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Bone conduction pathways confer directional cues to salamanders

G. Capshaw1,*, J. Christensen-Dalsgaard2, D. Soares3 and C. E. Carr1

ABSTRACT
Sound and vibration are generated by mechanical disturbances within the environment, and the ability to detect and localize these acoustic cues is generally important for survival, as suggested by the early emergence of inherently directional otolithic ears in vertebrate evolutionary history. However, fossil evidence indicates that the water-adapted ear of early terrestrial tetrapods lacked specialized peripheral structures to transduce sound pressure (e.g. tympana). Therefore, early terrestrial hearing should have required nontympanic (or extratympanic) mechanisms for sound detection and localization. Here, we used atympanate salamanders to investigate the efficacy of extratympanic pathways to support directional hearing in air. We assessed peripheral encoding of directional acoustic information using directionally masked auditory brainstem response recordings. We used laser Doppler vibrometry to measure the velocity of sound pressure-induced head vibrations as a key extratympanic mechanism for aerial sound reception in atympanate species. We found that sound generates head vibrations that vary with the angle of the incident sound. This extratympanic pathway for hearing supports a figure-eight pattern of directional auditory sensitivity to airborne sound in the absence of a pressure-transducing tympanic ear.

KEY WORDS: Amphibian, Hearing, Extratympanic, Sound localization

INTRODUCTION
Sound is generated by mechanical disturbances, often in the vicinity of the receiver, and thus the ability to localize sound sources generally confers a clear fitness advantage, for example to facilitate navigation, detection of prey and avoidance of potential predators. The emergence of inherently directional otolithic sense organs in aquatic gnathostomes during early vertebrate evolutionary history indicates that peripheral encoding of directional cues from acoustic energy may be an ancestral feature of the tetrapod ear. However, little is known about the evolution of directional hearing during the water-to-land transition. In this study, we show that the atympanic salamander ear is directional, even without impedance-matching tympanic middle ears, which are generally considered essential for sound source localization in terrestrial tympanate vertebrates.

The water-to-land transition imposed a novel challenge for early tetrapods: their tissue was now of greater density than the surrounding media (air) and therefore served as a reflective barrier preventing acoustic energy from reaching the sensory hair cells of the inner ear. The tympanic middle ear evolved separately in each major tetrapod lineage, at least five times during vertebrate evolutionary history as an adaptation to compensate for the air–skin impedance mismatch (Christensen-Dalsgaard and Carr, 2008; Clack, 1997, 2002; Kitazawa et al., 2015). However, fossil evidence demonstrates a 100 million year delay between the development of structural adaptations to support terrestrial living during the Carboniferous period and the emergence of sound-receptive tympanic ears in the Triassic. This indicates that the ancestral tetrapod ear was largely unspecialized for hearing on land (Clack, 1997, 2002). Despite this, the first terrestrial tetrapods may have had rudimentary aerial hearing capabilities similar to that observed in modern lungfish, with sensitivity to high amplitude, low frequency airborne sound pressure (Christensen-Dalsgaard et al., 2011; Christensen et al., 2015a; Clack, 2015). Recent studies in extant atympanate species indicate that aerial hearing in the absence of an impedance-matching tympanic middle ear may occur thanks to the detection of sound-induced head vibrations (in lungfish: Christensen et al., 2015a; in snakes: Christensen et al., 2012; and in salamanders: Capshaw et al., 2020; Christensen et al., 2015b). The broad phylogenetic distribution of this extratympanic pathway for hearing indicates that it may represent a generalized terrestrial hearing mechanism for species lacking adaptations for the detection of airborne sound.

In many tetrapod lineages, including amphibians, squamates and archosaurs, the tympanic ear was formed at the spiracular openings, which connected the two tympana via the pharynx. This is an important feature, because the resulting acoustical coupling of the tympana produces inherently directional ears (Bee and Christensen-Dalsgaard, 2016; Christensen-Dalsgaard and Manley, 2005, 2008). Within the coupled ears of these taxa, sound interacts with the internal and external surfaces of the tympanic membranes to generate location-dependent responses (reviewed in Christensen-Dalsgaard, 2010). Greater directional cues are extracted from sounds for which the wavelength exceeds the size of the animal’s head, hence the usual binaural cues – interaural time and level differences – are negligible. In anuran amphibians, the efficacy of these sound localization mechanisms is strongly frequency dependent. However, the anuran tympanic membrane is largely unresponsive to low frequencies, and directional responses of auditory nerve fibers to low frequency sound are therefore believed to originate through extratympanic pathways, i.e. bone conduction (Feng, 1980; Jørgensen and Christensen-Dalsgaard, 1997a,b; Wilczynski et al., 1987). The extent to which extratympanic pathways can confer directionality to an atympanate vertebrate in a terrestrial environment has never been tested. Although studies of directional hearing in frogs infer the ability of extratympanic pathways to transmit location cues to the ear, it is necessary to verify that extratympanic pathways alone are sufficient to enable terrestrial
auditory directionality in the absence of specializations for detecting aerial sound pressure.

In the present study, we explore the directionality of the atympanic salamander ear to evaluate the ability of extratympanic sound detection pathways to support directional hearing in air. Although salamanders, being atympanate, had been believed to be largely insensitive to acoustic cues, behavioral studies have reported the ability of terrestrial salamanders to use acoustic cues for navigation (Diego-Rasilla and Luengo, 2004, 2007; Pupin et al., 2007). Additionally, our previous study found that airborne sound waves are capable of generating translational vibrations in the salamander skull that are detectable to the auditory end organs of the inner ear, conferring pressure sensitivity to these atympanate animals (Capshaw et al., 2020).

Here, we tested the hypothesis that airborne sound interacts with the salamander head in a directional manner to generate directional cues at the peripheral level of the auditory system even in the absence of a terrestrially adapted tympanic ear. We used auditory brainstem response (ABR) recordings to investigate peripheral encoding of directional information in the eighth cranial nerve in response to free-field airborne sound pressure stimuli. Because extratympanic hearing in salamanders is mediated by the detection of sound-induced head vibrations (Capshaw et al., 2020), we further used laser Doppler vibrometry to assess directionality of the translational movement of the animal owing to sound pressure stimuli. We combined the results of the ABRs and laser vibrometry experimentation to provide insight into the ability of an atympanic ear that is unspecialized for terrestrial sound detection to extract directional cues from free-field airborne sound pressure.

**MATERIALS AND METHODS**

**Directional auditory brainstem response recording**

We investigated the directional sensitivity of the atympanic salamander ear using a directionally masked ABR recording technique. Our study incorporated six lungless species from the family Plethodontidae (*Desmognathus fuscus* (n=12), *Eurycea cirrigena* (n=11), *Eurycea lucifuga* (n=13), *Gyrinophilus porphyriticus* (n=10), *Plethodon cinereus* (n=15) and *Plethodon glutinosus* (n=14)). We incorporated these six ecologically diverse plethodontid species because previous study demonstrated habitat-related differences in the salamander low frequency response that may influence directionality sensitivity (Capshaw et al., 2020). Because air-filled body cavities such as the lungs may influence auditory directionality in amphibians (Bee and Christensen-Dalsgaard, 2016), we also included two salamander species with lungs (*Ambystoma opacum* (n=9), *Ambystoma tigrinum* (n=8)) to provide a natural comparison to the directional response measured in lungless plethodontids. Field-collected salamander species were collected with permission from the Tennessee Wildlife Resources Agency, permit numbers 1386, 1388, 1605 and 3838.

We anesthetized salamanders using 20% w/v benzocaine applied to the ventral body surface and placed three Teflon-coated silver wire electrodes subcutaneously at the left ear (recording electrode), on the midline of the head dorsal to the brainstem (inverting electrode), and in the tail (ground). We optimized electrode placement for each individual to maximize the signal to noise ratio for evoked potential recording. ABR measurements were performed in a custom-built anechoic chamber lined with 500 mm acoustic foam wedges and shielded by a Faraday cage to reduce electrical and acoustic noise. All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee of the University of Maryland, protocol numbers R-16-59 and R-SEP-19-41.

Masked ABR (mABR) recordings were performed using a broadband click stimulus in the presence and absence of an ongoing 200 Hz masking tone (Fig. 1) with click and masker emitted from separate loudspeakers to allow assessment of directional hearing. The mABR recording paradigm allows the use of a long duration pure tone stimulus as a simultaneous masker to ensure frequency specificity even at low frequencies (Brandt et al., 2018). The click stimulus was generated by a small speaker (ORB Audio, flat frequency response from 0.15 to 18 kHz) at a distance of 0.5 m from the left ear of the salamander. The click was one half-cycle of a 2000 Hz sinusoid with sufficient power across a frequency range of 10 to 1000 Hz and was presented at the lowest level necessary to evoke a neural response at 90% of the maximum amplitude from each individual. The evoked response was evaluated based on the presence of two to three peaks in the waveform occurring approximately 5 ms after stimulus onset that were discriminable from noise, verified by visual inspection of the ABR waveforms. The recorded response latencies, when corrected for the time required for sound to travel to the salamander, approximated the 2 ms responses from auditory nerve recordings in the grass frog (Jørgensen and Christensen-Dalsgaard, 1997b) and from ABR recordings in Cope’s grey treefrog (Schrode et al., 2015), indicating that the first wave of the salamander ABR may originate in the eighth cranial nerve. The masker tone was broadcast by a low frequency loudspeaker (Electro-Voice TL606A, flat frequency response from 50 to 400 Hz) placed 1.5 m from the salamander. We used a 200 Hz tone to ensure that the frequency of the masking tone was within range of peak sound pressure sensitivity for salamanders (Capshaw et al., 2020). Equipment was calibrated, stimuli were generated and data were recorded using Tucker-Davis Technologies system 2 hardware and custom software (QuickABR, Odense, Denmark) following methods described in Capshaw et al. (2020).

The anesthetized salamander was placed on an acoustic foam block on a rotating table within an anechoic chamber. The position of the click speaker was held constant 0.5 m from the salamander and oriented to present the click stimulus to the left ear, ipsilateral to the recording electrode (−90 deg). The animal and click speaker were rotated relative to the tone speaker to present the masker tone from eight sound incidence angles separated by 45 deg intervals (Fig. 2A). We increased the amplitude of the simultaneous tone until masking was observed as a reduction in the amplitude of the click response waveform (Fig. 1C). Masked and unmasked click responses were averaged over 800 presentations, and every second stimulus presentation was phase inverted to reduce artifacts. Auditory sensitivity to the 200 Hz tone was determined as its efficiency in attenuating the neural response to the click stimulus (e.g. its masking efficiency). Detection thresholds represent the lowest level of the masker tone that reduced the amplitude of the ipsilateral click response in the masked ABR relative to the unmasked ABR (Fig. 1C). Thresholds were verified by visual inspection of evoked potential waveforms using MATLAB vr2016b (MathWorks). We randomized the order of presentation for masker tone locations and used a coding scheme to ensure that detection threshold analyses could be performed blind to the location of the sound source for each trial. Although automated algorithms are useful to ensure objective and consistent threshold detection, several studies have indicated that visual inspection and automated algorithms perform equally well with no significant variation between thresholds determined using either method (Brandt et al., 2018; Lauridsen et al., 2021; Mooney et al., 2010; Schrode et al., 2015).
Directional sensitivity was determined by comparing sensitivity thresholds among different locations for the masker tone relative to the ipsilateral response (Fig. 2B). We normalized mABR thresholds by computing the difference between thresholds recorded at different masker tone locations and the ipsilateral response. From these normalized thresholds, we created polar plots of directional sensitivity to the 200 Hz tone. We evaluated the significance of threshold differences using a linear mixed model incorporating species and masker tone position as fixed effects and individual as a random effect using the R package nlme v3.1-131 (https://CRAN.R-project.org/package=nlme). We used type III tests of fixed effects to evaluate significant differences among factors and followed these analyses with post hoc pairwise testing with Bonferroni adjustments for multiple comparisons using the R package lsmeans v2.26-3 (https://CRAN.R-project.org/package=lsmeans). We assessed the influence of the air-filled lungs on the auditory directional response using linear mixed models incorporating masker tone position and presence/absence of lungs as fixed factors, and species and individual as nested random effects.

**Laser vibrometry**

We used laser Doppler vibrometry to measure the vibrations induced in the lateral surface of the salamander head overlying the left ear by free-field sound pressure stimuli. Vibration data were...
collected from *A. opacum* (*n*=4), *A. tigrinum* (*n*=4), *D. fuscus* (*n*=3), *E. cirrigera* (*n*=4), *E. lucifuga* (*n*=4), *G. porphyriticus* (*n*=4), *P. cinererus* (*n*=5) and *P. glutinosus* (*n*=3). Salamanders were placed on a heavy steel platform in an IAC Acoustics anechoic chamber covered with 500 mm foam rubber acoustic wedges to reduce reverberations. The salamander was positioned with the head facing 0 deg, the ipsilateral (recording) ear at −90 deg and the contralateral (right) ear at +90 deg (Fig. 2A). Sound pressure stimuli consisted of 100 ms frequency sweeps (0.1–10 kHz at 81 dB SPL) emitted by 12 JBL 1G loudspeakers located 1 m from the salamander and separated by 30 deg intervals. We measured vibration velocities using an OFV-5000 vibrometer with an OFV-505 sensor (Polytec, Waldbronn, Germany) at a sensitivity of 1 V mm$^{-1}$ s$^{-1}$. We used a probe microphone (BK 4182) to measure sound pressure levels at the location of the salamander head.

Stimuli were generated and data were recorded using Tucker-Davis Technologies system 2 hardware and custom software (DragonQuest, Odense, Denmark). Sound and vibration recordings were collected using a 22.64 kHz sampling rate and averaged over 10 presentations. We extracted vibration velocities measured in response to a 200–400 Hz subset of the sweep to enable more direct comparison with the physiological data of auditory directionality obtained via mABR. We calculated transfer functions as the ratio between the laser spectra measured by the vibrometer and the sound spectra recorded by the probe microphone. Vibration velocity transfer functions (in dB re. 1 mm s$^{-1}$ Pa$^{-1}$) were plotted as a function of the incident angle of the sound pressure stimulus to generate polar plots of sound-induced motion of the salamander head.

**RESULTS**

**Directionality of the neurophysiological response**

Directional mABR recordings revealed directional sensitivity to a 200 Hz sound pressure stimulus among eight atypompanic salamander species. Fig. 3 shows mean detection thresholds for the masker tone recorded from each species across eight different tone locations. Auditory sensitivity to the 200 Hz tone demonstrates species-specific variation consistent with patterns observed in our previous study (Capshaw et al., 2020), in which facultative cave salamander species-specific variation consistent with patterns observed in our previous study (Capshaw et al., 2020). We observed a significant main effect of the masker tone position on its masking efficiency, measured as mABR threshold difference relative to the ipsilateral response ($F_{7,585}=4.46$, $P<0.0001$). The masking efficiency of the 200 Hz tone was greatest when presented from the same direction as the click stimulus (−90 deg), and decreased as the direction of the masker tone input rotated relative to the recording ear. Contralateral thresholds (+90 deg) were slightly elevated relative to the ipsilateral response; however, pairwise comparisons of the data showed that these did not deviate significantly from ipsilateral thresholds ($P=0.94$). The highest sensitivity thresholds for the 200 Hz masker tone were obtained at the rostral (0 deg) and caudal (180 deg) ends, averaging 5–6 dB greater than the ipsilateral response; these threshold differences were significant across all species (Bonferroni-adjusted pairwise comparisons of least square means: $P<0.001$ when comparing each masker position to the ipsilateral response).

**Directionality of sound-induced head vibrations**

We evaluated variation in the amplitude of vibration velocity transfer functions (in dB re. 1 mm s$^{-1}$ Pa$^{-1}$) owing to the incident angle of the sound pressure stimulus at the lateral head surface overlying the left ear. The angle of sound incidence had a significant effect on amplitude of vibration transfer functions recorded at the ipsilateral ear for all species ($F_{11,385}=2.31$, $P<0.001$). Vibration velocity transfer functions were greatest in response to sound sources oriented toward the ipsilateral ear (Fig. 4B). Contralateral stimulation resulted in a reduction in transfer function amplitude averaging approximately 3 dB (range: 1–6 dB differences across species) compared with the ipsilateral response. Stimulation at the rostral and caudal ends of the animal resulted in significantly reduced transfer function amplitudes relative to ipsilateral stimulation (Bonferroni-adjusted pairwise comparisons: $P<0.0001$ for rostral and caudal stimulation). We observed no significant interspecific differences in sound-induced vibrations ($F_{7,34}=1.96$, $P=0.10$), and no significant effects of the air-filled lungs on measured head vibrations (lungs: $F_{1,6}=0.05$, $P=0.83$; lungs×tone position: $F_{11,43}=1.35$, $P=0.20$). Comparison of normalized sound pressure sensitivity thresholds and sound-induced head vibrations revealed a similar pattern in which detection thresholds and
vibration velocity transfer functions varied with the angle of the incident sound to generate auditory directionality characterized by a rostral and caudal null. We note, however, that the figure-eight directionality was more prominent in the measures of auditory sensitivity (Fig. 4A).

**DISCUSSION**

We have shown that the atympanic ear of salamanders is directional, and that this directionality is most likely generated by sound-induced vibrations of the head. To assess the directional sensitivity of the eighth cranial nerve, we used mABR recordings in response to free-field sound pressure emitted from eight locations around the animal. In salamanders, mABR detection thresholds show a figure-eight pattern of directional sensitivity in the eighth cranial nerve, reflecting greatest sensitivity to sound pressure along the mediolateral axis of the body and reduced sensitivity along the rostrocaudal axis. Salamanders had the lowest detection thresholds in response to ipsilateral stimulation, although this response did not differ significantly from thresholds obtained in response to contralateral stimulation.

We observed no significant interspecific differences in the patterns of directional sensitivity among the eight species incorporated in our study, and therefore found no evidence for variation in auditory directionality among diverse salamander species. Although head sizes differ among the species tested, this variation is small relative to the wavelength of the sound waves corresponding to peak auditory sensitivity (e.g. less than 250 Hz) in salamanders. It follows that bone conduction hearing, mediated by the detection of sound-induced head vibrations, may generate a consistent pattern of directionality among species, regardless of species-specific variation in size or auditory detection thresholds. Additionally, there were no significant differences in the directional response of species with or without lungs, indicating that the presence of air-filled body cavities such as the lungs does not contribute to auditory directionality in atympanic salamanders.

In atympanic species, auditory sensitivity to airborne sound is most likely achieved through the detection of sound-induced vibrations in the head (Capshaw et al., 2020; Christensen et al., 2012, 2015b). This extratympanic mechanism for aerial sound detection occurs when a sound pressure wave generates translational movement in the animal that is of sufficient amplitude to stimulate the auditory end organs in the inner ear. We used laser vibrometry to assess the directionality of the sound pressure-generated vibrations in the salamander head in response to sounds broadcast from 12 locations around the animal. We observed a figure-eight-like pattern of directionality encoded in the bone-conducted vibration velocities in all species similar to that obtained from mABR evoked activity in the eighth cranial nerve. However, the figure-eight pattern of sound-induced head vibrations was not symmetrical across the midline: sounds broadcast ipsilateral to the recording ear elicited the highest vibration velocities relative to all other sound source directions, including the contralateral direction. Comparison of the amplitude of sound-induced head vibrations measured by laser vibrometry and directional sensitivity measured by mABR recording reveals a notably sharper figure-eight pattern encoded by the auditory nerve (Fig. 5A). This suggests that peripheral processing of sound at the level of the inner ear may further refine the representation of sound source location to the atympanic auditory system, beyond the directional cues provided by sound source location-dependent variation in head vibrations.

**Common patterns of low-frequency directionality in eared and earless amphibians**

In anurans with a functional tympanic ear, the directionality of the ear is frequency dependent. For high frequency sounds, auditory directionality shows an ovoidal pattern with the greatest activity elicited in single unit auditory fibers in response to ipsilateral sound sources (Feng, 1980; Feng and Shofner, 1981; Jørgensen and Christensen-Dalsgaard, 1997a; Wang et al., 1996). This ovoidal pattern of directional sensitivity matches the ovoidal directionality of the eardrum and is indicative of the anuran tympanic middle ear acting as a combined pressure and pressure difference receiver for frequencies greater than 500 Hz.

At low frequencies, the directionality of the frog ear transitions to a figure-eight pattern comparable to the pattern we observed in the atympanic salamander ear (Fig. 5) (Feng, 1980; Feng and Shofner, 1981; Jørgensen and Christensen-Dalsgaard, 1997a; Wang et al., 1996). This transition is also seen in the directionality of individual fibers to low and high frequency stimulation (see Jørgensen and Christensen-Dalsgaard, 1997a, their fig. 7), indicating that the origin of the directional response is peripheral to the inner ear sensory cells. Similar to the salamanders in the present study, single

Fig. 4. Peripheral directionality of the auditory response in eight salamander species. (A) Directional auditory sensitivity represented by mean masking efficiency of the 200 Hz tone (in dB re. 20 μPa) broadcast from different locations around the salamander, normalized to the ipsilateral (–90 deg) response. (B) Directional characteristics of sound-induced head vibrations. Values represent the mean vibration velocity transfer function (in dB re. 1 mm s⁻¹ Pa⁻¹) in response to a free-field 200–400 Hz sweep broadcast from different locations around the salamander. Laser vibrometer data were collected from the lateral head surface overlying the left ear.

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Mechanisms for extratympanic auditory directionality in salamanders

The lung–ear pathway has previously been implicated for improving the directional response of the tympanic middle ear in amphibians (Ehret et al., 1990, 1994; Hetherington, 2001; Jørgensen et al., 1991; Narins et al., 1988). Here, we show that salamanders with and without lungs demonstrate comparable directional responses to low frequency sound pressure, indicating that the air-filled lungs do not contribute to peripheral auditory directionality in atypmanate species as well as in the terrestrially adapted tympanic ears of auditory specialists.

Fig. 5. Comparison of the low frequency directional response in atypmanate and tympanic amphibians. (A) Salamander auditory sensitivity thresholds (solid line) and head vibration transfer functions (dashed line) measured in response to free-field sound pressure stimuli. Values represent grand mean data across all species, normalized to the ipsilateral (−90 deg) response; thresholds are in dB relative to the ipsilateral response. (B) Directional response of a single auditory fiber in *Rana temporaria* recorded from the right ear (+90 deg) in response to low frequency (300 Hz) tone bursts. Values are equivalent dB, relative to the ipsilateral (+0 deg) response. Equivalent dB are calculated by referring spike rates elicited by directional stimulation to the rate-intensity function measured at +0 deg sound incidence. Redrawn from Jørgensen and Christensen-Dalsgaard (1997a, fig. 10).

Atypmanic sound localization and directional ambiguity

The problem with the figure-eight sensitivity of the salamander ear is that it is ambiguous with insufficient cues to distinguish ipsilateral from contralateral directions. This 180 degree ambiguity problem has been discussed extensively in the fish literature, and the general idea is that fish may require a secondary input to allow them to disambiguate ipsilateral and contralateral sound sources via the phase relationship between particle motion and sound pressure (for recent reviews, see Hawkins and Popper, 2018; Sisneros and Rogers, 2016). Access to phase comparison between the directly received acoustic stimulus and that re-radiated from a second source may also provide the basis for eliminating the ambiguity resulting from the figure-eight pattern of directional detection observed here in salamanders.

Potential second sources for phase comparisons in salamanders include the perilymphatic connections coupling the inner ears (Wever, 1978). The salamander otic capsule lacks a round window and, unlike other species that use a reentrant fluid circuit to provide pressure relief in the absence of a round window, fluid displacement...
in the salamander ear passes through the cranial cavity via the perilymphatic foramina to the oval window of the opposite ear (Smith, 1968; Wever, 1978). Wever (1978) noted that direct vibratory stimulation of one ear evoked cochlear potentials in both ears, with greater ipsilateral responses. Hearing in salamanders is therefore binaural, and binaural phase differences may contribute to sound localization. Other secondary input sources may include the air-filled lungs and the mouth cavity. These could potentially act as a monopole sensor that, together with input from dipole sensors such as the otolithic saccular maculae, could resolve the 180 degree ambiguity in a manner similar to the swim bladder in auditory specialist fish. Alternatively, the operculum – via its attachment to the scapula – may provide a secondary vibration input to the inner ear.

Although the neural substrates underlying the directional processing of acoustic information in salamanders are unknown, comparisons with bony fish and frogs are instructive. In these animals, intrinsically directional inputs from the eighth cranial nerve terminate in the medulla, and projections between the first order brainstem auditory nuclei may serve to sharpen the figure-eight directional response. In fish, auditory directionality is generally encoded by fibers that innervate saccular hair cells with similar best axes, and exhibit figure-eight directionality (Edds-Walton et al., 1999; Fay and Edds-Walton, 1997). Binaural projections between the descending octaval nuclei, the first site of central processing in the auditory medulla of the fish, refine the directional response (Edds-Walton, 1998; Edds-Walton and Fay, 1998, 2009), and in toadfish, recordings from the midbrain torus semicircularis reveal further sharpening (Edds-Walton and Fay, 2003). In frogs, contralateral inhibition between the two dorsal medullary nuclei converts the figure-eight low frequency directional response in the auditory nerve fibers into a more ovoidal (i.e. disambiguated) pattern biased towards ipsilateral sounds (Christensen-Dalsgaard and Kenneworff, 2005). The anuran torus semicircularis retains this directional information, with the best responses elicited by lower frequencies presented along the interaural axis (Pettigrew et al., 1981). Salamanders share similar ascending projections to frogs. Their torus semicircularis receives contralateral auditory projections from the intermediate nucleus of the octaval column, and auditory units in the torus are more sensitive to contralateral acoustic stimulation (Manteuffel and Naujoks-Manteuffel, 1990). Thus, it seems probable that directionality of the atympanic salamander ear is supported by binaural projections in the auditory brainstem. Species comparisons indicate that binaural connections mediating sound localization are likely ancestral to tetrapods (Carr and Christensen-Dalsgaard, 2016; Walton et al., 2017).

In summary, salamander responses to low frequency sound are directional, and should permit sound source localization to facilitate navigation. Directionality in these atympanate species likely originates from differential amplitudes of sound-induced vibrations of the head that vary with incident angle of the sound source. This extratympanic mechanism for the reception of airborne sound is capable of generating fluid inertial movement within the otic capsule of the atympanic ear, where maximal displacement occurs at the locations of the pressure-relief windows along the interaural axis. Bone conduction of sound therefore supports a figure-eight pattern of extratympanic sensitivity to sound pressure, which has been observed in tympanate and atympanate amphibian species in response to low frequency acoustic stimulation, and may reflect the capacity of the ancestral atympanic tetrapod ear to detect directional cues from aerial sound in a terrestrial environment. More research is needed to investigate how directional information is processed (and disambiguated) by the central nervous system. Also, these studies would be greatly informed by further investigations of sound localization behavior in atympanate species.

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Competing interests

The authors declare no competing or financial interests.

Author contributions


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