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Evolution of Sound Source Localization Circuits in the Nonmammalian Vertebrate Brainstem

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Abstract

The earliest vertebrate ears likely subserved a gravistatic function for orientation in the aquatic environment. However, in addition to the detection of acceleration created by the animal's own movements, the otolithic end organs that detect linear acceleration would have responded to particle movement created by external sources. The potential to identify and localize these external sources may have been a major selection force in the evolution of the early vertebrate ear and in the processing of sound in the central nervous system. The intrinsic physiological polarization of sensory hair cells on the otolith organs confers sensitivity to the direction of stimulation, including the direction of particle motion at auditory frequencies. In extant fishes, afferents from otolithic end organs encode the axis of particle motion, which is conveyed to the dorsal regions of first-order octaval nuclei. This directional information is further enhanced by bilateral computations in the medulla and the auditory midbrain. We propose that similar direction-sensitive neurons were present in the early aquatic tetrapods and that selection for sound localization in air acted upon pre-existing brain stem circuits like those in fishes. With movement onto land, the early tetrapods may have retained some sensitivity to particle motion, transduced by bone conduction, and later acquired new auditory papillae and tympanic hearing. Tympanic hearing arose in parallel within each of the major tetrapod lineages and would have led to increased sensitivity to a broader frequency range and to modification of the pre-existing circuitry for sound source localization.

Keywords

archosaurs; auditory; birds; crocodiles; fish; hearing; lepidosaurs; lizards; sound localization; tympanum

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Introduction

In all vertebrate groups investigated to date, the auditory system conveys information about both the nature of sound sources (e.g., conspecific versus predator or prey) and their relative locations. Sound localization accuracy varies among vertebrates, but the key neural elements required for directional analyses exist in all bony fish, suggesting that localization may have been an early function of the vertebrate auditory system that developed in a common ancestor or evolved within the lineages through parallel evolution. Here we consider the evolution of sound source localization circuits in the nonmammalian vertebrate brain stem. We focus on the commonalities of auditory circuits among nonmammalian lineages and on potential scenarios for the evolution of brain stem sound source localization circuits.

Directionality of Sound

Sound can radiate in all directions from a source and travel through air, water, and substrate. How can the source be located? The alternating pressure in the propagating sound wave (i.e., compression and rarefaction of the particles in the medium) creates a spatial pressure gradient that results in a forward-backward movement of the particles along the axis of propagation (particle motion). Therefore, although sound pressure is scalar (i.e., non-directional), particle motion has both direction and magnitude (a vector); however, the axis of particle motion alternately points toward and away from the source (180° ambiguity). How fish resolve the ambiguity is not known with certainty, but most current theories require simultaneous detection of sound pressure and particle motion [see discussion of the issues and theories by Fay, 2005; Sisneros and Rogers, 2016]. Sound pressure may be detected in bony fishes via gas-filled structures that can indirectly stimulate the ear [Radford et al. 2012; Ladich and Schulz-Mirbach, 2016]; however, the indirect stimulation of the ear consistently originates from the location of the gas bladder, independent of the location of the source. Therefore, central computations must resolve that issue as well.

In general, otolithic end organs respond directly to particle motion as inertial accelerometers with the greater relative motion of the sensory tissue (macula) versus the dense otolith (fig. 1A, B) resulting in stimulation of the sensory hair cells. Particle motion sensitivity was likely present in extinct jawed fishes through their otolithic end organs, which probably evolved as gravistatic sensors in the early vertebrates (Fritzsche and Straka 2014). Additionally, both ray-finned and lobe-finned fishes are thought to have possessed gas bladders early in their evolution, and some fishes may have used them for respiration. Those gas bladders also may have conferred some pressure sensitivity [Clack, 2015].

In air, the presence of external ears, either as simple openings or with external pinnae, provides a low-impedance pathway to the inner ear and provides the potential for left and right comparisons for sound sources off the midline of the recipient. There is a potential 180° ambiguity for midline sound sources (due to the equal stimulation of the two ears) that may be addressed by behavior (e.g., turning the body or the pinnae to reorient the ears with respect to the source) [Yost, 2013]. In the terrestrial groups that have acquired tympanic middle ears, the middle ear transforms an oscillating pressure at the tympanum into motion of the inner-ear fluid surrounding the sensory receptors (hair cells) on a papilla or basilar membrane, as described in the following section.

Encoding Direction

In water, directional information originates with the sensory hair cells, which are morphologically and physiologically polarized and are organized in specific patterns on different sensory end organs (fig. 1A, B). The apical surface of the hair cell has a distinctive array of vertical stereovilli that are oriented in ascending order, often with a single kinocilium at the tallest end of the bundle (fig. 2C). This morphological polarity dictates a single axis of maximum excitation that occurs during movement of the stereovilli toward the kinocilium (arrow in fig. 2C); maximum inhibition occurs during movement of the stereovillae away from the kinocilium. Overall, the response function is cosinusoidal with a central null (no response) perpendicular to the best axis and a graded response to stimuli located at angles between the best axis and the null [Hudspeth and Corey, 1977]. This feature is the basis for encoding stimulus direction in octavolateralis systems (ear and lateral line).

Hair cells encode particle motion via movement of the fluids surrounding the semicircular canal cristae (angular acceleration), otolith organs (linear acceleration) and other auditory maculae in the ears of vertebrates (e.g., macula neglecta, papillae, basilar membrane). The intrinsically directional hair cells are grouped in various, often highly conserved, patterns on the sensory maculae (fig. 1). In the bony fishes (Osteichthyes), the otolith organs have distinctive groupings of different hair cell orientations (bidirectional, orthogonal, and/or sweeping) that provide directional responsiveness for stimuli in the plane (x, y, z) of the macula (figs. 1B, 2C). In the end organs sensitive to airborne sound in tetrapods, hair cells are oriented in parallel rows along the length of the basilar membrane of archosaurs, the papilla of lepidosaurs, the caudal part of the amphibian papilla of anurans, and the cochlea of mammals (fig. 1B), where frequency mapping dominates the organization [Köppl and Manley, 1992; Mann and Kelley, 2011; Sienknecht, 2013].

The gravistatic organs and semicircular canals share the same physiologically polarized hair cells as other octavolateralis systems. Therefore, we suggest that the circuitry associated with orientation to gravity in an aquatic environment provided the starting point for vector analyses in the brain stem of the earliest fishes [see Duncan and Fritzsche, 2012; Straka and Baker, 2013; Fritzsche and Straka, 2014]. Furthermore, in the vertebrates studied thus far, central auditory pathways share a common octaval pattern of organization, which includes monaural and binaural nuclei in the medulla, convergence of multiple medullary projections in the auditory midbrain, and increased specificity of sound source location [e.g., Grothe et al., 2004; Carr and Edds-Walton, 2008]. The commonalities in the basic auditory pathway among lineages that diverged millions of years ago are consistent with a simple ancestral “template” for sound analyses that included source localization. In a larger view, these features are also consistent with an ancestral octavolateralis pattern exhibited by lateral line, electrosensory, and vestibular eighth nerve projections [Northcutt, 1980; Grothe et al, 2004].

In the Beginning: The Ear in Early Vertebrates

The earliest vertebrates were aquatic jawless fishes (agnathans) that appeared in the late Cambrian Period (Paleozoic Era). The fossil record for those earliest vertebrates with cartilaginous crania or otic regions is poor, however, and data on otic morphology are

limited [Friedman and Giles, 2017]. Some osteostracan fossils have cranial evidence of anterior and posterior semicircular canals on each side [Clack, 2017], which is similar to the organization found in some extant agnathan species. Although the extant agnathans, the hagfishes (*Myxine* sp.) and lampreys (*Petromyzon* and *Lampetra* spp.), have very different sensory maculae (fig. 1A), the differences in their ears are discussed here because they may illustrate evolutionary steps in the development of auditory maculae.

The hagfish ear has a pair of tubular sacs that are oriented $\pm 30^\circ$ angles with respect to the midline [Lowenstein and Thornhill, 1970]. Within each sac lie two cristae (anterior and posterior) and a single elongate macula (macula communis) between the cristae patches in the fluid-filled membranous labyrinth (right ear shown in figure 1A). The macula communis in hagfish has sensory hair cells that are oriented in various directions [fig. 15 in Lowenstein and Thornhill, 1970], and, unlike the end organs in bony fishes, there is no striola where hair cells are in 180° opposition. In contrast, the lamprey macula communis exhibits greater regional specializations than in hagfish: the rostral and caudal ends are oriented horizontally and the central region is oriented vertically (fig. 1A) with a distinct line of polarity reversal [Lowenstein et al., 1968]. Regionalization of hair cell orientations could have been an early step in organizing regional response characteristics of the macula when encoding stimulus direction (e.g., specifically forward versus backward movement, rather than the more general percept that the body is moving) became an important function of the macula. The nearly ubiquitous presence of consistent hair cell orientation patterns on the maculae of different lineages of vertebrates (illustrated in fig. 1B) suggests that these patterns are functionally significant [Sienknecht, 2013]. The primary afferents that contacted groups of similarly oriented hair cells would have provided the advantage of greater directional specificity, which also would have been reflected in their input to the target medullary nucleus.

Although the auditory processing capabilities in agnathans have not been assessed, recordings from the eighth cranial nerve (nVIII) suggest the ear encodes linear acceleration. Lowenstein and Thornhill [1970] noted that nVIII afferents in the hagfish (*M. glutinosa*) responded well to yaw (changes in azimuth) and pitch (nose up, nose down, elevation) but did not respond well or consistently to roll. In addition to orientation responses, portions of nVIII in the lamprey (*Lampetra fluviatilis*) responded to vertical substrate vibration at frequencies in the auditory range [methods in Lowenstein and Roberts, 1951; data described in Lowenstein, 1970]. Lowenstein and Roberts [1951] concluded that the lamprey ear responded similarly to the vibration-sensitive components (sacculi, macula neglecta, and utricular lacinia) of the isolated ear from a ray (*Raja clavata*) [Lowenstein and Roberts, 1951]. Therefore, the data collected for the lamprey are consistent with an end organ capable of encoding a variety of directional stimuli, including vibration.

The general projection pattern of nVIII afferents in the lamprey is consistent with the pattern in jawed fishes: afferents project to a medullary octaval column that is ventral to and distinct from the more dorsal lateral line and most dorsal electrosensory nuclei [fig. 17.1 in McCormick, 1992]. Based on the analyses of Straka and Baker [2013], the octaval circuitry in lampreys is consistent with bilateral coordination of “axis-specific” (azimuth, elevation) orientation responses. Crista and macular projections from the periphery are organized

centrally by the function of the target (motor) nuclei that coordinate orienting behaviors. Straka and Baker [2013] suggested that the developmental flexibility of those projections provided the potential for functional plasticity in the octaval nuclei among the jawed fishes. As described in the next section, the octaval nuclei of jawed fishes have functional subdivisions that are based on the physiological response properties of the otic end organs.

Directional Hearing in Osteichthyes

Bony fishes (Osteichthyes) appear in the fossil record from the Silurian about 440 million years ago (MYA), and the ray-finned fishes (Actinopterygii) appear during the diversification of vertebrates in the Devonian (beginning about 419 MYA). Evidence for the evolution of the otolith organs is based primarily on the presence of statoliths or otoconia (masses of small calcareous particles) within the braincase or the presence of dense calcareous otoliths that are often found separately from a skull [reviewed by Friedman and Giles, 2017]. Fossils of early ray-finned fishes from the late Devonian indicate that the ancestral ear had a single pair of large otoliths associated with a ventral chamber, in contrast to the smaller otolith associated with the utricle, or its precursor, located more dorsally as in most extant fishes. In the fossil actinopterygian *Melanecta* from the early Carboniferous period [Coates, 1998], there are three bony semicircular canals (a horizontal canal in addition to the anterior and posterior canals in agnathans) and a separate ventral chamber that enclosed two otolith organs, potentially a saccule and lagena, based on the ventral location and the presence of large and small otoliths, all of which indicate functional specializations in the ear. A single ventral chamber housing two otolith organs (saccule and lagena) is present among extant non-teleost fishes [e.g., Meyer et al. 2010]. For a detailed discussion of hypotheses proposed for the evolution of the structures in the vertebrate ear, see Fritzsch and Straka [2014].

Extant non-teleost actinopterygians include the Cladistia, bichir and reedfish; the Chondrostei, sturgeons and paddlefish; and the Holostei, commonly the gars (Lepisosteiformes) and the bowfins (Amiiformes). The Holostei are considered to be the living sister taxon of the Teleostei (the most recent radiation of fishes). Although these extant species have evolved along separate paths for millions of years, their shared features are relevant to a discussion of the ancestors of modern ray-finned fishes and tetrapods. The non-teleost actinopterygians have a distinctive saccule and lagena housed in the same ventral chamber, and both are oriented in the vertical plane. These two maculae have similar total areas and hair cell counts in most non-teleosts [see Fig. 30 in Popper, 1983], and each is associated with otoconia (calcareous particles embedded in a matrix) rather than a solid, calcareous otolith [Popper, personal communication]. These saccules are organized with a simple bidirectional hair cell orientation pattern, but the macula is curved or twisted and may provide directional sensitivity in the rostrocaudal and dorsoventral axes (fig 1B). The relative role(s) of the two maculae in audition and/or gravistatic responses have not been determined.

The directional response properties of nVIII afferents have been assessed only in the lake sturgeon (*Acipenser fulvescens*) [Meyer et al. 2010, 2012]. Primary auditory afferents in the posterior ramus of nVIII (after the convergence of afferents from the saccule, lagena, and

macula neglecta) responded to low frequencies (100–300 Hz), exhibited phase locking to the sinusoidal stimuli, and had the characteristic cosine directional response pattern of hair cells and primary afferents found in teleost fishes (fig. 2D)[Popper and Fay, 1999]. Therefore, the capability for otolithic end organs to encode the axis of linear acceleration (with the 180° ambiguity) at auditory frequencies is present in this lineage and may have been present in the common ancestor of non-teleosts and teleosts.

Teleost fishes have the three otolith organs, usually housed in separate (though sometimes connected) chambers in the otic capsule, and the sensory epithelia (maculae) are oriented in different planes (utricle, horizontal; saccule and lagena, vertical). As in the non-teleosts, the macula may have twists [e.g., goldfish, *Carassius auratus*; Platt and Popper, 1984] or edges that curve along a highly sculptured otolith out of the plane in which the majority of the macula lies [Edds-Walton et al., 1999]. The range of directional responses of an otolith organ is a product of the macula's relationship with the otolith, orientation in the otic capsule, and the orientations of the hair cells along the macula.

Many otolith organs have fan-like hair cell orientations (e.g., utricle, fig. 1B; saccule, fig. 2C) that provide sensitivity to essentially all stimulus directions in a particular plane [Popper 1983]. The wide variety of orientation “patterns” on the otolith organs across the osteichthyes cannot be covered here, but the interested reader is referred to a detailed discussion in Ladich and Schulz-Mirbach [2016]. One consistent feature among all otolith organs in species investigated are hair cells oriented in 180° opposition with a characteristic line of polarity reversal (figs. 1B, 2C) [Sienknecht, 2013; Wu and Kelley, 2012]. Significantly, the orientations at the polarity reversal in the utricle are opposite to that of the saccule, a characteristic that is retained in all tetrapods (fig. 1B).

Two important points must be made with regard to the role of otolith organs in encoding sound source direction in fishes. First, individual afferents are not omnidirectional, and the majority of primary afferents encode sound direction based on input from the same or similarly oriented hair cells [e.g., Fay and Edds-Walton, 1997; Lu et al. 1998; Edds-Walton, et al. 1999; Weeg et al., 2002]. Second, the regional variations in hair cell orientations within a single otolith organ provide sensitivity to a variety of stimulus angles rather than a single direction (in contrast to the crista ampullaris). Therefore, based on the response properties of the afferents and the organization of the maculae, directional encoding is a key function of the otolith organs.

With respect to binaural cues, fishes have a very small range of interaural *time* differences, because the otolith organs in most species lie immediately adjacent to the midline (e.g., figs. 2A, B), and the speed of sound is about five times faster in water than in air. However, hair cells in 180° opposition encode the direction of the particle motion vector at different phases of the stimulus cycle [see fig. 1 in Chagnaud and Coombs, 2014], which can contribute to directional analyses. Binaural differences can exist because received sound levels can vary significantly for the right versus left ear due to curvature of the macula and angled arrangement of the auditory end organs (e.g., saccules, fig. 2A, B) [Sand, 1974; Popper 1983]. For example, as illustrated for the detection of azimuth in the oyster toadfish (*Opsanus tau*) based on extensive physiological recordings, stimuli that maximally stimulate

the rostral saccule on the left side of the fish will not stimulate the rostral saccule on the right side (fig. 2E). It is also important to note here that the other otolith organs, the utricle or lagena, may play a role in directional processing, particularly in fishes with parallel saccules (e.g., goldfish) [Popper and Fay, 1999]. The diversity of otolith organs among major fish taxa (e.g., the unusual auditory utricle characteristic of the herrings) indicates that the peripheral organization can vary substantially, but the central nervous system is organized similarly among non-teleosts and teleosts (Northcutt, 1980; McCormick, 1999), consistent with a common ancestral template for conducting directional analyses, whether for orientation to gravity or orientation with respect to sound.

A series of behavioral studies indicated that bony fish from a variety of taxa could determine the direction of a sound source [see review by Sisneros and Rogers, 2016]. Two pivotal studies with cod (*Gadus morhua*) determined their minimum audible angle (the minimum detectable angle between two sound sources) and concluded they could distinguish two sources separated by 20° in azimuth and 16° in elevation [Schuijf, 1975; Hawkins and Sand, 1977] at a distance of 3–4m, which is comparable to the ability of some small mammals [Brown and May, 2005]. In addition, cod can solve the 180° directional ambiguity inherent in particle motion and distinguish sounds from opposing directions [Buwalda et al., 1983]. The cod ear receives both *direct* particle motion from the external sound source and *indirect* input (pressure transformed to particle motion) from an extension of the gas bladder that approaches the otic capsule [Dale, 1976; Buwalda et al., 1983]. As noted previously, the indirect input from the gas bladder would provide a consistent caudal stimulus to the ear independent of the actual location of the source. Schuijf and Buwalda [1975] proposed the “phase model” and Schellart and Buwalda [1990] developed the “orbital model” to explain how the dual inputs could provide information that allows fish to resolve the 180° ambiguity. Discussion of the phase model, the orbital model, and other models of sound source localization by fishes may be found in Schellart and Buwalda [1990] and Sisneros and Rogers [2016].

Finally, there is evidence that binaural processing is necessary in bony fishes. Behavioral observations of cod support the requirement for two saccules [e.g., Schuijf and Siemelink, 1974] and physiological data [Horner et al. 1980; Edds-Walton and Fay, 2008, 2009] support the presence of binaural processing in the ascending auditory pathway for cod and oyster toadfish, suggesting that monaural processing is insufficient for sound source localization in these teleost fishes. In the following two sections, we focus our discussion on how source direction is encoded in the auditory nuclei of the brain stem of bony fishes, with special focus on the oyster toadfish.

Organization of the Brain Stem

Primary saccular afferents exit the macula and merge into bundles that represent regions of the saccule with the same or similar hair cell orientations before joining the VIIIth cranial nerve. This nonrandom organization of afferents has been documented in teleosts from different taxonomic groups that also have different saccular hair cell orientation patterns [Saidel and Popper, 1983; Edds-Walton, 1998a; Ladich and Popper 2001]. We propose that regional organization of opposing hair cell orientations (in contrast to the agnathan macula

communis) creates more coherent afferent input to the octaval nuclei and allows for the separation of afferents encoding low frequency linear acceleration for vestibulo-spinal or vestibulo-ocular circuits (below about 10 Hz) from afferents encoding sound (generally defined as above 20 Hz) within the same target nucleus. In general, the octaval nuclei in the fish medulla receive some input from all end organs of the ear, though the *relative* contribution from each end organ varies within each nucleus [McCormick, 1999].

Afferents in nVIII project to the octaval column of nuclei in the medulla that is ventral to the two lateral line nuclei. In non-teleost fishes, there are four octaval nuclei (from rostral to caudal): anterior, descending, magnocellular, and posterior [McCormick 1992, 1999] (fig. 3A). Teleost fishes have an additional octaval nucleus, the tangential octaval nucleus; however, only the anterior and descending octaval nuclei contribute to the ascending auditory pathway in any bony fish. Since contributions from the anterior octaval nucleus appear to be species dependent among teleosts, McCormick [1999, 2011] has suggested that ascending projections from the anterior octaval nucleus and the descending octaval nucleus to the midbrain may be a basal vertebrate circuit and that the projections from the anterior octaval nucleus have been lost secondarily in some species.

In the non-teleost bowfin, *Amia calva*, the electrosensory nuclei and lateral line nuclei dominate the medulla. The descending octaval nucleus is relatively small with nearly equal areas in the dorsal (potentially auditory) and ventrolateral (vestibular) regions [McCormick, 1981]. Input from the saccule is most dorsomedial, and the lagenar and utricular inputs project progressively more laterally in the descending octaval nucleus [McCormick and Braford, 1988]. This pattern of dorsomedial saccular input is common in teleosts with an “auditory” saccule (responds to stimulus frequencies in what is defined by humans as auditory: > 20 Hz). Among clupeids studied to date, a portion of the divided utricle has an auditory role [e.g., Denton et al., 1979], and the central projections from the auditory subdivision of the utricle terminate in the dorsomedial region of the descending octaval nucleus [McCormick, 1997], which is consistent with the hypothesis that the physiological activity of afferents strongly influences the distribution of afferent inputs, not simply the site of origin.

The size of the descending octaval nucleus varies among fishes and may be described as having three patterns [McCormick, 1999; 2011]. The simplest, possibly basal, pattern is that seen in the bowfin, *Amia* (p1 in fig. 3B). The “derived” descending octaval nucleus extends more dorsally than the smaller nucleus in the bowfin (for example, includes p1 and p2 in fig. 3B) and is found in many teleosts [e.g., O’Marra and McCormick, 1999]. The third pattern seen in some teleosts is an expanded descending octaval nucleus that extends dorsally and/or medially to underlie the cerebellar crest (includes p1, p2, and p3 in fig. 3B). McCormick [1999, 2011] suggested that the dorsomedial extension (e.g., p3) of the descending octaval nucleus may represent an expansion for reception of inputs from afferents whose response properties are dominated by the pressure input (versus particle motion input from the otolith organs). The pressure-dominated inputs could provide the phase of the pressure wave for directional computations and/or encode the higher frequencies commonly detected by fishes with specializations for pressure detection (e.g., goldfish) [Ladich and Schulz-Mirbach,

2016]. The latter suggests that an expansion of the frequency response of the ear coincides with a dorsal/medial expansion of the processing area in the auditory nucleus.

Afferents from two or more otolith organs converge onto the same cell in the dorsal descending octaval nucleus in the goldfish [McCormick and Wallace, 2012], and there is anatomical evidence for convergence of otolith organ inputs in the descending octaval nucleus of the sleeper goby (*Dormitator latifrons*) [Tomchik and Lu, 2005]. Along with the ipsilateral convergence of auditory inputs in the descending octaval nucleus, a commissural tract links the octaval nuclei [Ariëns-Kappers et al., 1936; Nieuwenhuys and Pouwels, 1983], and the dorsal region of the descending octaval nucleus has reciprocal connections with its contralateral homologue, providing the opportunity for ipsilateral-contralateral comparisons (e.g., fig. 3A, B) [Edds-Walton, 1998b]. Among electroreceptive fishes, both ipsilateral and contralateral otolith organ inputs project directly to the descending octaval nucleus [Bell, 1981; Nieuwenhuys and Pouwels, 1983; Kozłowski and Crawford, 1998]. Given that the earliest fishes are likely to have been electroreceptive [Northcutt, 1980], the binaural nature of the auditory region of the descending octaval nucleus may have been established early in the evolution of fishes.

The descending octaval nuclei project to both the secondary octaval population in the medulla (fig. 3A–D) and to the auditory midbrain (nucleus centralis of the torus semicircularis). The secondary octaval population consists of 2–3 nuclei that are sometimes compared to the olivary nuclei in other vertebrates based on their location and involvement in the ascending auditory circuit [see McCormick and Hernandez, 1996], but no physiological studies have been conducted on any nucleus in the secondary octaval population of a fish, and no homology nor analogy with olivary nuclei is intended by their inclusion in this review. The dorsal secondary octaval population has large bipolar cells with long, highly branched dendrites that extend laterally into the descending octaval nucleus and dorsally to the cerebellar crest (SO in fig. 3B–D), and axons that join the lateral lemniscus or the internal arcuate tract to the contralateral medulla (fig. 3D). The secondary octaval population projects directly to the auditory midbrain [McCormick and Hernandez, 1996; Bass et al. 2000, 2001; Edds-Walton and Fay 2005b]. A single study in toadfish has shown that the dorsal octaval nucleus, the secondary octaval population, and the midbrain (nucleus centralis) exhibit GABAergic somata and puncta [Edds-Walton et al., 2010], suggesting inhibitory interactions within those nuclei. The midbrain of fishes is a major sensory integration center where audition and other sensory systems converge (e.g., lateral line, vision), but directional sensitivity is retained in all species investigated [Schellart et al., 1987; Ma and Fay, 2002; Edds-Walton and Fay, 2003; 2005b].

In summary, the brain stem octaval nuclei in fishes exhibit similar connections to other vertebrate auditory nuclei. These common features are consistent with a basal circuit for sound source analysis. The most obvious difference is in the organization of the octaval nuclei among fishes versus tetrapods. All first-order nuclei in fishes receive inputs from all end organs of the ear. The otic afferent inputs appear to be organized based on the functional role of the peripheral end organ, with vestibular processing most ventral and auditory processing most dorsal. Overlap of otolithic and canal cristae afferent projections may be attributable to mixed functions of the otolithic end organs (see Cortopassi and Lewis, 1998).

We hypothesize that the expansion of auditory processing was associated with separation of the dorsal regions of the octaval nuclei that process sound from the ventral regions that process angular/linear acceleration associated with orientation of the body. Furthermore, within the octaval column, neural populations that no longer fire in synchrony, such as afferents that respond to sound versus gravity, would be less likely to remain grouped by common developmental mechanisms.

Directional Hearing Circuit in the Oyster Toadfish

The neural circuit to encode source direction has been investigated from the periphery to the midbrain in the oyster toadfish. Directional responses to particle motion were evaluated systematically only in the saccule [Fay and Edds-Walton, 1997; Edds-Walton et al. 1999], although the lagena and utricle may respond to particle motion in the auditory range [see review by Edds-Walton, 2016]. Physiologically characterized auditory afferents were labeled to reveal their origins on the saccule and their pattern of inputs to the medulla. Some saccular afferents bifurcated at their entrance into the medulla and projected to both the anterior octaval nucleus and to the descending octaval nucleus; however, the majority of directional saccular afferents projected only to the descending octaval nucleus, and all of them branched to form terminal fields at multiple sites along the rostro-caudal axis of that nucleus [Edds-Walton et al. 1999].

Extracellular recordings in the dorsal descending octaval nucleus revealed that some auditory neurons had cosinusoidal directional responses like those of primary saccular afferents (fig. 4A), but the majority of cells (60%) had sharpened directional responses (narrower response areas; fig. 4A') in azimuth and/or elevation [Edds-Walton and Fay, 2008]. This sharpening may be mediated by binaural and/or local inhibitory computations [Fay, 2005]. Neurons in the descending octaval nucleus also display a wider range of best axes (particularly in azimuth) than was seen among saccular afferents (compare distribution of points in fig. 4B, B'), indicating that the left saccule was not the only source of directional information to the left descending octaval nucleus [Edds-Walton and Fay, 2008]. The presence of a commissural tract between the dorsal descending nuclei is the likely source of contralateral input required to account for the wide range of directional responses (fig. 3B).

Further support for the presence of binaural auditory cells in the descending octaval nucleus was obtained physiologically through tipping the contralateral (right) saccular otolith while recording single-unit activity in the left descending octaval nucleus. Mechanical tipping of the otolith away from the brain (by about 30°) altered the normal orientation of the sensory epithelium and altered the normal activity from that saccule [Edds-Walton and Fay, 2009]. A variety of changes in the directional responses of cells in the descending octaval nucleus when the contralateral saccular otolith was tipped confirmed that contralateral input contributes to the directional analyses (e.g., fig. 2D). The most common response during tipping was the loss of activity (regained post-tipping), indicating loss of contralateral input(s) required to reach threshold, which is consistent with contralateral excitatory input. Such cells are often abbreviated as EE cells for the presumed excitatory contra- and ipsi-lateral inputs; but note that the methods could not distinguish EE cells from cells that

received ipsilateral inhibitory input that required sufficient excitatory contralateral inputs to overcome the inhibition (an EI cell; see below). In other binaural cells, contralateral tipping resulted in an increase in the magnitude of the directional response (increased spiking) and other cells exhibited both an increase in spiking and a change in the directional response pattern, consistent with contralateral inhibitory inputs to those cells (abbreviated as IE cells) (fig. 2D). Thus, we can conclude that the commissural tract between the two dorsal regions of the descending octaval nuclei (fig. 3B) mediates directional auditory analyses. In addition, altering the *ipsilateral* saccular input caused an increase in the magnitude of the directional response of some dorsal descending cells, indicating inhibition could originate ipsilaterally as well as contralaterally [see fig. 6 in Edds-Walton and Fay, 2009]. Similar conclusions were reached by Horner et al. [1980] using ipsilateral, contralateral, and simultaneous anodal blocking on the posterior saccular nerve bundle in cod while recording from auditory cells at sites along the octaval column near nVIII.

Further directional computations were indicated by the response properties of cells in the midbrain target (nucleus centralis in the torus semicircularis) of the descending octaval nucleus and secondary octaval projection cells [Edds-Walton and Fay, 2003, 2005a]. Over 85% of midbrain auditory cells had sharpened directional responses, with the greatest increase occurring in the relative number of cells sharpened in azimuth [Edds-Walton and Fay, 2005a]. The saccular otolith-tipping experiments revealed greater convergence of inputs (only 5% lost activity during tipping) and greater dependence on binaural inputs for the directional auditory responses in the midbrain [fig. 5 in Edds-Walton and Fay, 2009]. Horner et al. [1980] also documented binaural auditory units in the midbrain of the cod using ipsilateral or contralateral anodal blocks.

Taken together, studies in toadfish and cod confirm that binaural interactions are involved in the directional computations in the brain stem of teleost fish from taxonomically diverse members of the Osteichthyes, which may indicate a common computational pathway in an ancestor of the bony fishes. The consistent presence of directional responses along the auditory pathway indicates that determining source direction is an important component of auditory processing in those teleost fishes examined. We suggest that the similarities in the circuit and complex auditory processing at the various levels of the ascending circuits of fishes and terrestrial vertebrates indicate that the computational circuitry necessary for sound source localization may have emerged early in the evolution of vertebrates (fig. 5).

The Transition onto Land: Early Tetrapods

Tetrapods evolved from the lobe-finned fishes around 390 MYA, in the middle Devonian [Clack, 2011]. The work on modern fishes described above provides a framework for how the auditory systems of these early aquatic tetrapods could have been organized. Although most of the physiological data on directional hearing come from teleosts, the data are consistent with anatomical and physiological results from other taxonomically distant fishes [McCormick 1999; Meyer, 2010, 2012] and thus may reflect the organization of the auditory systems of the early aquatic tetrapods [Carr and Christensen-Dalsgaard, 2016]. It seems unlikely that neural circuits sensitive to sound in air appeared *de novo* because sound sensitivity would have been based on the same auditory end organs as in the ancestors.

Instead, we propose that neural circuits for sound source localization were present in the ancestors of the early Osteichthyes (figs. 5A–C) and that end organs sensitive to particle motion endowed early aquatic tetrapods with directional hearing in water.

Below, we review the evolution of the tympanum and consider scenarios for the subsequent reorganization of neural circuits for sound localization in early amphibious tetrapods and in their diapsid descendants. We will not discuss scenarios for the development of the mammalian ear and auditory pathways. For recent reviews of mammalian ear evolution, the reader is directed to Manley [2012, 2016] and Fritzsche and Straka [2014], and for discussions of mammalian auditory pathways, see Grothe et al. [2005], Fritzsche et al. [2013], and Nothwang [2016].

What could early terrestrial tetrapods hear? As for the aquatic habitat, the sound wave in air acts as a force that imparts linear acceleration at low frequencies and generates structural vibrations in the cranium at higher frequencies. These effects are collectively referred to as bone conduction or extratympanic hearing. The main limitations in air compared to water are (1) the wavelengths are shorter, (2) there are density differences between animal and medium, and (3) the animal is not moving freely in the medium but usually is in contact with the substrate. Nonetheless, there is abundant evidence that atympanate tetrapods (salamanders, earless frogs, snakes) are relatively sensitive to low-frequency airborne sound [Christensen et al., 2012; Christensen et al., 2015b; Capshaw and Soares, 2016; Pereyra et al., 2016].

Lungfish can detect airborne sound through sound-induced head vibrations [Christensen et al., 2015a]. Several recent papers suggest that the first terrestrial tetrapods might have had similar hearing to modern lungfish and thus some sensitivity to low frequency airborne sound [Christensen et al., 2015a; Clack, 2015, Carr and Christensen-Dalsgaard, 2016] even though they lacked middle ear adaptations for hearing in air. Like lungfish, and as discussed in the previous paragraph, early aquatic tetrapods probably could hear underwater by sensing the particle motion component of sound [Christensen et al., 2011a, b]. In air, they may have heard low-frequency sound by non-tympanic mechanisms. The sound-induced vibrations of the skull might have produced directional responses to the particle motion component of sound. Resonance of air in their lungs or gill pouches could have conferred sensitivity to sound pressure in air and in water [Christensen et al. 2015a, b; Clack, 2015]. Because the vibration response would still reflect the 180° ambiguity inherent in particle motion, processing of sound pressure as well as particle motion would be as relevant as for the tetrapod ancestors for directional hearing.

Over time, tetrapod ears changed, developing increased sensitivity to sound in air. Christensen-Dalsgaard and Manley [2013] outlined a scenario with four stages of ear morphology leading to increased sensitivity of the nonmammalian ear prior to development of the tympanum, beginning with an early inner ear similar to the lungfish ear and sensitive to very low-frequency sound and substrate vibrations. Subsequently, high-frequency sensitivity of the inner ear could have been increased by reducing the mass of the otolith(s). Next, the columella (also termed the stapes) transformed from a structural element in the jaw to a flexible columella-inner ear attachment with the large columella acting as an inertial

element. Finally, a tympanic ear may have been achieved by joining a much smaller columella to the surface of the skull in the region where the skin covered the former spiracle [Christensen-Dalsgaard and Manley, 2013].

Both paleontological and developmental studies show that tympanic hearing appeared after the divergence of the major tetrapod groups from their common ancestor. Examination of the fossil record shows separate development of key middle ear elements in amphibians, synapsids (mammal-like reptiles), lepidosauromorph diapsids (snakes, lizards), archosauromorph diapsids (dinosaurs, birds, and crocodiles), and probably turtles [Clack, 1997]. Comparisons of jaw development in mouse and chick support the paleontological findings. The relative positions of the primary jaw joint and the first pharyngeal pouch led to the coupling of tympanic membrane formation with the lower jaw in mammals and with the upper jaw in diapsids [Kitazawa et al., 2015]. In the mammalian ancestors, the primary jaw joint moved dorsally, eventually associating the lower jaw with the tympanum and tympanic ring (a structure derived from lower jaw elements). Mammalian middle ear bones were derived from the hyomandibular bone and from both upper and lower jaw elements. In diapsids, the hyomandibular decoupled from the quadrate to form the columella, or stapes, and the position of the primary jaw joint did not move [Kitazawa et al., 2015]. Thus tympanic hearing is a true evolutionary novelty that arose in parallel multiple times [Clack 1997; Christensen-Dalsgaard and Carr, 2008], most likely in the Triassic, 100 MY after the origin of tetrapods.

Scenarios for Emergence of Directional Sensitivity in Air

We proposed above that early atympanate tetrapods possessed neural circuits sensitive to sound direction in water (fig. 5). The question is how these circuits would function in air. Studies of low frequency auditory nerve responses in extant amphibians may provide a clue. Below 400 Hz, the tympanum shows very little motion or directionality [Wilczynski et al., 1987], and auditory nerve responses are assumed to be extratympanic in origin. These low frequency responses are directional with an almost 180° phase shift between ipsilateral and contralateral stimulation and equal sensitivity to ipsi- and contra-lateral directions [Wilczynski et al., 1987, Jørgensen and Christensen-Dalsgaard, 1997a, b; Christensen-Dalsgaard and Kannevorf, 2005]. Atympanate frogs, the so-called earless frogs, have secondarily lost part of the middle ear apparatus [Jaslow et al., 1988; Pereyra et al., 2016]. Most of the atympanate species communicate by sound and, therefore, must hear solely through extratympanic pathways, but nothing is known about their ability to localize sounds.

Tetrapods are characterized by a new hearing organ, the basilar papilla or the cochlea. We do not know when it emerged or whether it is even homologous in modern tetrapods. The papilla generally lies within the lagenar recess of the inner ear/otic capsule, and its origin(s) are debated. Lombard and Bolt [1979] proposed that the basilar papilla in amphibians and amniotes was independently derived, while Fritzsche [1987] used his analysis of the coelacanth, *Latimeria*, to argue that the papilla arose once in the sarcopterygian ancestor of tetrapods [Fritzsche, 1999]. Regardless of which scenario is correct, it is likely that the early papilla was formed from patches of hair cells either without an otoconial covering or with an otoconial covering that was lost. Furthermore, formation of a new auditory papilla should

have exerted a reorganizing effect on its central auditory targets [Wilczynski, 1984; Fritzsche, 1997, 1999]. Afferents innervating these hair cells could have conveyed directional information to auditory nuclei in the medulla (fig. 5B).

One scenario, illustrated in figure 5, is the transformation of an Osteichthyan circuit (fig. 5A, B) to one found in diapsids via a hypothetical amphibious intermediate. In this scenario, afferents from the new papilla would have first overlapped with the saccular input to the “auditory” portion of the descending octaval nucleus (or its equivalent, fig. 5C). New, dorsal auditory targets might have formed later. This scenario requires relatively little reorganization since, in fish, auditory afferents from the saccule enter the medulla and terminate in the dorsal regions of the anterior octaval nucleus and the descending octaval nucleus (fig. 5A, B). We propose that these two octaval nuclei may correspond to the two diapsid first order nuclei. Based on both position and connections, the diapsid first order nucleus angularis may be derived from an anterior octaval population, like that in chondrosteans, holosteans, and teleosts, while the first order nucleus magnocellularis in diapsids may be derived from the dorsal, auditory portion of the descending octaval nucleus [fig. 5A–E]. Major changes would have included accommodation of afferents from the new papilla and the separation of the dorsal “auditory” portion of the descending octaval nucleus from the underlying “vestibular” portion.

What directional information could have been available to the hypothetical intermediate shown in fig. 5C? In amphibians and diapsids, the tympana originate at the location of the spiracles. The spiracles open directly in the pharynx through permanently open Eustachian tubes, coupling the ears acoustically. We have hypothesized that the ancestral tympanic ears may have been similar to the ears of recent frogs and lizards with regard to coupling of the two tympana to each other and to the lungs through wide Eustachian tubes and the mouth cavity. The coupling is not as strong in frogs as in lizards (see below), but it does generate an effective directionality (here and in the following defined as the difference between ipsi- and contra-lateral eardrum vibrations) of up to 10 dB with a strongly lateralized directivity pattern [Christensen-Dalsgaard and Manley, 2005; reviewed in Christensen-Dalsgaard, 2005].

In frogs, directionally sensitive inputs project to the auditory dorsal medullary nucleus. Its origins and possible homology to the amniote cochlear nuclei remain uncertain [Wilczynski and Capranica, 1984], but it is reasonable to assume that the dorsal medullary nucleus is derived from an “auditory” portion of the octaval column since the connections are similar. All four acoustic end organs (saccule, lagena, and the evolutionarily “new” amphibian and basilar papillae) project within, or near, the dorsal acoustic zone in at least some anurans [reviewed in McCormick, 1999]. Interestingly, the dorsal medullary nucleus also receives extensive commissural projections from the contralateral dorsal medullary nucleus, indicating significant binaural interactions in a primary medullary nucleus [Feng, 1986; Christensen-Dalsgaard, 2005]. This mirrors the projection patterns (figs. 3B, 5) [Edds-Walton, 1998b] and physiological responses observed in fishes [Edds-Walton and Fay, 2009].

Binaural neurons in frogs are present in the first order dorsal medullary nucleus and generally respond to specific combinations of interaural time and level differences when stimulated dichotically [Christensen-Dalsgaard and Kannev, 2005]. Binaural responses are mainly EI [Feng and Capranica, 1976], although both ipsilateral and contralateral inputs are excitatory when presented monaurally. Binaural EI/IE neurons in the medulla, like those found in oyster toadfish, could transform and sharpen directional responses of auditory nerve fibers by suppression. The first-order and olivary nuclei project predominantly to the contralateral midbrain torus, where most of the cells show directional responses [Christensen-Dalsgaard, 2005]. It appears that directionality is computed via a population code mediated in part by inhibition by the opposite halves of the torus.

Directional Hearing in Modern Diapsids

Diapsids are a diverse group that includes lepidosaurs, turtles, and archosaurs (crocodiles and birds), as well as a number of extinct animals. They display a variety of mechanisms to enhance directional hearing that are also reflected in the organization of the first-order auditory nuclei. Lizards have middle ears that are coupled across the pharynx and thus are inherently directional [Christensen-Dalsgaard et al., 2011b], while recent archosaurs have less directional ears and well-developed binaural auditory nuclei devoted to sound localization [Carr and Christensen-Dalsgaard, 2015; Carr et al., 2016]. Turtles do not show notable adaptations for directional hearing. Their middle ears are connected to the mouth cavity by thin Eustachian tubes, and their hearing may be adapted for an amphibious life [Putman et al., 2010; Christensen-Dalsgaard et al., 2012; Willis et al., 2013b]. Snakes have lost their tympanum and each stapes is attached to the lower jaw. Snakes are hypothesized to have the ability to localize substrate-borne vibration [Friedel et al., 2008], but little is known about processing of sound source location in their central auditory systems. Therefore, we will focus on a scenario for the evolution of brain stem circuits for sound source localization, first in lepidosaurs because of their coupled ears, and then in archosaurs (figs. 5, 6). The emergence of tympanic hearing in both groups would have led to increased sensitivity to higher frequency sounds and to an expanded representation of sound in the central auditory system as sound became more salient.

Directional Hearing in Lepidosaurs

We have hypothesized that early tympanate diapsids had coupled ears since the diapsid tympanum forms by roofing over the spiracle, which couples the tympana through the pharynx [Christensen-Dalsgaard and Carr, 2008]. Coupling allows sound from the ipsi- and contra-lateral ears to interact at the eardrum and cancel or increase its motion depending on the phase difference between the two components, producing a strongly directional response (fig. 6B) [Christensen-Dalsgaard, 2011]. It is not clear how strong this coupling would have been in early tympanate diapsids, but modern lepidosaurs have strong acoustical coupling of the eardrums due to unattenuated transmission of sound from the contralateral ear with ensuing significant directionality (20–30 dB) in a certain frequency range [Christensen-Dalsgaard and Manley, 2008; Christensen-Dalsgaard et al., 2011b; review in Carr et al., 2016].

The inherent directionality provided by coupled ears resembles the situation in fishes. Both receive directional input from their periphery: fishes via directional hair cells, and lepidosaurs via coupled tympana. The auditory brain stem in early tympanate diapsids and lepidosaurs may have functioned like that of fishes, where bilateral comparisons act to sharpen and differentiate between sounds from different locations. Anatomically, the brain stem auditory circuits of both groups are characterized by commissural projections (fig. 5B, C, E), which may be a conserved feature of the octavolateralis system, as discussed above [Straka and Baker, 2013]. In lepidosaurs, binaural (bilateral) comparisons could be used to differentiate between loud sounds from a “poor” location and quiet sounds from a “good” location. For example, monaural responses are directional, with higher spike rates from ipsilateral stimulus directions and lower rates from contralateral stimulus directions (fig. 6B). However, spike rate responses also depend on sound level and, therefore, binaural comparisons are needed to disambiguate the effects of level and location. These computations should require inhibitory interactions, much like the EI processing observed in the oyster toadfish’s descending octaval nucleus and the frog’s dorsal medullary nucleus discussed above [Christensen-Dalsgaard and Kannev, 2005; Edds-Walton and Fay, 2008]. Since eardrum directionality is strongly asymmetric across the midline, lateralized responses could be generated by rate-based binaural comparisons from each ear. This coding strategy would also be similar to the rate-based or population code comparisons in two lateralized channels proposed for mammals [Stecker et al., 2005; McAlpine et al. 2001, see review in Grothe et al., 2010].

Directional Hearing Circuit in Geckos

The connections of the central auditory nuclei of lepidosaurs and archosaurs are similar, although the lepidosaur nuclei are not as large (fig. 6A, C). Studies of geckos nevertheless have revealed well-differentiated monaural and binaural ascending pathways. In all tympanate diapsids studied, the first order nucleus magnocellularis projects bilaterally to the nucleus laminaris. The ipsilateral projection from the nucleus magnocellularis innervates the dorsal dendrites of laminaris neurons, while the contralateral projection innervates the ventral dendrites [Yan et al., 2010; Tang et al., 2012]. This connection pattern is very similar to that found in fishes (fig. 5B) and the same as that in archosaurs, but with the major difference that the canonical map of interaural time differences found in birds is not found in lizards.

In lizards with well-developed high-frequency hearing, like the gecko, the first-order nucleus angularis is comparatively large, presumably reflecting the additional projections of afferents from a new population of hair cells sensitive to high frequencies [Szpir et al., 1990; Tang et al., 2012]. Projections of the olivary nuclei are complex in gecko, consistent with a well-developed central auditory system, while extensive GABAergic connections suggest a role for inhibitory interactions [Tang et al., 2012].

Directional Hearing in Archosaurs

In the evolution of directional hearing, lepidosaurs and archosaurs took separate paths [Manley, 2016]. Archosaur middle ears are more isolated from the mouth but are connected by sinuses, resulting in attenuation of interaural transmission and a weaker directionality (of

up to 10 dB) in the periphery. There also appears to be an increased role for binaural comparisons in the brain. In archosaurs, auditory nerve fibers enter the brain and bifurcate to terminate in the first order nucleus angularis and nucleus magnocellularis (fig. 5C; 6A, E). The neurons of the nucleus magnocellularis project bilaterally to the nucleus laminaris in a prominent dorsal tract, similar to that in fishes (fig. 5E). The nucleus laminaris is large, often extends across most of the dorsal surface of the brain stem, and contains a place map of interaural time differences [Köppl and Carr, 2008; Carr et al. 2009, 2015; Palanca-Castan and Köppl, 2015a, b]. These structures all receive descending inhibitory inputs from the superior olive. We will review these sound source localization circuits below, first for crocodilians, then for birds, and finally for a sound localization specialist, the barn owl.

Directional Hearing Circuit in Crocodilians

Crocodilians have a large, thin tympanum coupled to a columella (or stapes). This configuration is consistent with adaptations for hearing in air, although they also hear underwater [Higgs et al., 2002]. Paratympanic sinuses couple the middle ears and are believed to contribute to the increased range of interaural time differences [Carr et al., 2009] and to the directional sensitivity observed in the auditory brain stem response [Bierman et al., 2014]. Phase locking in the crocodilian auditory nerve is improved when compared to lizards and frogs, in which phase locking is largely absent above 1,000 Hz. In the caiman, the auditory nerve phase locks to frequencies up to at least 1,500 Hz [Smolders and Klinke, 1986; Carr et al., 2009]. These improvements in temporal coding are relevant for a system that computes interaural time differences.

In crocodilians, the projections of the nucleus magnocellularis to the nucleus laminaris appear to act as delay lines to create maps of interaural time difference (fig. 6) [Carr et al., 2009]. This scheme is termed the Jeffress model or place code, and it assumes that delay line inputs synapse on arrays of coincidence-detector neurons that respond maximally when phase-locked inputs coincide [for reviews, see Grothe et al, 2010; Ashida and Carr, 2011]. Crocodilian nucleus laminaris neurons are bitufted and receive input from the ipsilateral nucleus magnocellularis on their dorsal dendrites and input from the contralateral nucleus magnocellularis on their ventral dendrites. These laminaris neurons act as coincidence detectors (EE) and are sensitive to interaural time differences [Carr et al., 2009]. This circuit will be discussed in more detail below.

The computation of interaural time difference in the nucleus laminaris represents a major change in neural coding strategy when compared to lepidosaurs. The brain stem nuclei in birds and crocodilians also appear proportionally larger than those of lizards and turtles [Bierman and Carr, 2015], which may reflect the greater range of neural computations taking place there.

Directional Hearing in Birds

The auditory systems of birds are similar to those of crocodilians. The nucleus magnocellularis and nucleus angularis form the starting points of at least two parallel ascending auditory streams with varying degrees of specialization for processing different aspects of sound. The nucleus magnocellularis is the simpler of the two, containing one

predominant neuron type adapted to maintain the temporal information conveyed through phase locking from the auditory nerve. The nucleus magnocellularis is thus regarded as the starting point of a “time pathway”. The best-known function of this pathway is the processing of interaural time differences used in sound localization [reviewed by Konishi, 2003; Burger and Rubel, 2008]. Nucleus angularis appears to be the origin for everything else, although commonly referred to as the “intensity pathway”, which does not do justice to the complexity already seen at this level. The nucleus angularis contains a range of cell types having distinct anatomical and physiological properties [reviews in Fukui and Ohmori, 2003; Grothe et al., 2004; MacLeod and Carr, 2007]. Although cell-specific connection patterns have not been investigated, nucleus angularis neurons project to the superior olive and lemniscal nuclei, as well as directly to the auditory midbrain (inferior colliculus). Thus, the potential for differentiated processing streams is present at the level of the first order auditory nuclei.

Interaural time differences are computed in the nucleus laminaris, as described above for alligators [reviewed by Ashida and Carr, 2011]. The physiological range of interaural time differences is determined both by head size and by coupling between the ears. In birds, air-filled interaural canals connect the middle ears and may increase directionality by allowing sound transmission from the contralateral ear to the internal surface of the eardrum [Larsen et al., 2016]. The nucleus laminaris neurons are bitufted, with segregated inputs from ipsi- and contra-lateral nucleus magnocellularis. Bitufted laminaris neurons are found in turtles [Willis et al., 2013a], geckos [Tang et al., 2012], alligators [Carr et al., 2009] and birds [Ramon y Cajal, 1908; Smith and Rubel, 1979], and resemble the bitufted neurons of the fish descending octaval nucleus and in the dorsal secondary octaval population (fig. 3C, D). The bitufted organization may reflect computational demands, as well as evolutionary history [for discussion, see Carr and Soares, 2002]. The biophysics of binaural processing has not been investigated in most vertebrate groups, but in archosaurs and mammals, segregation of binaural inputs onto separate sets of dendrites improves coincidence detection [Agmon-Snir et al., 1998]. Interestingly, this bitufted morphology may have originated in a common ancestor of the Actinopterygii and the Sarcopterygii. A similar conservation of cell types has been proposed for the elements of the vocal hindbrain-spinal compartment [Bass et al., 2008].

Directional Hearing Circuit in the Barn Owl

Barn owl auditory pathways are organized similarly to other archosaurs but exhibit many features that may be associated with accurate sound source localization. These include hypertrophy of brain stem auditory nuclei with respect to other birds [Kubke et al., 2004; Kubke and Carr, 2006], high frequency hearing [Konishi, 1973], increased ability to phase lock to higher frequency sounds [Köppl, 1997], reorganization of the circuits for computation of interaural time differences in the nucleus laminaris [Carr et al., 2015], parallel processing of interaural time and level differences [Moiseff and Konishi, 1983], and the presence of a map of auditory space in the midbrain [Knudsen and Konishi, 1978]. Most of these adaptations may be related to high-frequency hearing and accurate localization of the high-frequency sounds made by prey (above 4 kHz). At these frequencies, interaural

coupling is greatly reduced, and the ears are functionally not connected, so directional information relies on neural processing of both interaural level and time differences.

Owls are the only birds studied to date with strongly asymmetrical ears [Volman and Konishi, 1990]. This asymmetry produces interaural level difference cues that are used for localization of sound source elevation, while interaural time differences are used to determine sound source azimuth. The separate, bi-coordinate processing of interaural time and level differences allows for parallel computation of interaural time differences in the nucleus laminaris and for emerging sensitivity to interaural level differences in the nuclei of the lateral lemniscus [Manley et al., 1988]. The two parallel channels converge on the midbrain, where they form a matrix of neurons tuned to different combinations of binaural cues [reviewed in Konishi, 2003]. This tuning to binaural cues is responsible for the spatial selectivity of the midbrain space-specific neurons, which respond maximally to stimuli located in a small, restricted area termed the receptive field [reviewed in Takahashi et al., 2003].

The well-developed map of auditory space in the barn owl midbrain may be an adaptation for nocturnal predation. Less nocturnal owls with symmetric ears have auditory responses that are less restricted in elevation, and they have a map only of sound source azimuth [Volman and Konishi, 1990]. The maps of auditory space in the barn owl inferior colliculus may provide speed and accuracy and permit precise alignment of auditory and visual maps in the optic tectum to mediate orientation to prey. Thus, barn owl auditory circuits reflect numerous adaptations of a basal circuit mediating sound source localization.

Analysis and Conclusions

Bilateral comparisons probably evolved with the earliest bilaterians [Renier et al., 2010] and are a key feature of octaval systems [Straka and Baker, 2013]. In this review, we propose that sensitivity to sound direction, supported by bilateral comparisons, characterized the auditory systems of the earliest aquatic vertebrates and provided a template for auditory processing in modern tetrapods. In extant fishes, the intrinsic physiological polarization of sensory hair cells and their various orientations on the otolith organs confer sensitivity to the direction of particle motion originating from locations all around the fish [Popper and Fay 1999]. This wide range of hair cell orientations is found in all lineages of jawless and jawed fishes (agnathans, chondrichthyes, and osteichthyes; fig. 1) and is consistent with a common ancestral form with otolith organs capable of encoding stimulus direction. Binaural comparisons are a consistent component of sound localization circuits. In fish, binaural differences may be generated primarily by the different orientations (and resultant best response directions) of the paired auditory maculae, whereas in tetrapods, acoustical coupling of the middle ears and/or interaural time and level differences may generate binaural cues.

There are organizational differences between the central auditory circuits in Osteichthyes and tetrapods. In fishes, octaval nuclei receive inputs from all end organs. There is some regional specialization: inputs from the canal cristae tend to terminate most ventrally, and inputs from the three otolithic end organs are distributed more dorsally. The otolith organ

with a primary auditory role projects to the most dorsal and medial portions of the descending octaval nucleus [McCormick, 1999; Edds-Walton et al., 1999], and the descending nucleus is consistently a major contributor to the ascending auditory circuit. Binaural connections and binaural computations that result in sharpened directional responses characterize the descending octaval nuclei and the auditory midbrain [Edds-Walton and Fay, 2009; Edds-Walton et al., 2010].

With the movement onto land, early tetrapods may have retained some sensitivity to particle motion transduced by bone conduction. It is not clear when new auditory papillae emerged, although tympanic hearing certainly evolved multiple times after branching of the major tetrapod lineages [Clack, 1997; Christensen-Dalsgaard and Carr, 2008]. New papillae with broader and/or higher frequency responses than the otolith organs may have exerted a reorganizing effect on the central auditory nuclei [Wilczynski, 1984]. The differences between the response properties of the otolith organs and the new papillae (e.g., frequency range and overall sensitivity) may have been associated with greater separation of otolith inputs versus papilla inputs, resulting in a gradual shift from nuclei with indistinct functional borders, as found in fishes, to functionally distinct nuclei and increased regionalization of the dorsal (auditory) versus ventral (vestibular) medulla found in modern tetrapods (fig. 5A–E). One scenario (papilla first) is that afferents innervated by the new auditory basilar papilla terminated in the dorsal, auditory portion of the descending octaval nucleus, which expanded in response to the new input (fig. 5). A second scenario (brain first) is that the new papilla followed the formation of new first order auditory targets, such as the dorsal medullary nucleus in amphibians or the auditory portion of the descending octaval nucleus in diapsids. Subsequently, perhaps in the early diapsids, the dorsal auditory portion of the piscine-like descending octaval nucleus could have separated to form the first order nucleus magnocellularis, while the anterior octaval nucleus became the first order nucleus angularis. Similar changes should have occurred in the ancestors of mammals [for a recent review of rhombic lip derivatives, see Wullman, 2010].

The commissural connections between the first-order auditory nuclei present in fishes are not present in diapsids, while there are commissural connections to the second order nuclei in both groups (fig. 5B, C, E). There are (at least) two potential scenarios. One is that the ancestral tetrapods may not have had homotopic connections between first order octaval nuclei. However, as discussed by Straka and Baker [2013], commissural connections are characteristic of the octaval nuclei among all vertebrates, so we regard this as unlikely. The second scenario is that commissural connections were lost between first order nuclei, but retained between first and second order nuclei for the requisite binaural comparisons.

Binaural comparisons allow disambiguation of location and level cues. This would have been important in the earliest tympanate diapsids, whose tympana were likely coupled. Coupled ears are inherently directional, and simple, rate-based comparisons (EI) generate strongly lateralized responses on each side of the brain. In lizards, these responses resemble the “two-channel” model proposed for mammals in which location is computed by comparing the lateralized output of the two channels [for review, see Grothe et al., 2010]. In archosaurs, the middle ears are more isolated from the mouth than in lizards and thus less well coupled. Instead, the ears are connected by sinuses, resulting in attenuation of interaural

transmission and a weaker directionality in the periphery. In this group, sensitivity to interaural time difference is mediated by neural computations in the second order nucleus laminaris. This circuit is consistent with the Jeffress model, which assumes arrays of coincidence-detector neurons that form a map of interaural time differences.

In summary, peripheral end organs vary among vertebrates, and the mechanisms for stimulating the ear vary, but the computations for sound source localization involve comparable steps along an ascending neural pathway that is similar in extant fishes and diapsids. Given the potential advantages of locating various sound sources, we hypothesize that faithfully encoding stimulus direction was an early function of the ascending auditory system. We have proposed that an ancient neural circuit could have been modified within the various lineages of nonmammalian vertebrates to retain sensitivity to sound source location while the peripheral auditory system responded to the requirements of life on land.

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Fig. 1.

Sensory receptor orientations on maculae in the ear of vertebrates. **A** In the relatively more ancient lineage of jawless fishes, the hair cells on a single macula communis are oriented with various best directions. In the hagfish (right ear shown), the macula communis is an elongate patch of hair cells with an overlying statoconia (not shown) that lies in the same membranous labyrinth as the two cristae (dashed line indicates midline). In lamprey, hair cell orientations occur in 180° opposition in the middle segment of the macula communis. Arrows indicate best direction (particle motion producing maximum excitatory response) for hair cells in that region of the sensory epithelium (modified from Lowenstein, 1968). **B** In the Osteichthyes and their descendants, hair cells are oriented in patterns that have 180° opposition (along the line shown). Arrowheads indicate best direction for hair cells in that

region of the sensory epithelium; red, green emphasize the general differences illustrated among the vertebrates shown. (Portions redrawn from Sienknecht, 2013).

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Fig. 2.

Auditory periphery and directional auditory processing in a teleost fish, the oyster toadfish (*Batrachoidide: Opsanus tau*). **A** Topogram of living adult; relative density is indicated from white (greatest density: bone and otoliths) to black (lowest density: gas bladder). The paired saccular otoliths are visible in the box rostral to the vertebral column (V) and the gas bladder (GB). [scale bar = 10 cm] **B** Box in A enlarged to show all three otoliths. Note the parallel arrangement of the utricles versus the curved saccules ($\pm 40^\circ$ of the midline rostrally) and the small lagenar otoliths. U, utricle; S, saccule; L lagena (indicated with solid white arrow on right). **C** Hair cell structure: stair-step array of stereovilli and a single kinocilium, filled black, seen in side view; the axis of maximum excitation (= best direction for the cell) shown by the arrow. The arrow notation is used to illustrate the sweeping array of hair cell orientations on the saccular macula of an adult toadfish (~ 2 mm long); two-headed arrows are used because the location of the 180° reversal in orientation varied slightly among individuals. **D** Results of tipping the contralateral (= right) saccular otolith while recording directionality of a cell in the left descending octaval nucleus (DON) with 100 Hz particle motion stimuli at 30° intervals in the mid-sagittal plane (dots in plot are at stimulus angles). Altering the contralateral saccular input changed the best axis (BA) by 30° . **E** Each saccular afferent has a 'best azimuth' and a 'best elevation' in response to sinusoidal particle motion. Due to the angled orientations ($\pm 30^\circ$ off midline) of the saccular maculae, the right (red) and left (blue) saccules will be stimulated differentially by sounds from different azimuths around the fish as shown. Binaural computations comparing left and right inputs (plotted in green) could provide sufficient information to identify the location of a source in azimuth (particle motion axes shown with 180° ambiguity). [A, B modified from Edds-Walton et al., 2015; C modified from Edds-Walton and Popper, 1995; D original drawing from data presented in Edds-Walton and Fay, 2009; E modified from Fay, 2005].

Fig. 3.

The dorsal descending octaval nucleus and dorsal secondary octaval population in a teleost fish (oyster toadfish, *Opsanus tau*). **A** Neurobiotin injected into the dorsal descending octaval nucleus (dDON) labeled cells via their processes (methods in Edds-Walton and Fay, 2008). The dorsal secondary octaval population was labeled either via their dendrites or transneuronal transport. CC crista cerebellaris; dDON, dorsal descending octaval nucleus; dor dorsal; LLNM lateral line nucleus medialis; SO dorsal division of the secondary octaval population; Vdesc, descending tract of trigeminal cranial nerve; ven ventral; vDON ventral descending octaval nucleus. **B** Diagrammatic cross-section of medullary nuclei at the entrance of cranial nerve VIII showing projections from the descending octaval nucleus to the lateral lemniscus (to auditory midbrain), ipsilateral and contralateral secondary octaval population (only dorsal population shown), and contralateral DON. [IA, internal arcuate tract; LL, lateral lemniscus; LLN, lateral line nerve; p1, p2, p3, areas of descending (not functional subdivisions); RF reticular formation; VIII vestibuloacoustic cranial nerve] **C** A different experiment with same methods as in A that revealed the extent of dorsal and ventral dendrites of secondary octaval cells of the dorsal population. Asterisk indicates location of enlarged view in D; vertical arrow indicates approximate location of neurobiotin injection in an adjacent section. **D** Enlargement of cells in the dorsal population of the secondary octaval nuclei; asterisk indicates location from C; arrows indicate axons that turn ventrally to join either the lateral lemniscus or the internal arcuate fibers that cross to the contralateral medulla.

Fig. 4.

Directional responses of primary saccular afferents compared with afferents in the descending octaval nucleus (DON) of the toadfish (*Opsanus tau*). **A** Typical directional response pattern in the horizontal plane (azimuth) for a single saccular afferent stimulated at three levels. The outer circle represents 100 spikes/sec; center = 0 spikes. The directional response pattern is plotted with respect to the center of the stimulus dish that held the fish; dots represent stimulus angles (Fay and Edds-Walton, 1997). The best axis was calculated based on the relative response to adjacent stimuli and the location of the null. This saccular afferent saturated at the highest level and the cosine function flattened. **A'** A typical directionally sharpened cell in the descending octaval nucleus (DON). The directional response area is smaller and the response to the best direction is substantially greater than to the adjacent stimulus angles. **B** Each afferent has a best azimuth and a best elevation that together represent an axis that pierces three-dimensional space as illustrated in this visually flattened 'globe' plot, showing only the northern hemisphere; outer circle at head = 0° azimuth & elevation (= "equator"); concentric circles indicate elevation (= "latitude") with 90° elevation (straight up = north pole) in the middle of the fish head; degrees of elevation in italics. Saccular afferent best directions are plotted with different symbols to represent different experiments, including intracellular and extracellular recordings. The distribution of saccular afferents reflects some bias in sampling the more accessible rostral saccule (refer to fig. 2) but indicate the range of responses from that otolith organ [see Edds-Walton et al., 1999 for regional afferent data]. **B'** Best directions for left dorsal DON cells obtained from extracellular recordings plotted as in B. The left dorsal DON receives ipsilateral saccular input and reciprocal connections from the contralateral DON. The auditory population in

DON responds to a great range of azimuths and elevations consistent with convergence of left and right saccular inputs [B, B' modified from Edds-Walton and Fay, 2008].

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Fig. 5.

Proposed scenario for formation of the first order auditory nuclei in an early tetrapod. Ancestral osteichthyan structures and connections in the auditory brain stem nuclei are assumed to have resembled those of some extant fishes. Auditory structures in blue, major changes in connections in red. **A** Parasagittal view of the bowfin (*Amia calva*) brain illustrating the octaval column nuclei and the overlying cerebellar crest (modified from McCormick, 1981). The lateral line nuclei (medialis and caudalis) that overlie the octaval column are not shown. Dashed line indicates location of cross section at entrance of cranial nerve VIII. **B** A cross-sectional view through a generalized teleost medulla. DON projects across the midline via the internal arcuate tract (IA; dashed gray lines) to the contralateral DON (dashed red line) and to the bitufted neurons of the dorsal secondary octaval nucleus (SOd). The homotopic connections between the DONs may have been present in the aquatic ancestor or may have developed more recently. In this scenario, we assume that the lateral line nuclei (nucleus medialis, M) and electrosensory components (not shown) of the octavolateralis system were also present. Both DON and secondary octaval nuclei (SOd and ventral SOv) project to the midbrain torus semicircularis via the lateral lemniscus (LL, in dashed grey circle). **C** Hypothetical intermediate, with auditory input from a new papilla (red). The new input is illustrated as terminating in the dorsal DON, but it is also possible that new auditory input led to the formation of new, dorsal auditory targets, like the first order nucleus magnocellularis (NM). In the intermediate, we propose that the homotopic

connections between first order auditory nuclei were not present, and the connections from the first order nuclei to a secondary octaval nucleus, via the IA, were retained. **D** Parasagittal view of a diapsid brain illustrating the octaval column nuclei and the underlying vestibular column, as in (A). One first order nucleus, nucleus angularis (NA), is located rostral to NM. The second order nucleus laminaris (NL) is located above NM. **E** Diapsid pattern with distinct NM and NL (in blue) above the dorsal vestibular nucleus (VeD). The NM has a red border to show its separation, as compared with the aquatic intermediate (in C).

Fig. 6.

Sound source localization circuits in the auditory brain stem of lepidosaurs and archosaurs for comparison with fig. 5E. **A** Lepidosaurs show a similar pattern to the diapsid in fig. 5E, with NM and NL in blue. NM and NA receive auditory nerve input, and NM projects bilaterally to NL (dashed grey line). Both NA and NL project to the superior olivary nuclei (SO) and to the midbrain via the lateral lemniscus (blue arrow) [modified from Tang et al., 2012]. **B** Physiological recordings from the auditory nerve revealed interaural delay sensitivity that is consistent with strong coupling between the eardrums. Three nerve recordings with best frequencies of 1900, 2900, and 3800 Hz are shown. All three responses show a common *minimum* ITD (at arrow, 245 μs) that most likely reflects the cancellation of eardrum motion by direct and indirect sound components impinging on both sides of the eardrum, as shown by the close correspondence between the response minima and the biophysical measures of interaural delay [modified from Christensen-Dalsgaard et al., 2011]. **C** Schematic cross section and matching photomicrograph through the alligator brain stem at the level of NL and NA. These dorsal auditory structures extend into the IVth ventricle. A small rostral extension of NM lies most medial to NL. NM projects to NL (black/dashed line), and NA and NL project to the superior olive and midbrain (blue arrows). **D** ITD responses within alligator NL reveal a wide range of best ITDs. Interaural delay curves plot the response of a 1kHz NL neuron against changing ITDs at three frequencies around best frequency. This neuron had a best ITD of -162 μs, i.e., in the contralateral hemifield. Note the similar response functions in B and D.