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Accessory pathway for sound transfer in a neotropical frog

(auditory system/hearing/amphibian/laser Doppler vibrometry)

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ABSTRACT A portion of the lateral body wall overlying the lung cavity of the arboreal frog, *Eleutherodactylus coqui*, vibrates in response to free-field sound. Peak displacement amplitude of the body wall in response to a natural call note presented at 73 decibels sound pressure level is 1.70 × 10^{-9} m, roughly 8 decibels less than that of the ipsilateral eardrum, as measured by laser Doppler vibrometry. We show that the vibration magnitude varies predictably across the body profile and is posture and frequency dependent. Two routes to the inner ear are described for sounds impinging on the body wall; either of these accessory pathways could modify direct input from the peripheral auditory system and enhance sound localization in these small vertebrates.

It is generally accepted that insects, small anuran amphibians, and birds use a pressure-gradient system to localize sounds (1–3). Such a system responds to the resultant force on a vibrating membrane, which receives acoustic input from both sides. In frogs, sounds impinging on the two sides of the tympanic membrane result in a directionally dependent acoustic input to the inner ear (1–4). Although sound reaches the internal side of the tympanic membrane via the pathway consisting of the air-filled, middle-ear cavity and the large permanently opened Eustachian tubes leading to the flexible buccal cavity, the only demonstrated input to this pathway in frogs to date has been via the contralateral ear (2, 3). We now report a second input to this pathway via the lungs, larynx, and buccal cavity, which could serve to modulate direct tympanal input.

MATERIALS AND METHODS

Animals. Ten healthy adult male arboreal frogs, *Eleutherodactylus coqui*, were captured in their natural habitat in the Caribbean National Forest, Puerto Rico, and transferred within 2 days to the laboratory. Pairs of animals were housed in closed terraria (temperature: 23–25°C; humidity: 90%) containing fresh soil and green plants (*Pothos* spp.). Within minutes of transfer the frogs assumed normal postures and moved about freely.

A single awake unrestrained animal was then placed in a Plexiglas terrarium containing a vertical post (diameter = 10 cm). Before being measured, the animal was gently prodded with a dull probe, which initiated leaping and caused the frog to tire. After ≈60 sec the animal jumped or was placed, partially exhausted, either in a horizontal position on the post top, or in a vertical face-up position on the post side.

Vibration Measurements. Body velocity profiles of seven frogs were made to determine whether any external body parts (besides the tympanum) vibrated in response to an externally delivered sound stimulus. A point on the frog's body was directly illuminated by a 5 mW He/Ne laser (DISA 55X; Skovlunde, Denmark); the spot size was ≈1 mm^2. Direction of the laser was facilitated by interposing a mirror in the beam path; post rotation and mirror adjustment allowed focus of the laser beam at any point on the frog's profile. With the laser in Doppler vibrometer mode, we computed the velocity spectrum of the modulated backscattered beam by Fourier analysis (Nicolet model 446A spectrum analyzer).

Frogs were measured in the darkened laboratory with the terrarium lid removed, thereby providing the laser beam an unobstructed 120-cm path to the frog's body. Because the animal was artificially tired and the photoreceptors were backlighted with light scattered by his body, the frog's jumping tendency was reduced. Thus, animals often remained stationary for up to 90 min enabling the collection of a complete uninterrupted set of velocity measurements.

Tympanic membrane velocity was measured in response to playback of either a tape recording of a series of 130 natural calls of *E. coqui* presented at behaviorally relevant levels (5) or continuous broadband noise (spectrum flat ± 2 decibels (dB) from 0.2–5.0 kHz; level: 82 dB sound pressure level (SPL) at tympanum). Stimuli were presented through a free-field loudspeaker (Isophon model KM13/150, Berlin) located 45 cm from the animal along an axis through the two tympana. The frequency spectrum of the advertisement "coqui" call used as an acoustic stimulus, measured at the position of the animal's eardrum [using a 0.5-inch (1 inch = 2.54 × 10^{-2} m) microphone and measuring amplifier (Brüel & Kjaer models 4133 and 2606, respectively, Naerum, Denmark)], contained two principal peaks: the "*co*" peak at 1075 Hz and the "*qui*" peak at 2075 Hz (6). Durations of the *co* and *qui* notes were 110 and 165 msec, respectively.

Pressure Measurements. We measured changes in the buccal pressure in response to 110-dB SPL tone bursts presented via a closed acoustic delivery system sealed against the lateral body wall over the ventrolateral portion of the lung cavity. The system consisted of a calibrated headphone (Beyer DT-48, Heilbronn, F.R.G.) mounted in a specially constructed brass housing designed to accept (*i*) a short length of rubber tubing sealed over the lateral body wall and (*ii*) a 0.5-inch condenser microphone (Brüel & Kjaer model 4134) for stimulus monitoring. Before these measurements, the ears were covered with a thick layer of silicon grease to attenuate tympanal input by roughly 40 dB at the test frequencies (7). Buccal pressure was continuously monitored using a custom-made transducer (8) based on a solid-state sensor (Omega model PX160, Stamford, CT). The transducer was attached via a short flexible length of Tygon tubing to a 24-gauge hypodermic needle inserted through the lower jaw into the buccal space. A small hole drilled in the shaft of the needle near the tip helped ensure that it remained patent during the measurements. The buccal pressure in response to at least 2000 repetitions of a 1-kHz or 2-kHz,

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Abbreviations: dB, decibel(s); SPL, sound pressure level.
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50-msec tone burst (rise–fall times: 5 msec) presented with a repetition period of 300 msec was averaged (EG&G PARC model 4202, Princeton, NJ) and plotted (Tektronix 4662; Beaverton, OR).

RESULTS

During this study we discovered two bilaterally symmetrical regions of the frog's body, ≈25 mm^2, the centers of which are located ≈8 cm caudal to the tympana, which exhibit a velocity profile in response to playback of the natural call of *E. coqui* similar to that of the tympanum. The magnitude of vibration in these areas was significantly greater than that of the surrounding tissue and only slightly less than that of the ipsilateral tympanic membrane (Fig. 1). In response to playback of a series of natural calls, we measured both tympanic-displacement amplitudes and lateral-body-wall responses (Table 1). Control regions located dorsomedial to the hind leg or near the dorsal midline showed little or no response (Fig. 1 c and l). Skin removal from freshly killed specimens revealed that the area of the lateral body wall that responded most vigorously to the call overlay the ventrolateral surface of the lung cavity in each of the seven frogs tested (see shaded areas, corresponding to the location of the underlying lung cavity, in Figs. 1 and 2).

On several occasions, a frog appeared so exhausted after his bout of forced jumping that, upon leaping to the top of the post, the animal would initially take on a low, or water-conserving, posture (13) with >80% of his ventral surface in contact with the substrate. Measurements taken with the frog in this low body posture revealed body-wall velocities in response to 1-kHz stimuli reduced by 10–20 dB relative to those taken from the upright posture. Furthermore, response to 2-kHz call notes was found only on the most ventral portion of the lateral body wall (Fig. 2). In contrast, in intermediate postures (in which ≈30% of the ventral surface remained in contact with the substrate) or fully upright postures, body-wall velocities increased to both 1- and 2-kHz stimuli over a relatively wider area of the lateral body wall (Figs. 1 and 2). Moreover, when a frog assumed a vertical face-up position on the post, the area of greatest vibration velocity shifted toward the midline relative to the horizontal postures (Fig. 2).

The transfer function derived from the velocity spectrum of the body wall narrows in the vertical posture, relative to that measured in the horizontal posture (Fig. 3); the velocity spectrum of the tympanum includes higher frequencies than that for the body wall when the frog is in the horizontal posture. Specifically, for the record illustrated in Fig. 3, the low-frequency response limit (that frequency for which velocity is 3 dB above background) was 1.29 kHz for both the tympanum and the body wall, independent of body posture. In contrast, the tympanic high-frequency response limit was 3.38 kHz, compared with 2.58 kHz and 1.95 kHz

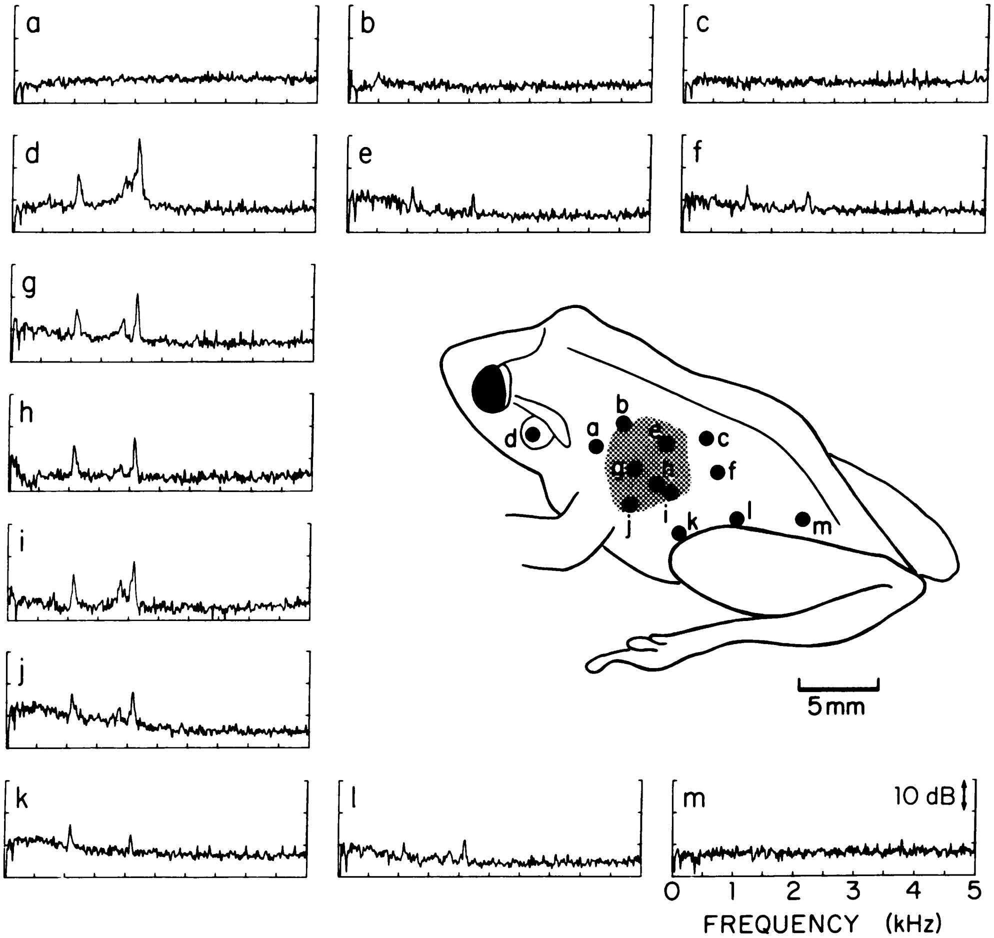

FIG. 1. Left profile of frog 7 showing points of velocity spectrum measurements and the corresponding mean velocity spectra (n = 130). Each spectrum is the difference between the average velocity of the measured point in response to the acoustic stimulus and the average background velocity in the absence of a stimulus. This animal spontaneously assumed the natural low-alert posture as indicated; his vocal sac was elevated above the substrate. The shaded area indicates extent of the frog lung cavity in this position, determined by rapid dissection of a freshly killed animal. Average velocity spectra for points overlying the most ventrolateral portion of the lateral lung cavity closely resemble those obtained from the typanum (point d). Note the lack of response at point a, located between tympanum and lung.

Table 1. Tympanic and body-wall displacements in response to the two-note advertisement call of *E. coqui*

	Tympanum			Body wall		
Stimulus	*n*	Range, nm	Mean ± SD, nm	*n*	Range, nm	Mean ± SD, nm
Co note	13	0.53–3.92	1.88 ± 1.13	8	0.28–1.42	0.71 ± 0.44
Qui note	14	0.60–4.75	2.56 ± 1.32	9	0.15–1.70	0.61 ± 0.61

The *co* and *qui* notes have spectral peaks at 1.075 and 2.075 kHz, have durations of 110 and 165 ms, and were presented at 66 and 73 dB SPL measured at the animal's tympanum, respectively. Mean tympanic-displacement amplitudes are consistent with previous measurements of other anuran species (8–12, 17).

for the body wall in the horizontal and vertical postures, respectively. These values were typical for the seven animals measured. Both the shift toward the midline of the location of greatest vibration velocity and the 0.4-octave reduction in high-frequency cutoff frequency seen in the vertical posture are consistent with the relatively large extent (≈45%) of ventral surface in contact with the substrate in this position.

Measurements of buccal pressure changes induced by sounds impinging directly on the animal's lateral body wall revealed a small but consistent correlated increase with stimulus presentation (Fig. 3, *Inset*). The closed-field acoustic stimuli were inaudible to a normal human observer standing inside the sound-attenuated chamber. Moreover, any ambient sound would be subject to roughly 40-dB attenuation by the silicon grease, and thus external tympanal input from airborne sounds during these measurements can be considered negligible. Additional control experiments were done, in which free-field acoustic stimuli were presented to the pressure sensor alone, both with the Tygon tubing open and plugged with modeling clay; in neither case was a pressure change recorded in response to sound, eliminating the possibility that the pressure-transducer apparatus was directly responding to the stimulus. Thus, we believe that body-wall vibrations produced in response to external sounds generate intrapulmonary pressure changes that are transmitted through the larynx to the buccal cavity.

DISCUSSION

Discovery of a lateral portion of the frog's body wall that responds to airborne sound is unexpected and is potentially significant in understanding directional hearing in small vertebrates. We have demonstrated that the regions exhibiting the highest vibrational velocities directly overlie the ventral portions of the lung cavities. Moreover, frequencies giving rise to the greatest vibrational velocities of the body wall lie in the range of 1.3–2.6 kHz, corresponding to

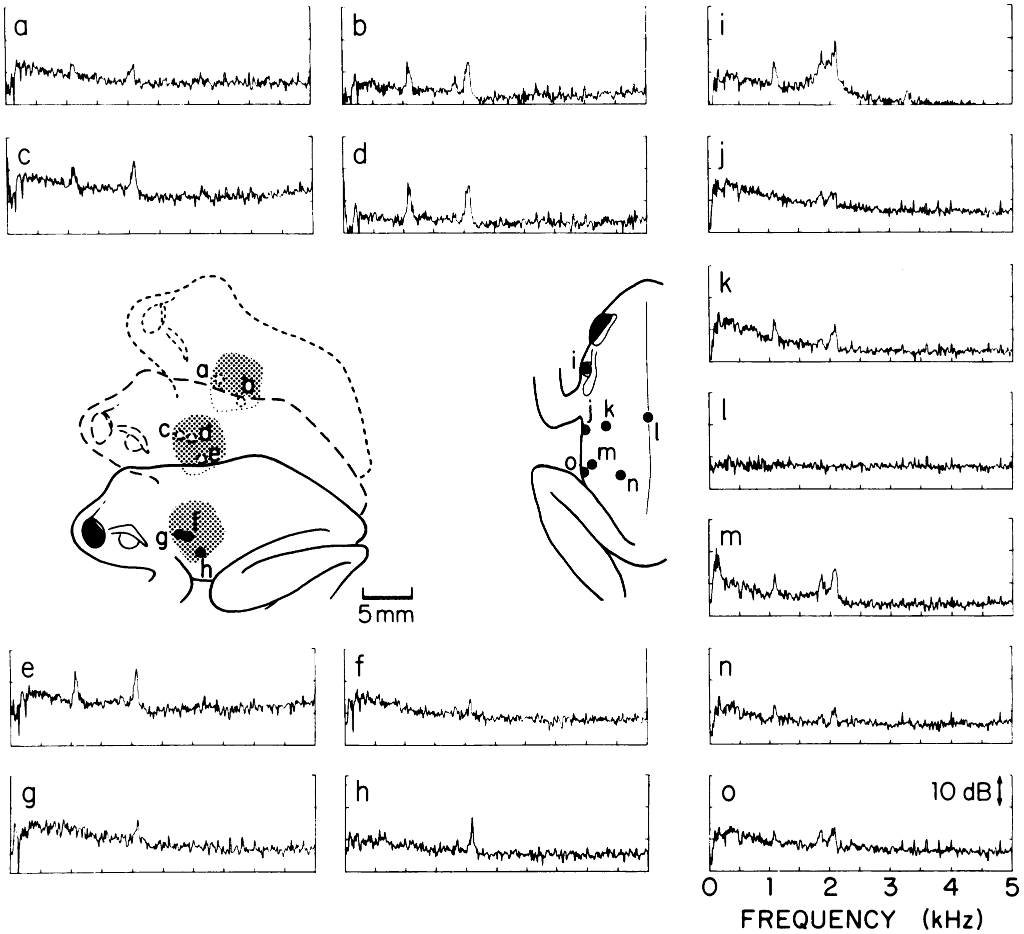

FIG. 2. Frog 9 shown in three horizontal postures—high-alert, low-alert, and water-conserving—and in a vertical face-up posture. The region of greatest body wall sensitivity shifted with changes in lung position consistent with postural modifications. The body-wall sensitivity persists in the vertical face-up position, the most common calling posture assumed by males in their natural habitat (5). Spectral properties of the call are the same as for Fig. 1.

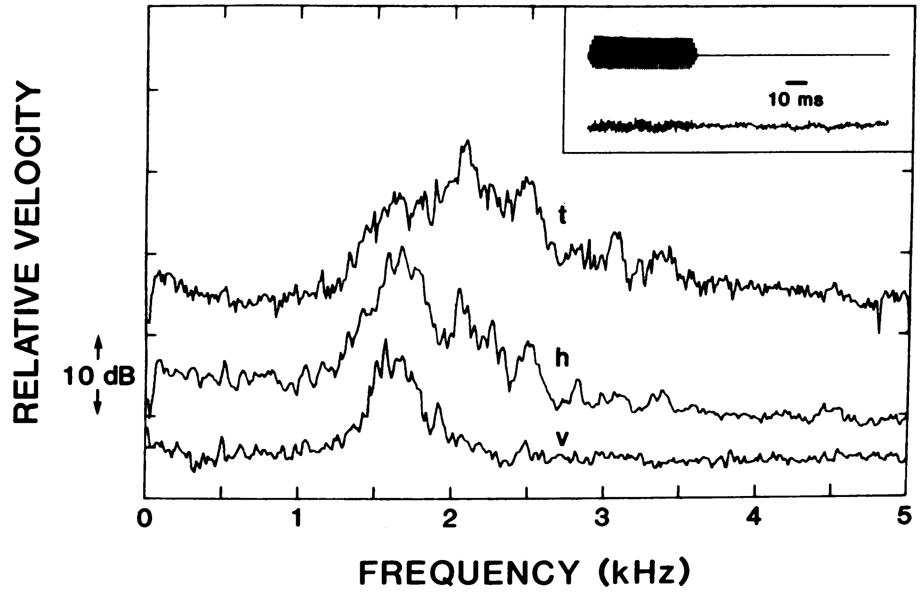

FIG. 3. Velocity spectrum, t, of left tympanum of frog 3 as determined by his response to free-field broadband noise. Spectra h and v are the velocity spectra of a point on the lateral body wall overlying the ventrolateral portion of the lung cavity, with the frog in a horizontal and vertical face-up posture, respectively. *Inset* (*Upper*) A 50-msec, 2-kHz tone burst presented at a level of 110 dB SPL, measured at the lateral body wall. (*Lower*) Record of average buccal pressure change in response to 2755 presentations of the tone burst delivered to the lateral body wall overlying the lung cavity. This pressure record illustrates a typical signal-to-noise ratio of 10–12 dB; average background noise level corresponded to a voltage of 40 mV peak-to-peak.

frequencies in the *qui* note of the species advertisement call and the region of greatest sensitivity of the basilar papilla in the inner ear (6).

Does the animal make use of this additional information for encoding directional information of a sound source? We think this is a question that can be best addressed with behavioral techniques. A more tractable question at this point is whether information from the lateral body wall could physically reach the inner ear and then potentially be used in evaluating position, or the acoustic nature, of a sound source. A light microscope examination of skin tissue overlying the lung cavity revealed no specialized mechanoreceptors that might mediate this function, but an exhaustive EM study of this region has not been done.

Because a pressure-gradient detector critically relies on the pressure *difference* across a vibrating membrane, complete understanding of the function of such a system requires that the sound pathways and all inputs to these pathways be defined. We therefore directly tested whether sounds impinging on the lateral body wall could affect pressure in the buccal cavity and thus provide an additional modulating input to the inside of the tympanic membranes.

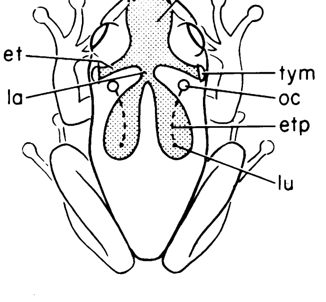

FIG. 4. Schematic diagram of existing and proposed pathways for sound transfer to the inner ear in the frog. tym, tympanum; oc, otic capsule; buc, buccal cavity; n, nares; lu, lung; et, Eustachian tube; la, larynx; etp, extratympanal pathway (putative). The buccal cavity receives acoustic input from the contralateral ear as well as from the lungs via the larynx.

Our results suggest that a second point of entry into the buccal cavity does exist—namely, via the body wall, lung cavity, and larynx. Buccal pressure recordings indicate that in the resting *E. coqui* the larynx is open during ≈25% of the ventilatory cycle (P.M.N., unpublished data), thus providing direct communication between lungs and the buccal space and, ultimately, the tympanic membranes. This internal-tympanal acoustic pressure could, in theory, modify input to the inner ear in a directionally dependent manner, thus affecting sound localization.

In addition, it has been suggested (14, 15) that an, as yet undefined, extratympanal pathway to the inner ear of the frog exists for low-frequency sounds (Fig. 4). One candidate for such a pathway might be from lung to inner ear via the endolymphatic sacs, a complex tubular system that is an extension of the posterior division of the saccule, running out into the vertebral canal (16). In any event, the mechanisms underlying sound localization in these small vertebrates are apparently more complex than has been previously imagined.

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