Table 1. Compilation of fluorescence emission and excitation data of various Chls and Chlides in acetone. Experimental conclusion c.f. Fig. 2

Chls and Chlides	Excitation max.	Emission max.
Chl a	428	670
Chlide a	428	670
Chl b	452	653
Chlide b	450	653
Chl RC I	433	677
Chlide RC I	433	677
Chl γ	–	–
Chlide γ	462	662

products of Chlide a (band 3) and a mixture of Chlide a and PChlide (band 4).

The evidence of the intermediates confirms the idea that Chl b does not derive from Chl a [7], but has a separate pathway. We must also assume that the chlorination of Chl RC I occurs at the stage of Chlide or earlier.

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Directional Hearing of Awake, Unrestrained Treefrogs

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Behavioral experiments have demonstrated that frogs are able to locate sound sources with a remarkable accuracy [1–3], but biophysical and electrophysiological studies have not resulted in a clear understanding of the peripheral mechanism underlying their directional hearing [4–7]. Furthermore, the directionality observed in those studies have varied considerably. Recently, mathematical models have been used to analyze the frog's auditory periphery [6, 7]. The predicted directionality is small and critically dependent on the values assumed for the models' parameters, only few of which can be measured with any reasonable accuracy.

The anuran's tympanal membrane receives sound at its external surface and, likely, at its internal surface as well; it therefore acts as an inherently directional pressure difference receiver. Sound may arrive at the inner surface of the tympanum from the opposite ear and/or the mouth cavity via the wide and permanently open Eustachian tube. The soft and flexible internal sound pathways in the frog's head are likely to change during anaesthesia and thus affect measurements of the directionality of the ears [5]. This is probably one reason for the different experimental results that have been reported in the past. There is thus a need for reliable physiological and biophysical data on the directionality of the auditory periphery in *unanaesthetized* frogs.

We have measured the vibration velocity of the centre of the frog's tympanum using a laser vibrometer at a distance of about 60 cm (the use of laser vibrometry for such measurements is discussed in detail in [8]). The amplitude spectrum of the tympanal vibration velocity in response to acoustic clicks was computed by means of a Fast-Fourier-Transform (HP 3582A). The vibration velocities at various frequencies were plotted as a function of the direction of the sound.

Awake, unrestrained green treefrogs (*Hyla cinerea*) received sound from various directions in a free sound field, which was obtained in the following way: The frog was placed on a wooden plate, about 1 m above the floor and at the centre of a roundabout carrying a loudspeaker (Philips AD 5060). A brief electrical pulse was generated and sent to the loudspeaker, causing it to emit its impulse response (duration about 0.3 ms). The sound travelled about 70 cm to the frog, where it caused the frog's tympanum to vibrate for about 3 ms. The experimental setup included several surrounding objects (e.g., the laser vibrometer and a microscope), but the distances from the frog to these objects were sufficiently long so that any "echoes" from them arrived at the frog's head well after the initial tympanal vibration had decayed. These objects, therefore, did not affect the sound field around the frog during the short time interval of our impulse measurement.

We discovered that the tendency of our treefrogs to jump during an experimental session could be reduced by increasing the ambient illumination. Some of our animals sat motionless for more than 1 h, sufficient for 7 measurements at each of 12 sound directions. In each measurement, a time average of 128 tympanal impulse responses was computed, and the averaged vibrational waveform was then used in computing the amplitude spectrum. Throughout these measurements, the frogs sat upright with their heads lifted, and the floor of the mouth (which moved up and down during successive bouts of buccal respiration) did not touch the substrate. In separate experiments, a microphone probe (Brüel & Kjær 4170, exponential horn, external diameter 1 mm) was

placed close to the ear in order to monitor any possible, small pressure change due to head diffraction.

The amplitude spectrum of the tympanal vibration (Fig. 1) was similar to that earlier reported for this species [9]. In the frequency range 1–3 kHz, the vibration velocity of the tympanum was large, and here the 7 measurements at each direction had very little scatter (Fig. 1). The standard deviation was often less than 1 dB.

In the averaged directional diagram (Fig. 2A) the distance from the centre indicates the vibration velocity (here normalized relative to the velocity at the frontal sound direction of 0°). The average difference between the vibration amplitudes for ipsi- and contralateral sound input (90° and 270°) is 9 dB in the frequency range 1–3 kHz. While the directional diagrams for individual frogs and at different frequencies deviated somewhat from this average, no clear dependence on frequency in the range of 1 to 3 kHz could be seen in the data. The bars in Fig. 2A include 50% of the data (the standard deviation would not be a proper measure of scatter in this case).

As expected, the sound pressure amplitude close to the external surface of the tympanum changes very little with the direction of the sound. In Fig. 2B, the average values have been plotted for the frequency range 1–3 kHz and normalized relative to 0° (in order to allow a comparison with Fig. 2A). At 2.5–3 kHz, a surplus pressure of about 1 dB is found for 30°–90° sound, and at 180°–210° the sound pressure in this frequency range is about −1 dB. At 4 kHz these values are about 2.5 dB and −2 dB, respectively.

Obviously, these treefrogs have sufficient cues to distinguish between sounds arriving from the ipsilateral (90°) and contralateral (270°) directions (Fig. 2A). It is also obvious from our results that the accuracy of locating sound sources in the frontal direction (0°) would be enhanced whenever they moved. This likely is why female green treefrogs exhibit lateral head scanning movements of about 30° right or left during phonotaxic approaches [2, 3]. In addition, when approaching a sound source on the ground, they frequently perform a "zig-zag hopping" trajectory [1, 2], i.e., they move directly towards the sound source, but land with their body axis about 30° relative to the target axis in alternating jumps.

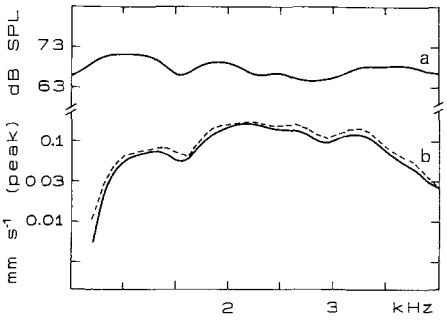

Fig. 1. The amplitude spectrum of the sound pressure (*a*) and the vibration velocity of the centre of the tympanal membrane (*b*). In *b*, the *solid line* is the mean velocity, and the *broken line* indicates the mean plus one standard deviation (calculated from 7 consecutive measurements; sound direction (cf. Fig. 2) 90°)

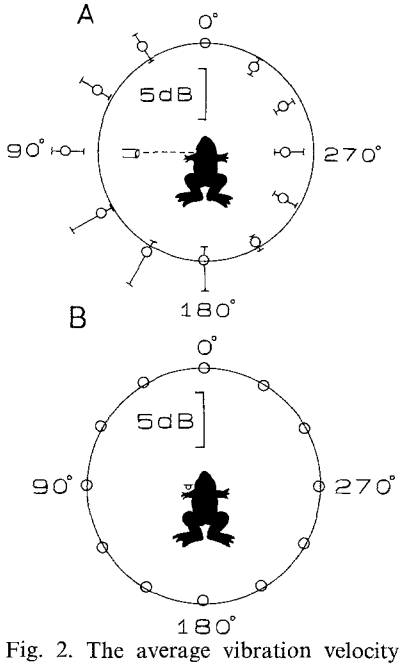

Fig. 2. The average vibration velocity of the centre of the tympanal membrane (A) and the average sound pressure 1 mm from the eardrum (B), plotted as a function of the direction to the sound source (0° is frontal, 90° is ipsilateral). The data in (A) are median values of data from 4 frogs and 4 frequencies (1, 1.5, 2, and 3 kHz)

Binaural directional information is processed in the anuran's central nervous system. In the torus semicircularis, which is an important midbrain auditory centre, EI-neurons are excited by input from one ear and inhibited by input from the other. Many of these cells are sensitive to interaural intensity differences of 1–2 dB or less [10]. The directionality shown in Fig. 2A may thus be sufficient, even without head scanning, for localizing frontal sound sources in a free sound field in the laboratory. It remains to be learned whether these binaural intensity cues are sufficient for a frog in its natural environment where the directional information may fluctuate [11]. It has been proposed experimentally [5] and theoretically [7] that directional hearing in anurans may be improved if they deliberately control the volume and/or shape of their mouth cavity. So, it is conceivable that frogs and toads could obtain an even better directionality than that shown in Fig. 2A by such an active adjustment. But that has yet to be verified.

The technique that we have employed in this study on awake, unrestrained anurans hopefully will clarify the physical basis for binaural directional cues in these small animals. Further measurements are in progress.

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