LETTERS

Ultrasonic communication in frogs

Albert S. Feng[1]*, Peter M. Narins[2]*, Chun-He Xu[3], Wen-Yu Lin[1], Zu-Lin Yu[4], Qiang Qiu[4], Zhi-Min Xu[4] & Jun-Xian Shen[4]*

Among vertebrates, only microchiropteran bats, cetaceans and some rodents are known to produce and detect ultrasounds (frequencies greater than 20 kHz) for the purpose of communication and/or echolocation, suggesting that this capacity might be restricted to mammals[1,2]. Amphibians, reptiles and most birds generally have limited hearing capacity, with the ability to detect and produce sounds below ~12 kHz. Here we report evidence of ultrasonic communication in an amphibian, the concave-eared torrent frog (*Amolops tormotus*) from Huangshan Hot Springs, China. Males of *A. tormotus* produce diverse bird-like melodic calls with pronounced frequency modulations that often contain spectral energy in the ultrasonic range[3,4]. To determine whether *A. tormotus* communicates using ultrasound to avoid masking by the wideband background noise of local fast-flowing streams, or whether the ultrasound is simply a by-product of the sound-production mechanism, we conducted acoustic playback experiments in the frogs' natural habitat. We found that the audible as well as the ultrasonic components of an *A. tormotus* call can evoke male vocal responses. Electrophysiological recordings from the auditory midbrain confirmed the ultrasonic hearing capacity of these frogs and that of a sympatric species facing similar environmental constraints. This extraordinary upward extension into the ultrasonic range of both the harmonic content of the advertisement calls and the frog's hearing sensitivity is likely to have co-evolved in response to the intense, predominantly low-frequency ambient noise from local streams. Because amphibians are a distinct evolutionary lineage from microchiropterans and cetaceans (which have evolved ultrasonic hearing to minimize congestion in the frequency bands used for sound communication[5] and to increase hunting efficacy in darkness[2]), ultrasonic perception in these animals represents a new example of independent evolution.

We recorded the vocalization patterns of eight male frogs in the field under three experimental conditions for a period of three minutes each: (1) an NS period, during which no sound was presented, (2) a US period, during which we presented the ultrasonic components of a previously-recorded conspecific vocal signal at ~77 dB sound pressure level (SPL) (root mean squared or r.m.s. reading), a sound level that is behaviourally relevant, and (3) an AUD period, during which we presented the audible components (<20 kHz) of the same vocal signal at a similar sound level. For five frogs (asterisks in Fig. 1a), the male's calling rate was markedly increased during the AUD and/or US period, compared to spontaneous calling rates during the NS period. Three frogs (601-4, 602-1, 602-2) showed no overt evoked vocal responses to any playback stimulus. The stimulatory effect of the US components was most robust for frogs 531-1 and 601-2. Frog 531-1 did not produce any calls during the NS period, but emitted 11 calls during the US period. Frog 601-2 produced six calls during the NS period, and emitted 18

calls during the US period, including four antiphonal responses that were precisely time-locked (within 30 ms of the stimulus offset) to the US stimulus (Fig. 1b)—the probability of all four occurrences by chance is 7.4×10^{-7} (binomial probability). These results show that males of *A. tormotus* detect and respond to ultrasound.



a Evoked vocal responses

Frog no.	NS	US	AUD
*531 - 1	0	11	10 (2)
*531 - 2	2	6	–
*601 - 2	6	18 (4)	–
*531 - 3	0	0	18
*601 - 5	6	6	14
601 - 4	0	1	1 (1)
602 - 1	3	5 (1)	1
602 - 2	0	0	1
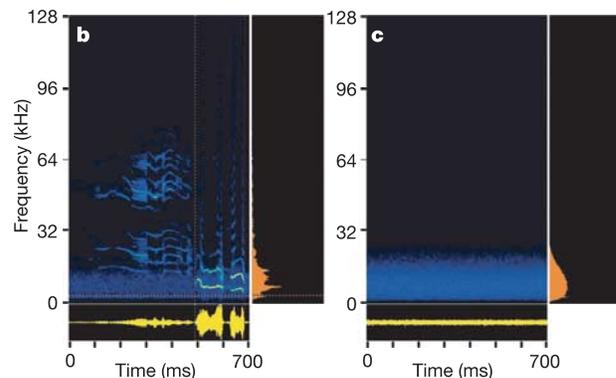
Figure 1 | *A. tormotus* can detect and respond to ultrasound. a, Evoked vocal responses from playback studies of eight male frogs. Shown are the numbers of calls produced during NS, US and AUD periods (3 min each). The NS column represents control data before playback trials. Numbers in parentheses represent frog antiphonal responses that were precisely time-locked to the stimulus. Cells without values correspond to incomplete trials owing to the frog escaping from its calling site. b, Sound spectrogram (top left panel), waveform (bottom panel) and average amplitude spectrum (right panel) of a representative antiphonal response (produced 401 ms after the stimulus onset—shown between the two vertical cursors in the left panel), stimulated by the US components of the stimulus (390-ms long, to the left of the white vertical cursor). c, Ambient background noise recorded within 3 m of Tau Hua Creek in the absence of frog calls, recorded with a custom-made ultrasonic microphone having a high-pass cutoff frequency of 15 kHz with a roll-off of 10 dB per octave (hence the appearance of a progressive decrease in spectral energy from 0.1 to 15 kHz; see Methods). The noise has a nearly flat spectrum below 10 kHz when measured with a sound-level meter[3].

[1]Department of Molecular and Integrative Physiology & Beckman Institute, University of Illinois, Urbana, Illinois 61801, USA. [2]Departments of Physiological Science and Ecology & Evolutionary Biology, University of California, Los Angeles, California 90095, USA. [3]Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China. [4]State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.
*These authors contributed equally to this work.
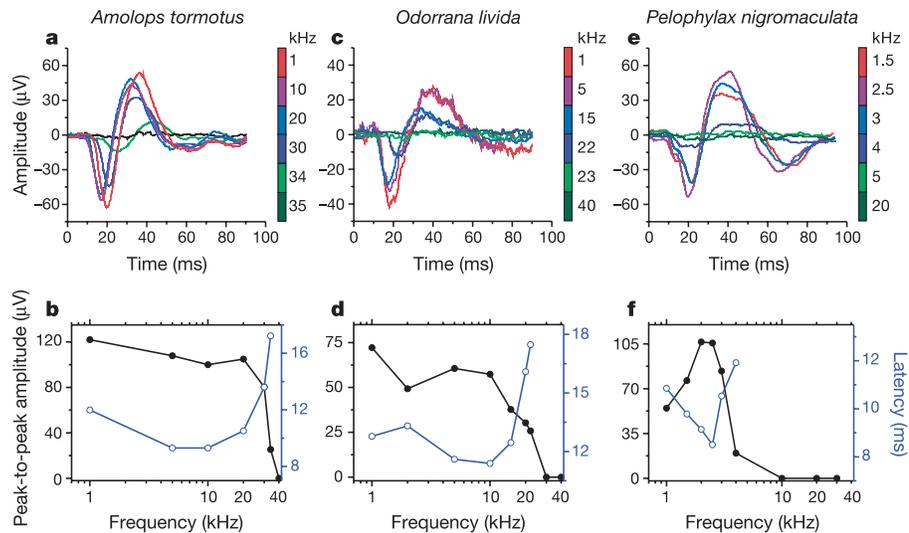
Figure 2 | Averaged auditory-evoked potential (AEP) data from the torus semicircularis validate the ultrasound sensitivity of *A. tormotus*. a–f, Shown are AEPs recorded from the torus semicircularis of *A. tormotus* (**a, b**), *O. livida* (**c, d**), and *P. nigromaculata* (**e, f**) in response to ten tone bursts over 1–40 kHz presented at a rate of 0.5 bursts s⁻¹.

a, c, e, Representative AEP waveforms. **b, d, f**, Corresponding peak-to-peak amplitudes ($N_1 - P_1$) of the AEPs (black) and N_1 latencies (blue) as a function of tone frequency. The N_1 latencies of the AEPs for the upper end of the species' hearing ranges were not measurable. N_1 and P_1 refer to the first negative and positive peaks of the AEP, respectively.

To validate the ultrasonic sensitivity of *A. tormotus* physiologically, we recorded auditory-evoked potentials (AEPs) from the torus semicircularis, the dominant midbrain auditory processing centre in the frog central nervous system. AEPs were consistently observed in response to tone bursts presented at 89 dB SPL from 1 to 34 kHz (Fig. 2a); no AEP was detectable for stimuli ≥35 kHz. The peak-to-peak AEP amplitudes were inversely correlated with latency (Fig. 2b). Because AEP latency decreases with increasing sound level above threshold, the shape of the latency curve approximates that of the species' audiogram.

We next isolated 30 single units from the torus semicircularis of 16 frogs and observed their tone-burst responses. Of these, 12 cells responded to tone bursts over a wide range of frequencies, including tones >20 kHz. For example, one tonic unit (Fig. 3a, right panels) fired maximally to a 20-kHz tone but also responded to tone bursts up to 27 kHz (Fig. 3a); a phasic-burst unit (Fig. 3b, right panels) responded best to a 10-kHz tone, with observable responses to tone bursts at 1–30 kHz (Fig. 3b). The distribution of the units' best frequencies is shown in Fig. 3c. Together, the AEP and single-unit data demonstrate the extraordinary ultrasonic sensitivity of *A. tormotus*.

The intense background noise from fast-flowing streams in Huangshan Hot Springs has a broad energy spectrum[3], with a peak around 0.1 kHz, substantial amplitudes over 0.2–10 kHz, and a progressive decline from 11–22 kHz (Fig. 1c). Extending call frequencies into the ultrasonic range probably represents an adaptation to prevent the frog's vocal signals from being masked by the background noise[4], similar to the high-frequency shift in the song of great tits living in urban areas[6]. Results of the present behavioural and electrophysiological studies support this hypothesis.

To determine whether sympatric frog species also show ultrasonic sensitivity, we recorded AEPs from the torus semicircularis of a large odorous frog (*Odorrana livida*) living in Huangshan Hot Springs. The AEP data from *O. livida* revealed that this frog also had the ability to detect ultrasound up to 22 kHz (Fig. 2c, d), but ultrasonic communication in this species remains to be shown.

In contrast, AEP recordings from the torus semicircularis of a black-spotted pond frog (*Pelophylax nigromaculata*), commonly found in rice fields and ponds throughout much of China[7], revealed that its upper range of hearing was limited to 4–5 kHz (Fig. 2e, f). On the basis of these results and previously published studies[1,2,8], we suggest that ultrasonic hearing in frogs is: (1) probably limited to frog species living in noisy environments, and (2) probably not due to artefacts in the acoustic stimulation system used in the physiological studies. Spectral analysis of the loudspeaker outputs revealed that the stimulus delivery system was quite linear; any subharmonics
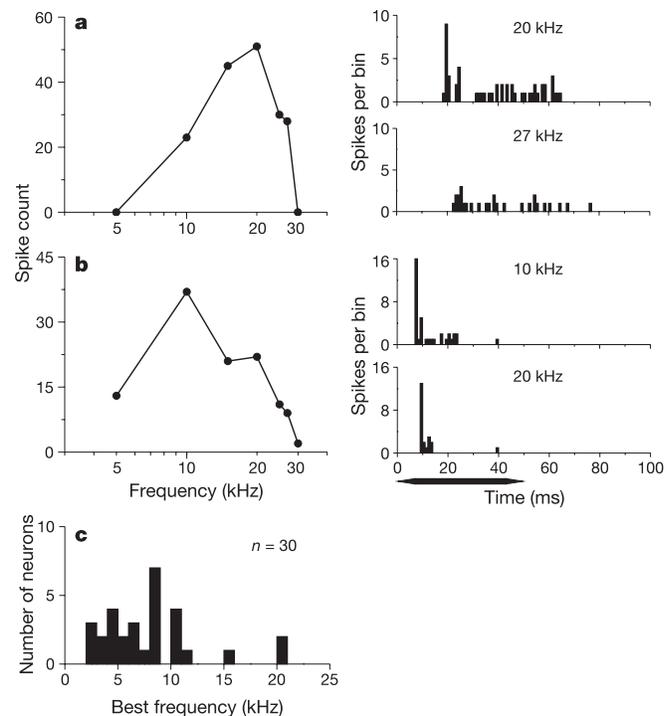
Figure 3 | Single-unit data from the torus semicircularis further confirm the ultrasound sensitivity of *A. tormotus*. a, Tone-burst responses from a tonic unit (see post-stimulus time histograms (PSTHs) in right panels) that responds to 10–27 kHz, with a best frequency of 20 kHz. **b**, Tone-burst responses from a phasic-burst unit (see PSTHs in right panels) that responds to 5–30 kHz, with a best frequency of 10 kHz. Vertical axes in **a, b** represent the total number of spikes in a 100-ms measurement window (with respect to the onset of tone bursts) at different frequencies. The horizontal bar below the four right panels represents the 'stimulus' starting at time zero. **c**, The distribution of best frequencies from single units.

generated by this system were at least 80 dB below the amplitude of the fundamental frequency of the stimulus.

In humans, ultrasound can be detected via bone conduction-mediated stimulation of the ear[9–11] or auditory cortex[12,13]. To determine whether the frog's ear is responsible for the ultrasonic sensitivity in *A. tormotus*, we carried out AEP recordings from the torus semicircularis of one frog under: (1) an 'intact' condition, with both ears unobstructed, or (2) an 'occluded' condition, with modelling clay covering the openings of both ear canals. The AEPs recorded under the intact condition (pre- and post-occlusion) were normal, with clear evidence of ultrasonic sensitivity (Fig. 4a), whereas ear occlusion abolished AEPs (Fig. 4b). Thus, ultrasonic sensitivity in *A. tormotus* is mediated by acoustic stimulation of the ear.

Frogs have two primary auditory organs: the amphibian papilla, which is sensitive to low and intermediate frequencies, and the basilar papilla, which is sensitive to high frequencies[14,15]. In previous studies, the highest upper limit recorded for frog hearing as determined by the auditory sensitivity of nerve fibres innervating the basilar papilla is 8.2 kHz (ref. 16). As such, the particular organ in the frog's ear that contributes to ultrasonic sensitivity is unclear.

It has been suggested that the restricted hearing in frogs is largely attributed to the limited high-frequency response of their middle ear ossicles[17–20], owing to transmission loss resulting from flexion in the extracolumella–columella link[19]. Furthermore, with increasing frequency, the tympanic membrane vibration breaks up into higher vibration modes[21,22]. Thus, for frogs to detect ultrasound they must circumvent these problems.

Males of *A. tormotus* have several highly unusual morphological features. First, their tympana are recessed (hence their common name) and invisible from the outside (Fig. 4c). Like mammals and unlike nearly all other frogs, they possess ear canals (Fig. 4c)—these have a resonant frequency of ~4.3 kHz (see Methods). Because the actual shape of the ear canal is complex, with distinct recesses, it might support secondary resonant frequencies, which may facilitate

Figure 4 | The ear is responsible for ultrasound sensitivity in *A. tormotus*. a, b, AEP data recorded from the torus semicircularis of one *A. tormotus* in the intact condition (**a**) and occluded condition (**b**) show that ear occlusion abolishes the AEPs. **c,** Low-power photomicrograph (×2) of a horizontal section (stained with haematoxylin and eosin) through the right ear of a male of *A. tormotus*. See http://www.beckman.uiuc.edu/profiles/feng/videofiles/ for three-dimensional (3D) reconstruction of the ear canal. Tym, tympanum; EC, ear cavity; XC, extracolumella. The opening of the ear cavity on the body surface is posterior to the retina (R); this opening was covered for the ear occlusion experiment described in **b**. Scale bar, 400 μm.

high-frequency hearing. Second, recessed tympana shorten and therefore reduce the mass of the middle-ear ossicles. Third, the frog's tympanic membranes (Fig. 4c) are extremely thin (3–4 μm at the rim, 17–18 μm towards the centre, and 30–40 μm at the anchor point of the columellar footplate). Similarly, the eardrums of *O. livida* are thin and transparent, even though they are not embedded deep inside the ear cavities. Both low-mass ossicles and thin membranes facilitate transmission of high-frequency sounds to the inner ear.

At present, the specific contributions of the external, middle and inner ear (including the hair cells, the basilar papilla and the tectorial membrane) to the frog's auditory sensitivity, as well as the precise transmission characteristics of the external and middle ears, have yet to be determined. Elucidating these contributions represents fertile ground for research in comparative hearing and evolutionary biology. Additionally, although *A. tormotus* can detect and use ultrasound to communicate during male–male territorial interactions, it is unclear whether or not ultrasound is also involved in male–female interactions. Unlike the males, females of *A. tormotus* do not have recessed ears[23], and thus further research will be necessary to determine whether this ultrasonic hearing ability is sexually dimorphic.

METHODS
Acoustic playback experiments were carried out from 25 May to 2 June 2005, between 19:00 and 24:00 along the Tau Hua Creek in Huangshan Hot Springs, China. The WAV file of a pre-recorded call of *A. tormotus* (Fig. 1c in ref. 4) was stored on the flash memory of a custom-made playback unit, digitally filtered (low-pass or high-pass with a cutoff frequency of 20 kHz; slope 100 dB per octave), and then broadcast through an audible loudspeaker (Visaton DSM25FFL; pass band 2–22 kHz) or an ultrasonic loudspeaker (Polaroid; pass band 22–120 kHz) or both, at various playback levels. Ten stimuli were presented at a rate of 1 per 15 s over a 3-min period. The loudspeakers were mounted on a tripod and positioned 40–50 cm away from a calling frog.
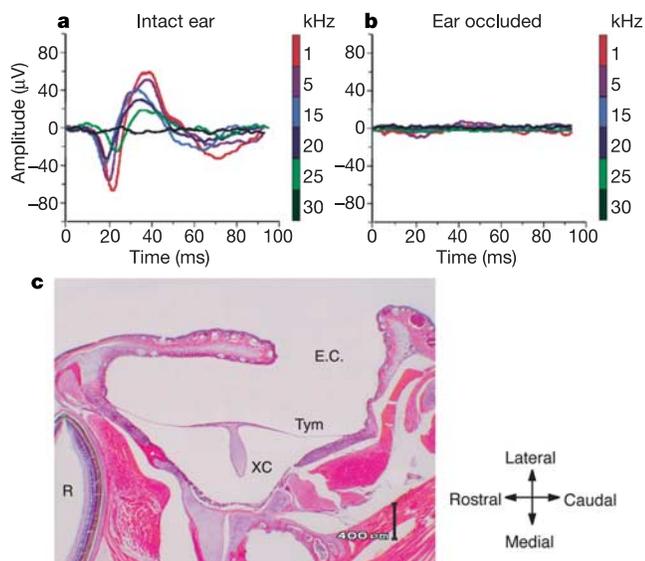
The sound pressure levels (SPLs) of natural frog calls ranged from 65 to 92 dB SPL (fast reading r.m.s.) at a distance of 32 cm and an ambient noise level of 63 dB SPL (measured with a sound-level meter; GenRad 1982)[3], depending on the call type and the centre frequency of the octave-band filter. At a distance of 40 cm, the r.m.s. SPL of the AUD components of the stimulus was 72.7 dB SPL, and that of the US components was 84.1 dB SPL; these were measured in the laboratory using a condenser microphone (Brüel and Kjaer 4135) and a precision measuring amplifier (Brüel and Kjaer 2610).

Frog movements were monitored visually under dim light, and their vocalizations were recorded using a PC-based digital recorder (PC-Tape) and a custom-made ultrasonic microphone having a flat frequency response from 15–120 kHz, with a roll-off of 10 dB per octave and 6 dB per octave at <15 kHz and >120 kHz, respectively[24]; use of an ultrasonic microphone allowed detailed characterization of the spectral profile of the background noise and vocal signals in the ultrasonic range. Signals were digitized (16-bit A/D conversion) at a sampling rate of 256 kHz (8× oversampling), saved as WAV files, analysed (fast Fourier-transformed with 1024 points) and displayed using a custom-designed program[4].

Electrophysiological experiments were carried out to determine the hearing range and sensitivity of the auditory system of several frog species: the concave-eared torrent frog (*A. tormotus*), the large odorous frog (*O. livida*) and the black-spotted pond frog (*P. nigromaculata*). Frogs were deeply anaesthetized by immersion in a 0.5% solution of tricaine methanesulphonate[25] and wrapped in cotton gauze. The skin on the dorsal surface of the head was incised, and a small hole made in the skull above the torus semicircularis. After surgery, the animal was placed inside a soundproof room and immobilized during the recording session with periodic injections of D-tubocurarine chloride (10 μg per g body weight).

Tone bursts (50–100-ms duration, 5-ms rise and fall times, presented at a rate of 0.5–1 pulse s^{-1}) were generated by a computer using A/D and D/A converters (Tucker Davis Technologies System 3), and broadcast from an ultrasonic loudspeaker (1–100 kHz; Tucker Davis Technologies ES-1) attached to a post positioned 10 cm from the frog's contralateral eardrum. The frequency response of the stimulation system was equalized to ±6 dB over 2–40 kHz. SPLs were measured with a condenser microphone (Brüel and Kjaer 4135) and a sound-level meter (Brüel and Kjaer 2610).

Glass micropipette electrodes (tip diameter 1–2 μm) were used to record auditory-evoked potentials (AEPs) and single-unit activities from the torus

semicircularis in response to tone bursts at a constant and behaviourally relevant sound level of 80–90 dB SPL. Neural signals were amplified, monitored visually, extracted using BrainWare (Tucker Davis Technologies), stored on a hard drive and analysed off-line. AEPs were averaged over 10 trials; these measurements were repeated every 100 μm along the dorsoventral extent of the torus semi-circularis (3–5 electrode penetrations per frog). No attempt was made to determine the existence of tonotopy. Single-unit recordings involved a similar stimulation paradigm; each tone/intensity was presented 20 times to construct a post-stimulus time histogram (PSTH).

To evaluate the morphology of the ear canal of *A. tormotus*, three frogs were anaesthetized and decapitated. Heads were immersed in 10% formalin for 10 days, embedded in paraffin, sectioned (6-μm slices) in the transverse, para-sagittal and horizontal planes, and alternate slices were stained with luxol fast blue–cresyl violet and haematoxylin–eosin. Every fifth photographic image of the frog's transverse section was digitized. The digital files were stacked and aligned using the frog's sagittal profile as a guide to create a three-dimensional (3D) reconstruction of the ear canal (see video display at http://www.beckman.uiuc. edu/profiles/feng/videofiles/). The volume of the ear canal was calculated with Analyse AVW, an image-analysis software package. Assuming the ear canal is a simple Helmholtz resonator, its resonant frequency was calculated using the measured volume, and the area, radius and height of the opening (equations (8.1) and (8.16) in ref. 26).

1. Hauser, M. D. *The Evolution of Communication* 124–175 (MIT Press, Cambridge, 1996).
2. Bradbury, J. W. & Vehrencamp, S. L. *Principles of Animal Communication* 161–173 (Sinauer Associates, Sunderland, 1998).
3. Feng, A. S., Narins, P. M. & Xu, C. H. Vocal acrobatics in a Chinese frog, *Amolops tormotus. Naturwissenschaften* **89**, 352–356 (2002).
4. Narins, P. M. *et al.* Old world frog and bird vocalizations contain prominent ultrasonic harmonics. *J. Acoust. Soc. Am.* **115**, 910–913 (2004).
5. Sales, G. & Pye, D. *Ultrasonic Communication by Animals* 23–68 (Chapman and Hall, London, 1974).
6. Slabbekoorn, H. & Peet, M. Birds sing at a higher pitch in urban noise. *Nature* **424**, 267 (2003).
7. Fei, L. *Atlas of Amphibians of China* 162–163, 188–189, 230–231 (Henan Science and Technical Publisher, Henan, 1999).
8. Rand, A. S. in *The Evolution of the Amphibian Auditory System* (eds Fritzsch, B., Ryan, M. J., Wilczynski, W., Hetherington, T. E. & Walkowiak, W.) 415–431 (John Wiley & Sons, New York, 1988).
9. Pumphrey, R. J. Upper limit of frequency for human hearing. *Nature* **166**, 571 (1950).
10. Corso, J. F. Bone-conduction thresholds for sonic and ultrasonic frequencies. *J. Acoust. Soc. Am.* **35**, 1738–1743 (1963).
11. Dieroof, H. G. & Ertel, H. Some thoughts on the perception of ultrasonics by man. *Arch. Oto-Rhino-Laryn.* **209**, 277–290 (1975).
12. Hosoi, H., Imaizumi, S., Sakaguchi, T., Tonoike, M. & Murata, K. Activation of the auditory cortex by ultrasound. *Lancet* **351**, 496–497 (1998).
13. Imaizumi, S. *et al.* Ultrasound activates the auditory cortex of profoundly deaf subjects. *Neuroreport* **12**, 583–586 (2001).
14. Feng, A. S., Narins, P. M. & Capranica, R. R. Three populations of primary auditory fibers in the bullfrog (*Rana catesbeiana*): their peripheral origins and frequency sensitivities. *J. Comp. Physiol.* **100**, 221–229 (1975).
15. Lewis, E. R. & Narins, P. M. in *Comparative Hearing: Fish and Amphibians* (eds Fay, R. R. & Popper, A. N.) 218–268 (Springer, New York, 1999).
16. Loftus-Hills, J. J. & Johnstone, B. M. Auditory function, communication, and the brain-evoked response in anuran amphibians. *J. Acoust. Soc. Am.* **47**, 1131–1138 (1970).
17. Manley, G. A. The middle ear of the tokay gecko. *J. Comp. Physiol.* **81**, 239–250 (1972).
18. Jørgensen, M. B. & Kanneworff, M. Middle ear transmission in the grass frog, *Rana temporaria. J. Comp. Physiol.* **182**, 59–64 (1998).
19. Mason, M. J. & Narins, P. M. Vibrometric studies of the middle ear of the bullfrog *Rana catesbeiana* I. The extrastapes. *J. Exp. Biol.* **205**, 3153–3165 (2002).
20. Mason, M. J. & Narins, P. M. Vibrometric studies of the middle ear of the bullfrog *Rana catesbeiana* II. The operculum. *J. Exp. Biol.* **205**, 3167–3176 (2002).
21. Anson, M., Pinder, A. C., Keating, M. J. & Chung, S. H. Acoustic vibration of the amphibian eardrum studied by white noise analysis and holographic interferometry. *J. Acoust. Soc. Am.* **78**, 916–923 (1985).
22. Purgue, A. P. Tympanic sound radiation in the bullfrog *Rana catesbeiana. J. Comp. Physiol.* **181**, 438–445 (1997).
23. Chen, B.-H. *The Amphibian and Reptilian Fauna of Anhui* (Anhui Publishing House of Science and Technology, Heifei (Anhui), 1991).
24. Siemers, B. M. & Schnitzler, H.-U. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* **429**, 657–661 (2004).
25. Ohr, E. A. Tricaine methanesulfonate-I: pH and its effects on anesthetic potency. *Comp. Biochem. Physiol. C* **54**, 13–17 (1976).
26. Kinsler, L. E. & Frey, A. R. *Fundamentals of Acoustics* 186–200 (John Wiley, New York, 1962).