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ORIGINAL PAPER

T.E. Hetherington

Laser vibrometric studies of sound-induced motion of the body walls and lungs of salamanders and lizards: implications for lung-based hearing

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Abstract A laser Doppler vibrometer was used to measure the acoustic responses of different body surfaces of several species of salamanders and lizards. The lateral body wall over the lung displayed sound-induced motion up to 30 dB greater than the lateral head surface from 300–1,000 Hz in salamanders and from 200–2,500 Hz in lizards. The lateral body wall of lungless plethodontid salamanders showed no such enhanced motion to sound. The lateral body wall of lizards was more responsive than their tympanum to sound frequencies below about 1,250–2,000 Hz. The frequency of the peak response of lizard body walls matched the resonant frequency of a Helmholtz resonator with the volume and dimensions of their lungs. In contrast, the frequency of peak response of salamander body walls was well below the resonant frequencies calculated for both Helmholtz resonators and closed tubes with the dimensions and volumes of their lungs. Nonetheless, filling the lungs with saline dramatically reduced the responsiveness of the lateral body walls of both the lunged salamanders and the lizards. As previously demonstrated in anuran amphibians, the lateral body wall and lungs of salamanders and lizards may function in sound reception, especially at relatively low frequencies.

Keywords Hearing · Lizard · Lung · Middle ear · Salamander

Abbreviations *LDV* laser Doppler vibrometer · *SPL* sound pressure level

T.E. Hetherington
Department of Evolution,
Ecology, and Organismal Biology,
Ohio State University, 1735 Neil Avenue,
Columbus, OH 43210–1293, USA
E-mail: hetherington.1@osu.edu
Tel.: +1-614-2920832
Fax: +1-614-2922030

Introduction

Most tetrapod vertebrates possess a tympanic middle ear that is specialized for reception of air-borne sound and consists of a tympanum, air-filled middle ear cavity, and auditory ossicle(s) (Wilczynski and Capranica 1984; Jaslow et al. 1988; Lewis and Narins 1999). However, reduction and loss of a tympanic middle ear is observed in many groups of amphibians and reptiles. For example, many species of anuran amphibians (frogs and toads) and lizards show a loss of a specialized tympanum and, to varying degrees, reduction of the middle ear cavity (Wever 1976, 1985; Jaslow et al. 1988). Complete absence of a tympanum and middle ear cavity, and in some cases auditory ossicle, is observed in salamanders, caecilian amphibians, some anuran amphibians, snakes, and some lizards (Wever 1976, 1985; Jaslow et al. 1988). Because of the lack of tympanic middle ear elements, these latter groups sometimes are described as being "earless," although most retain well-developed auditory receptor organs within the inner ear. Many "earless" species of amphibians and reptiles are fossorial in habits and never vocalize, but others, such as certain species of frogs and toads, are surface dwelling forms that produce a variety of vocalizations and respond behaviorally to sound (Jaslow et al. 1988; Lindquist and Hetherington 1996). The latter behaviors suggest the utilization of non-tympanic pathways of sound reception in these species.

Recent research has focused on the lungs as an alternative system for hearing airborne sound in "earless" species. Hartline (1971) first suggested that lungs serve an auditory function in snakes. Many studies have now demonstrated that the lateral body wall over the lungs of anuran amphibians is very responsive to sound (Narins et al. 1988; Ehret et al. 1990; Jørgensen et al. 1991; Hetherington 1992). Several researchers have examined the functional significance of lung-borne sound reception in anurans with well-developed tympanic middle ears. For example, sound entering the lungs can, by passing through the respiratory pathways and into the middle ear cavity, improve the directional response of the tympanic middle ear to low frequency sounds (Narins et al. 1988; Ehret et al. 1990, 1994; Jørgensen 1991; Jørgensen et al. 1991) or function as a low frequency channel for conduction of underwater sound to the eardrum of the aquatic frog *Xenopus laevis* (Christensen-Dalsgaard and Elepfandt 1995). In addition, Hetherington and Lindquist (1999) directly tested the role of lung-borne sound reception in a species that entirely lacks a tympanic middle ear, the fire-bellied toad *Bombina orientalis*, and found that disruption of the lung pathway dramatically reduced hearing sensitivity. The precise pathway of sound transfer from the lungs to the inner ear has not been determined, although transmission through the respiratory pathways likely is involved (Hetherington and Lindquist 1999).

The purpose of this study is to examine the potential generality of lung-borne sound reception in tetrapod vertebrates by extending observations to other amphibians (salamanders) and reptiles (lizards). It has been proposed that lung-based hearing may represent the original auditory mechanism of tetrapods (Hetherington and Lindquist 1999), so this mechanism may be generally distributed in tetrapods. However, it is possible that lung-based hearing is restricted to or best developed in frogs and toads because the lateral body wall over the lungs of the latter often consists only of skin and thin layers of cutaneous muscle. In most other terrestrial vertebrates, such as salamanders and lizards, the lateral body wall is supported by ribs and contains thicker layers of axial musculature that might impede sound transmission into the lungs.

Salamanders are especially interesting to investigate with regard to lung-based hearing for two reasons. First, they entirely lack tympanic middle ears, and therefore may rely largely on such an alternative route of sound reception. Second, this group provides a natural experiment because some species, including all the species of the family Plethodontidae, lack lungs. This study, therefore, examined body wall responses to sound in both lunged and lungless species of salamanders.

Materials and methods

Animals

Four species of salamanders were used in laser vibrometric tests in this study: five spotted salamanders (*Ambystoma maculatum*; family Ambystomatidae), 14.8–23.9 g; five red-spotted newts (*Notophthalmus viridescens*; family Salamandridae) 3.3–5.1 g; five redback salamanders (*Plethodon cinereus*; family Plethodontidae), 1.8–2.5 g; and five northern dusky salamanders (*Desmognathus fuscus*; family Plethodontidae), 2.2–4.6 g. In addition, lung dimensions and volumes were measured in two red-spotted newts (3.7 g and 4.4 g) and two spotted salamanders (16.2 g and 21.8 g). Salamanders were captured in the field and used in this study within 5 days of capture.

Three species of lizards were used in laser vibrometric tests in this study: five green anoles (*Anolis carolinensis*, family Polychridae) 2.9–4.5 g; five western fence lizards (*Sceloporus occidentalis*, family Phrynosomatidae) 6.7–11.8 g; and three western skinks (*Eumeces skiltonianus*, family Scincidae) 2.6–4.8 g. In addition, lung dimensions and volumes were measured in two green anoles (3.9 g and 4.2 g). Lizards were purchased or captured in the field and maintained in captivity on a diet of crickets for up to 6 weeks before testing.

Laser doppler vibrometry

Measurements of the sound-induced motion of different body surfaces were made with a Polytec OFV 1000 laser Doppler vibrometer (LDV). Salamanders were anesthetized by immersion in a 1% tricaine methanosulfonate salt (MS-222) solution and lizards were anesthetized by a few subcutaneous injections of sodium pentobarbitol along the ventral body surface for a total dosage of approximately 15–20 mg/kg. At the anesthesia levels used, the salamanders typically showed occasional buccal pumping and the lizards showed sporadic lung ventilation. Care was taken not to deflate the lungs of the animals during handling, and all animals appeared to have normally inflated lungs during tests.

After anesthesia, animals were placed in a dark sound attenuating chamber used for the tests. The chamber was approximately 3.2 m long, 2.2 m wide, and 2.3 m high and lined with acoustic foam. Animals were positioned on a heavy metal table and a full range 25.4 cm speaker was suspended 1 m above them. A Polytec OFV-100 optical head was positioned about 20 cm to the side of the animal and the neon-helium laser beam was focused on a small metallic reflector (0.3 × 0.3 mm and <0.02 mg) placed on various body surfaces. At this distance the focused beam was approximately 35 μm in diameter. Measurements were made on the center of the tympanum (lizards only), on the lateral head surface overlying the inner ear region (in lizards, just anterior to the tympanum), on the anterolateral body wall directly over the lung (or thoracic area of the lungless salamanders), and on the posterolateral body wall over the abdominal region.

Preliminary tests were done to determine the most responsive point of the anterolateral body wall over the lungs of the lizards and lunged salamanders. The lungs of the lizards extended, depending on the species, from about one-half to two-thirds the distance from the forelimbs to the hindlimbs. However, more anterior body wall areas nearer the forelimbs displayed the most conspicuous motion during breathing, and preliminary measurements verified that this area showed the greatest responses to sound. The anterolateral body wall point eventually chosen for measurements in the lizards was about one-fifth (20%) the distance from the forelimbs to the hindlimbs. The lungs of the spotted salamanders and red-spotted newts extended about two-thirds to three-quarters the length of the distance between the forelimbs and hindlimbs. Motion of the lateral body wall during lung ventilation was less conspicuous in these salamanders than in the lizards. However, sampling discovered that, as found in the lizards, points about one-fifth (20%) the distance from the forelimbs to the hindlimbs typically showed the highest responses to sound, so this area was also chosen for measurements of anterolateral body wall motion in salamanders. In both lizards and salamanders, the posterolateral body wall point (over the abdominal area and caudal to the lung) chosen for measurements was about 80% the distance from the forelimbs to the hindlimbs.

Salamanders were moistened frequently to keep the skin surfaces from drying. Continuous sound stimuli (at 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,250, 1,600, 2,000, 2,500, 3,000, 4,000, and 5,000 Hz) were delivered at a sound pressure level (SPL) of 90 dB. SPL was monitored with a 1.27-cm condenser microphone (Brüel and Kjaer Type 4155) positioned 2 cm above and just anterior to the head of the animals and connected to a sound level meter (Brüel and Kjaer Type 2230) and third-octave/octave filter set (Brüel and Kjaer Type 1625). Vibrometer output voltage for each tested frequency was measured on a wave analyzer (Hewlett Packard 3581A) and converted to relative vibration velocity ($\mathrm{dB} = 20 \log V/V_o$; $V_o = 1$ μm/s).

Vertical and horizontal vibrations of the animal platform under experimental conditions were measured with the LDV. At 90 dB SPL, vibratory amplitudes generally did not rise above a background level of approximately 1 μm/s or less. Transient signals up to about 6 μm/s were occasionally observed at low frequencies below about 200 Hz, but these did not interfere with voltmeter readings of tissue responses at those frequencies. Background noise at the lowest frequency tested (100 Hz) was around 1 μm/s. At higher frequencies the baseline noise was below 1 μm/s.

Effects of filling of the lung

To further investigate the potential role of the lungs in hearing, the effects of filling the lungs with oxygenated saline were investigated. These tests were done on three red-spotted newts and three green anoles and included LDV measurements of sound-induced motion of the anterolateral body wall, posterolateral body wall and (in the case of lizards) the tympanum. A control series of measurements were made on the animals, and then saline was injected though the glottis and into the lungs by a syringe (Hetherington and Lindquist 1999). Another set of measurements of sound-induced motion was then made. Subsequently, the lungs were emptied of saline by pressure applied to the sides of the animals, the lungs re-inflated with air using a syringe, and a last set of measurements made.

Measurements of lung volume

The dimensions and volumes of the lungs of two red-spotted newts, two spotted salamanders, and two green anoles were measured to allow comparison of body wall LDV responses with calculated resonant properties of the lungs. Salamanders were killed by overanesthesia by immersion in a 1% tricaine methanosulfonate salt (MS-222) solution and lizards were killed by subcutaneous injections of an overdose of sodium pentobarbitol (approximately 100 mg/kg lizard). The trachea, bronchi, and lungs were surgically exposed. The bodies of the animals were suspended with the head up, and a needle on a water-filled 1-cc syringe (salamanders) or 2-cc syringe (lizards) was placed into the exposed trachea. The tip of the needle was positioned just above the divergence of the left and right bronchi. The water inside the syringe was colored with toluidine blue dye so that its level within the bronchi and trachea could easily be seen. The lungs were first compressed to force air out, and then water was injected into the lungs until the level reached the tip of the needle at the point of divergence of the bronchi. (The volumes of the bronchi were very small compared to those of the lungs and were ignored in these measurements). Filling the lungs in this manner appeared to fully inflate the lungs. The volume of water injected into the lungs was measured to the nearest 0.01 cc, and lung volume was calculated as half of the total water injected into both lungs. The length and maximum diameter of the inflated lungs and the length and diameter of the bronchi were also measured to the nearest 0.1 mm with a set of digital calipers. Mean values for lung volume and lung and bronchial dimensions were then calculated for each species and used in the formulas below to estimate resonant properties.

The frequency of Helmholtz resonance (f) of the lungs of each species was calculated using the following formula (Fletcher 1992):

$$f = (c/2\pi)[S/(L + 0.6a)V]^{1/2} \quad (1)$$

where f is the resonant frequency, c is the speed of sound (344.2 m/s at 22 °C), S is the cross-sectional area of the bronchus, a is the radius of the bronchus, L is the length of the bronchus, and V is the volume of the lung.

Salamander lungs were relatively long and narrow compared to those of the green anoles, so the resonant frequencies of closed tubes of comparable dimensions also were calculated using the following formula (Resnick and Halliday 1966):

$$f = c/4(L + 0.4d) \quad (2)$$

where f is the resonant frequency, c is the speed of sound (344.2 m/s at 22 °C), L is the length of the lung, and d is the average diameter of the lung.

Results

Salamanders

The anterolateral body walls overlying the lungs of both red-spotted newts (*N. viridescens*) and spotted salamanders (*Ambystoma maculatum*) showed enhanced motion to sound compared to the lateral head tissues and posterolateral body wall. Figure 1 shows results from one newt that are generally representative of data from all individuals of both lunged species. All three body surfaces showed similar motion at frequencies below about 300–400 Hz. The anterolateral body wall over the lung showed increased responsiveness at higher frequencies. The variation in acoustic responsiveness of the anterolateral body wall and lateral head surface observed between individual newts is demonstrated in Fig. 2. There was a clear and consistent difference between the responses of these two surfaces in all of the individuals tested. Responses of the anterolateral body wall at the frequency of peak motion were at least 24 dB greater than those of the lateral head surface at the same frequency in all of the newts. A comparable pattern was observed in spotted salamanders. Responses of the anterolateral body wall at the frequency of peak motion were at least 19 dB greater than those of the lateral head surface at that same frequency in all of the spotted salamanders tested.

The frequency response of the anterolateral body wall of newts and spotted salamanders differed, presumably in relation to body size. Peak motion was observed from 1,600–2,500 Hz in the newts (Fig. 2) and from

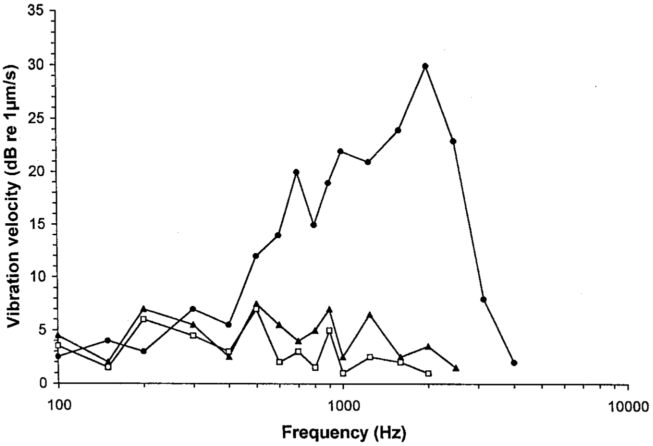

Fig. 1 Laser Doppler vibrometer (LDV) measurements of the acoustic responsiveness of the lateral head surface (*open squares*), anterolateral body wall (*solid circles*), and posterolateral body wall (*solid triangles*) of a red-spotted newt (*Notophthalmus viridescens*). Note the significant sound-induced motion of the anterolateral body wall over the lung compared to the other body surfaces

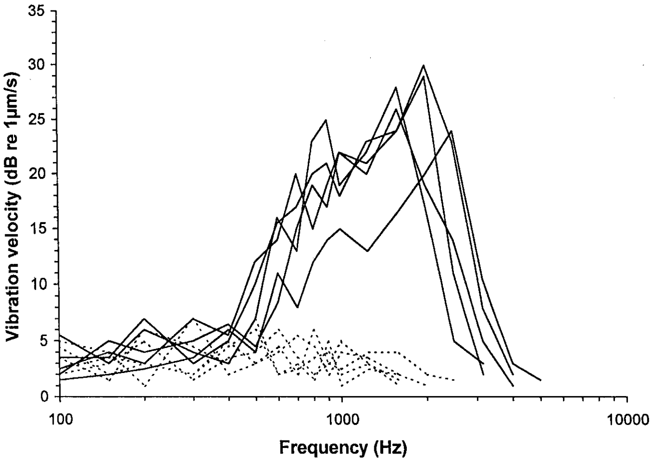

Fig. 2 LDV measurements of the acoustic responsiveness of the lateral head surface (*dashed lines*) and anterolateral body wall (*solid lines*) of five red-spotted newts (*N. viridescens*). Each *line* represents data from one individual. Note the consistent difference in responsiveness between the lateral head and anterolateral body wall over the lung

1,250–1,600 Hz in the larger spotted salamanders. Responses of the anterolateral body wall could be measured up to 5,000 Hz in newts, but only up to about 4,000 Hz in spotted salamanders. Within species, body size was inversely related to frequency of peak motion. For example, in Fig. 2, the smallest newt tested (3.3 g) displayed peak motion of the anterolateral body wall at 2,500 Hz, whereas the largest newt (5.1 g) displayed peak motion at 1,600 Hz.

The amplitude of sound-induced motion of the anterolateral body wall typically was higher in the newts (see Fig. 4 below). At the most responsive frequencies, motion of the body wall of newts was generally 4–7 dB greater than that of spotted salamanders. Responses of the lateral head surface and the posterolateral body wall over the abdominal region were generally similar in both species.

The pattern of sound-induced motion of the body surfaces of a lungless redback salamander (*P. cinereus*) is shown in Fig. 3. As expected, the anterolateral body wall showed no pronounced responses to sound and all three body surfaces tested showed comparable patterns of low responsiveness. No observable peaks in motion were evident in any body surface, and no measurable motion was observed above about 3,000 Hz. The same pattern was observed in the other lungless species examined, the dusky salamander (*D. fuscus*).

Figure 4 compares the mean acoustic responsiveness of the anterolateral body walls of the four species of salamanders examined and clearly shows the difference in sound-induced motion of the body walls of the lunged versus the lungless species. The anterolateral body walls of the lunged species become more responsive to sound above about 500 Hz. Near the presumed resonant frequencies of the lungs (about 1,250–2,500 Hz, depending on the size of the animal), responses of the anterolateral body walls of the lunged species usually were more than 20 dB greater than those of the lungless species.

Lizards

The anterolateral body walls overlying the lungs of all three species of lizards tested, the green anole (*Anolis carolinensis*), the western fence lizard (*S. occidentalis*), and the western skink (*E. skiltonianus*) showed significant motion to sound comparable to that of the tympanum but was restricted to lower frequencies than the latter. Figure 5 shows data from one green anole that is representative for that species and displays the same general pattern observed in the other two species as well. The lateral head surface anterior to the tympanum and the posterolateral body wall over the abdominal region showed low responsiveness to sound. No motion of these surfaces could be measured at frequencies above about 2,000 Hz. The anterolateral body wall showed elevated responses to sound beginning at about 300–600 Hz. Peak motion of the anterolateral body wall occurred from about 1,000–2,000 Hz, depending on the individual and species, and motion could be measured up to 4,000 Hz in some animals.

The tympanum of all three species responded to higher frequencies than the anterolateral body wall (Fig. 5). Tympanic motion peaked from 2,000–3,000 Hz, depending on species and body size, and motion usually could be measured up to the highest frequency examined (5,000 Hz). The tympanum was more responsive to sound than the anterolateral body wall at frequencies beginning around 1,250–2,000 Hz.

Figure 6 compares the mean acoustic responsiveness of the anterolateral body walls of all three species of lizards. The body walls of all the species show a very similar pattern of responsiveness, although slight,

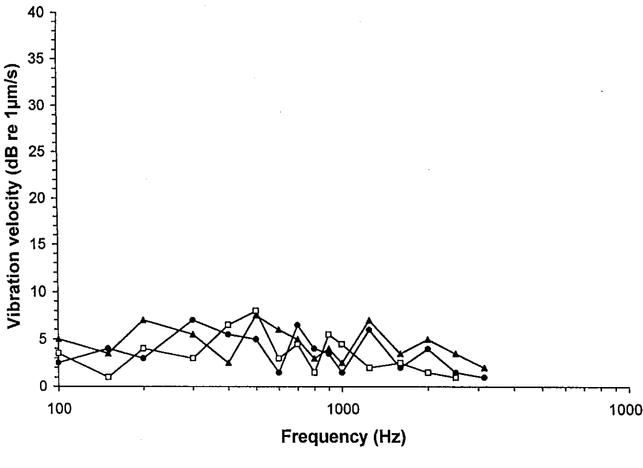

Fig. 3 LDV measurements of the acoustic responsiveness of the lateral head surface (*open squares*), anterolateral body wall (*solid circles*), and posterolateral body wall (*solid triangles*) of a redback salamander (*Plethodon cinereus*). There is no pronounced responsiveness of the anterolateral body wall in this lungless species

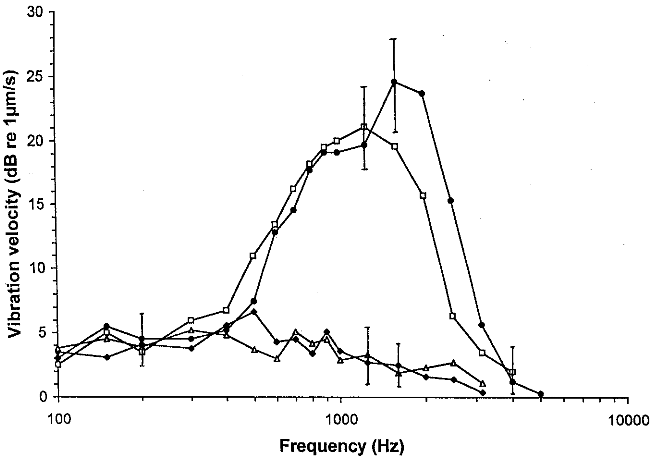

Fig. 4 Comparison of the mean acoustic responsiveness of the anterolateral body walls of the four species of salamanders examined ($n = 5$ for all species), the red-spotted newt (*N. viridescens*; *solid circles*), the spotted salamander (*Ambystoma maculatum*; *open squares*), the redback salamander (*P. cinereus*; *open triangles*), and the northern dusky salamander (*Desmognathus fuscus*; *solid diamonds*). The pronounced responsiveness of the anterolateral body wall in the newt and tiger salamander is lacking in the lungless redback and dusky salamanders. SDs (*vertical bars*) are shown for mean values at certain frequencies for certain species, namely 200 Hz for newts, 1,250 Hz for spotted salamanders and redback salamanders, 1,600 Hz for newts and dusky salamanders, and 4,000 Hz for spotted salamanders

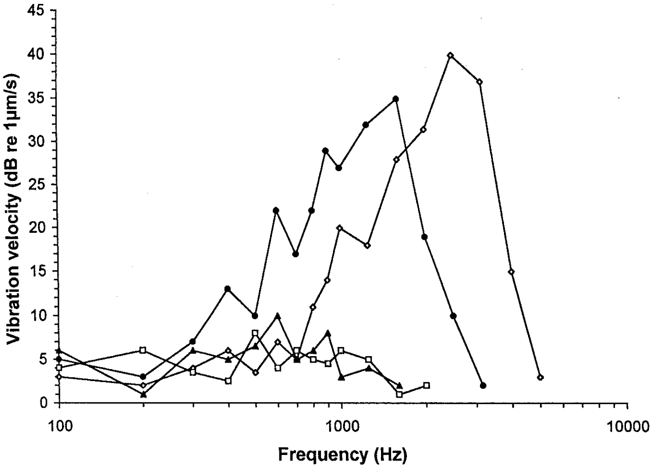

Fig. 5 LDV measurements of the acoustic responsiveness of the lateral head surface anterior to the tympanum (*open squares*), tympanum (*open diamonds*), anterolateral body wall (*solid circles*), and posterolateral body wall (*solid triangles*) of a green anole (*Anolis carolinensis*). Both the tympanum and the anterolateral body wall over the lung show pronounced responsiveness compared to the other body surfaces. The frequency response of the anterolateral body wall is shifted to lower frequencies compared to the tympanum

apparently size-related differences in the mean frequency responses can be observed. For example, mean motion of the anterolateral body wall of western fence lizards, the largest species, peaked at 1,000 Hz, whereas those of the smaller green anoles and western skinks peaked at 1,250 and 1,600 Hz, respectively. The mean amplitude of the peak motion in western fence lizards also was a few decibels less than that observed in green anoles and western skinks, but again, overall, the body wall responses were very similar.

Effects of filling the lung

Filling the lungs with oxygenated saline had a dramatic effect on the acoustic responsiveness of the anterolateral body walls of both salamanders and lizards. Figure 7 shows the effect of filling the lung of a red-spotted newt. Filling the lung caused a clear reduction in responsiveness above about 300 Hz in this individual. When the filled lung was emptied and re-filled with air, sound-induced motion returned to initial levels (Fig. 7). This effect was consistent among the three newts tested (Fig. 8). At the frequency of peak motion (2,000 Hz), filling the lungs suppressed motion by 22–25 dB among the three newts.

The effect was very similar in the green anoles (Fig. 9). Filling the lungs significantly decreased motion above about 300 Hz, and re-filling the lungs with air restored the pattern of acoustic responsiveness. This effect of filling the lungs was consistent across the three individuals tested (Fig. 9). At the frequency of peak motion (1,000–1,600 Hz), filling the lungs suppressed motion by 21–28 dB among the three anoles.

Filling the lungs of the green anoles also had an effect on tympanic responses to sound, although the effect was more subtle than that observed for the anterolateral body wall. Figure 10 shows tympanic responses of all three anoles tested before and after the lungs were filled with oxygenated saline. Tympanic responses from about

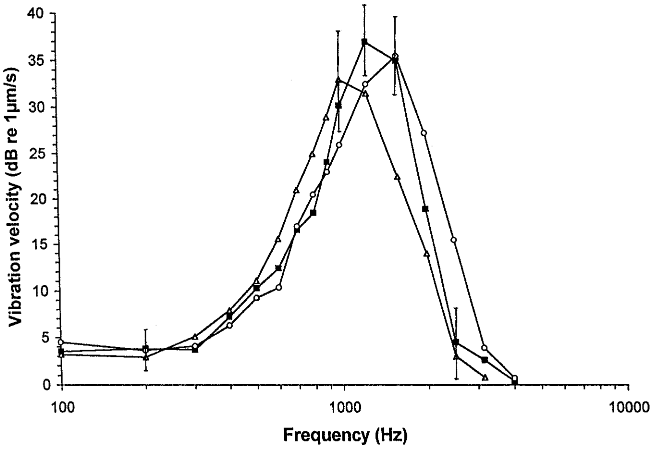

Fig. 6 Comparison of the mean acoustic responsiveness of the anterolateral body walls of the three species of lizards examined, the green anole (*Anolis carolinensis*; *solid squares*; $n = 5$), western fence lizard (*Sceloporus occidentalis*; *open triangles*; $n = 5$), and western skin (*Eumeces skiltonianus*; *open circles*; $n = 3$). SDs (*vertical bars*) are shown for mean values at certain frequencies for certain species, namely 200 Hz, 1,250 Hz, and 2,500 Hz for anoles, 1,000 Hz for fence lizards, and 1,600 Hz for skinks

500–1,500 Hz were reduced by up to about 15 dB after filling the lung. Responses of the tympanum at higher frequencies were unaffected. The range of frequencies over which filling of the lung reduced tympanic motion generally matched the frequency range of significant acoustic responsiveness of the anterolateral body wall (Figs. 5, 6).

Lung dimensions and calculated resonant frequencies

The inflated lungs of both green anoles examined were approximately 20 mm long and 10 mm wide with bronchi about 2 mm long and 1.5 mm wide, and their lung volumes were 0.80 cc and 0.86 cc (mean 0.83 cc). The lungs of the salamanders were relatively smaller and narrower. The inflated lungs of the newts were approximately 20 mm long and about 1.5 mm in diameter, and those of the spotted salamanders were about 25 mm long and about 2 mm in diameter. Bronchi of both salamander species were about 1 mm long and about 1 mm in diameter. The lungs of both red-spotted newts had volumes of about 0.04 cc and the lungs of both spotted salamanders had volumes of about 0.08 cc.

The corresponding Helmholtz resonances calculated using these values were 6,733 Hz for red-spotted newts, 5,201 Hz for spotted salamanders, and 1,113 Hz for green anoles. Comparison of these values with mean LDV measurements in Fig. 4 finds that the Helmholtz resonances calculated for both species of salamanders fall well above frequency of peak vibration velocity of the anterolateral body wall. In newts, the peak of mean body wall motion occurred at 1,600 Hz (range 1,600–2,500 Hz) and in spotted salamanders the peak of body wall motion occurred at 1,250 Hz (range 1,250–1,600 Hz). In contrast, the Helmholtz resonance of the anole lung matches the frequency of peak motion of the anterolateral body well more closely (Fig. 6). In green

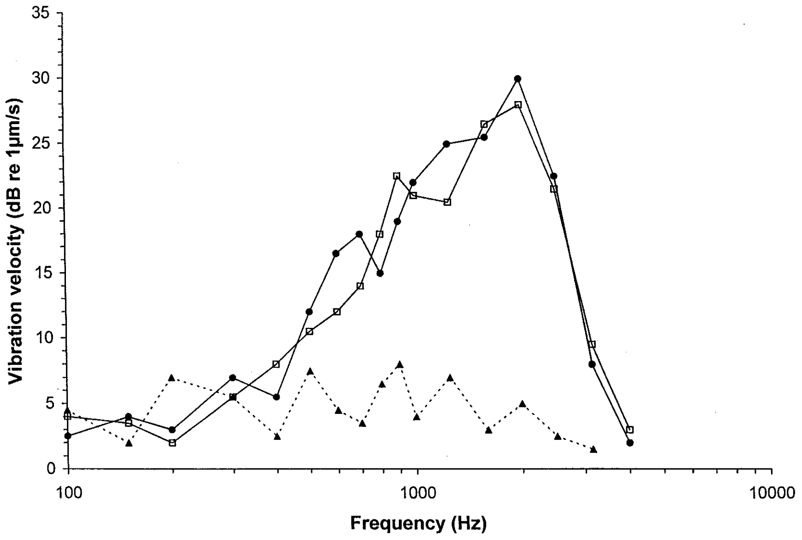

Fig. 7 The effect of filling the lungs of a red-spotted newt (*N. viridescens*) with oxygenated saline on the acoustic responses of the anterolateral body wall over the lung. The two *solid lines* represent data before the lungs were filled (*solid circles*) and after the lungs were emptied of saline and refilled with air (*open squares*). The *dashed line* (*solid triangles*) represents data when the lungs were filled with saline. Note the dramatic decrease in body wall responses when the lungs were filled with saline

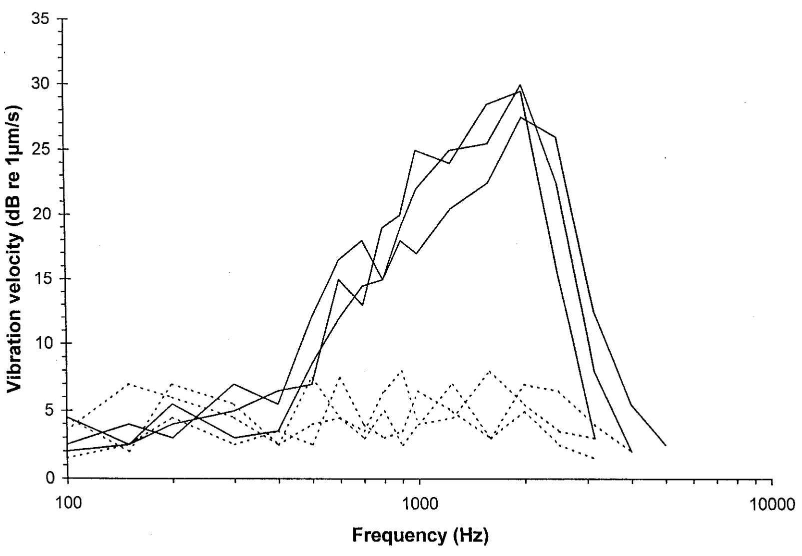

Fig. 8 The effect of filling the lungs of three red-spotted newts (*N. viridescens*) with oxygenated saline on the acoustic responses of the anterolateral body wall over the lung. The *solid lines* represent measurements before filling the lungs and the *dashed lines* represent measurements after the lungs were filled

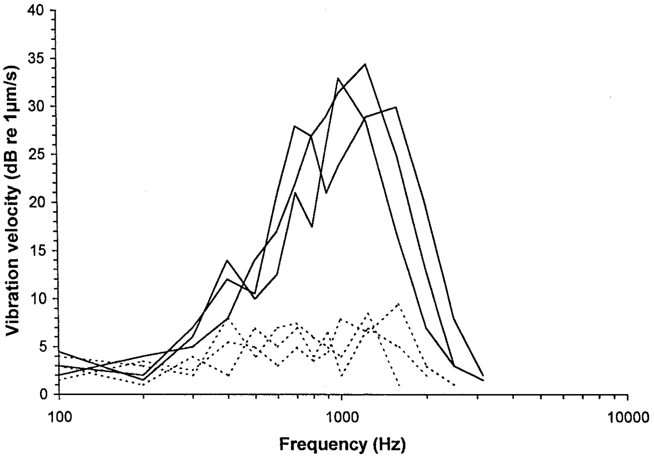

Fig. 9 The effect of filling the lungs of three green anoles (*Anolis carolinensis*) with oxygenated saline on the acoustic responses of the anterolateral body wall over the lung. The *solid lines* represent measurements before filling the lungs and the *dashed lines* represent measurements after the lungs were filled. Note the dramatic decrease in body wall responses when the lungs were filled with saline

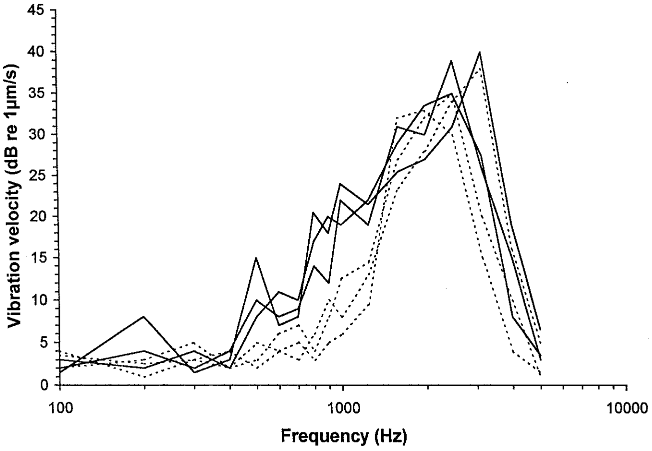

Fig. 10 The effect of filling the lungs of three green anoles (*Anolis carolinensis*) with oxygenated saline on the acoustic responses of the tympanum. The *solid lines* represent measurements before filling the lungs and the *dashed lines* represent measurements after the lungs were filled. Filling the lungs with saline produced some decrease in tympanic motion at frequencies from about 500–1,250 Hz

anoles, the peak of mean body wall motion occurred at 1,250 Hz (range 1,000–1,600 Hz).

Calculations of the resonant frequencies of closed tubes with the same dimensions as the relatively long and narrow lungs of the salamanders provided a closer match with the frequency of peak motion of their body walls. Resonant frequencies of 4,137 Hz and 3,164 Hz were calculated for the newts and the spotted salamanders, respectively. Both values, however, are still more than double the frequencies of peak motion of the anterolateral body walls of the two species (Fig. 4).

Discussion

The results of this study suggest that the lateral body wall and lungs of many terrestrial vertebrates may function in sound reception. In addition to previous work on anuran amphibians, this study has now shown that the lateral body walls of both salamanders and lizards display sound-induced motion significantly greater than that of unspecialized head tissues. The lizards examined in this study possessed tympanic middle ears, and the lateral body wall displayed greater motion than the tympanum over a broad range of relatively low frequency sounds. The importance of air-filled lungs in generating body wall responses to sound in both salamanders and lizards was demonstrated by the significant decreases in body wall motion following filling of the lungs with oxygenated saline. The lack of significant lateral body wall motion in the lungless plethodontid salamanders further demonstrated the role of air-filled lungs.

The lungs of the green anole lizards fit a Helmholtz resonator model fairly well. Sound-induced motion of the lateral body walls of the anoles peaked around 1,250 Hz, and the calculated Helmholtz resonance for the lungs of this species was 1,113 Hz. In contrast, the frequencies of the peak response of the body walls of both species of lunged salamanders were well below the calculated Helmholtz resonances. Calculation of the resonant frequencies of closed tubes with the dimensions of the long and narrow lungs of the salamanders produced a better match, although the frequencies of peak body wall motion were still less than half that of the calculated resonances. The reasons for this mismatch are unclear. Potentially, morphological features of the lungs, such as the thickness and pliability of their walls, the pattern of internal partitioning, their relationship with surrounding tissues, etc., might affect resonant properties. Despite the mismatch in the case of salamanders, filling the lungs of these animals abolished the pronounced responsiveness of the lateral body wall, strongly implicating the lung as the important factor in generating the acoustic response.

Measurement of lung dimensions and volumes helped to explain the similarity in body wall resonances of the relatively small red-spotted newts and larger spotted salamanders. Sound-induced motion of the lateral body walls of newts peaked around 1,600 Hz whereas body wall motion of spotted salamanders peaked around 1,250 Hz (Fig. 4). Although the latter weighed close to five times the former, the newts had proportionately larger lungs about half rather than one-fifth the volume of those of the spotted salamander. The Helmholtz and closed tube resonances calculated from the lung dimensions of these two salamanders also were closer than would be expected in species of such different body sizes.

The demonstration that the lateral body wall of salamanders and lizards can respond to sound is especially significant because these animals have stouter body walls, with ribs and thicker layers of axial musculature, than most anuran amphibians that have been studied to date. These findings therefore increase the likelihood that this phenomenon may be widespread among tetrapod vertebrates.

The acoustic responsiveness of the lateral body wall and lungs of salamanders and lizards suggests that they might serve as a pathway of sound transfer to the inner ear. Hartline (1971) suggested that the lungs were involved in hearing in snakes, a group that lacks a tympanic middle ear, and Hetherington and Lindquist (1999) showed experimentally that the lateral body wall and lungs were important for hearing in fire-bellied toads (*Bombina orientalis*), an anuran species that also lacks a tympanic middle ear. The auditory sensitivity of both snakes and *Bombina* is restricted to relatively low frequencies, suggesting that a lung pathway functions effectively only over this frequency range. The acoustic responses of the body wall and lungs of the salamanders and lizards in this study also were limited to relatively low frequencies, at least in comparison to the acoustic responses of tympanic middle ears in comparably sized animals. For example, at frequencies above about 1,250–2,000 Hz, the tympana of the lizard species studied were more responsive than the anterolateral body wall. The findings of this study provide further circumstantial evidence that the body wall and lungs may serve as a low frequency channel in many tetrapod vertebrates.

Potential pathways of sound transfer from the lungs to the inner ear remain speculative (Hetherington and Lindquist 1999). Because sound from the lungs can modify tympanic motion, sound pressure can clearly pass from the lungs to the middle ear cavity, probably via the respiratory passages (Ehret et al. 1990; Jorgensen 1991). Hetherington and Lindquist (1999) speculated that in anurans sound from the lungs could enter the round window, which typically is covered with thin layers of muscle and opens ventrally toward the pharynx. In this case, sound energy would flow into the inner ear in a direction opposite that of sound entering the oval window via the tympanum and auditory ossicle. An attractive aspect of this "reverse flow" hypothesis, at least for frogs and toads, is that it probably accurately describes energy flow in the ear of tadpoles. Tadpoles of several families of anurans have a direct structural connection (the bronchial columella) from each bronchus to each round window (Witschi 1949; T.E. Hetherington, personal observation), and this arrangement likely conducts sound energy within the lungs to the inner ear in a manner analogous to that of fishes that use air bladders for underwater hearing (Witschi 1949; Schellart and Popper 1991). The use of the round window for transfer of lung-borne sound into the inner ear of metamorphosed anurans would therefore represent a retention of the larval pattern. A problem for this proposed pathway is the impedance mismatch at the round window that would decrease transfer of air-borne sound energy from the pharynx into the inner ear fluids. Why a structural connection like the bronchial columella would not be retained in metamorphosed anurans for purposes of overcoming this impedance mismatch is a reasonable question. Perhaps, at least in the case of anurans with tympanic middle ears, such an ossicular element would interfere with the pressure-release function of the round window when higher frequency sound energy conducted via the tympanum and columella enters at the oval window.

There are other potential routes of sound transfer from the lungs to the inner ear that also would fit the "reverse flow" pattern. For example, Narins et al. (1988) speculated that sound energy from the lungs might travel along the vertebral column and enter the inner ear medially from the endolymphatic sac within the cranial cavity. The periotic (perilymphatic) systems of the inner ears of many amphibians and reptiles also extend medially into the cranial cavity, and also might provide an entry point for sound. In most of these "reverse flow" hypotheses, however, there is the problem of a pressure release pathway. In tadpoles the laterally positioned oval window is empty and could serve as a pressure release point for sound entering the round window, but in metamorphosed amphibians and other tetrapods the oval window is largely occluded by auditory ossicle(s). However, much remains to be understood about energy flow in the inner ear of vertebrates such as amphibians (Purgue and Narins 2000), and these "reverse flow" hypotheses of inner ear sound flow should be tested.

Although the lungs of lizards may be involved in the direct transfer of sound energy to the inner ear of these reptiles, this study suggests that the lungs might also contribute to the function of the tympanic middle ear. Studies on anuran amphibians have demonstrated that lung-borne sound can modify tympanic responses to low frequency sound (Narins et al. 1988; Ehret et al. 1990, 1994; Jørgensen 1991; Jørgensen et al. 1991), and this study has found comparable results in lizards. Filling the lungs of green anoles with saline depressed tympanic responses to sound below about 1,500 Hz. This range of frequencies corresponds generally to the frequency range of significant acoustic responsiveness of the anterolateral body wall, suggesting that part of the low frequency response of the tympanum was produced by sound energy passing from the lungs through the respiratory passages to the inner surface of the tympanum. Given the small size of the heads of these species of lizards, directional information on sounds may very well depend on their middle ears acting as pressure-gradient receivers (Aertsen et al. 1986; Eggermont 1988). The middle ear cavities of all of the species of lizards examined in this study have broad Eustachian tubes connecting to the pharynx, so it is likely that their middle ears can serve as pressure-gradient receivers. As suggested for anurans (Narins et al. 1988; Ehret et al. 1990, 1994; Jørgensen 1991; Jørgensen et al. 1991), the body wall/lung pathway may improve the directional responses of the tympanic middle ear of lizards to low frequency sounds.

To date, the acoustic responsiveness of the lateral body wall and lung has only been studied in relatively small vertebrates. It may be that the lungs can effectively serve in reception of a reasonable range of frequencies only in small animals. An inverse relationship between the frequency of peak responsiveness and body size was found in this study, and presumably this is related to resonant properties of lungs of different sizes. Hetherington (1992) documented similar size-related changes in body wall responses in anurans amphibians. The frequency response of the lateral body walls and lungs of substantially larger tetrapods may be restricted to only very low frequencies and therefore be less effective for detection of a range of sound frequencies. Potentially, selective pressures driving the evolutionary development of a tympanic middle ear may have been most intense in lineages of larger animals in which extra-tympanic pathways such as the lung are effective for only a very limited range of low frequencies. However, a variety of selective pressures may have been involved in the evolution of the tympanic middle ear (e.g., selective pressures to improve the ability to obtain directional information from sound signals).

In summary, the lung pathway of sound reception first documented in anuran amphibians may be found in many terrestrial vertebrates. This pathway likely represents a retention of a primitive auditory system of aquatic ancestors that utilized a lung for transducing underwater sound pressure signals. This primitive system may have been the first auditory system of terrestrial vertebrates, and may remain important for hearing in many tetrapods today, especially those lacking standard tympanic middle ears. In those tetrapods with well-developed tympanic middle ears, the body wall and lungs may serve as a low frequency channel of sound reception and may contribute to the directional characteristics of the middle ear.

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