Current Zoology, 2019, 65(3), 333–341
doi: 10.1093/cz/zoy089
Advance Access Publication Date: 6 December 2018
Article

Article

Behavioral and neural auditory thresholds in a frog

Ryan C. Taylor[a,b,c,*], Karin Akre[a], Walter Wilczynski[d], and Michael J. Ryan[a,c]

[a]Department of Integrative Biology, University of Texas at Austin, Austin, TX, USA, [b]Department of Biological Sciences, Salisbury University, Salisbury, MD, USA, [c]Smithsonian Tropical Research Institute, Balboa, Republic of Panama, and [d]Neuroscience Institute and Center for Behavioral Neuroscience, Georgia State University, Atlanta, GA, USA

*Address correspondence to Ryan C. Taylor. E-mail: rctaylor@salisbury.edu.
Handling editor: Klaudia Witte

Received on 13 July 2018; accepted on 27 November 2018

Abstract

Vocalizations play a critical role in mate recognition and mate choice in a number of taxa, especially, but not limited to, orthopterans, frogs, and birds. But receivers can only recognize and prefer sounds that they can hear. Thus a fundamental question linking neurobiology and sexual selection asks—what is the threshold for detecting acoustic sexual displays? In this study, we use 3 methods to assess such thresholds in túngara frogs: behavioral responses, auditory brainstem responses, and multiunit electrophysiological recordings from the midbrain. We show that thresholds are lowest for multiunit recordings (ca. 45 dB SPL), and then for behavioral responses (ca. 61 dB SPL), with auditory brainstem responses exhibiting the highest thresholds (ca. 71 dB SPL). We discuss why these estimates differ and why, as with other studies, it is unlikely that they should be the same. Although all of these studies estimate thresholds they are not measuring the same thresholds; behavioral thresholds are based on signal salience whereas the 2 neural assays estimate physiological thresholds. All 3 estimates, however, make it clear that to have an appreciation for detection and salience of acoustic signals we must listen to those signals through the ears of the receivers.

Key words: anurans, auditory brainstem responses, auditory thresholds, mate choice, Physalaemus pustulosus, signal recognition thresholds, sexual selection, túngara frogs, vocalizations

Mate choice can generate sexual selection (Darwin 1871; Rosenthal 2017), but mate choice can only proceed if choosers can detect signalers. So understanding the basic mechanisms of signal detection is crucial to understanding the tempo and mode of sexual selection. Acoustic communication is an ideal system for such studies; it is a requisite for social behavior across a diversity of taxonomic groups as it mediates interactions such as mate choice and territory defense (Ryan 2001; Gerhardt and Huber 2002; Greenfield 2002). Hearing threshold, the lowest sound pressure level (SPL) detectable by a receiver, is an important factor for all functions of acoustic communication because a receiver must first detect an auditory signal prior to responding. It is especially critical for mate choice because if an animal is calling or singing for mates but receivers do not hear it, we can ask rhetorically, is the sender even sending a signal?

Hearing threshold is also a critical variable for determining a signal's active space, the distance over which receivers can detect a signal (Marten and Marler 1977; Brenowitz 1982; Lohr et al. 2003; Bernal et al. 2009b). Active space can vary substantially depending on the amplitude at the source, receiver sensitivity, ambient noise, competing acoustic signals, and attenuation due to habitat structure (Wiley and Richards 1982; Dusenbery 1992; Bee and Micheyl 2008). Measuring hearing threshold relative to the source amplitude can provide good estimates of the active space of a signal under ideal conditions and can form a basis for understanding perception under the more

© The Author(s) (2018). Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

acoustically complex conditions that many animals experience during communication (Gerhardt and Huber 2002; Bee and Schwartz 2009).

Hearing thresholds can be estimated in several ways. First, evoked action potentials measured from the 8th nerve or specific brain nuclei can provided response threshold estimates for specific cells or regions of the nervous system (Capranica and Moffat 1975; Walkowiak 1980; Zelick and Narins 1985). We refer to these as electrophysiological thresholds. Neural thresholds can also be estimated from evoked potentials recorded from the auditory brainstem response (ABR; Kenyon et al. 1998). We refer to these as ABR thresholds. ABR provides information on acoustic processing as a result of summed activity across auditory neurons. It is less invasive than other neurophysiological measures, but recording is typically limited to short tone bursts or clicks, and threshold estimates are likely to be higher than some other measures due to the trans-cranial recordings (Lohr et al. 2013). Both types of neurophysiological measures provide information about auditory processing, but the relationship between neural processing and perception is often unclear (Eggermont and Ponton 2002). Thresholds can also be tested through behavioral assays (Fay 1988) whereby individuals are scored based on absolute responses or relative to particular recognition criteria (Beckers and Schul 2004; Bee and Schwartz 2009). Behavioral measures likely provide better insight into ecologically relevant auditory perception, but may still underestimate thresholds in non-human animals due to variation in motivation.

Behavioral auditory thresholds measure a particular whole-organism response. For example, male túngara frogs produce a mating call that consists of a whine followed by 0–7 chucks (Ryan 1985). The female's behavioral threshold for exhibiting phonotactic preferences for multiple-chuck calls versus whines without chucks is around 72 dB SPL, whereas the threshold for discriminating between 3-chuck and 1-chuck calls is around 90 dB SPL (Akre and Ryan 2010). It should be noted that in these preference tests there are several explicit tasks involved, including but not limited to: detection, perception, analysis, decision-making, and motor output, all of which can be modulated by external factors, such as the presence of predators, and internal factors such as levels of circulating hormones. We follow Bee and Schwartz (2009) and use the term signal recognition threshold for this behaviorally derived threshold, which is related to speech reception threshold in human psychophysics (Plomp and Mimpen 1979).

Several studies have compared ABR thresholds and signal recognition thresholds; behavioral responses can yield lower threshold values than ABR (Buerkle et al. 2014). For example, human listeners exhibit ABR thresholds that are 10 – 30 dB higher than behavioral thresholds, depending on frequency (Gorga et al. 1988; Goshorn et al. 2017). Likewise, ABR thresholds are ~20 dB greater in rhesus monkeys (Laskey et al. 1999) and typically 30 dB greater in birds (Dyson et al. 1998; Brittan-Powell et al. 2002) when compared with behavioral thresholds. In Orca whales and seals, however, there is better correspondence between the 2 methods (Szymanski et al. 1999; Wolski et al. 2003), and in fish ABRs can have lower thresholds than behavioral responses (Kenyon et al. 1998). But how can animals behaviorally respond to a sound if the neural data, specifically the ABR, show that the animal does not hear the sound?

There are a number of technical factors associated with ABR recordings that influence the measurement of thresholds, which can restrict ABRs from measuring the absolute lowest threshold to either simple stimuli or complex sounds (Kraus and Nicol 2009; Skoe and Kraus 2010). For example, the signal-to-noise ratio in the recording determines the estimate of thresholds (Norrix and Velenovsky 2018). The amplitude of the evoked signal will be influenced by electrode position (King and Sininger 1992), distance from the source to the recording electrodes, as well as the conductivity of intervening tissue. Cancellation of action potentials that are out of phase or when the neural response as a whole is not well synchronized to the stimulus will also decrease signal amplitude (Kraus and Nicol 2009). The level of electrical noise picked up by the surface electrodes related to random neural activity as well as external electrical signals is a major issue as it sets a floor beneath which the evoked signal cannot be detected (Elberling and Don 1987; Kraus and Nicol 2009). As a result, the signal and noise levels determining the detected threshold could be very different in ABRs compared with behavioral tests.

Studies that measure hearing threshold by behavioral response often use protocols where animals are conditioned to respond to tones or clicks. This artificial context gives a consistent measure of threshold for behavioral response, but it does not yield information about thresholds for natural unlearned behavioral tasks (Fay and Popper 1999). For example, data from human psychoacoustics show that the addition of another stimulus modality (e.g., flash of light) can alter responses and can lower hearing thresholds (Lovelace et al. 2003) suggesting that anticipated or conditioned responses may overestimate ecologically relevant thresholds.

Anuran amphibians are good models for measuring auditory thresholds because many species are acoustic specialists. Male vocalizations are necessary for female mate attraction and females express robust phonotactic preferences for specific call properties (Ryan 2001; Gerhardt and Huber 2002; Bee 2015). Male vocalizations consist of simple, repeated notes and are easily synthesized for playback studies. On the other hand, frogs do not respond well to classical or operant conditioning techniques (Bee and Schwartz 2009), and thus behavioral responses are rarely used to measure thresholds in these animals (but see Simmons et al. 1985; Simmons 1988). Neurophysiological approaches, using single or multiunit brain recordings or ABRs, also have been employed to estimate thresholds. These thresholds are better indicators of detection of a stimulus and do not involve the series of tasks that are often evoked by signal recognition. Nonetheless, these responses can also be influenced by internal factors such as hormone levels and recent experience (Wilczynski and Burmeister 2016).

In this study we compare signal recognition thresholds, ABR thresholds, and electrophysiological thresholds in túngara frogs, *Physalaemus* (= *Engystomops*) *pustulosus*. Male túngara frogs call in choruses to attract females (Ryan 1985). As noted above, males can produce a simple call (the whine) or a complex call (the whine plus 1 to 7 chucks appended to the call). Females express a strong mating preference for complex calls (86%, $N = 3662$; Gridi-Papp et al. 2006).

All frogs have 2 inner ear organs that are sensitive to airborne sound, and their sensitivities tend to match the emphasized frequencies in the conspecific mating call (Capranica and Moffat 1983; Wilczynski and Ryan 2010). The túngara frog's whine is a downward frequency sweep from 1000 to 400 Hz, stimulating mostly the amphibian papillae (AP), whose best excitatory frequency (BEF), estimated from midbrain recordings, is 516 Hz. The chucks occur in short bursts with the peak energy at 2,552 Hz, stimulating mostly the basilar papillae (BP), whose BEF, also estimated from midbrain recordings, is 2,133 Hz (Wilczynski et al. 2001). As with most anurans studied to date, the threshold for peak sensitivity in the BP is substantially higher than it is for the AP (Capranica and Moffat 1983; Ryan et al. 1990).

Our goals for this study were to: 1) measure the signal recognition threshold of female túngara frogs as an estimate of the threshold for signal salience, 2) determine whether signal recognition

thresholds vary with different call types (simple versus complex), and 3) compare the signal recognition threshold for mating calls with electrophysiological and ABR thresholds in response to simple stimuli (tones).

Materials and Methods

Signal recognition thresholds

For all 3 measures of thresholds we estimated the threshold as the lowest amplitude that elicits a response. Thus we follow the standard textbook definition of threshold as the minimum intensity of a physical stimulus that can just be detected by an observer (Yantis and Abrams 2017).

We estimated the signal recognition thresholds to male advertisement calls for 20 reproductive adult female túngara frogs captured from the field in Gamboa, Panama. Females were captured in amplexus in early evening, usually between 19:00 and 20:00 h, and then tested, usually between 20:00 and 04:00 h, and released at the end of the night. We determined thresholds by testing for female phonotaxis in response to advertisement calls played at varying amplitudes. For each test, females were placed under a cone in the center of a 2.7×1.8 m Acoustic Systems sound chamber in our laboratory at the Smithsonian Tropical Research Institute. Two speakers at equal distance, ca. 1.35 m from the cone, broadcast acoustic stimuli: 1 speaker played an advertisement call every 2 s, and the other speaker antiphonally broadcast a burst of white noise. We shaped the white noise to mimic the sound envelope of a túngara frog call. White noise stimuli were used to control for the possibility that females would respond to any sound; in fact, no females responded to the speaker broadcasting the white noise.

Stimuli were switched between speakers after each test. Females were restrained under the cone whereas the speakers played for 3 min. At this time, we raised the cone from outside the chamber and the speakers continued playing. The female could then respond to the stimuli by moving to a speaker. We scored the female as making a choice when she approached to within 10 cm of the speaker. If she did not move from the center of the chamber within 5 min of raising the cone, if she remained stationary for 2 min at any point after leaving the center of the chamber, or if she did not choose a speaker within 15 min, the test was scored as no choice. An infrared light illuminated the chamber, and an infrared sensitive video camera projected an image of the chamber to a screen outside the chamber where female activity was scored.

Thresholds were determined for 3 variants of the advertisement call: a whine only (W), a whine with 1 chuck (WC), and a whine with 2 chucks (WCC). All call stimuli were recordings of a natural call that was near the centroid of the multivariate distribution of 300 calls of a túngara frog population in Gamboa (Ryan and Rand 2003). As all females heard the same calls, variation in thresholds could not result from difference among the stimuli. Each female was tested for thresholds to all 3 variants of the call. The order of experiments was varied such that 3 females' thresholds were determined for first W, then WC, and finally WCC; 3 females were tested in the order W, WCC, WC; 5, WC, W, WCC; 2, WC, WCC, W; 5, WCC, WC, W; and 2, WCC, W, WC.

The stimuli beginning each testing series consisted of a call broadcast at 70 dB SPL (re. 20 μPascals) in the center of the arena versus a burst of white noise always played at 82 dB SPL as this amplitude is supra-threshold for mating call recognition. Stimulus amplitude was measured with peak amplitude and flat weighting settings on a GenRad 1982 SPL meter (General Radio Corporation, West Concord, MA). If the female exhibited phonotaxis to the call, she was tested again with the call played at 3 dB lower amplitude. We continued to lower the call amplitude by 3 dB until she failed to exhibit phonotaxis to the call. The lowest amplitude to which she responded was determined as her threshold response. If she did not choose the call broadcast at 70 dB SPL, she was tested again with the call played at 3 dB higher amplitude. We continued to increase the call amplitude by 3 dB until she chose the call, and again the lowest intensity to which she responded was determined as her threshold response. After her threshold to the first call type was determined, the same process was used to determine her threshold for the second and then the third call types.

Before and after testing a female for the signal recognition threshold, we tested her response to a synthetic W versus WC at 82 dB SPL to establish that she was motivated for phonotaxis to an advertisement call. The data from the female were included in the study only if she exhibited phonotaxis in both of these pre- and post-tests. Groups were compared with analysis of variance.

ABR thresholds

We elicited ABRs of 6 male and 10 female túngara frogs. The frogs were obtained from a breeding colony at the University of Texas, descended from wild–caught frogs in the general area of Gamboa, Panama. We maintained colony frogs in 38 L terraria on a 12 L:12 D light cycle at 28° C. All frogs were sexually mature adults ($1.37\,g \pm 0.09$ SE). For each test, frogs were immobilized using an intramuscular injection of d–tubocurarine chloride (3 mg/mL) at a volume of 15 μL per gram of frog mass.

Pure tones, ranging from 250–3,000 Hz, were generated using SigGen software (Tucker-Davis Technologies, Alachua, FL, USA). We broadcast the tones at a rate of $19\,s^{-1}$. Each tone was 10 ms in duration with a linear 2 ms rise and 2 ms fall time. The initial SPL was 105 dB SPL (re. 20 μPa) and stepped down in increments of 5 dB until the ABR waveform was no longer apparent. The waveform of the signal was alternated 180° to cancel stimulus artifacts. We broadcast the stimulus tones through TDT BioSig software to a Realistic amplifier (model MPA-40) connected to a Cambridge Soundworks speaker (North Andover, MA, USA). The speaker was placed 5 cm above and 15 cm in front of the subject. SPLs were recorded with a Brüel and Kjær (model 2238 mediator) SPL meter with the detector placed within 2 cm of the ear of the frog (C weighting). All ABR recordings were conducted with the frog placed on an anti–vibration table located within a sound attenuation chamber (ETS-Lindgren, Austin, TX). The frog was placed on a moistened paper towel that rested on a cork board.

We recorded responses through 2 stainless steel electrodes inserted just under the skin of the frogs. The recording electrode was placed subcutaneously on the dorsal mid-line, slightly anterior to the caudal edge of the cranium; the reference electrode was placed subcutaneously in the hind leg. A ground electrode was attached to the leg of the anti-vibration table using copper wiring. We amplified evoked potentials through a headstage DB4 amplifier (gain = 30 K, high pass filter 100 Hz, low pass filter 3,000 Hz, notch filter at 60 Hz).

Each recorded ABR wave represented the averaged response to 500 stimulus presentations (250 in each phase). As is standard for ABR studies, we determined threshold responses as the lowest intensity at which a response was visually detectable above the background noise (Figure 1; Walsh et al. 1986; Hall 1992; Boettcher et al. 1993; Higgs et al. 2002).

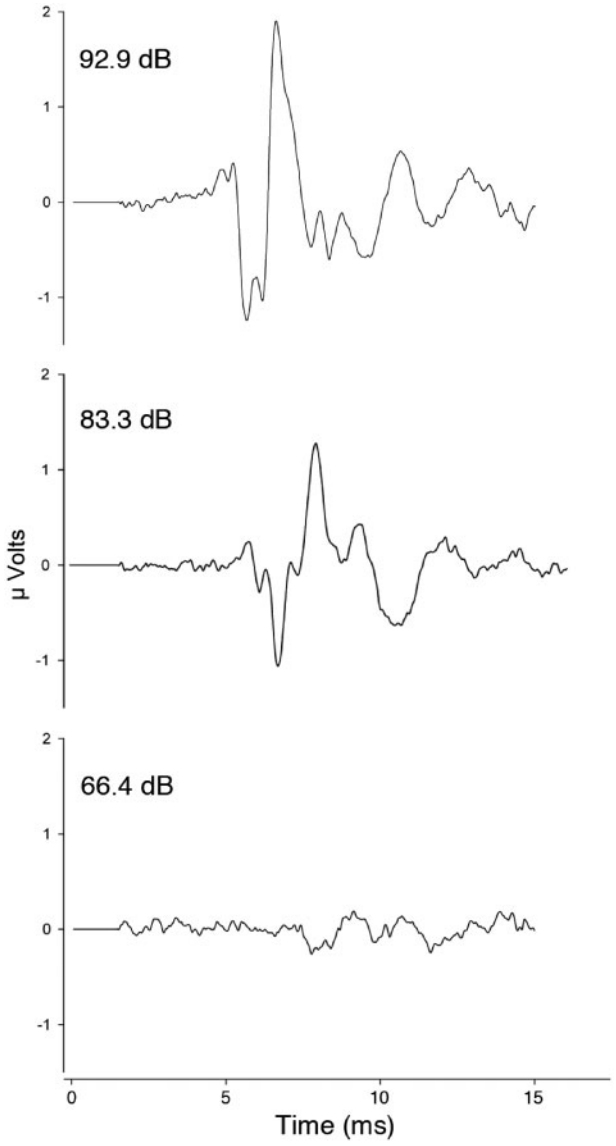

Figure 1. Representative ABR recordings from a female using a 750 Hz tone.

Electrophysiological thresholds

Data were taken from recordings used to assess BEFs in the frequency ranges of the amphibian (AP) and basilar (BP) papillae (as in Lombard and Straughan 1974; Hubl and Schneider 1979; Penna et al. 2013) which were reported in Wilczynski et al. (2001) as part of a multispecies study of auditory system tuning in *Physalaemus* species. Thresholds were not reported in that paper but its figure 4a presents a representative audiogram which does contain some information on thresholds.

Midbrain multiunit recordings from the torus semicircularis were obtained from 5 male and 1 female adult túngara frogs. Individuals were collected in Gamboa, Panama, then transported back to the University of Texas at Austin for the electrophysiological recordings. Frogs were housed in 38 l aquaria under a 12 L:12 D cycle with an average room temperature of 23°C. Animals were fed fruit flies or small crickets. They were left undisturbed for at least 2 weeks to allow recovery from transportation stress. Following this housing period, individuals were prepared for midbrain recording.

Each frog was anesthetized by immersion in a 2.5% aqueous solution of urethane. The skin on the head over the midbrain was incised and retracted, and a small hole was drilled in the skull. The skin was repositioned and the animal allowed to recover from the anesthesia and surgery for 2 days with careful monitoring for signs of distress and any other adverse reactions.

On the day of recording the animal received an intramuscular injection of d-tubocurarine chloride (7.5 μg/g body weight in 20 mg/mL aqueous solution) and the surgical area swabbed with 2% lidocaine. The animal was placed on a cork platform and draped with moist gauze atop a vibration-reducing table inside an Industrial Acoustics sound attenuating booth with the dorsal surface of the midbrain exposed. A custom designed earphone assembly was sealed around the ear contralateral to the midbrain targeted for recording. The earphone was calibrated with a B&K 2230 precision digital sound level meter after each recording session. A glass microelectrode filled with 3 M KCl (impedance of 3–5 MOhms) was lowered with a hydraulic microdrive until robust multiunit auditory activity could be discerned in response to a multitone search stimulus.

Thresholds were then determined to single frequency tone bursts (150 ms duration, repeated every 1.5 s) presented in 100 Hz intervals from 100–4,000 Hz. Once approximate threshold minima were observed, smaller frequency steps were used around those points to estimate AP BEF and BP BEF more accurately. Thresholds were determined by monitoring the neural activity through earphones and visually via an oscilloscope trace. Signal amplitude was adjusted up and down in first 10 dB, then 1 dB, increments using manually controlled resistive attenuators until acoustically driven multiunit activity was no longer observed. We only included animals for which we obtained robust auditory evoked activity from at least 2 different electrode positions in the midbrain, each of which yielded consistent frequency–threshold data at least twice. We did not observe evidence of tonotopy in our multiunit responses. Thresholds at measured frequency points were averaged for each animal to obtain an estimate of that individual's threshold to frequencies from 100–4,000 Hz in 100 Hz increments. The AP BEF estimate was considered the frequency below 1,000 Hz with the lowest threshold; the BP BEF estimate was considered the frequency above 1,000 Hz with the lowest threshold. Average thresholds across individuals were determined using GraphPad Prism (version 7.04).

Results

Signal recognition thresholds

The mean signal recognition threshold to an advertisement call was 61.3 dB SPL. Adding chucks to the advertisement call did not change the threshold response. Across females, thresholds did not differ significantly between calls (mean ± SE: W = 61.4 ± 1.65 dB SPL; WC = 60.8 ± 1.95 dB SPL; WCC = 61.7 ± 2.12 dB SPL; $F_{2,38} = 0.057$, $P = 0.944$). Also, the means of threshold levels for call types tested first, second, and third did not differ significantly ($F_{2,38} = 1.917$, $P = 0.122$). Finally, threshold levels did not consistently increase or decrease as a chuck was added to the whine (Sign test: W vs. WC, 2-sided probability = 1.0) or to the whine-chuck (WC vs. WCC, 2-sided probability = 0.424).

ABR thresholds

Males were most sensitive to tones broadcast at 750 Hz, exhibiting a mean threshold of 70.7 dB SPL ± 3.32 SD; females showed the greatest mean sensitivity at 500 Hz with a threshold of 71.9 dB

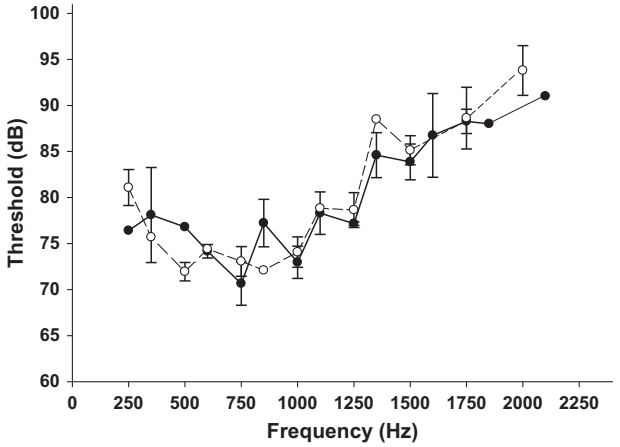

Figure 2. Audiogram based on ABR recordings for males and females. SPL measurements: Closed circles = males. Open circles = females. Error bars indicate ± 1 SD. Points lacking error bars indicate that only 1 recording was obtained from an individual at that frequency.

SPL ± 2.00 SD. Both males and females generally showed the greatest sensitivity between 500–1,000 Hz, the range covered by the dominant, and the most salient, harmonic of a male's whine. There was little overall difference between the sexes, however; both showed rapidly increasing threshold levels at frequencies >1,000 Hz (Figure 2). The lowest SPL at which we were able to obtain an ABR for a female was 66.4 dB SPL, using a 750 Hz tone. The lowest SPL which evoked an ABR in a male frog was 68.3 dB SPL, also at 750 Hz.

The frequency range over which evoked potentials were recorded largely covered the range that is attended to by the AP. Previous data (Ryan et al. 1990) showed that the BP has a BEF of 2,133 Hz estimated by our midbrain recordings; however, we were unable to record ABR waveforms at frequencies >2,100 Hz. As noted above, thresholds for the BP are always substantially higher than thresholds for the AP in all frogs that have been tested (Capranica and Moffat 1983).

Electrophysiological thresholds

Frequency-threshold curves had a bimodal appearance often found in electrophysiological characterizations of frog auditory tuning using various approaches (Feng et al. 1975; Capranica and Moffat 1983; Wilczynski et al. 1984, 2001; Penna et al. 2008; Miranda and Wilczynski 2009; Wilczynski and Ryan 2010; Buerkle et al. 2014; Schrode et al. 2014). A broad area of sensitivity was seen between 100 and 1,000 Hz, which is considered reflective of AP activity. An area of relatively elevated thresholds was apparent at higher frequencies leading to a second, narrower, zone of sensitivity between 2,000 and 2,500 Hz, which is thought to reflect BP tuning. Above 2,500 Hz, thresholds rose steadily. The most sensitive point in the lower, AP, band averaged 516 Hz with a mean (±SEM) threshold of 45.3 dB SPL (± 2.97; range 38.1–58.2 dB SPL). The most sensitive point in the upper, BP, region averaged 2,133 Hz with a mean (±SEM) threshold of 59.3 dB SPL (± 1.44; range 55.2–65.1 dB SPL). AP and BP threshold estimates correlated with each other across the 6 animals (Spearman $r = 0.886$, $P = 0.033$). Because we had only 1 female in our sample we did not statistically assess sex differences in any tuning parameter. However, the 1 female was not noticeably different in BEFs or thresholds from the 5 male subjects.

Discussion

Our study reveals substantial differences among signal recognition thresholds, electrophysiological thresholds, and ABR thresholds. There are a number of experimental factors that could contribute to this variation. For example, phonotaxis and ABR assays used open field sound playbacks, but the multiunit assay used closed field sound playback through a sealed earphone. ABR is notoriously sensitive to electrode placement. Different stimuli were used: the behavioral tests employed mating calls, the ABR recordings used short tone bursts whereas the midbrain recordings used longer artificial stimuli. Immediately prior to midbrain and ABR recordings subjects were treated with curare which can influence sensitivity, but not in the behavioral tests. Finally, the sex ratios differed amongst the 3 tests: only females were tested with phonotaxis and mostly males for midbrain recordings, whereas ABR utilizes both males and females.

A review of the literature (Table 1) shows that not only is there substantial variability in reported anuran auditory thresholds, but there are also differences among techniques using the same species. Thus it is likely that some of this variation results from inter–individual and inter–specific differences in hearing sensitivity, whereas other differences are due to the method used to estimate the threshold. For example, neurophysiological recordings from the midbrain or 8th nerve yield thresholds as low as 20 dB SPL in *Hyla aurea* (Loftus-Hills and Johnstone 1970) and 25 dB SPL in *Rana pipiens* (Fuzessery and Feng 1982) to ca. 45 dB SPL in *Scaphiopus couchii* (Capranica and Moffat 1975) and nearly 60 dB SPL in several other species. ABR estimates range from 45 to 50 dB in *Hyla cinerea* to 55–60 dB SPL in *Xenopus laevis, Hyla chrysoscelis*, and *Babina daunchina* (Table 1). Behavioral thresholds are rare; an estimate of ca. 40 dB SPL is reported for female phonotaxis in *Hyla versicolor* (Beckers and Schul 2004) and 43 and 60 dB SPL for male evoked calling in *Pleurodema thaul* (Penna et al. 2008) and *Eupsophus emiliopugini* (Penna et al. 2005), respectively (Table 1).

Despite this existing body of work in anurans, there are very few species in which more than 1 type of threshold estimate is available. *Hyla cinerea* thresholds have been reported based on midbrain recordings and ABRs in different publications; in that species ABR estimates appear slightly higher than estimates from midbrain recordings. Thresholds from both midbrain recordings and behavioral tests of evoked calling are available for *Pleurodema thaul* (Penna et al. 2008) showing them to be similar. As reported here, *Physalaemus pustulosus* is the only species for which midbrain recordings, ABRs, and behavioral tests have been combined to estimate/compare thresholds. For this species, thresholds are lowest for midbrain multiunit recordings, highest for ABRs, with the behavioral measure intermediate.

Our behavioral results from túngara frogs found a mean signal recognition threshold to advertisement calls of 61.3 dB SPL, higher than most behavioral and neurophysiological thresholds reported for many other species (Table 1). Previous work, however, has also shown a trend whereby smaller frogs exhibit higher threshold values (Loftus-Hills 1973; Wilczynski et al. 1984); our data are commensurate with those findings.

We found a high degree of variation in behavioral responses with some females exhibiting thresholds as low as 42 dB SPL. If this is the true absolute threshold for túngara frogs, it would place it very close to the midbrain threshold obtained in this species. Measured variation using ABR was lower than our phonotaxis results, but the means were higher, at ~71 dB SPL.

Table 1. Auditory thresholds for a variety of anurans measured with different techniques

Species	Threshold	Method	Reference
Acris crepitans	50 dB	Single unit recordings from 8th nerve	Keddy-Hector et al. (1992)
Alytes cisternasii	45–50 dB	Multiunit recording from torus	Bosch and Wilczynski (2003)
Alytes cisternasii	39–47 dB	Multiunit recording from torus	Penna et al. (2015)
Alytes dickhilleni	42–46 dB	Multiunit recording from torus	Penna et al. (2015)
Alytes obstetricans	42 dB	Multiunit recording from torus	Penna et al. (2015)
Babina daunchina	50–60 dB	ABR	Zhang et al. (2012)
Bombina bombina	45–60 dB	Single neuron recordings from torus	Walkowiak (1980)
Bufo arenarum	47 dB	Multiunit recording from torus	Penna et al. (1990)
Bufo chilensis	49 dB	Multiunit recording from torus	Penna et al. (1990)
Bufo spinulosus	63 dB	Multiunit recording from torus	Penna et al. (1990)
Crinia parainsignifera	35 dB	Multiunit recording from torus	Loftus-Hills and Johnstone (1970)
Eleutherodactylus coqui	35–40 dB	Single unit recordings from 8th nerve	Zelick and Narins (1985)
Eupsophus calcaratus	55–59 dB	Multiunit recording from torus	Penna et al. (2013)
Eupsophus emiliopugini	60 dB	Evoked calling ♂	Penna et al. (2005)
Eupsophus emiliopugini	43–48 dB	Multiunit recording from torus	Penna and Moreno-Gómez (2014)
Eupsophus roseus	37 dB	Multiunit recording from torus	Moreno-Gómez et al. (2013)
Hyla arborea	42 dB ♂, 38 dB ♀	Multiunit recording from torus	Hubl and Schneider (1979)
Hyla aurea	20 dB	Multiunit recording from midbrain	Loftus-Hills and Johnstone (1970)
Hyla chrysoscelis	38 dB	Multiunit recording from midbrain	Hillary (1984)
Hyla chrysoscelis	41 dB	Behavioral phonotaxis ♀	Bee and Schwartz (2009)
Hyla chrysocelis	43 dB	Behavioral phonotaxis ♀	Nityananda and Bee (2012)
Hyla chrysoscelis	58–61 dB	ABR	Schrode et al. (2014)
Hyla chrysoscelis	43 dB	Behavioral phonotaxis ♀	Lee et al. (2017)
Hyla cinerea	35 dB	Multiunit recording from midbrain	Lombard and Straughan (1974)
Hyla cinerea	30 dB	Reflex modification procedure	Simmons et al. (1985)
Hyla cinerea	40 dB	Multiunit recording from torus	Penna et al. (1992)
Hyla cinerea	39 dB ♂, 45 dB ♀	Multiunit recording from torus	Miranda and Wilczynski (2009)
Hyla cinerea	55 dB	Behavioral phonotaxis ♀	Velez et al. (2012)
Hyla cinerea	50–52 dB	ABR	Buerkle et al. (2014)
Hyla cinerea	45–50 dB	ABR	Gall and Wilczynski (2015)
Hyla crucifer	58 dB	Single unit recordings from 8th nerve	Wilczynski et al. (1984)
Hyla ebraccatus	70 dB ♂, 42 dB ♀	Multiunit recording from torus	McClelland et al. (1997)
Hyla microcephala	65 dB ♂, 49 dB ♀	Multiunit recording from torus	McClelland et al. (1997)
Hyla regilla	41 dB	Multiunit recording from midbrain	Lombard and Straughan (1974)
Hyla savignyi	41 dB ♂, 40 ♀	Multiunit recording from torus	Hubl and Schneider (1979)
Hyla versicolor	25 dB	Multiunit recording from torus	Lombard and Straughan (1974)
Hyla versicolor	37–43 dB	Behavioral phonotaxis ♀	Beckers and Schul (2004)
Leptodactylus melanonotus	40 dB	Multiunit recording from torus	Lombard and Straughan (1974)
Limnodynastes tasmaniensis	36 dB	Multiunit recording from torus	Loftus-Hills and Johnstone (1970)
Odorrana schmackeri	47 dB	Evoked potential recording from torus	Yu et al. (2006)
Physalaemus pustulosus	45 dB	Multiunit recording from midbrain	This study
Physalaemus pustulosus	61 dB	Behavioral phonotaxis ♀	This study
Physalaemus pustulosus	71 dB	ABR	This study
Pleurodema thaul	43 dB	Evoked calling ♂	Penna et al. (2008)
Pleurodema thaul	41–51 dB	Multiunit recording from torus	Penna et al. (2008)
Rana catesbeiana	25	Single unit recording from 8th nerve	Frishkopf and Goldstein (1963)
Rana catesbeiana	10–20 dB	Reflex modification procedure	Simmons et al. (1985)
Rana pipiens	25–40 dB	Single unit recordings	Fuzessery and Feng (1982)
Rana ridibunda	29 dB ♂, 23 dB ♀	Multiunit recording from torus	Hubl and Schneider (1979)
Rana temporaria	30–35 dB	Single neuron recordings from torus	Walkowiak (1980)
Rana temporaria	41 dB ♂, 41 dB ♀	Multiunit recording from torus	Brzoska et al. (1977)
Rana temporaria	70 dB ♂	Galvanic skin response	Brzoska et al. (1977)
Scaphiopus couchii	40–50 dB	Single unit recording from 8th nerve	Capranica and Moffat (1975)
Smilisca baudinii	25 dB	Multiunit recording from torus	Lombard and Straughan (1974)
Xenopus laevis	55 dB	ABR	Katbamna et al. (2006)

Information derived from a Google scholar search using various combinations of search terms related to taxonomy and auditory physiology, behavior, or communication, supplemented by survey of references cited in the resulting papers. In all cases, dB refers to dB SPL re. 20 μPascals, torus refers to the auditory midbrain nucleus, the torus semicircularis, and ABR is auditory brainstem response. The way in which thresholds are obtained and reported in studies based on electrophysiological recordings from the auditory nerve or midbrain varies greatly; readers are referred to the referenced publication for details. Thresholds from those studies listed here are the lowest thresholds reported or illustrated, the average of the lowest thresholds obtained across individuals at the most sensitive frequency of the auditory response, or the range of such thresholds across individuals in the study.

Behaviorally, túngara frogs exhibited ~10 dB greater sensitivity compared with ABR; this is consistent with a number of other behavioral versus ABR comparisons (Gorga et al. 1988; Yan and Popper 1991; Kenyon et al. 1998; Kojima et al. 2005; Yuen et al. 2005). In our ABR study, 10 ms tone bursts were presented to females at a rate of 19 s^{-1}, whereas male vocalizations lasting 350 ms, or longer, were presented to females at intervals of one every 1 s in the behavioral tests. We were unable to obtain ABR responses with full–length calls, thus it is possible that the difference in our threshold estimates were at least partially a result of differences in stimulus presentation (Gorga et al. 1988). Thresholds obtained from midbrain multiunit recordings were much lower than both behavioral and ABR measures. In general, direct electrophysiological measures from the midbrain or auditory nerve yield the lowest reported threshold measurements (Table 1). The shape of the audiogram generated by ABR did, however, correspond to that obtained from midbrain recordings (Ryan et al. 1990; Wilczynski et al. 2001). This is also seen for *Hyla cinerea* and *H. chrysoscelis* the only other species for which both ABRs (*H. cinerea*, Buerkle et al. 2014; Gall and Wilczynski 2015; *H. chrysoscelis*, Schroder et al. 2014) and midbrain multiunit recordings (*H. cinerea*, Penna et al. 1992; Miranda and Wilczynski 2009; *H. chrysoscelis*, Hillary 1984) have been described. Although these 2 methods may not yield the same absolute thresholds, they do appear to be consistent in measuring relative thresholds across frequencies to reveal the basic hearing range and frequency–threshold relationship of the auditory system.

Signal recognition as verified through phonotaxis occurs after a great deal of neural processing, including signal detection, auditory processing, and coding the motor response (Wilczynski and Ryan 2010). One factor that can influence signal recognition thresholds is motivation. Although we controlled for motivation by giving female frogs full volume phonotaxis tests before and after the threshold tests, females may not respond to distant males even though they can detect the sound if the perceived search cost is high (Rand et al. 1997; Baugh and Ryan 2010). Another factor unique to signal recognition thresholds is the evaluation of a signal's properties. Specific signal properties (e.g., simple versus complex calls) could potentially influence threshold for response; our results do not show this to be a significant effect, however. In spite of this, we found a lower threshold response in behavioral tests than in ABR recordings. This result could be an effect of gross differences in auditory signals (e.g., tones versus calls) or technology sensitivity (e.g., trans-cranial recording with ABRs) rather than the individuals tested. More interestingly, it may also suggest that cognitive processing of ecologically salient signals alters threshold responses. Such a process could occur in several ways, including alteration of attention or midbrain auditory nuclei differentially propagating signals to motor centers (Hoke et al. 2008).

Another difference between these methods is that the animals tested using ABR were not sexually receptive (lab animals) while field–caught frogs were. Circulating levels of sex hormones in lab animals were likely lower, potentially affecting hearing threshold as has been shown in fish (Sisneros et al. 2004), and for visual sensitivity in túngara frogs (Cummings et al. 2008). In their review, Wilczynski et al. (2005) outlined a number of hormonal axes likely to play a critical role in anuran reproductive behavior. For example, exposure to calling conspecifics elevates androgen levels in male green tree frogs (Burmeister and Wilczynski 2000) and gonadal steroids in female túngara frogs (Lynch and Wilczynski 2006). These data suggest an intriguing path for future studies on the interaction of circulating hormone levels with auditory processing and hearing thresholds.

We also found little difference between males and females in ABR thresholds, suggesting no sex differences in hearing sensitivity (Figure 2). We did not record from a sufficient number of túngara females to test for a sex difference in midbrain sensitivity, although it has been found in some, but not all, anuran species (Table 1). Male túngara frogs exhibit similar phonotaxis behaviors to females (Bernal et al. 2009a), suggesting that sensitivity differences in the auditory system between the sexes are minimal for typical conspecific signals (Hoke et al. 2008). So although ABR underestimates hearing thresholds, it is a valuable technique for generating audiograms and estimating peak hearing sensitivity in sexually non-receptive (e.g., non-behaving) frogs.

Testing the signal recognition threshold for an unlearned behavioral response allowed us to apply threshold measures to an ecological setting and calculate an active space for túngara frog mating calls. Our behavioral data suggest that females will detect and respond to male vocal calls at a distance of ~15 m (based on sound attenuation rate of 6 dB per doubling of distance [Berenak 1954]); we do know, however, that there is excess attenuation of the túngara frogs' call in nature thus the true active space might be smaller (Ryan 1986; Kime et al. 2000). In other frogs, and certainly in túngara frogs as well, background noise will also limit the distance over which frogs can detect their calls (Bee and Schwartz 2009; Lee et al. 2017). Our estimated signal recognition threshold is different from the findings of Marsh et al. (2000) where females failed to show a significant response to playbacks at 64 dB SPL, a SPL that would be experienced by a female ~10 m from a calling male. Marsh et al. (2000) tested female response at 64 dB SPL specifically, without using a gradual decrease/increase method to reach an actual threshold. The different results between that study and our current data could mean that thresholds are altered relative to the acoustic environment (Bee et al. 2012) or other ecological conditions (Baugh and Ryan 2010). Regardless of the differences between these studies, our results reinforce the point that although humans can perceive túngara frog choruses over hundreds of meters, we must listen to the distant frogs through the ears of the receivers to have an accurate evaluation of a signal's active space.

Acknowledgments

We are grateful to Kim Hoke for helpful comments on an early version of this manuscript. The manuscript also benefited greatly from the comments of 3 anonymous reviewers. The Smithsonian Tropical Research Institute provided lab space and logistical support for behavioral studies. Kim Hunter provided help with figures.

Funding

This work was supported by National Science Foundation grant # IBN 98–16564 to MJR. All necessary permits were obtained from the government of Panama for collection of frogs and the research was approved by the IACUC of the University of Texas.

Authors' Contributions

R.C.T., K.A., W.W., and M.J.R. conceived the study; K.A. determined behavioral thresholds; T.R.C. determined ABR thresholds; W.W. determined multiunit neural thresholds; R.C.T., K.A., W.W., and M.J.R. wrote the paper.

References

Akre KL, Ryan MJ, 2010. Proximity-dependent response to variably complex mating signals in túngara frogs *Physalaemus pustulosus*. *Ethology* **116**: 1138–1145.

Baugh AT, Ryan MJ, 2010. Ambient light alters temporal-updating behaviour during mate choice in a Neotropical frog. *Can J Zool* **88**:448–453.

Bee MA, 2015. Treefrogs as animal models for research on auditory scene analysis and the cocktail party problem. *Int J Psychophysiol* **95**:216–237.

Bee MA, Micheyl C, 2008. The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it? *J Comp Psychol* **122**:235–251.

Bee MA, Schwartz JJ, 2009. Behavioral measures of signal recognition thresholds in frogs in the presence and absence of chorus-shaped noise. *J Acoust Soc Amer* **126**:2788–2801.

Bee MA, Vélez A, Forester JD, 2012. Sound level discrimination by gray treefrogs in the presence and absence of chorus-shaped noise. *J Acoust Soc Amer* **131**:4188–4195.

Beckers OM, Schul J, 2004. Phonotaxis in *Hyla versicolor* (Anura, Hylidae): the effect of absolute call amplitude. *J Comp. Physiol* **190**:869–876.

Berenak LL, 1954. *Acoustics*. New York: McGraw-Hill.

Bernal XE, Rand AS, Ryan MJ, 2009a. Task differences confound sex differences in receiver permissiveness in túngara frogs. *Proc R Soc B* **276**: 1323–1329.

Bernal XE, Page RA, Ryan MJ, Argo TF, Wilson PS, 2009b. Acoustic radiation patterns of mating calls of the túngara frog *Physalaemus pustuosus*: implications for multiple receivers. *J Acoust Soc Amer* **126**:2757–2767.

Boettcher FA, Mills JH, Norton BL, Schmiedt RA, 1993. Age-related changes in auditory evoked potentials of gerbils. II. Response latencies. *Hear Res* **71**: 145–156.

Bosch J, Wilczynski W, 2003. Auditory tuning of the Iberian midwife toad *Alytes cisternasii*. *J Herpetol* **13**:53–57.

Brzoska J, Walkowiak W, Schneider H, 1977. Acoustic communication in the grass frog (*Rana t. temporaria* L.): calls, auditory thresholds and behavioral responses. *J Comp Physiol* **118**:173–186.

Brenowitz EA, 1982. The active space of red-winged blackbird song. *J Comp Physiol* **147**:511–522.

Brittan-Powell EF, Dooling RJ, Gleich O, 2002. Auditory brainstem responses (ABR) in adult budgerigars *Melospsittacus undulates*. *J Acoust Soc Amer* **112**:999–1008.

Buerkle NP, Schrode KM, Bee MA, 2014. Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*). *Com Biochem Physiol* **178**:68–81.

Burmeister SS, Wilczynski W, 2000. Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). *Horm Behav* **38**:201–209.

Capranica RR, Moffat AJM, 1975. Selectivity of the peripheral auditory system of spadefoot toads (*Scaphiopus couchi*) for sounds of biological significance. *J Comp Physiol* **100**:231–249.

Capranica RR, Moffat AJM, 1983. Neurobehavioral correlates of sound communication in anurans. In: Ewert JP, Capranica RR, editors. *Advances in Vertebrate Neuroethology*. New York: Plenum Press. 701–730.

Cummings ME, Bernal X, Reynaga R, Rand AS, Ryan MJ, 2008. Visual sensitivity to a conspicuous male cue varies by reproductive state in *Physalaemus pustulosus* females. *J Exp Biol* **211**:1203–1210.

Darwin C, 1871. *The Descent of Man and Selection in Relation to Sex*. London: Murray.

Dyson ML, Klump GM, Gauger B, 1998. Absolute hearing thresholds and critical masking ratios in the European barn owl: a comparison with other owls. *J Comp Physiol* **182**:695–702.

Dusenbery DB, 1992. *Sensory Ecology*. New York: Freeman.

Elberling C, Don M, 1987. Threshold characteristics of the human auditory brain stem response. *J Acoust Soc Amer* **81**:115.

Eggermont JJ, Ponton CW, 2002. The neurophysiology of auditory perception: from single units to evoked potentials. *Audiol Neuro-Otol* **7**:71–99.

Fay RR, 1988. *Hearing in Vertebrates: A Psychophysics Databook*. Winnetka (IL): Hill–Fay.

Fay RR, Popper AN, 1999. *Comparative Hearing: Fish and Amphibians*. New York: Springer.

Feng AS, Narins PM, Capranica RR, 1975. Three populations of primary auditory fibers in the bullfrog *Rana catesbeiana*: their peripheral origins and frequency sensitivities. *J Comp Physiol* **100**:221–229.

Frishkopf LS, Goldstein MH, Jr1963. Responses to acoustic stimuli from single units in the eighth nerve of the bullfrog. *J Acoust Soc Amer* **35**: 1219–1228.

Fuzessery ZM, Feng AS, 1982. Frequency selectivity in the anuran auditory midbrain: single unit responses to single and multiple tone stimulation. *J Comp Physiol* **146**:471–484.

Gall MD, Wilczynski W, 2015. Hearing conspecific vocal signals alters peripheral auditory sensitivity. *Proc Roy Soc Lond B* **282**:20150749.

Gerhardt HC, Huber F, 2002. *Acoustic Communication in Insects and Anurans*. Chicago (IL): University of Chicago Press.

Gorga MP, Kaminski JR, Beauchaine KA, Jesteadt W, 1988. Auditory brainstem response to tone bursts in normally hearing subjects. *J Speech Hear Res* **31**:87–97.

Goshorn EL, Marx CG, Ward K, 2017. Relationship between behavioral and electrophysiological hearing thresholds. *J Acoust Soc Amer* **142**:2612.

Gridi-Papp M, Rand AS, Ryan MJ, 2006. Complex call production in túngara frogs. *Nature* **441**:38.

Greenfield MD, 2002. *Signalers and Receivers, Mechanisms and Evolution of Arthropod Communication*. Oxford: Oxford University Press.

Hall JW, 1992. *Handbook of Auditory Evoked Responses* Boston (MA): Allyn and Bacon.

Higgs DM, Brittan-Powell EF, Soares D, Souza MJ, Carr CE et al., 2002. Amphibious auditory responses of the American alligator *Alligator mississippiensis*. *J Comp Physiol* **188**:217–223.

Hillary CM, 1984. Seasonality of two midbrain auditory responses in the treefrog *Hyla chrysoscelis*. *Copeia* **1984**:884–852.

Hoke KL, Ryan MJ, Wilczynski W, 2008. Candidate neural locus for sex differences in reproductive decisions. *Biol Lett* **4**:518–521.

Hubl L, Schneider H, 1979. Temperature and auditory thresholds: bio-acoustic studies of the frogs *Rana r. ridibunda, Hyla a. arborea* and *Hyla a. savignyi* (Anura, Amphibia). *J Comp Physiol* **130**:17–27.

Katbamna B, Brown JA, Collard M, Ide CF, 2006. Auditory brainstem responses to airborne sounds in the aquatic frog *Xenopus laevis*: correlation with middle ear characteristics. *J Comp Physiol* **192**:381–387.

Keddy-Hector A, Wilczynski W, Ryan MJ, 1992. Call patterns and basilar papilla tuning in cricket frogs. II. Intrapopulational variation and allometry. *Brain Behav Evol* **39**:238–246.

Kime NM, Turner WR, Ryan MJ, 2000. The transmission of advertisement calls in Central American frogs. *Behav Ecol* **11**:71–83.

King AJ, Sininger YS, 1992. Electrode configuration for auditory brainstem response audiometry. *Amer J Audiol* **1**:63–67.

Kenyon TN, Ladich F, Yan HY, 1998. A comparative study of hearing ability in fishes: the auditory brainstem response approach. *J Comp Physiol* **182**: 307–318.

Kojima T, Ito H, Komada T, Taniuch T, Akamatsu T, 2005. Measurements of auditory sensitivity in common carp *Cyprinus carpio* by the auditory brainstem response technique and cardiac conditioning method. *Fish Sci* **71**: 95–100.

Kraus N, Nicol T, 2009. Auditory evoked potentials. In: Binder MD, Hirokawa N, Windhorst U, editors. *Encyclopedia of Neuroscience*. Heidelberg, Berlin: Springer Berlin Heidelberg. 214–218.

Lasky RE, Soto AA, Luck ML, Laughlin NK, 1999. Otoacoustic emission, evoked potential, and behavioral auditory thresholds in the rhesus monkey *Macaca mulatta*. *Hear Res* **136**:35–43.

Lee N, Ward JL, Vélez A, Micheyl C, Bee MA, 2017. Frogs exploit statistical regularities in noisy acoustic scenes to solve cocktail-party-like problems. *Curr Biol* **27**:743–750.

Loftus-Hills JJ, 1973. Comparative aspects of auditory function in Australian anurans. *Aust J Zool* **21**:353–367.

Loftus-Hills JJ, Johnstone BM, 1970. Auditory function, communication, and the brain-evoked response in anuran amphibians. *J Acoust Soc Amer* **47**: 1131–1138.

Lohr B, Wright TF, Dooling RJ, 2003. Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. *Anim Behav* **65**:763–777.

Lohr B, Brittan-Powell EL, Dooling RJ, 2013. Auditory brainstem responses and auditory thresholds in woodpeckers. *J Acoust Soc Amer* **133**:337–342.

Lombard RE, Straughan IR, 1974. Functional aspects of anuran middle ear structures. *J Exper Biol* **61**:71.

Lovelace CT, Stein BE, Wallace MT, 2003. An irrelevant light enhances auditory detection in humans: a psychophysical analysis of multisensory integration in stimulus detection. *Cog Brain Res* **17**:447–453.

Lynch KS, Wilczynski W, 2006. Social regulation of plasma estrogen concentration in a female anuran. *Horm Behav* **50**:101–106.

Marsh DM, Rand AS, Ryan MJ, 2000. Effects of inter-pond distance on the breeding ecology of túngara frogs. *Oecologia* **122**:505–513.

Marten K, Marler P, 1977. Sound transmission and its significance for animal vocalization. I. Temperate habitats. *Behav Ecol Sociobiol* **2**:271–290.

McClelland BE, Wilczynski W, Rand AS, 1997. Sexual dimorphism and species differences in the neurophysiology and morphology of the acoustic communication system of two neotropical hylids. *J Comp Physiol* **180**:451–462.

Miranda JA, Wilczynski W, 2009. Sex differences and androgen influences on midbrain auditory thresholds in the green treefrog *Hyla cinerea*. *Hear Res* **252**:79–88.

Moreno-Gómez FN, Sueur J, Soto-Gamboa M, Penna M, 2013. Female frog auditory sensitivity, male calls, and background noise: potential influences on the evolution of a peculiar matched filter. *Biol J Linn Soc* **110**:814–827.

Nityananda V, Bee MA, 2012. Spatial release from masking in a free-field source identification task by gray treefrogs. *Hear Res* **285**:86–97.

Norrix LW, Velenovsky D, 2018. Clinicians' guide to obtaining a valid auditory brainstem response to determine hearing status: signal, noise, and cross-checks. *Amer J Audiol* **27**:25–36.

Penna MN, Capranica RR, Somers J, 1992. Hormone-induced vocal behavior and midbrain auditory sensitivity in the green treefro, *Hyla cinerea*. *J Comp Physiol* **170**:73–82.

Penna M, Palazzi C, Paolinelli P, Solís R, 1990. Midbrain auditory sensitivity in toads of the genus *Bufo* (Amphibia – Bufonidae) with different vocal repertoires. *J Comp Physiol* **167**:673–681.

Penna M, Plaza A, Moreno-Gómez FN, 2013. Severe constraints for sound communication in a frog from the South American temperate forest. *J Comp Physiol* **199**:723–733.

Penna M, Narins PM, Feng AS, 2005. Thresholds for evoked vocal responses of *Eupsophus*. *Herpetologica* **61**:1–8.

Penna M, Moreno-Gómez FN, 2014. Ample active acoustic space of a frog from the South American temperate forest. *J Comp Physiol* **200**:171–181.

Penna M, Velásquez NA, Bosch J, 2015. Dissimilarities in auditory tuning in midwife toads of the genus *Alytes* (Amphibia: anura). *Biol J Linn Soc* **116**: 41–51.

Penna MN, Velásquez N, Solís R, 2008. Correspondence between evoked vocal responses and auditory thresholds in *Pleurodema thaul* (Amphibia; Leptodactylidae). *J Comp Physiol* **194**:361–371.

Plomp R, Mimpen AM, 1979. Improving the reliability of testing the speech reception threshold for sentences. *Audiology* **18**:43–52.

Rosenthal GG, 2017. *Mate Choice, the Evolution of Sexual Decision-Making from Microbes to Humans*. Princeton (NJ): Princeton University Press.

Rand AS, Bridarolli ME, Dries L, Ryan MJ, 1997. Light levels influence female choice in túngara frogs: predation risk assessment? *Copeia* **1997**:447–450.

Ryan MJ, 1985. *The Túngara Frog, a Study in Sexual Selection and Communication*. Chicago (IL): University of Chicago Press.

Ryan MJ, 1986. Environmental bioacoustics: evaluation of a commonly used experimental technique. *Anim Behav* **34**:931–933.

Ryan MJ, 2001. *Anuran Communication*. Washington (DC): Smithsonian Institution Press.

Ryan MJ, Fox JH, Wilczynski W, Rand AS, 1990. Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature* **343**:66–67.

Ryan MJ, Rand AS, 2003. Sexual selection and female preference space: how female túngara frogs perceive and respond to complex population variation in acoustic mating signals. *Evolution* **57**:2608–2618.

Schrode KM, Buerkle NP, Brittan-Powell EF, Bee MA, 2014. Auditory brainstem responses in Cope's gray treefrog *Hyla chrysoscelis*: effects of frequency, level, sex and size. *J Comp Physiol* **200**:221–238.

Simmons AM, 1988. Selectivity for harmonic structure in complex sounds by the green treefrog *Hyla cinerea*. *J Comp Physiol* **162**:397–403.

Simmons AM, Moss CF, Daniel KM, 1985. Behavioral audiograms of the bullfrog *Rana catesbeiana* and the green tree frog *Hyla cinerea*. *J Acoust Soc* **78**: 1236–1244.

Sisneros JA, Forlano PM, Deitcher DL, Bass AH, 2004. Steroid-dependent auditory plasticity leads to adaptive coupling of sender and receiver. *Science* **305**:404–407.

Skoe E, Kraus N, 2010. Auditory brainstem response to complex sounds: a tutorial. *Ear Hear* **31**:302.

Szymanski MD, Bain DE, Kiehl K, Pennington S, Wong S et al., 1999. Killer whale *Orcinus orca* hearing: auditory brainstem response and behavioral audiograms. *J Acoust Soc Amer* **106**:1134–1141.

Velez A, Höbel G, Gordon NM, Bee MA, 2012. Dip listening or modulation masking? Call recognition by green treefrogs *Hyla cinerea* in temporally fluctuating noise. *J Comp Physiol* **198**:891–904.

Walkowiak W, 1980. The coding of auditory signals in the torus semicircularis of the fire-bellied toad and the grass frog: responses to simple stimuli and to conspecific calls. *J Comp Physiol* **138**:131–148.

Walsh EJ, McGee J, Javel E, 1986. Development of auditory-evoked potentials in the cat. II. Wave latencies. *J Acoust Soc Amer* **79**:725–744.

Wilczynski W, Zakon HH, Brenowitz EA, 1984. Acoustic communication in spring peepers: call characteristics and neurophysiological aspects. *J Comp Physiol* **55**:577–584.

Wilczynski W, Rand AS, Ryan MJ, 2001. Evolution of calls and auditory tuning in the *Physalaemus pustulosus* species group. *Brain Behav Evol* **58**: 137–151.

Wilczynski W, Lynch KS, O'Bryant EL, 2005. Current research in amphibians: studies integrating endocrinology, behavior, and neurobiology. *Horm Behav* **48**:440–450.

Wilczynski W, Ryan MJ, 2010. The behavioral neuroscience of anuran social signal processing. *Curr Opin Neurobiol* **20**:754–763.

Wilczynski W, Burmeister SS, 2016. Effects of steroid hormones on hearing and communication in frogs. In: Bass AH, Sisneros JA, Popper AN, Fay RR, editors. *Hearing and Hormones, Springer Handbook of Auditory Research*. Vol. **57**. Switzerland: Springer. 53–75.

Wiley RH, Richards DG, 1982. Adaptations for acoustic communication in birds: sound transmission and signal detection. In: Kroodsma DE, Miller EH, editors. *Acoustic Communication in Birds*. Vol. **1**. New York: Academic Press. 131–181.

Wolski LF, Anderson RC, Bowles AE, Yochem PK, 2003. Measuring hearing in the harbor seal *Phoca vitulina*: comparison of behavioral and auditory brainstem response techniques. *J Acoust Soc Amer* **113**:629–637.

Yantis S, Abrams RA, 2017. *Sensation and Perception*. 2nd edn. New York: MacMillan Learning.

Yan HY, Popper AN, 1991. An automated positive reward method for measuring acoustic sensitivity in fish. *Behav Res Methods Instrum Comput* **23**: 351–356.

Yu Z-L, Qiu Q, Xu Z-M, Shen J-X, 2006. Auditory response characteristics of the piebald odorous frog and their implications. *J Comp Physiol* **192**: 801–806.

Yuen MML, Nachtigall PE, Breese M, Supin AY, 2005. Behavioral and auditory evoked potential audiograms of a false killer whale *Pseudorca crassidens*. *J Acoust Soc Amer* **118**:2688–2695.

Zelick R, Narins PM, 1985. Temporary threshold shift, adaptation, and recovery characteristics of frog auditory nerve fibers. *Hear Res* **17**:161–176.

Zhang D, Cui J, Tang Y, 2012. Plasticity of peripheral auditory frequency sensitivity in Emei music frog. *PLoS ONE* **7**:e45792.