have focused on the saccule. Our data indicate that convergence of saccular input could be "sufficient" for the fish to determine the location of a sound source, but we have not investigated the possibility that the horizontally oriented utricle and/or vertically oriented lagena contribute important information (gravistatic, postural or auditory) that facilitate behavioral responses to biologically relevant sounds. Projections from the utricle overlap with those of the canal cristae (particularly the horizontal canal cristae) at multiple sites in the octaval column in a variety of fishes, which ultimately contribute to circuits that control posture and coordinate head and eye movements (Straka and Baker 2011).

3.4.1 The Roles of DON

As noted in Sect. 3.2.3, retrograde transport of label injected into the torus semicircularis to fill the somata of projection cells in the medulla consistently labeled primarily the dDON with contralateral predominance. The medial region of the AON (where bifurcating saccular afferents sometimes terminated; Edds-Walton et al.

1999) and the secondary octaval populations (that project to the auditory midbrain) were located in medial areas of the medulla that were obscured by a large blood sinus, and we were never able to confirm auditory recordings in any of those sites. Therefore, we have focused our studies on the dDON (Fig. 3a). The research questions addressed in the dDON included: how are the response characteristics different from the saccular afferent responses? Is there evidence for convergence of inputs and directional computations?

Edds-Walton and Fay (2008) found similarities and differences in the auditory response characteristics of dDON cells when compared with saccular afferents. The frequency response of dDON cells was similar to that of saccular afferents (best frequencies of 84–185 Hz for 79 % of cells), however bandwidth of the responses varied greatly, which was not a characteristic of saccular afferents. Most of the dDON cells had iso-level frequency response functions with an inverted-V shape (with various slopes), indicating narrowing of the frequency response (= tuning) when compared to saccular afferents. However, given the breadth of the bandwidths measured at 50 % of maximum response, the majority of afferents in dDON could not be considered sharply tuned (see Figs. 4 and 6 in Edds-Walton and Fay 2008). Thus, we conclude that the brain is capable of processing broadband sounds such as the pulsed sounds produced by conspecifics. In addition, the observed responses to pure tones (Winn's behavioral studies and our physiological studies) can be attributed to the broad nature of tuning in the ear (Edds-Walton and Fay 2008). For example, many dDON cells not tuned to 100 Hz will respond well to it.

The majority of cells in dDON exhibited good phase-locking. As in saccular afferents, Edds-Walton and Fay (2008) found a subset of cells for which phase-locking was level-dependent (improved with increasing levels above threshold). Another subset of dDON cells phase-locked consistently at all levels *and* exhibited level-dependent phase shifts (mean 2.7°/dB, maximum of 6°/dB; Fig. 4) as was seen in saccular afferents. However, there was not a dichotomy of phase-locking accuracy in dDON, but rather an array of variations in the strength of phase-locking and the degree of phase advance in the cells that exhibited it. Edds-Walton and Fay (2008) concluded that within dDON there appears to be the potential for the generation of parallel computational pathways: one pathway in which phase is encoded independent of level, and another pathway that could contribute to stimulus level comparisons. Both of these pathways would contribute to soundscape analyses and, potentially, to sound source localization.

Edds-Walton and Fay (2005b) determined that the convergence of auditory inputs in the dorsal division of DON does not result in a loss of directional information. On the contrary, the majority of cells in dDON exhibited DRPs in both the horizontal and vertical planes that tended to be less broad with respect to the level of response to stimulus angles adjacent to the best axis. This narrowing of the directional response area is similar to frequency tuning and has been called "sharpening" of the directional response (Edds-Walton and Fay 2005b). Quantification of this narrowing of the directional response was achieved by employing a sharpening ratio (SR):

$$SR = [(R_1 + R_2)/2]/R_3$$

where R_3 is the maximum response at the best stimulus angle (or best axis, BA); R_1 is the response at the adjacent stimulus axis that is −30° from the BA; and R_2 is the response at the adjacent stimulus axis that is +30° from the BA (illustrated in Fig. 5a, b). This calculation was done separately for the horizontal plane (= azimuth) and for the vertical plane (or mid-sagittal plane = elevation). For a perfect cosine response function, the calculated SR is 0.866. The maximum SR possible is 1, which would be for a cell that has an equivalent response to three adjacent stimulus axes (and no distinct single best stimulus angle). An SR of 1 was never present in any of our data sets and would not be expected from saccular afferents unless the cell's ability to respond to a stimulus is "saturated" by stimulus levels well above threshold. An SR near "0" would indicate a very sharpened directional response wherein the cell responds well to only one axis. Due to the natural variations in spike counts for stimulus repetitions, particularly for cells with background activity, a minimum data set at two stimulus levels was required, and the critical value for considering a cell to be sharpened was an average SR ≤ 0.75 (Fig. 5c). The median SR value for DON cells was 0.67 in azimuth and 0.62 in elevation (Edds-Walton and Fay 2005b).

In a subset of DON cells (n = 73), 64 % exhibited sharpening (SR < 0.76) in azimuth and 67 % exhibited sharpening in elevation (Fig. 5c). Moreover, some of the DON cells were sharpened greatly (SR < 0.56, Fig. 5c) in both planes. An equally important observation was that there were cells for which sharpening occurred in one plane only (azimuth or elevation) or for which sharpening was unequal in the two planes. Taken together, the evidence indicates that sharpening is an important computation in DON, which occurs by various ways (likely the weighting of inputs) that result in different degrees of sharpening in different planes. In addition, directional sharpening and frequency tuning appear to be separate computational processes, as one is not predictive of the other (Edds-Walton and Fay 2003, 2008).

The best direction in three-dimensional space was calculated for afferents in DON and plotted on a flattened globe (northern hemisphere only) to compare the distribution around the fish with the best directions plotted for saccular afferents (Fig. 6). The globe's outer perimeter (equivalent to the equator of the flattened globe) represents 0° in elevation, and directly above the fish (shown at the center of the globe) is 90° elevation. Elevation rings (similar to latitude lines on a globe) are shown for 30 and 60° in elevation around the fish. Azimuth is represented around the fish with 0° in azimuth at the head of the fish; 30° in azimuth is labeled on the left side of the fish for the saccular data to identify the angle around which most of the left saccular data were found. Note that the best direction is shown as the point on the globe at which the characteristic axis would pierce the northern hemisphere of a globe.

Comparing the best directions for afferents from the left saccule and cells in the left DON reveals very different distributions (Fig. 6). The directional plot for saccular afferents reflects the orientation of the saccule in the otic capsule of the fish. The large number of overlapping data points around 30° left azimuth is consistent with the orientation of hair cells on the rostral saccule, where much of the physiological recording was done due to the accessibility of the rostral bundle of the saccular
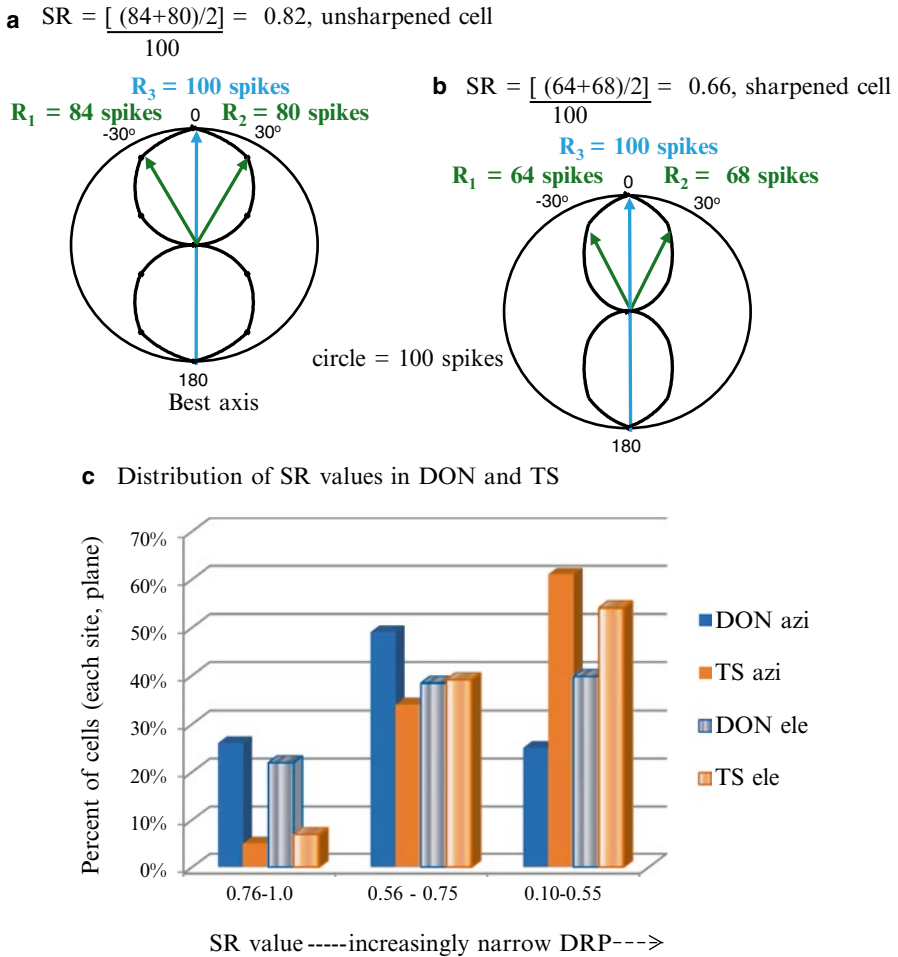
a SR = $\frac{[(84+80)/2]}{100}$ = 0.82, unsharpened cell

R₃ = 100 spikes
R₁ = 84 spikes 0 **R₂ = 80 spikes**
-30° 30°

b SR = $\frac{[(64+68)/2]}{100}$ = 0.66, sharpened cell

R₃ = 100 spikes
R₁ = 64 spikes 0 **R₂ = 68 spikes**
-30° 30°

circle = 100 spikes

180
Best axis

180

c Distribution of SR values in DON and TS

Fig. 5 Sharpening ratios (*SR*) for cells in the dorsal division of the descending octaval nucleus (*DON*) and cells in the torus semicircularis (*TS*). The SR calculation is illustrated and calculated from the relative spike rate at the best axis (*R₃, blue line*) and the spike rates for the two stimuli ±30° (*green arrows*) from the best axis, as revealed by the DRP for the directional stimulus set. SR was calculated separately for azimuth and elevation. SR for a perfect cosinusoidal response (shown in Fig. 1) is 0.866. (**a**) Calculation and illustration of the DRP of an unsharpened cell; the hypothetical values shown are average spikes per stimulus angle; *blue line* is the best axis in that plane, with only half of the adjacent stimulus axes shown for simplicity. The length of the *axis line* indicates relative spike rates at that angle, with the *outer circle* representing 100 spikes/s. The *blue line* touches the circle, thus the average spike rate at that stimulus direction was 100 spikes/s. (**b**) Calculation and illustration of the DRP of a sharpened cell; the values shown are realistic, but hypothetical. (**c**) Histogram illustrating the distribution of SR values for DON cells, in azimuth and elevation, to compare with the SR of TS cells in each plane. The distribution of SR in TS is shifted to the right as cells in TS were more sharpened than in the DON. The DRP is plotted with regard to the cell's response to each stimulus axis (modified from Edds-Walton and Fay 2005)
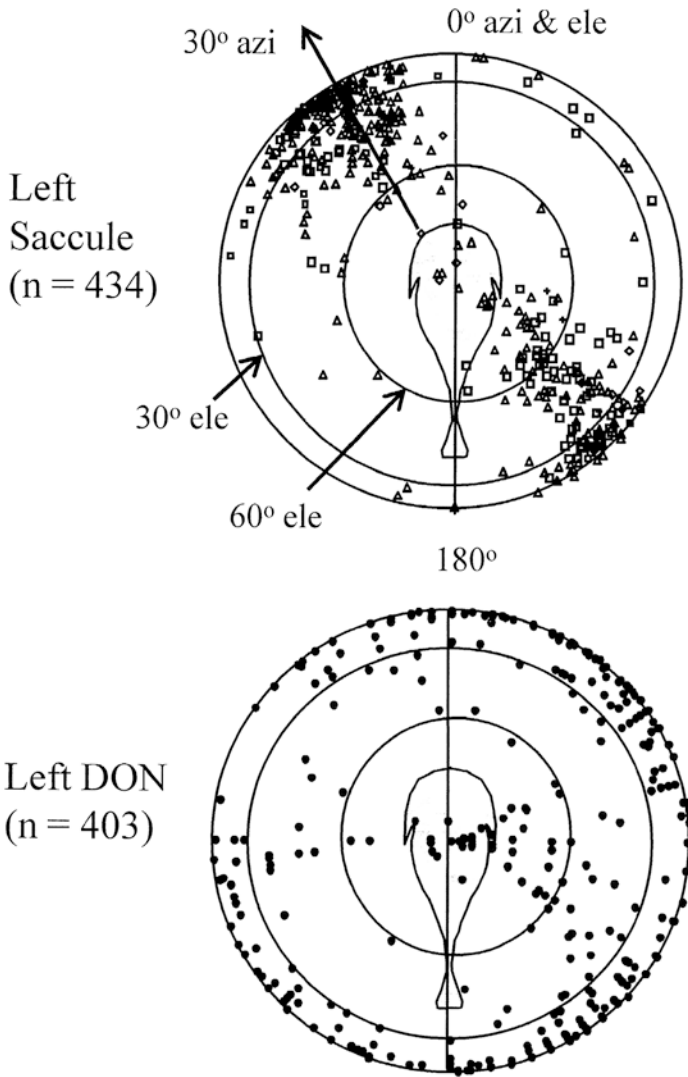
Fig. 6 Flattened globe illustrating the best directions for left saccular afferents (*top*) and for cells in the dorsal division of the left descending octaval nucleus (*DON*, *below*). Direction is represented by a point on the northern hemisphere of a globe at the appropriate azimuth and elevation where the vector for best direction would pierce the northern hemisphere. The *outer circle* is equivalent to the equator (0° elevation) and directly above the fish is the equivalent of the North Pole (90° elevation). *Concentric circles* indicate 30 and 60° elevation. Azimuth is displayed with respect to the fish silhouette, 0° at the head and 180° at the tail. Each point is a different afferent or cell; different shapes indicate different data sets and data points overlap. The *arrow* at 30° azimuth indicates the stimulus angle closest to the angle of the left saccule with respect to the midline of the fish. The distribution of best directions in the saccule reflects the orientation of the endorgan and the hair cell orientations on the sensory epithelium. The DON contains cells that represent the acoustic space all around the fish; the distribution is consistent with the convergence of inputs from the left and right saccules. Directional stimuli were presented at 30° intervals in the horizontal and mid-sagittal planes, and the best directions shown here were interpolated based on the distribution of responses to the stimulus angles in both planes (modified from Edds-Walton and Fay 2005, 2008)

nerve. Note also the relatively small number of cells with best directions directly above the fish, which is consistent with the relatively small and inaccessible area of the saccule, the middle, where purely vertical cells are located (Fig. 1b). The best directions for afferents from the caudal saccule overlap the locations of afferents from the rostral saccule (see Edds-Walton et al. 1999 for details on the caudal afferents). In contrast, the directional plot for DON cells reflects the acoustic space all around the fish. There are two potential ways to achieve this difference: convergence of inputs from the left and right saccules or convergence of input from other ipsilateral otolithic endorgans. At present, anatomical data can be used to support either circuit, but there are physiological data to support the convergence of left and right saccular data in the DON (and the TS, see Sect. 3.4.3).

Edds-Walton and Fay (2009) argued that the distribution of responses in DON could be the result of convergence of left and right saccular inputs based on the following data. Although saccular afferents do not cross the midline to the contralateral DON, there is a topographic tract that connects the dorsal divisions of the left and right DONs (Edds-Walton 1998b). In a unique study, Edds-Walton and Fay (2009) altered saccular inputs (by tipping one of the two saccular otoliths) while recording from directional auditory cells in DON. Data were difficult to obtain because three complete sets of frequency and directional data were needed: Pre-tipping, Tipping, and Post-tipping data (with results consistent with the pre-tipping data). Ipsilateral tipping confirmed that the method worked and was reversible. Often tipping eliminated spike activity in an ipsilateral or contralateral DON cell, which was consistent with removal of essential excitatory input from the saccule. Most importantly, Edds-Walton and Fay (2009) showed that altering contralateral inputs can alter the DRP (and sometimes frequency response) of a cell in DON. As was apparent in the sharpening analyses, there were a range of differences in the DRP during tipping, consistent with a variety of computations. The data clearly show that contralateral saccular input contributes to the computations that occur in DON (Edds-Walton and Fay 2009), and therefore, at least some of the computations in DON are binaural.

3.4.2   Other Targets of the Saccule in the Octaval Column

As noted earlier, MON and TON do not appear to be involved in the ascending auditory circuit in toadfish (consistent with other teleosts, McCormick 1999, 2011), but both receive input from the saccule as well as the utricle, lagena, and canal cristae. The saccular input to MON and TON is of interest because of the potential for dual function of the saccule as an auditory and vestibular (gravistatic) endorgan (Platt and Popper 1981). Highstein et al. (1992) suggested the MON as the origin of the ipsilateral descending vestibulospinal tract, which is consistent with a role in gravistatic orientation. Physiological recordings in MON confirmed that a subset of cells (in two of the three subdivisions designated by Highstein et al. 1992) respond well and phase-lock to auditory frequencies (Edds-Walton et al. 2013). The TON receives heavy input from all known vestibular structures and very little input from the

saccule (Highstein et al. 1992; Edds-Walton 1998a). However, these data provide further evidence that some region of the saccule could contribute to circuits that modulate orientation with respect to gravity (yaw, pitch, roll) as well as circuits involved in behavioral responses to sound.

3.4.3 Torus Semicircularis

As in other teleosts, the midbrain of the oyster toadfish is a major integration site of sensory information. Visual input converges with other senses important for appropriate behavioral responses, as in other vertebrates (Tricus and Highstein 1990; Carr and Edds-Walton 2008; Straka and Baker 2013). Multimodal cells are to be expected, though sorting out the particular range of responses is a daunting task. The studies by Edds-Walton and Fay focused on auditory processing and the potential for integration of hearing and components of the lateral line sense (also known as Svenning; for a review, see Braun and Sand 2014).

The midbrain torus semicircularis of teleost fishes consists of two divisions: the more dorsal nucleus centralis (NC) and the more ventral nucleus ventrolateralis (NVL). Anterograde transport of neurobiotin from dDON revealed projections to the dorsal division of the secondary octaval population (SOdor) and to the auditory midbrain (NC). Retrograde transport of neurobiotin injected at characterized auditory sites in NC confirmed that a subset of dDON cells and SOdor cells projected to those sites (Edds-Walton and Fay 2003). Clearly, these inputs provide a multitude of possibilities for physiological computations in the midbrain. In addition, cells in NC and NVL have extensive processes, and interactions between the auditory input to NC and the lateral line input to NVL provide further opportunities for converging the inputs from those two sensory systems. Bimodal cells with a variety of response characteristics appear to be present in both nuclei (Fay and Edds-Walton 2001; Edds-Walton and Fay 2005a).

Auditory cells in the midbrain lack phase-locking, are broadly tuned as in DON, and exhibit sensitivity to temporal codes (interpulse intervals, Fay and Edds-Walton 2002). The bandwidth indicates that most cells that respond best to 100 Hz will also respond well to harmonics of 50 Hz (the lowest frequency tested) or 200 Hz if they are present at a similar level. Therefore, at least some of the cells in the NC respond very well to the pulses in conspecific vocalizations.

The auditory (and bimodal) cells also exhibit sharpened DRPs; however, the data indicated that the computations that produce sharpening continue along the ascending pathway (e.g., via SOdor) or occur in the TS. The distribution of SR values for cells in the TS is shifted toward lower values, indicating greater sharpening, particularly in the horizontal plane, in the TS (Fig. 5c). More than half of TS cells were in the highly sharpened category. The median SR value for azimuth among TS cells was 0.49 (DON median SR in azimuth = 0.67) and the median SR value for elevation among TS cells was 0.54 (DON median SR in elevation = 0.62). In addition, there was an even greater variety in the relative sharpening of the two planes in TS (coefficient of determination = 0.1) compared to DON (coefficient of determination = 0.4),

which further indicates that the computations are the result of a variety of excitatory and inhibitory interactions among the various sources of input to the TS.

Edds-Walton and Fay (2009) demonstrated that binaural cells result from the convergence of excitatory (EE) and inhibitory (EI or IE) inputs to cells in both DON and NC (and see Edds-Walton et al. 2010). Therefore, there are potential sites in fishes for bilateral comparisons that may function similarly to binaural sites in the ascending auditory pathway of other vertebrates (e.g., nucleus laminaris in birds or nuclei of the superior olivary complex in mammals).

The otolith tipping experiments described for cells in the DON (Sect. 3.4.1) were also conducted with cells in the TS. The only otolithic input that was altered during these experiments was from the saccule. As in the DON, altering saccular input altered the DRPs and/or spiking activity of cells in the TS (Edds-Walton and Fay 2009). Unlike the DON, spike activity was rarely eliminated in a TS cell during tipping, consistent with complex interactions of converging inputs from various sources.

Lastly, plotting the characteristic axis for cells in the TS on the flattened globe (as in Fig. 6) provided confirmation that acoustic space around the fish is well represented in the midbrain as it is in DON (Edds-Walton and Fay 2003). Given that the directionality of auditory cells improves (becomes sharpened) along the ascending auditory pathway to the level of the midbrain, Edds-Walton and Fay (2005b) concluded that encoding the location of a sound source is an important component of auditory processing in the toadfish.

4   Conclusions

Studies in the oyster toadfish combined anatomical tract-tracing and physiological recordings from identified sites on the saccule, in the DON, and in the torus semicircularis to document auditory processing at each level of the ascending auditory pathway. The toadfish saccule encodes frequency, sound level, and sound source direction in phase-locked activity conveyed via auditory afferents ipsilaterally to nuclei of the octaval column. The large DON plays a major role in auditory processing and contributes bilaterally to the ascending auditory circuit. Binaural convergence of auditory information provides the fish with information about sound sources at locations all around the fish. Furthermore, a series of related physiological studies showed that the auditory system of the toadfish consistently encodes frequency, temporal pattern, sound level, and the axis of particle motion for sound sources. The response characteristics in the midbrain indicate that frequency tuning tends to be broad with little narrowing of the frequency response in the ascending auditory circuit. However, computations in the medulla and the midbrain narrow directional responses and should allow the fish to locate a vocalizing conspecific, to determine the locations of multiple sound sources around the fish based on direction and relative sound levels, and, in general, evaluate the soundscape. The toadfish ear tells the toadfish brain "what" and "where"—we are just beginning to understand "how."

5   Future Directions

1. Do the sensory roles of the lagena and the saccule differ substantially for otophysines (fishes with otophysic connections that enhance detection of the pressure component of underwater sound) versus non-otophysines (like the oyster toadfish) that lack otophysic connections? This seems likely, but requires carefully conducted comparative studies. Fay (1984) provided the first data on the directional responses from the utricle, saccule, and lagena in the goldfish, using the same frequency stimulus for all three. Those data are a clear indication that all three endorgans can have overlapping frequency responses, and that each could contribute to directional sound analyses. However, there are distinct differences in the size and shape of the saccule and lagena in different fish species. The overall areas of the sensory epithelia for the lagena and the saccule in goldfish are nearly equivalent and the endorgans lie directly adjacent to each other (Platt 1977; Edds-Walton and Popper 2000). In most teleosts investigated thus far, the lagena is the smallest of the three otolithic endorgans and is located caudal to the saccule. In toadfish, the lagenar nerve joins VIII with the afferents from the posterior canal crista. Does the relative size and/or location of the lagena reliably reflect its role in audition versus gravistatic/postural functions?
2. What are the roles of the nuclei in the secondary octaval populations? Although they are clearly involved in the ascending auditory circuit, and maybe other sensory systems as well (McCormick 2011), the location and small size of these nuclei (though the individual cells can be large, particularly in the dorsal division) provide a huge technical challenge for electrophysiology. Answering this particular research question may best be approached by the use of a slice preparation centered at the entrance of VIII.
3. Can otophysine fishes determine the direction of a sound source, despite enhanced "unidirectional" input from the gas bladder? Zeddies et al. (2012) have shown that local particle motion is the key parameter used by the midshipman fish (a non-otophysine) to localize a sound source (conspecific vocalization from a speaker). A similar study (with a carefully quantified sound field) is needed, ideally using a vocal otophysine fish, but also using goldfish or carp. There are anecdotal stories of trained goldfishes or carp coming to a feeding site when a "dinner bell" attracts them. In that context, there are multiple cues for the fish, including visual observations of the feeder (human or mechanical) and chemical cues in the water. Will the goldfish localize a sound source without other cues to attract them in a natural setting?
4. Does input from the gas bladder facilitate sound source localization in fishes that lack a mechanical connection between the gas bladder and the ear? Coffin et al. (2014) have provided evidence that midshipman fish use the indirect input from the gas bladder to compute the direction of a sound source. In addition, a recent model of hearing in toadfish (Rozin et al. 2013) that incorporates the proximity of the gas bladder and the morphology of the otic capsule (Edds-Walton et al. 2015) provides mathematical evidence that indirect input from the gas bladder

could be detected by the toadfish ear. Although the indirect input to the toadfish ear would not improve hearing sensitivity (nor extend the frequency range), the indirect input could provide phase information; therefore, the phase model of sound source localization first described for cod (Schuijf 1976) may apply to other teleost species as well. A better understanding of the potential role of the gas bladder in sound source localization in teleosts should be explored, ideally as a multi-species comparison of morphological variations in the gas bladder-ear association with the ability to localize a sound source under natural conditions.

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