Neural Mechanisms of Auditory Scene Analysis in a Non-Mammalian Animal Model

A Dissertation
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

Katrina Marie Schrode

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Advised by Mark A. Bee

September 2014

Acknowledgements

I am grateful to Madeleine Linck, Don Pereira, Ed Quinn, Gary Calkins and Christopher Maldonado for access to the wonderful field sites in which I spent my spring and summer nights and for collection permissions. Funding support was provided by training grants from NIH to Tim Ebner, grants from NSF and NIH to Mark Bee, a doctoral dissertation fellowship from the UMN graduate school and a doctoral dissertation improvement grant from NSF. Additional small grants were provided by the International Society for Neuroethology, the Acoustical Society of America, the Graduate and Professional Student Assembly, and the Marine Biological Laboratory at Woods Hole.

Many people have helped me in the pursuit of my degree and been extremely generous with their time. First and foremost, I am indebted to my advisor, Dr. Mark Bee. He has been supportive, thoughtful, demanding, patient, critical, and encouraging, and I couldn't have asked for a better advisor. I am also grateful to have had a wonderful committee throughout this process, and I'd like to thank all the past and current members, Dr. Geoff Ghose, Dr. Karen Mesce, Dr. Teresa Nick, Dr. Andrew Oxenham, and Dr. David Redish, for their guidance, advice, and mentorship. I'd also like to acknowledge the behavior group and the acoustic communication group, both of which provided attentive audiences and good discussion. I would also like to acknowledge Drs. Raquell Holmes and Steve Negus for teaching me how to ask questions and Dr. Amanda Lauer for her immense patience during my final weeks of graduate school.

In addition to having a great advisor, I've had a great lab to work with. I enjoyed my time working with everyone in the lab and benefited from the time I spent with each member. Many thanks to Michael Caldwell, Noah Gordon, Norman Lee, Elliot Love, Vivek Nityananda, Beth Pettitt, Jessie Tanner, Sandra Tekman, James Tumulty, and Jessica Ward. Also thanks to the many undergrads who helped collect and test frogs, in particular Nate Buerkle, Camille Herteux, Desiree Schaefers, and Dylan Verden.

I'd like to thank my loving family who have always been supportive of me in whatever I do. Finally, I thank Andrew, my dear friend and accomplice, for riding this coaster with me.

Table of Contents

List of Tables

List of Figures

Introduction

Healthy auditory systems perform well in quiet places where there are no overlapping sounds, but are greatly challenged in noisy environments (Brumm and Slabbekoorn, 2005; Hulse, 2002). In these environments, all of the sounds in the "acoustic scene" combine to create a single waveform that impinges on the receiver's ear, from which the auditory system must extract some meaningful signal (Bregman, 1990). A particular example of this auditory scene analysis occurs in multi-talker environments, where the acoustic scene consists of the overlapping sounds of competing signalers. The problem of communicating in multi-talker environments has been well-studied in the human hearing literature, where it is known as the cocktail party problem (Cherry, 1953), but it is not unique to humans. Many non-human animals also encounter noisy social environments and have evolved to solve cocktail-party-like problems of vocal communication (Bee and Micheyl, 2008).

However, the mechanisms that humans and other animals use to solve the problem may differ. While human and other vertebrate auditory systems share ancestral traits from their most recent common ancestor, there is evidence for divergence of auditory systems between the separate tetrapod lineages (Christensen-Dalsgaard and Carr, 2008; Manley, 2001). The tympanic middle ear, for example, has evolved *independently* in at least three major terrestrial vertebrate lineages, including the one leading to birds and reptiles, the lineage leading to modern amphibians, and the lineage leading to humans and other mammals (Christensen-Dalsgaard & Carr 2008). The structure of the inner ear also varies between auditory systems, with different numbers and types of sensory papillae across taxa. Amphibians appear to have evolved a sensory papillae unique to the lineage, which functions in some species as the sole sensory papilla and in others (especially in anurans) in conjunction with a second papilla, the basilar papilla (Lewis and Lombard, 1988; Manley et al., 2004).

The independent evolution of auditory systems suggests that vertebrates may have evolved a diversity of novel solutions to cocktail-party-like problems (Bee and Micheyl, 2008). However, traditionally, research into similar problems in other non-human animals has been limited. Research on the more general problem of auditory scene analysis has been primarily in birds (Bee and Klump, 2004; Bee and Klump, 2005; Bee et al., 2010; Itatani and Klump, 2009; MacDougall-Shackleton et al., 1998) and mammals (Chiu et al., 2009; Fishman et al., 2001; Fishman et al., 2004; Izumi, 2002; Ma et al., 2010; Micheyl et al., 2005; Moss and Surlykke, 2001; Pressnitzer et al., 2008). The aim of my dissertation research was to investigate

mechanisms that enable a non-mammalian vertebrate, specifically a frog, to navigate noisy, multi-signaler environments.

I chose frogs as subjects because they are an important and well-studied model system for the investigation of acoustic communication (reviewed in Gerhardt and Huber, 2002; Narins et al., 2007; Ryan, 2001; Bee, 2014) and their auditory systems differ from those of reptiles, birds and mammals in several interesting ways. During the breeding season, hundreds of males, usually of multiple species, gather in ponds and compete for females with loud (80 - 105 dB SPL @ 1 m; Gerhardt, 1975), species-specific advertisement calls. Males also use aggressive calls to mediate disputes over territories and calling sites (reviewed in Gerhardt & Bee, 2007). In order to reproduce, a female frog must successfully detect, recognize, localize and discriminate between advertisement calls in the chorus. Females exhibit strong preferences for particular signal traits that identify high-quality males of the correct species and will exhibit phonotaxis (approach behavior) toward preferred calls (reviewed in Gerhardt & Bee, 2007). Female fitness is thus strongly tied to the ability to communicate in noisy social environments (Gerhardt 2001).

One unique property of the frog auditory system regards how sound frequency is processed. While most terrestrial vertebrates have one sensory organ in the inner ear (e.g. the cochlea), frogs have two distinct organs, the amphibian papilla (AP), which is unique to the amphibian lineage, and the basilar papilla (BP) (Geisler et al., 1964). In all frogs examined to date, the tonotopically-organized AP is sensitive to lower frequencies than the BP; additionally, nerve fibers arising from the AP tend to have narrow tuning curves, while those from the BP are homogeneously tuned to a broad frequency range (Zakon and Wilczynski, 1988). The frequencies to which the sensory organs are most sensitive often coincide with spectral peaks in species-specific advertisement calls such that they are thought to function as “matched filters” by filtering out unwanted background sounds (Capranica and Moffat, 1983; Gerhardt and Schwartz, 2001).

Directionality of the frog auditory system (and most other terrestrial vertebrates) arises through internal coupling of the ears via the Eustachian tubes, such that the ears function as pressure-gradient receivers (Christensen-Dalsgaard, 2005; Christensen-Dalsgaard, 2011). This is in contrast to the mammalian auditory system in which directionality is achieved through comparisons in the central auditory system of the sounds at the two ears. In addition to providing directionality, internal coupling of the ears also reduces the independence of the ears. The anuran inner ear can also receive sound through a number of extratympanic pathways including through the body wall and lungs (Mason, 2007).

I selected two species of treefrog for my studies, Cope's gray treefrog (*Hyla chrysoscelis*) and the green treefrog (*H. cinerea*). There is a large literature on hearing and sound communication in both of these species (reviewed in Bee, 2014), but much more is known about the physiology of the green treefrog auditory system. The advertisement calls of gray treefrogs are composed of a series of about 30 short (~10 ms) pulses, produced with a pulse rate of 40 to 65 pulses/s (Ward et al., 2013). Those of green treefrogs, on the other hand, consist of a single pulse, 120-200 ms in duration, that contains a waveform periodicity that ranges between 200 and 500 Hz (Oldham and Gerhardt, 1975). The calls of gray treefrogs have a bimodal frequency spectrum with peaks around 1.3 and 2.6 kHz (Schrode et al., 2012a). Green treefrogs have calls with a more broadband spectrum, but synthetic versions elicit strong responses from females when they contain energy in a band between 0.9 and 1.1 kHz and a band between 2.5 and 3.6 kHz (Gerhardt, 1974c). I chose to work with these two species of treefrog because of these striking difference in their advertisement calls, which suggest that sound processing and hearing may differ strongly between the species as well. As an example, previous comparative work with gray and green treefrogs found a difference in the abilities of these species to detect signals in temporally fluctuating background noise (Vélez and Bee, 2010; Vélez and Bee, 2011; Vélez and Bee, 2013; Vélez et al., 2012).

While there is a growing literature investigating the neural mechanisms underlying the general problem of auditory scene analysis (Carlyon, 2004; Shamma and Micheyl, 2010; Snyder and Alain, 2007a), there has been much less focus on the study of neural mechanisms in the context of communication in real-world scenarios. In particular, we know very little about the neural mechanisms underlying communication of non-human animals in cocktail party-like-environments. More extensive use of minimally invasive electrophysiological techniques, such as auditory evoked potentials (AEPs), could facilitate comparative experimental research into the neural mechanisms involved in communicating in noisy environments. A major focus of my dissertation was geared toward developing the technique of recording AEPs in frogs. AEPs represent a relatively non-invasive, efficient and effective method of evaluating auditory sensitivity and processing. While they have been widely used to study sensitivity in a variety of animals including birds, reptiles, and even cephalopods (Brittan-Powell et al., 2010a; Gall et al., 2012b; Gorga et al., 1984; Hu et al., 2009; Song et al., 2006), they had been used only twice to investigate hearing in frogs prior to my work (Katbamna et al., 2006a; Zhang et al., 2012). The first two chapters of my dissertation are characterizations of a type of AEP, the auditory brainstem response (ABR), in Cope's gray treefrogs and green treefrogs. The ABR is an onset

response derived from the summed activity of the auditory nerve and brainstem nuclei. In both species, I found properties of the ABR that were consistent with features of anuran auditory physiology. This consistency of results served to validate the method. I was further able to estimate the frequency ranges of the two sensory papillae in gray treefrogs, which had not been previously determined in this species. The chapter on gray treefrogs has been published (Schrode et al., 2014). I trained an undergraduate student in the ABR technique. He completed most of the recordings in green treefrogs and I elected to give him first authorship on the corresponding manuscript. However, I developed the techniques, analyzed the data and wrote the paper, which has been through revision and is under review at *Comparative Biochemistry and Physiology* (Buerkle et al., 2014).

In the third chapter, which will be submitted to the *Journal of Experimental Biology*, I investigate an evolutionary mechanism potentially involved in detecting signals of interest in noisy environments. Sensory systems are tasked with processing a continuous and complex stream of input. In many cases, sensory systems seem to be evolutionary adapted to processing commonly encountered or biologically relevant stimuli (Rieke et al., 1995; Singh and Theunissen, 2003; Smith and Lewicki, 2006; Suga, 1989; Woolley et al., 2005). Emphasizing specific stimulus properties can improve the efficiency of processing in the sensory system by quickly filtering out irrelevant background stimuli (Rieke et al., 1995). In anurans, it has been proposed that the auditory system may function as a "matched filter" in which tuning of the auditory physiology closely matches that of the species-specific communication signals (Capranica and Moffat, 1983; Gerhardt and Schwartz, 2001). A number of examples of "spectral matched filters" have been found in which the peripheral and central auditory systems are tuned to frequencies prominent in species-specific communication signals. While there are also some examples of temporal matched filters in the central auditory system (Rose and Capranica, 1984; Rose and Capranica, 1985), there is little evidence of temporal matched filters in the peripheral auditory system. In this comparative study, I used two kinds of AEPs to test the hypothesis that temporal processing in each species and sex was selectively adapted to the temporal properties of species-specific advertisement and aggressive calls. In one method, known as the auditory steady state response (ASSR), I recorded responses to stimuli containing temporal amplitude modulations. The response largely represents phase-locking of the auditory nerve to the modulations in the stimulus. The second method was a paired-click paradigm, in which recordings are made of ABRs to clicks separated by varying amounts of silence. I found evidence in support of species-specific adaptation and weak evidence of sex-specific adaptation.

In the fourth chapter, I investigate the mechanisms by which listeners sort sounds in the acoustic scene into perceptual auditory "streams." In general, sounds tend to be integrated into a single stream if they share similarity in some feature. Similarity in frequency, for example, is a particularly strong integration cue. Conversely, the tendency to segregate sounds increases as a function of the distance between frequencies. In the chapter, I test a long-standing hypothesis of stream segregation, the channeling hypothesis (Hartmann and Johnson, 1991), which posits that segregation into separate streams occurs whenever sounds excite distinct "channels." These are either tonotopic channels based on frequency coding in the auditory system, or lateral channels based on the independence of the two ears (Hartmann and Johnson, 1991; Moore and Gockel, 2002). The channeling hypothesis can explain much of the stream segregation data, but it has become increasingly evident that it cannot account for all aspects of stream segregation (Cusack and Roberts, 2000; Grimault et al., 2002; Micheyl et al., 2007; Roberts et al., 2002; Shamma and Micheyl, 2010; Vliegen and Oxenham, 1999). Traditionally, tests of the channeling hypothesis have been limited to mammalian auditory systems and have used artificial stimuli such as tones. My aim was to test the channeling hypothesis in the context of vocal communication in an animal with an auditory system in which tonotopic and lateral channels are implemented differently than in the mammalian ear. Subjects were tested with communication signals having frequencies that targeted either one or both of the auditory papillae. The results were consistent with the channeling hypothesis in some instances, but not others. The responses of subjects indicated that they integrated sounds across papillae, despite the organs being tonotopically distinct channels. Sounds that stimulated only the AP were segregated as a function of frequency. Sounds that stimulated only the BP were likely segregated using level cues. My results suggested that the channeling hypothesis cannot fully describe how auditory systems achieve stream segregation. This chapter is currently in manuscript form and will be submitted to *Behavioral Neuroscience*.

The fifth chapter is a review of neural codes in the brain and focuses specifically on processing channels. Peripheral channels were introduced in the fourth channel as part of the channeling hypothesis. In this chapter I describe some of the history leading up to the discovery of channels in the visual system that underlie perception of color. This is followed by a more general characterization of channels and how their existence is tested. I then provide a number of examples of channels that have been discovered in the visual and auditory systems and describe the experiments that led to their discovery. This section ends with a brief look at the notion of channels in the somatosensory and olfactory systems. In the next section of this chapter, I discuss types of neural codes that differ from channels, providing some examples of these codes and

some consideration of their merits and weaknesses. In the next section, I consider neural processing in peripheral and central sensory systems, providing a specific example in the auditory system. This is followed by some discussion of the transformations that occur in neural codes as information is processed through ascending levels of a sensory system. The final section describes the significance of my dissertation work. I also suggest some future directions for the work, based on the results of my experiments in the first four chapters.

Chapter 1 Auditory brainstem responses in Cope's gray treefrog (*Hyla chrysoscelis*): effects of frequency, level, sex and size[1]

Our knowledge of the hearing abilities of frogs and toads is largely defined by work with a few well-studied species. One way to further advance comparative work on anuran hearing would be greater use of minimally invasive electrophysiological measures, such as the auditory brainstem response (ABR). This study used the ABR evoked by tones and clicks to investigate hearing in Cope's gray treefrog (*Hyla chrysoscelis*). The objectives were to characterize the effects of sound frequency, sound pressure level, and subject sex and body size on ABRs. The ABR in gray treefrogs bore striking resemblance to ABRs measured in other animals. As stimulus level increased, ABR amplitude increased and latency decreased, and for responses to tones, these effects depended on stimulus frequency. Frequency-dependent differences in ABRs were correlated with expected differences in the tuning of two sensory end organs in the anuran inner ear (the amphibian and basilar papillae). The ABR audiogram indicated two frequency regions of increased sensitivity corresponding to the expected tuning of the two papillae. Overall, there was no effect of subject size and only small effects related to subject sex. Together, these results indicate the ABR is an effective method to study audition in anurans.

[1] This chapter is published as Schrode, K. M., Buerkle, N. P., Brittan-Powell, E. F., & Bee, M. A. (2014). Auditory brainstem responses in Cope's gray treefrog (*Hyla chrysoscelis*): effects of frequency, level, sex and size. *Journal of Comparative Physiology A*, *200*(3), 221–238.

Introduction

The ability of relatively simple, species-specific vocal signals to elicit stereotyped behaviors in noisy environments makes the anuran auditory system an important model in neuroethology and sensory biology (Bee, 2012; Gerhardt and Huber, 2002; Kelley, 2004; Narins et al., 2007; Wilczynski and Ryan, 2010). However, we still lack a well-developed understanding of both species differences in anuran hearing and the influences such differences potentially have on the species specificity of vocalizations and behaviors in a broad, comparative framework. One reason for this is because the vast majority of anatomical, biomechanical, and electrophysiological studies of anuran hearing have been conducted using a relatively small number of model species, such as northern leopard frogs, *Rana pipiens* (Fuzessery and Feng, 1981; Fuzessery and Feng, 1982; Ho and Narins, 2006; Ratnam and Feng, 1998; Simmons et al., 1992), North American bullfrogs, *R. catesbeiana* (e.g., Feng and Capranica, 1976; Schwartz and Simmons, 1990; Simmons and Ferragamo, 1993; Simmons et al., 1993, 2000), grass frogs, *R. temporaria* (Bibikov, 2002; Christensen-Dalsgaard and Jorgensen, 1996; Christensen-Dalsgaard and Walkowiak, 1999; Christensen-Dalsgaard et al., 1998), African clawed frogs, *Xenopus laevis* (Bibikov and Elepfandt, 2005; Christensen-Dalsgaard et al., 1990; Edwards and Kelley, 2001; Elliott et al., 2007; Elliott et al., 2011), and green treefrogs, *Hyla cinerea* (e.g., Ehret and Capranica, 1980; Feng and Capranica, 1978; Klump et al., 2004; Miranda and Wilczynski, 2009a, 2009b; Mudry and Capranica, 1987). Efforts to assess audition in frogs and toads using relatively fast, minimally invasive procedures, such as dermal or subdermal recordings of auditory evoked potentials (AEPs) (Hall, 2007), could significantly enhance experimental neuroethological research on this group by facilitating comparisons among a much greater diversity of species.

AEPs have been widely used to study hearing and sound communication in a broad diversity of nonhuman vertebrates, including mammals (Aitkin et al., 1996; D'angelo et al., 2007; Katbamna et al., 1992; McFadden et al., 1999; Nachtigall et al., 2007a; Nachtigall et al., 2007b; Ramsier and Dominy, 2010; Song et al., 2006; Supin and Popov, 1995b; Supin et al., 1993; Szymanski et al., 1999; Uetake et al., 1996; Uzuka et al., 1998; Walsh et al., 1986), birds (Brittan-Powell and Dooling, 2004; Brittan-Powell et al., 2002; Brittan-Powell et al., 2005; Brittan-Powell et al., 2010b; Caras et al., 2010; Gall et al., 2011; Henry and Lucas, 2008; Henry and Lucas, 2009; Henry and Lucas, 2010a; Henry and Lucas, 2010b; Lohr et al., 2013; Lucas et al., 2002; Noirot et al., 2011), reptiles (Bartol et al., 1999; Brittan-Powell et al., 2010a; Higgs et al., 2002; Martin et al., 2012), and fish (Amoser and Ladich, 2005; Horodysky et al., 2008; Kenyon et al., 1998; Ladich and Yan, 1998; Lugli et al., 2003; Smith et al., 2004; Wysocki and Ladich, 2001;

Wysocki and Ladich, 2003), as well as a few invertebrates (Hu et al., 2009; Lovell et al., 2005; Mooney et al., 2010). While a few previous studies used AEPs to investigate the auditory systems of frogs, these studies used invasive recording procedures requiring surgery (Bibikov and Elepfandt, 2005; Carey and Zelick, 1993; Corwin et al., 1982; Hillery, 1984a; Katbamna et al., 2006b; Seaman, 1991; Yu et al., 2006). To our knowledge, only two previous studies have recorded AEPs in frogs using less invasive subdermal procedures (Katbamna et al., 2006a; Zhang et al., 2012).

In the present study, we used subdermal recordings of the auditory brainstem response (ABR) to investigate auditory sensitivity in Cope's gray treefrog (*Hyla chrysoscelis*). The ABR is a form of AEP that represents the summed output of synchronized neural activity in the auditory nerve and brainstem. ABR waveforms are typically characterized by a series of positive and negative deflections, whose presence or absence can be used to determine auditory thresholds (reviewed in Hall 2007). Neither of the two previous studies that used minimally invasive methods to record ABRs in frogs (Katbamna et al. 2006a; Zhang et al. 2012) systematically investigated the effects on the ABR of stimulus properties, such as frequency and sound pressure level, or subject characteristics, such as sex and size. We had three objectives in this study. First, we sought to characterize the ABR in gray treefrogs and describe its dependence on sound pressure level and different sound frequencies. Typically, ABR amplitude and latency are directly and inversely related, respectively, to sound pressure level, whereas changes in tone frequency can have complex effects on the waveform (reviewed in Hall 2007). The directional effects of sound level on amplitude and latency are quite consistent across species, but the effects of frequency can vary (e.g., Higgs et al. 2002; Kenyon et al. 1998; Popov and Supin 1990; Song et al. 2006). Second, we investigated the extent to which the ABR in gray treefrogs differs according to sex and body size. There is some evidence for sex-differences in ABRs from some animals (Church et al., 1984; Gall et al., 2011; Hall, 2007; Jerger and Hall, 1980), and previous invasive studies of frogs have revealed differences in auditory processing related to sex (Keddy-Hector et al., 1992; Narins and Capranica, 1976; Wilczynski and Capranica, 1984; Wilczynski et al., 1992; Zakon and Wilczynski, 1988) and body size (Keddy-Hector et al., 1992; Shofner and Feng, 1981; Zakon and Wilczynski, 1988). Third, we generated an ABR audiogram to quantify variation in auditory sensitivity across frequency, and we assessed the influence of sex and body size on sensitivity. Furthermore, we compared our ABR audiogram to one previously derived for this species from invasive multiunit recordings from the auditory midbrain (Hillery, 1984b) to verify the utility of ABRs as a method for assessing auditory sensitivity in frogs. Taking into

account results from the first three objectives, we discuss the possibility that the ABR might be useful for describing the frequency ranges of sensitivity of the different auditory end organs in the frog inner ear (Simmons et al., 2007; Zakon and Wilczynski, 1988), which vary by species and are unknown for most anurans.

Materials and methods

Subjects

Thirty-five adult Cope's gray treefrogs (17 females, 18 males) of the western mitochondrial DNA lineage (Ptacek et al., 1994) were used as subjects. We collected pairs of frogs in amplexus between 15 May and 30 June, 2011, from the Carver Park Reserve (Carver County, MN), the Crow-Hassan Park Reserve (Hennepin County, MN), and the Lake Maria State Park (Wright County, MN). All of our ABR recordings were made during the annual breeding season (mid-May to early-July) and within five days of the animal's collection. Collected frogs were brought to the lab at the University of Minnesota, placed in small containers with conditioned tap water, and kept at 2 °C until they were used in behavioral tests not described here. After behavioral testing, we maintained the frogs at ambient room temperature (near 20 °C) until ABR experiments began. We waited until females had released their eggs prior to recording so that we could administer a correct size-dependent dose of paralytic (see below). Subject body mass ranged between 2.8 g and 8.3 g ($\overline{X}$ ± s.d.; females: $\overline{X}$ = 5.5 ± 1.1 g; males: $\overline{X}$ = 4.4 ± 0.9 g). Body temperatures during ABR recordings were measured by placing a Miller & Weber quick-reading thermometer against the abdominal body wall; temperatures ranged between 17.0 and 20.0 °C, which is within the range of wet-bulb air temperatures at which this species breeds. After the completion of recordings, we released animals at their location of capture. All animals were collected with permission from the Minnesota Department of Natural Resources (permit #17031) and treated according to protocols approved by the Institutional Animal Care and Use Committee of the University of Minnesota (#1103A97192, last approved 04/16/2013).

ABR recordings

All ABR recordings were made inside a radio-shielded mini-acoustical chamber (MAC-3, Industrial Acoustics Corporation, Bronx, NY; inside dimensions: 81.3 cm × 61 cm × 61 cm, W × H × D). The sound chamber was equipped with a breadboard floor, and the ceiling and walls were

covered with acoustic foam to reduce reverberations. The sound pressure level (SPL re. 20 μPa) of the ambient noise in the chamber was measured with a Larson Davis System 831 sound-level meter (Larson Davis Inc., Depew, NY) and ranged between 10 and 13 dB SPL (fast RMS, flat weighting) in the 1/3-octave bands between 250 and 5000 Hz, which span the frequency range of our test stimuli.

For recordings, subjects were immobilized by an intramuscular injection of d-tubocurarine chloride (2.5 to 4 μg/g body mass). Subjects were able to regulate their lung volume as the paralytic took effect over several minutes and maintained what appeared upon visual inspection to be a normal lung volume throughout neural recordings. We did not manually manipulate lung volume. During recordings, subjects were draped with moist surgical gauze to facilitate cutaneous respiration and placed on an acoustically transparent pedestal made of plastic mesh (2-cm height, 4-mm mesh grid) positioned on the breadboard floor of the sound chamber. We positioned subjects in a natural sitting posture directly facing an Orb Mod 1 speaker (Orb Audio, New York, NY) also located on the breadboard so that the rostral edges of both tympanic membranes were 30 cm from the sound source. Note that for frequencies below about 1.1 kHz, this distance was less than one wavelength from the sound source; thus there was also some potential for particle motion to influence responses to these sounds. Prior to electrode placement, the subject's head was treated with liberal application of a topical anesthetic (lidocaine HCl 2.5%). Platinum alloy, subdermal needle electrodes (Grass F-E2; West Warwick, RI) were inserted 2-3 mm under the skin of the subject's head between the eyes (non-inverting) and adjacent to the right (ground) and left (inverting) tympanic membranes (Fig. 1-1a). Electrode impedance ranged from 1 to 5 kΩ. The electrode wires were twisted together to reduce electrical noise, connected to a TDT (Tucker-Davis Technologies, Alachua, FL) RA4LI low-impedance headstage and TDT RA4PA pre-amp that amplified (20×) and digitized the biological signal before sending it via fiber optic cable to a TDT RZ5 digital signal processor located outside the chamber. We used a computer running TDT BioSig software to sample the biological signal at a sampling rate of 25 kHz (16 bit). Responses were notch filtered at 60 Hz, bandpass filtered between 0.03 kHz and 3 kHz, and stored on hard disk for offline analyses using MATLAB v2010b (Mathworks, Natick, MA). Recording sessions lasted approximately 1.5 hrs.

We synthesized digital sound files (50 kHz sampling rate, 16-bit) using TDT SigGen software. Stimuli comprising short trains of clicks or tones were output via a TDT RM2 signal processor, attenuated by a TDT PA5 programmable attenuator, amplified by a Crown XLS 202 amplifier (Crown Audio, Inc., Elkhart, IN) and broadcast through the Orb Mod 1 speaker located

30 cm in front of the frog. Examples of click stimuli and tone stimuli broadcast through this setup are shown in Appendix 1, Fig A1-1.

Each recording session began and ended by verifying the presence of a biological signal in response to sound. To do this, we presented a stimulus train consisting of five 0.1-ms rectangular-pulse, broadband clicks (24.9-ms inter-click intervals) at either 85 dB or 90 dB pSPL (peak equivalent SPL re. 1 kHz tone), followed by a 100-ms silent interval, as illustrated in Fig. 1-1b (top). When output through our setup, the spectrum of the clicks was broadband, with a center frequency of approximately 1.6 kHz and 6-dB down points of approximately 0.345 and 2.8 kHz. Together, these procedures allowed us to verify the presence of an ABR in response to a suprathreshold stimulus (the clicks), to measure the biological signal in the absence of a stimulus (during the 100-ms silent interval), and to verify that neural responses to sound did not change during a recording session. If signals were very small or noisy, electrodes were repositioned until a robust, repeatable signal was acquired. Visual inspection of these click-evoked ABRs indicated no change in responses over the duration of recording sessions.

After initially verifying the presence of a robust ABR, we next investigated the effects of stimulus frequency and intensity on the ABR by presenting subjects with stimulus trains that consisted either of nine tone bursts or nine clicks, examples of which are depicted in Fig. 1-1b (middle and bottom, respectively). Tone trains consisted of 5-ms pure tones (1-ms rise/fall $\cos^2$) separated by 20-ms inter-stimulus intervals. The frequency of tones was held constant within a tone train. In different tone trains, frequency was varied across 21 values ranging from 0.35 kHz to 5.0 kHz. These frequencies included (in kHz) 0.35, 0.5. 0.75, 0.875, 1.0 to 1.5 (in 0.1 kHz steps), 1.625, 1.75, 1.875, 2.0 to 2.8 (in 0.2 kHz steps), 3.0, 4.0, and 5.0. Click trains consisted of clicks (as described above) that were 0.1 ms in duration and separated by 24.9 ms inter-click intervals.

The nine consecutive tones or clicks in the train increased in 5-dB steps over a 40-dB range (see Fig. 1-1b), starting at either 45, 50, or 55 dB and ending at 85, 90, or 95 dB (SPL re 20 μPa, fast RMS, c-weighting for tones; pSPL for clicks). Because of variation in sensitivity across both subjects and frequencies, we selected an absolute range for each stimulus train that included values above and below the visually detected ABR threshold (see below). For all stimuli, we obtained two replicate averages of the ABR, each based on averaging responses to 400 consecutive presentations of each stimulus train (800 presentations total across both replicates). Stimulus trains were presented at a rate of 4 train/s. All consecutive sounds within a stimulus train and between each repeated presentation of the train alternated in phase (tones) or polarity

(clicks) to reduce the microphonic potential. During acquisition of the first set of replicate averages of the ABR, presentations of click trains preceded presentations of tone trains; this order was reversed for acquiring the second replicates. Within each replicate, the order of tone trains

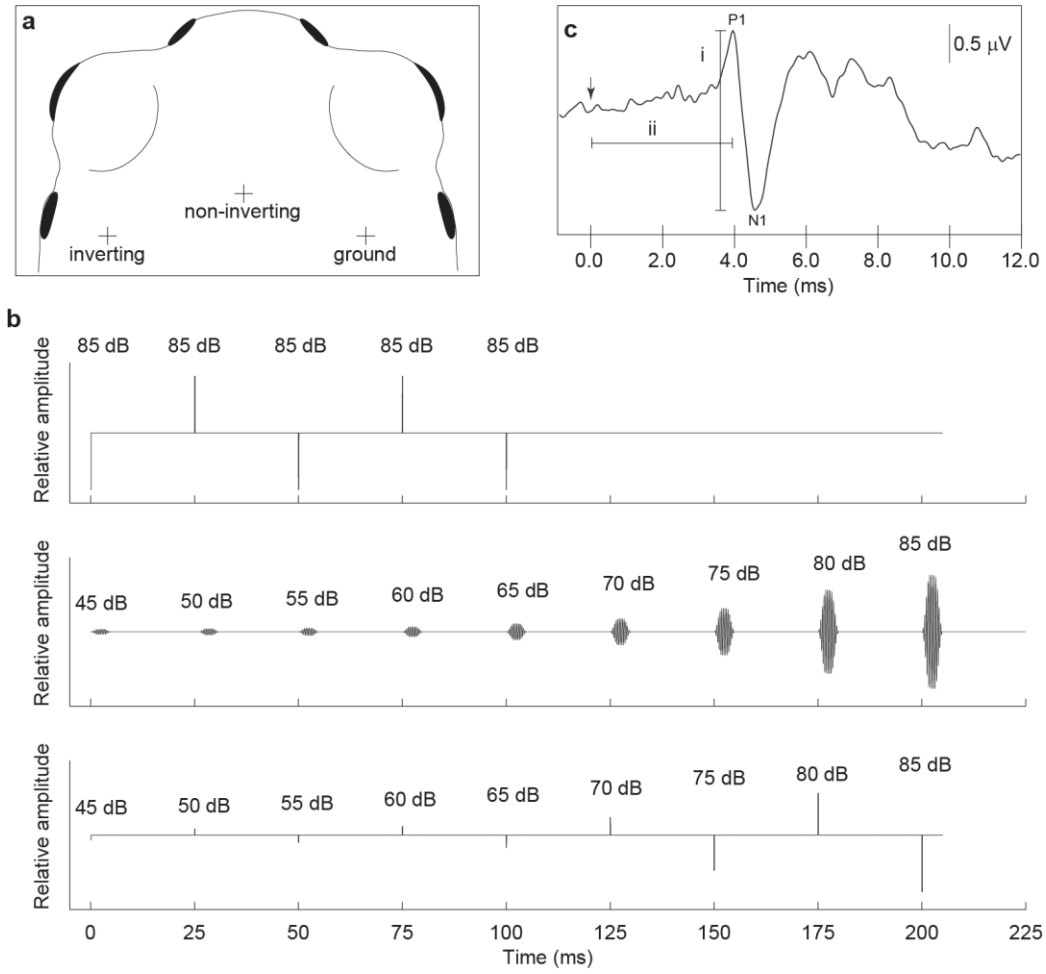


Figure 1-1 Recording procedures

a *Placement of recording electrodes depicted by "+"s.* ***b*** *Schematics of stimulus trains used in experiments. Depicted are examples of (top) a train of 5 clicks (0.1 ms, 24.9 ms inter-click interval) followed by a 100-ms period of silence broadcast at the beginning and end of a session to verify the presence of a signal (during clicks) and to obtain a recording in the absence of stimulus (during silence), (middle) a train consisting of 9 tones (5 ms in duration, 20-ms inter-tone interval) of increasing intensity, and (bottom) a train of 9 clicks of increasing intensity. Stimulus level is indicated above each sound in the train, in dB SPL (for tones) or dB pSPL (for clicks).* ***c*** *A typical ABR waveform indicating the three measures quantified in this study, including (i) amplitude, measured from maximum of the first positive deflection (P1) to minimum of the subsequent negative deflection (N1) and (ii) latency, measured from the time sound impinged on the tympanic membranes, indicated by the arrow, to the time of P1.*

was randomized for each subject. On rare occasions, a subject would produce an isolated buccal pumping movement that produced a large artifact in the biological signal. Recordings disrupted by such artifacts were immediately rejected and repeated.

The sound levels of tone trains were calibrated by placing the 1/2" condenser microphone of a Larson Davis System 831 sound-level meter 30 cm from the speaker at the approximate position of a subject's head during a recording session. The tone of highest intensity in each train was calibrated by matching its peak-to-peak voltage to that of a 1-s tone of the same frequency calibrated to the highest level in the train (85, 90, or 95 dB SPL). The voltage of subsequent tones in each train was digitally adjusted to achieve the appropriate nominal sound level. The frequency response of our system was ± 2 - 2.5 dB, based on recordings of the tone trains in the chamber. We used the peak-to-peak voltage of the calibrated 1 kHz tone to calibrate the pSPL of clicks.

ABR characterization

We characterized the morphology of the ABR waveform across stimuli using descriptive cross-correlation analyses and standard quantitative measurements of ABR amplitude and latency. The cross-correlation analysis was designed to assess how the general shape of the ABR varied between responses to clicks and tones and across different tone frequencies. We focused these cross-correlation analyses on tones with frequencies of 1.3, 1.625, and 2.6 kHz. We chose frequencies of 1.3 and 2.6 kHz for two reasons. First, they are close to the average frequencies present in the bimodal spectrum of male advertisement calls in our study populations (Schrode et al. 2012). Second, according to the "matched filter hypothesis" of anuran hearing (Capranica and Moffat 1983), they are presumed to be encoded primarily by the amphibian papilla (AP) and the basilar papilla (BP), respectively (Gerhardt, 2005; Hillery, 1984b). We chose 1.625 kHz as an additional frequency for further investigation because it was intermediate between the expected ranges encoded by each papilla. For cross-correlation analyses, we removed the DC offset from each response by subtracting its baseline mean amplitude and then positioned a 10-ms analysis window over the response extending from 2 ms before the peak of the first positive deflection (P1) to 8 ms after this peak (i.e., from -2 ms to +8 ms relative to P1 at 0 ms). We then averaged windowed responses across both replicates obtained for each individual before determining the average response across all individuals. We used MATLAB's *xcorr* function to compute the maximum cross-correlation coefficient comparing the average responses to tones of 1.3, 1.625, and 2.6 kHz and clicks to the average responses to tones at each frequency tested. These analyses were replicated at stimulus levels of 70, 75, 80, and 85 dB SPL. We selected these particular

levels for analysis because they reliably elicited robust responses from most individuals at most frequencies. To rule out the possibility that differences in cross correlations resulted from frequency-dependent differences in sensation level (SL), we performed additional cross-correlation analyses using the average responses recorded at a common sensation level of approximately 10 dB SL. We defined 10 dB SL as the stimulus level nearest to 10 dB above the average visually detected threshold across all individuals for that stimulus (see below).

We measured ABR amplitude and latency in responses to all combinations of stimulus and level at which a visually detectable ABR waveform was present. The first peak (P1) and the subsequent trough (N1) constituted the only visible, event-related deflection of the ABR waveform that were observed consistently in responses to both clicks and tones across all subjects. An example of these measurements is illustrated in Fig. 1-1c. We measured ABR amplitude (hereafter "amplitude") as the absolute voltage difference (in μV) between P1 and N1 (i.e., the peak-to-trough voltage; Fig. 1-1c). We measured ABR latency (hereafter "latency") as the time from when the stimulus arrived at the frogs' ears to P1 (Fig. 1-1c). We calculated 0.88 ms as the time required for sound to travel the 30-cm distance between the speaker and the frogs' ears given the range of temperatures recorded in the test chamber. Values of amplitude and latency in response to each stimulus were averaged across the two replicates for statistical analysis.

Threshold determination and ABR audiograms

We assessed auditory sensitivity using two methods of threshold estimation. First, two experienced observers independently determined ABR thresholds based on visual detection (e.g., Brittan-Powell et al. 2002, 2005, 2010b; Brittan-Powell and Dooling 2004; Lohr et al. 2013). We plotted the responses to each tone or click within a stimulus train in order of descending stimulus level, as illustrated for a single individual in Fig. 1-2. We operationally defined threshold as the arithmetic mean of the lowest stimulus level at which an ABR waveform was visible and the next lowest intensity (Fig. 1-2, arrowheads). Since stimulus level varied in 5-dB steps, threshold was effectively the sound pressure level 2.5 dB (one-half step) below the lowest stimulus level at which a response could be visually detected. We used a 10-ms window beginning 2 ms after stimulus onset for visual detection of responses. Threshold differences between the two observers were small (across all estimates: mode = 0.0 dB, median = 0.0 dB, and mean = 0.9 ± 3.8 dB), and the agreement between observers was quite high (intraclass correlation: r = 0.89). Below, we report thresholds that were averaged over the two stimulus replicates and across both observers.

As a second method of threshold estimation, we performed an automated, objective analysis in which we compared the predicted RMS amplitude of the ABR in response to a stimulus to the RMS amplitude of the biological signal determined when no stimulus was presented. We separately computed predicted RMS amplitudes for each frog and each stimulus as follows. Using MATLAB's *fminsearch* optimization function, we minimized the sum of squares to find the best-fit sigmoid curve fitting the actual RMS amplitudes of the biological signal computed over a 10-ms window between 2-12 ms after stimulus onset, averaged between replicates, and plotted as a function of stimulus level (see Fig. 1-5a in the Results section for an example). These fits had a mean (± s.d.) R^2 of 0.94 ± 0.13 across subjects (N = 35). We determined the ABR threshold as the lowest stimulus level at which the fitted curve of predicted RMS amplitudes first exceeded a fixed threshold criterion. This criterion was based on the mean and standard deviation of the RMS amplitudes of the biological signal recorded from the same

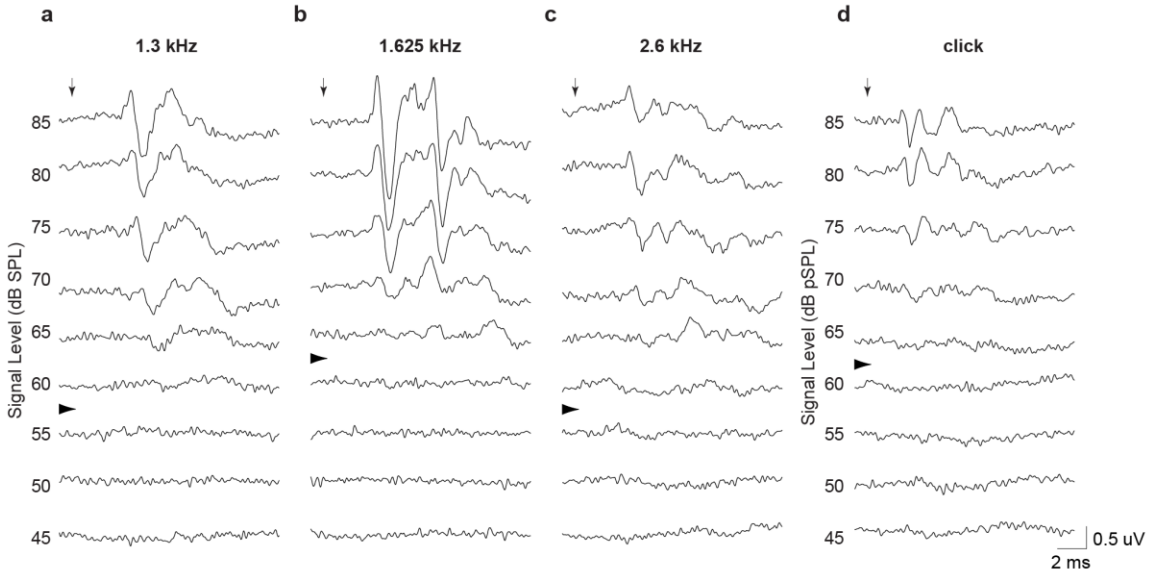


Figure 1-2 Responses from a single individual
Representative recordings of ABRs from a single individual in response to tones at ***a*** *1.3 kHz,* ***b*** *1.625 kHz,* ***c*** *2.6 kHz, and in response to* ***d*** *clicks presented at different sound pressure levels.* Downward pointing arrows *depict arrival times of sound at the tympanic membranes. The* right-pointing arrowheads *depict the visually detected thresholds for each frequency or for clicks*

animal when no acoustic stimulus was broadcast. We estimated these two statistical parameters separately for each individual by computing the RMS amplitudes of the biological signal in six 10-ms analysis windows (60 ms total) that were recorded in the absence of a stimulus at the beginning (three windows) and ending (three windows) of each recording session. This procedure allowed us to estimate for each subject a mean and standard deviation for the "baseline" RMS

amplitude of the neural recording in the absence of acoustic stimulation by tones or clicks. We explored three criterion values corresponding to 0.5, 1.0, and 2.0 standard deviations above the mean baseline RMS amplitude and calculated the threshold for each as the minimum predicted RMS amplitude value exceeding each criterion value.

Statistical analyses

We used repeated measures analysis of covariance (ANCOVA) to investigate the effects of frequency, level, sex, and size on tone-evoked and click-evoked responses. Estimates of threshold were available for all subjects in response to clicks and to tones at all 21 frequencies tested. This was not the case for our quantitative measures of ABR amplitude and latency, for which measures were only available for stimuli that produced a visually detectable ABR. Hence, sub-threshold stimulus levels resulted in missing values of amplitude and latency, which we dealt with using a two-step procedure. First, we limited our statistical analyses of ABR amplitude and latency to reduced datasets that included stimuli with which all subjects were tested and that elicited visually detectable responses from a majority of subjects. For tone-evoked responses, we included only the 17 frequencies between 0.75 kHz and 3.0 kHz (inclusive). For both tone-evoked and click-evoked responses, we included only the five stimulus levels between 65 dB and 85dB SPL (inclusive). This procedure reduced the proportion of missing values of amplitude and latency to 10% for tone-evoked responses and 14% for click-evoked responses. Second, we used multiple imputation, in which Monte Carlo methods are used to simulate the remaining missing values m times (Rubin 1976). A small number of imputations (e.g. m = 3-5) is generally used, as increasing this number does not significantly increase the accuracy of the estimated values (Rubin, 1976; Schafer, 1999; Schafer and Olsen, 1998). However, a larger number of imputations is associated with greater statistical power (Graham et al., 2007), and so we used 20 imputations (m = 20) for each of our four reduced datasets.

We performed separate factorial ANCOVAs for amplitude and latency and for each imputed dataset. For tone-evoked responses, each analysis consisted of a 17 frequency (within) × 5 level (within) × 2 sex (between) ANCOVA with subject size included as the covariate. For click-evoked responses each analysis consisted of a 5 level (within) × 2 sex (between) ANCOVA, with size as the covariate. Values of amplitudes and latencies were log-transformed to achieve normality prior to analysis. For each response variable, we report the mean and range of the resulting F statistics, effect sizes (partial η^2), and P values calculated over the 20 imputed datasets for that variable. We compared visually detected ABR thresholds in response to tones and clicks

using separate repeated measures ANCOVAs. Our analysis of tone-evoked responses consisted of a 21 frequency (within) × 2 sex (between) ANCOVA with subject size as the covariate. We compared thresholds for click-evoked responses between sexes with a univariate ANCOVA having sex as the single between-subjects factor (2 levels) and subject size as the covariate.

For all repeated measures analyses, we report *P*-values for omnibus tests having more than a single numerator degree of freedom based on the Greenhouse and Geisser (1959) correction method. In all ANCOVAs, the covariate of size was based on subject mass and was centered around the mean mass by subtracting the mean from each subject's mass prior to analysis. We employed a significance criterion of $\alpha = 0.05$ for all ANCOVAs. For multiply imputed data, an effect was considered significant if the mean *P*-value was below 0.05.

Results

ABR characterization

Average ABR waveforms evoked by 75-dB broadcasts of tones (1.3, 1.625, and 2.6 kHz) and clicks are illustrated in Fig. 1-3a-d. In many cases, the N1 deflection was much larger (relative to baseline) than that of the preceding P1 deflection (Fig. 1-3a-d; see also Fig. 1-2). The presence of additional peaks after P1-N1 was variable, both across animals and across stimuli (Fig. 1-3a-d). For example, responses to the 1.3 kHz tone (Fig. 1-3a) commonly had one prominent peak (P1) followed by a broader peak or plateau, while responses to the 2.6 kHz tone (Fig. 1-3c) typically included P1 and two additional prominent peaks. Responses at the intermediate frequency of 1.625 kHz (Fig. 1-3b) had a prominent P1 followed by a multi-peaked plateau and resembled a combination of the responses observed at 1.3 and 2.6 kHz. Responses to clicks were generally more similar to those elicited by the 2.6 kHz tones in having a pronounced P1 followed by two or more additional peaks (cf. Fig. 1-3c, d).

The results of the cross-correlation analyses are depicted in Fig. 1-3e-h. The patterns of cross-correlations were broadly similar across the range of 70 dB to 85 dB signal levels and at ~10-dB SLs. As depicted in Fig. 1-3e-h, comparisons of tone-evoked responses revealed the presence of two different waveform shapes, one characteristic of responses to lower tone frequencies (between 0.75 kHz and 1.5 kHz) and a second characteristic of responses to higher frequencies (between 1.75 kHz and 4 kHz), with a sharp transition between these two shapes (between 1.5 kHz and 1.75 kHz). Consider first responses to the 1.3-kHz tone (Fig. 1-3e). The ABRs evoked by the 1.3-kHz tone were most similar to those elicited by tones with frequencies ranging from 0.75 kHz to about 1.5 kHz, as indicated by cross-correlation coefficients near 1.0. Correlations were markedly weaker at frequencies below 0.75 kHz (Fig. 1-3e). Between 1.5 and 1.75 kHz, there was a sharp transition to somewhat lower correlation coefficients that remained similar up to about 4.0 kHz, above which correlations became even weaker (Fig. 1-3e). A near mirror image of this general pattern was found in correlations with responses to the 2.6-kHz tone

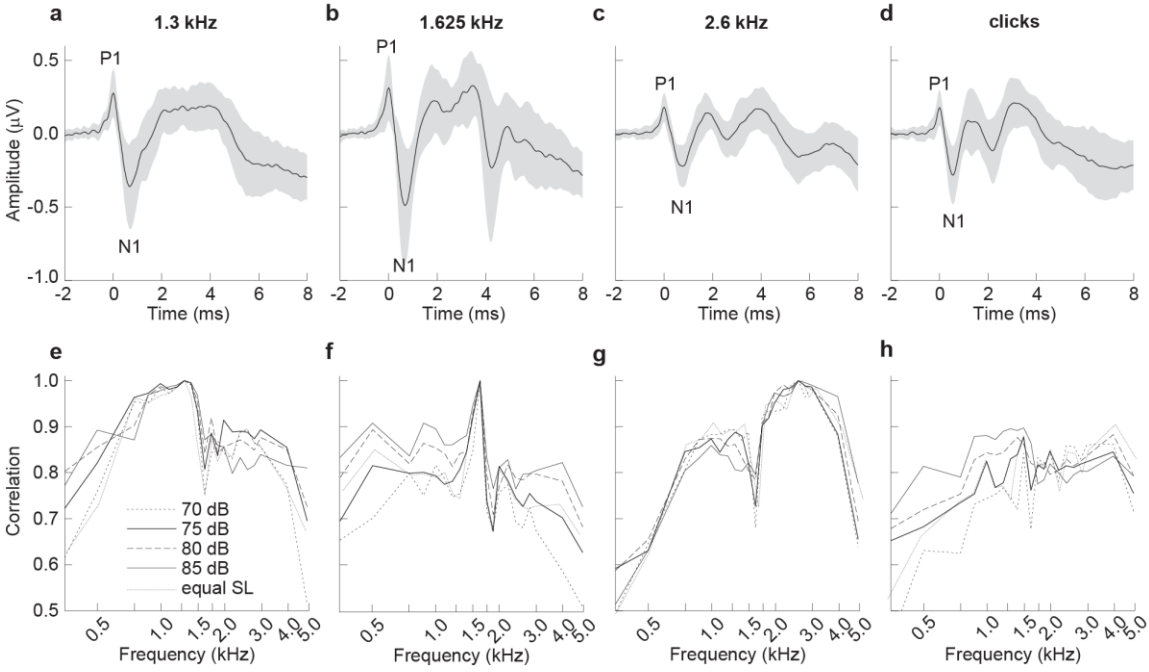


Figure 1-3 Average traces and cross-correlation analyses
a-d *Average traces temporally aligned to P1 (time = 0 ms) in response to tones of 75 dB SPL at frequencies of* ***a*** *1.3 kHz,* ***b*** *1.625 kHz, and* ***c*** *2.6 kHz and to* ***d*** *clicks of 75 dB pSPL.* Shaded areas *depict ± 1 s.d.* ***e-h*** *Cross correlation coefficients between the average response to tones presented at frequencies of* ***e*** *1.3 kHz,* ***f*** *1.625 kHz, and* ***g*** *2.6 kHz or to* ***h*** *clicks and average responses to all tone frequencies. Legend in* ***e*** *applies to* ***e-h*** *and is in units of dB SPL (for tones) or dB pSPL (for clicks)*

(cf. Fig. 1-3e and 1-3g). Responses to the 2.6-kHz tone (Fig. 1-3g) were most similar to those across a range of frequencies extending between about 1.75 and 4.0 kHz. Cross-correlation coefficients dropped off sharply above 4.0 kHz, and there was, again, a distinctive transition to lower correlation coefficients below 1.5 kHz. Coefficients decreased even further at frequencies below about 0.75 kHz (Fig. 1-3g). The ABR waveforms evoked by the intermediate tone frequency of 1.625 kHz (Fig. 1-3f) were most similar to those at 1.5 kHz, with somewhat lower coefficients at frequencies below 1.5 kHz. Correlations were generally even weaker at frequencies of 1.75 kHz and above (Fig. 1-3f). Coefficients for the cross-correlations between click-evoked responses and the responses to tones at different frequencies (Fig. 1-3h) were somewhat more variable as a function of stimulus level than the correlations observed between tones (Fig. 1-3e-g). Generally, correlations between click-evoked and tone-evoked responses (Fig. 1-3h) tended to be somewhat higher and more consistent across stimulus levels for responses to higher tone frequencies.

Effects of frequency, level, sex, and size

ABR amplitude

Repeated measures ANCOVAs revealed significant differences in the amplitude of tone-evoked responses due to the main effects of frequency and level (Table 1). The main effect of frequency was moderately large (partial $\eta^2 = 0.45$, Table 1), while the main effect of stimulus level had the largest effect size in the ANCOVA model (partial $\eta^2 = 0.84$, Table 1). The two-way interactions of frequency × level and frequency × sex were also significant, but were associated with smaller effect sizes (partial $\eta^2 \leq 0.12$, Table 1).

The effects of frequency, level, and sex on ABR amplitudes are illustrated in Figure 4 for responses averaged over all subjects in contour plots (Fig. 1-4a) and for each sex separately as iso-intensity plots (Fig. 1-4b). For responses to tones across levels, ABR amplitudes varied between 0.4 and 2.1 μV. There was a sharp discontinuity in ABR amplitude in an intermediate frequency range from about 1.5 kHz to 1.75 kHz (indicated by the solid arrows in Fig. 1-4a, c). At a given stimulus level, amplitudes tended to be larger for frequencies below this frequency range (< 1.5 kHz) compared with frequencies above it (> 1.75 kHz) and highest within this intermediate frequency range (Fig. 1-4a, b). As illustrated in Figure 4a-b, ABR amplitudes increased as a function of level varied across frequencies, which accounts for the significant

Table 1-1 Repeated measures analysis of covariance (ANCOVA) for ABR amplitude.
Shown are means (and ranges) for *F* statistics, *P* values, and effect sizes (partial η^2) from analysis of the 20 imputed datasets. Bold values indicate variables in which the mean *P*-value ≤ 0.05

Stimulus	Effect	df	*F*	*P*	Partial η^2
Tones	**frequency**	**16, 512**	**26.6 (22.7 - 29.8)**	**<0.001 (all <0.001)**	**0.45 (0.42 - 0.48)**
	level	**4, 128**	**169.8 (137.6 - 204.4)**	**<0.001 (all <0.001)**	**0.84 (0.81 - 0.86)**
	sex	1, 32	1.1 (0.8 - 1.4)	0.306 (0.243 - 0.370)	0.03 (0.03 - 0.04)
	size	1, 32	0.1 (<0.1 - 0.2)	0.752 (0.653 - 0.825)	<0.01 (<0.01 - 0.01)
	frequency x level	**64, 2048**	**4.3 (3.7 – 5.0)**	**<0.001 (all <0.001)**	**0.12 (0.10 - 0.14)**
	frequency x sex	**16, 512**	**3.8 (2.9 - 4.6)**	**0.015 (0.006 - 0.042)**	**0.11 (0.08 - 0.13)**
	frequency x size	**16, 512**	0.9 (0.6 - 1.4)	0.440 (0.245 - 0.603)	0.03 (0.02 - 0.04)
	level x sex	4, 128	0.2 (<0.1 - 0.6)	0.745 (0.496 - 0.962)	0.01 (<0.01 - 0.02)
	level x size	4, 128	0.6 (0.3 - 1.2)	0.531 (0.299 - 0.692)	0.02 (0.01 - 0.04)
	frequency x level x sex	64, 2048	1.4 (1.1 - 1.8)	0.156 (0.040 - 0.362)	0.04 (0.03 - 0.05)
	frequency x level x size	64, 2048	1.0 (0.7 - 1.3)	0.473 (0.194 - 0.767)	0.03 (0.02 - 0.04)
Clicks	**level**	**4, 128**	**86.3 (31.6 - 155.9)**	**<0.001 (all <0.001)**	**0.70 (0.50 - 0.83)**
	sex	1, 32	1.3 (0.2 - 2.6)	0.291 (0.117 - 0.651)	0.04 (0.01 - 0.08)
	size	1, 32	0.2 (<0.1 - 1.0)	0.723 (0.321 - 0.972)	0.01 (<0.01 - 0.03)
	level x sex	4, 128	1.9 (0.3 - 5.5)	0.253 (0.011 - 0.658)	0.05 (0.01 - 0.15)
	level x size	4, 128	1.1 (0.1 - 6.6)	0.534 (0.005 - 0.898)	0.03 (<0.01 - 0.17)

two-way interaction between frequency and level (Table 1). There was generally little difference between the ABR amplitudes of males and females in response to tones at most frequencies, though a more notable difference occurred at intermediate frequencies (Fig. 1-4b). This trend accounts for the relatively weak (partial $\eta^2 = 0.11$) but significant two-way interaction between frequency and sex (Table 1). We found no indication that the amplitudes of tone-evoked responses varied as a function of subject body size (Table 1).

Repeated measures ANCOVAs for the amplitudes of click-evoked responses revealed a large and significant effect of stimulus level (Table 1). No other effects or interactions in the ANCOVA models were significant. On average, click-evoked responses were typically smaller than tone-evoked responses. At a given stimulus level, the amplitudes of click-evoked responses were similar to the amplitudes of the smallest tone-evoked responses to tones (e.g. tone frequencies > 2.0 kHz). Mean amplitudes increased as a function of stimulus level from 0.5 μV at 65 dB to 1.0 μV at 85 dB (Fig. 1-4a, b). The effects of subject sex and the covariate of body size were quite small compared with the effect of stimulus level (Table 1).

ABR latency

In our ANCOVAs for the latency for tone-evoked responses, there were significant main effects of frequency, level, and sex, and significant two-way interactions of frequency × level and frequency × sex (Table 2). Evaluation of effect sizes, however, indicated that the main effects of frequency (partial $\eta^2 = 0.77$) and level (partial $\eta^2 = 0.89$) were much more important than other effects in the model (partial $\eta^2 \leq 0.18$) in determining the latency of tone-evoked responses (Table 2).

Contour plots and iso-intensity plots of ABR latency are depicted in Figure 4c and 4d, respectively. In response to tones, latencies typically ranged between 3 and 7 ms and decreased as a function of increasing frequency, particularly in the range of frequencies between 0.35 and 1.5 kHz. As with amplitudes, a discontinuity in the latencies of tone-evoked responses occurred in the frequency region between 1.5 and 1.75 kHz (Fig. 1-4c, d). For a given stimulus level, latencies below 1.5 kHz were generally longer than those above 1.75 kHz. Latencies decreased as stimulus level increased (Fig. 1-4c, d). Latencies were slightly shorter for females compared to males, particularly at tone frequencies below ~1.75 kHz (Fig. 1-4d), resulting in the significant main effect of sex and its interaction with frequency. There was no indication that ABR latencies varied as a function of subject body size.

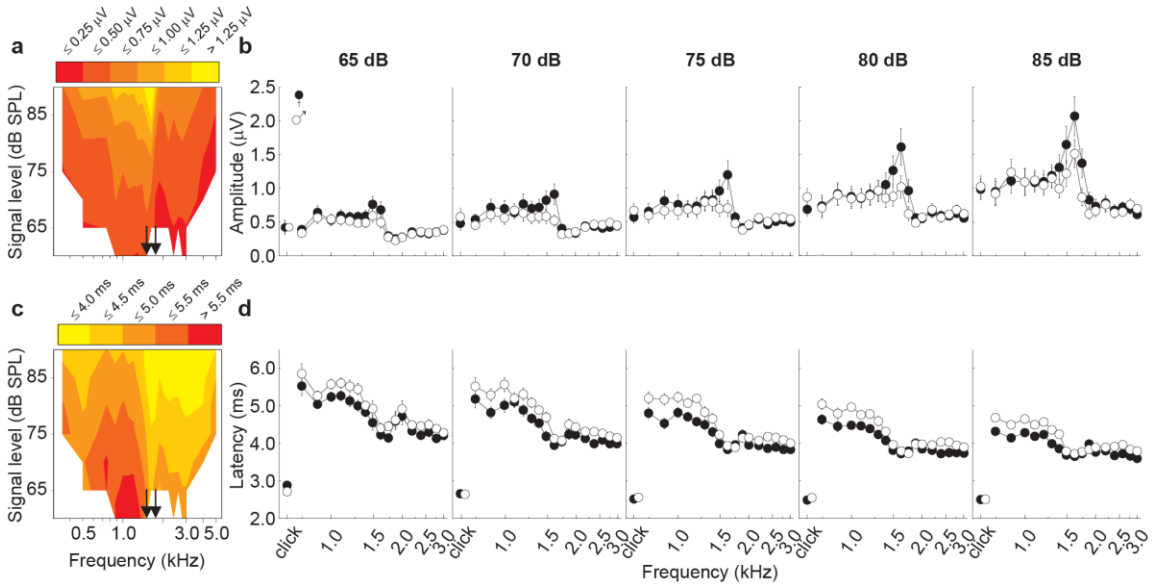


Figure 1-4 Amplitudes and latencies in response to tones and clicks
Characterization of the ABR in terms of ***a-b*** *amplitude (absolute voltage difference between P1 and N1) and* ***c-d*** *latency (time to P1 from sound arrival at tympanic membranes).* ***a & c*** *Mean amplitude and latency (averaged over all individuals) depicted in the form of a contour plot across all frequencies and all levels.* Arrows *indicate the range of frequencies (1.5 – 1.75 kHz) within which there is a sharp discontinuity in the values of the response measure.* ***b & d*** *Mean (±s.e.m.) amplitude and latency of click-evoked responses and tone-evoked responses across frequencies at five stimulus levels. Data are shown separately for males (*open circles*) and females (*filled circles*). The data depicted in* ***b*** *and* ***d*** *represent reduced datasets that included responses to clicks at levels of 65 to 85 dB pSPL and tones of frequencies from 0.75 kHz to 3.0 kHz presented at levels of 65 dB to 85 dB SPL. Plotted data were pooled across multiple imputations of the reduced datasets (see text). Values for some click-evoked responses are slightly displaced along the x-axis to reveal symbols otherwise hidden*

In the ANCOVA models for the latency of click-evoked responses, only the effect of stimulus level was significant (Table 2). Latencies for click-evoked responses were shorter than those for tone-evoked responses and were in the range of 2 to 3 ms (Fig. 1-4c, d). As with tone-evoked responses, the latencies of click-evoked responses decreased with increasing stimulus level (Fig. 1-4c, d). Neither sex nor body size influenced latencies in response to clicks.

ABR thresholds

General patterns of threshold differences across most frequencies were broadly similar between the different methods of threshold estimation examined here; that is, all resulting audiograms had

Table 1-2 Repeated measures analysis of covariance (ANCOVA) for ABR latency.
Shown are means (and ranges) for F statistics, P values, and effect sizes (partial η^2) from analysis of the 20 imputed datasets. Bold values indicate variables in which the mean P-value ≤ 0.05

Stimulus	Effect	df	*F*	*P*	Partial η^2
Tones	**frequency**	**16, 512**	**106.3 (96.4 - 115.7)**	**<0.001 (all <0.001)**	**0.77 (0.75 - 0.78)**
	level	**4, 128**	**258.6 (192.7 - 365.1)**	**<0.001 (all <0.001)**	**0.89 (0.86 - 0.92)**
	sex	**1, 32**	**6.9 (5.1 - 8.3)**	**0.014 (0.007 - 0.031)**	**0.18 (0.14 - 0.21)**
	size	1, 32	<0.1 (<0.1 - 0.1)	0.904 (0.792 - 0.997)	<0.01 (all <0.01)
	frequency x level	**64, 2048**	**2.7 (1.7 - 3.9)**	**0.012 (<0.001 - 0.079)**	**0.08 (0.05 - 0.11)**
	frequency x sex	**16, 512**	**2.3 (1.4 - 3.1)**	**0.041 (0.004 - 0.201)**	**0.07 (0.04 - 0.09)**
	frequency x size	16, 512	0.8 (0.4 - 1.2)	0.569 (0.316 - 0.897)	0.03 (0.01 - 0.04)
	level x sex	4, 128	0.7 (0.1 - 2.5)	0.569 (0.087 - 0.948)	0.02 (<0.01 - 0.07)
	level x size	4, 128	1.1 (0.2 - 2.7)	0.417 (0.079 - 0.868)	0.03 (0.01 - 0.08)
	frequency x level x sex	64, 2048	1.2 (0.8 - 1.8)	0.345 (0.062 - 0.649)	0.04 (0.02 - 0.05)
	frequency x level x size	64, 2048	1.1 (0.7 - 1.8)	0.365 (0.056 - 0.713)	0.03 (0.02 - 0.05)
Clicks	**level**	**4, 128**	**23.2 (6.7 - 34.9)**	**<0.001 (<0.001 - 0.006)**	**0.41 (0.17 - 0.52)**
	sex	1, 32	0.1 (<0.1 - 0.7)	0.768 (0.410 - 0.991)	<0.01 (<0.01 - 0.02)
	size	1, 32	3.6 (1.4 - 6.5)	0.087 (0.016 - 0.238)	0.10 (0.04 - 0.17)
	level x sex	4, 128	3.2 (0.8 - 6.7)	0.114 (0.002 - 0.474)	0.09 (0.02 - 0.17)
	level x size	4, 128	2.2 (0.1 - 5.7)	0.265 (0.011 - 0.941)	0.06 (<0.01 - 0.15)

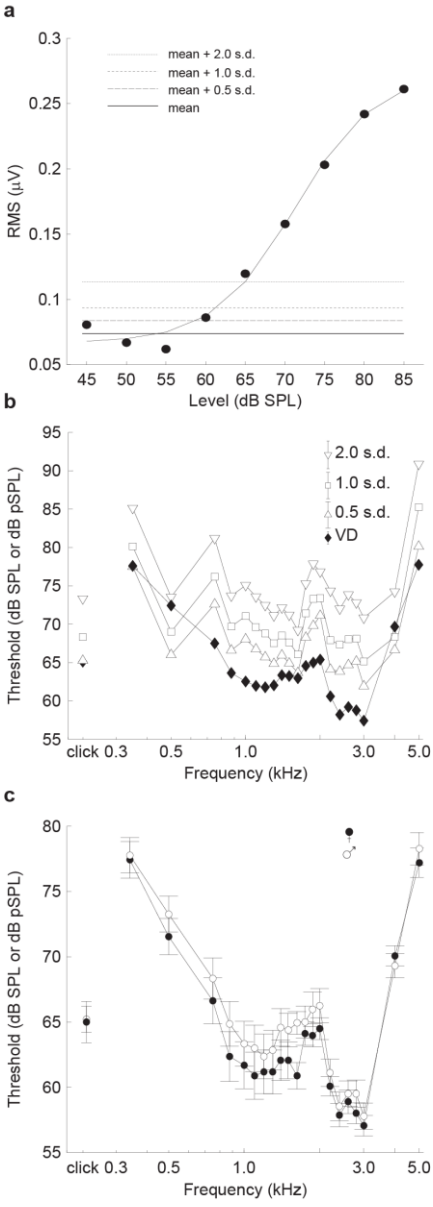
the same general shape (Fig. 1-5b). An exception to this generalization occurred at 0.5 kHz, which corresponded with a "dip" in automated thresholds that was not apparent in the visually detected thresholds. At present, it is not clear what is responsible for this difference between detection methods. As would be expected, increasing the threshold criterion for automated detection of responses from 0.5 s.d. to 2.0 s.d. resulted in progressively higher threshold estimates (Fig. 1-5a, b). The audiogram generated using a threshold criterion of 0.5 s.d. most closely matched that generated using visual threshold detection, with thresholds averaging about 5 dB higher across frequencies for automated thresholds (Fig. 1-5b). The mean (± s.e.m. here and elsewhere) threshold in response to clicks using a criterion of 0.5 s.d. was 65.3 ± 0.9, which was close to the average visually detected threshold of 65.1 ± 0.9 (Fig. 1-5b).

For clarity and brevity, and because of general similarities in shape, we focus here on interpreting the audiogram based on visually detected thresholds. One region of best sensitivity was broadly centered around 1.2 kHz (between 0.875 and 1.5 kHz), and a second was centered around 2.6 kHz (between 2.2 and 3.0 kHz). Visually detected thresholds in the lower frequency region ranged between 61 to 63 dB and were generally 2.5 to 4.4 dB higher than thresholds for the higher frequency region. Visually detected thresholds in a mid-frequency region between 1.5 and 2.0 kHz were higher than the most sensitive frequencies by about 5 to 8 dB. The slopes of the changes in thresholds between this less sensitive mid-frequency region and the adjacent, more sensitive regions at lower and higher frequencies were, respectively, 3.9 dB/octave (computed between 1.1 to 2.0 kHz) and -13.6 dB/octave (computed between 2.0 to 3.0 kHz). At the extreme low (< 0.875 kHz) and high (> 3.0 kHz) frequencies tested, visually detected thresholds increased to 15 to 20 dB over peak sensitivity. The changes in threshold occurred with a slope of -10.6 dB/octave below 0.875 kHz and 27.6 dB/octave above 3.0 kHz.

For tone-evoked ABRs, a repeated measures ANCOVA revealed significant differences in threshold related to differences in frequency ($F_{20, 640} = 87.8$, $P < 0.001$, partial $\eta^2 = 0.73$), but not sex ($F_{1, 32} = 1.0$, $P = 0.338$, partial $\eta^2 = 0.03$) or size ($F_{1, 32} = 0.2$, $P = 0.701$, partial $\eta^2 = 0.01$). The frequency × sex ($F_{20, 640} = 0.7$, $P = 0.595$, partial $\eta^2 = 0.02$) and frequency × size ($F_{20, 640} = 0.9$, $P = 0.447$, partial $\eta^2 = 0.03$) interactions were also not significant. Averaged across frequencies, females had slightly lower visually detected thresholds compared with males (Fig. 1-5c; females: 63.8 ± 0.4 dB; males: 64.9 ± 0.4 dB). This non-significant trend for a sex difference was slightly more pronounced at frequencies below 2.2 kHz (Fig. 1-5c). For click-evoked responses, average thresholds for males and females differed by less than 1.5 dB and there was no significant effect

of sex (Fig. 1-5c; $F_{1, 32} < 0.1$, $P = 0.898$, partial $\eta^2 < 0.01$) or the covariate of size ($F_{1, 32} = 0.1$, $P = 0.821$, partial $\eta^2 < 0.01$).

Figure 1-5 Audiograms



a *A representative example of threshold detection based on predicted values of RMS amplitudes of ABRs in response to stimuli presented at different levels. RMS amplitudes of responses were computed over a 10 ms analysis window that began when the stimulus arrived at the tympanic membranes. Depicted here is the best-fit sigmoid curve fit to RMS data for responses from one frog to tones of 2.6 kHz. Thresholds determined from the fits for each stimulus for each frog were averaged to construct the audiogram based on automatically detected thresholds plotted in* ***b****. Different threshold criteria based on the mean (*solid bold line*) and s.d. of the RMS amplitude of the biological signal recorded in the absence of a stimulus are indicated by* dashed lines*.* ***b*** *Comparison of audiograms and click-evoked response thresholds based on visually detected (VD) thresholds (*filled diamonds*) and automatically determined thresholds based on different criteria (*open triangles and squares*). To improve clarity of the plot, error bars are not shown for individual data. Error bars in the legend depict the s.e.m. averaged across frequencies and clicks for each threshold determination method.* ***c*** *Comparisons of mean ± s.e.m. visually detected thresholds for males (*open circles*) and females (*filled circles*) for responses to tones of different frequencies and to clicks*

Discussion

ABR characterization

The ABRs we recorded in Cope's gray treefrogs were characterized by a series of positive and negative deflections in amplitude. This general morphology, which is consistent with ABRs recorded across a wide range of taxa (Boettcher et al., 1993; Brittan-Powell et al., 2002; Brittan-

Powell et al., 2010a; Higgs et al., 2002; Lucas et al., 2002; Popov and Supin, 1990; Walsh et al., 1986), is also similar to that described from invasive studies of evoked potentials in frogs (Carey and Zelick 1993; Corwin et al. 1982; Katbamna et al. 2006b; Seaman 1991). While all ABRs in gray treefrogs shared the features of a distinctive P1 and N1, cross-correlation analyses suggested two discrete classes of tone-evoked waveforms, typified by responses to 1.3 kHz tones and 2.6 kHz tones. A low-frequency class included responses to frequencies below about 1.5 kHz, while a high frequency class included responses to frequencies above about 1.75 kHz. To rule out the possibility that differences in waveforms resulted from frequency-dependent differences in sensation level (SL), we compared the results for responses at a constant stimulus level to those at a common sensation level (~10 dB SL). The general patterns were still evident in the analysis of responses at a common sensation level. This result confirms that frequency-dependent differences in shape of the ABR waveform were not merely a reflection of frequency-dependent differences in sensitivity. Quantitative analyses of the effects of stimulus frequency and level on ABR amplitudes and latencies revealed discontinuities at intermediate frequencies that also support a division of responses into two classes. We suggest these two classes reflect differences in responses evoked by frequencies encoded primarily by the separate sensory papillae in the frogs' inner ear most sensitive to airborne sounds, the amphibian papilla (AP) and basilar papilla (BP). In most anuran species studied thus far, the AP tends to be sensitive to frequencies up to 1.0-2.0 kHz and the BP tends to be sensitive to frequencies higher than 1.0-2.0 kHz (Gerhardt and Schwartz 2001; Zakon and Wilczynski 1988). Waveforms for click-evoked responses were somewhat more similar to the 2.6 kHz frequency class. This result is consistent with reports from human studies that higher frequencies (1.0 to 4.0 kHz) are responsible for the generation of the click-evoked ABR (Hall 2007). The waveforms of the responses to the intermediate frequency of 1.625 kHz were not as easily classified as belonging to the low-frequency or high-frequency class, appearing instead to be intermediate in shape between waveforms of these two classes. We suggest suprathreshold tones at this intermediate frequency were able to excite both inner ear papillae simultaneously. Such an interpretation is consistent with previous behavioral data examining the preferences of female gray treefrogs for spectral call properties (Gerhardt 2005). From these ABR waveform data, we deduce the gray treefrog AP is sensitive to frequencies less than approximately 1.75 kHz and the BP is sensitive to frequencies above about 1.5 kHz.

ABR latencies are the main evidence used in determining the generators of ABR waves. The generator for P1 of the ABR in all animals is generally considered to be the VIIIth nerve

(Brittan-Powell et al. 2002; Lucas et al. 2002; Carey and Zelick 1993; Seaman 1991). Consistent with this view, the ABR latencies we recorded in gray treefrogs ranged from 3 to 7 ms for tone-evoked responses and 2 to 3 ms for click-evoked responses. These latency values are similar to latencies reported from single-unit recordings of VIIIth nerve fibers in other frog species (Feng, 1982; Hillery and Narins, 1984; Stiebler and Narins, 1990; Zakon and Capranica, 1981). ABR latencies in this study were also similar to those reported in more invasive studies of evoked potentials of other frogs, including *R. catesbeiana* (2.5 ms to 4 ms; Seaman 1991), *R. pipiens* (2 ms to 4 ms; Carey and Zelick 1993), and *X. laevis* (5 ms to 8 ms; Katbamna et al. 2006b). The ABR latencies reported here are also within the range of those reported for fish and reptiles, but perhaps slightly longer, on average, than those reported for mammals and birds. For example, our range of tone-evoked latencies (3 to 7 ms) is similar to the 2 to 7 ms range reported for fish (Kenyon et al. 1998) and overlaps the 6 to 10 ms range reported for alligators (Higgs et al. 2002), while latencies were 1.5 to 3 ms in gerbils (Boettcher et al. 1993) and cats (Walsh et al. 1986) and 1.5 to 4 ms in birds (Brittan-Powell et al. 2002; Henry and Lucas 2009). Our click-evoked latencies of 2 to 3 ms in gray treefrogs were similar to the 2 to 4 ms latencies reported for Tokay geckos and green anoles (Brittan-Powell et al. 2010b), but were generally longer than those reported for mammals (Klishin et al., 1990; Walsh et al., 1986) and birds (Brittan-Powell et al. 2002; Lucas et al. 2002). Previous studies showed a negative correlation between ABR latency and body temperature, which suggests that the shorter latencies of birds and mammals might, in part, be attributable to endothermy (Higgs et al. 2002).

Effects of frequency, level, sex, and size

ABR amplitude and latency

For responses to tones at a given frequency and to clicks, amplitude increased with increasing level while latency decreased, consistent with ABR recordings in many other animals (Brittan-Powell et al. 2002, 2005; Kenyon et al. 1998; Nachtigall et al. 2007; Zhang et al. 2012) and with invasive recordings of brainstem evoked potentials in other frogs (Carey and Zelick 1993; Katbamna et al. 2006b; Seaman 1991). At a given signal level, ABR amplitudes tended to be higher and latencies longer for responses to frequencies within the putative range of the AP (< 1.75 kHz) compared with those within the putative range of the BP (> 1.5 kHz). These differences were highlighted by sharp discontinuities in both measures between the values for

frequencies below about 1.5 kHz and those above 1.75 kHz. The largest amplitudes were found within this intermediate frequency range (1.5 – 1.75 kHz). ABR amplitude should depend, in part, on the number of units that respond to a stimulus. Frogs have a larger number of fibers innervating the AP than BP (Will and Fritzsch, 1988), which might lead one to speculate that this difference in numbers of fibers could account for the differences in amplitudes. However, only a subset of the fibers innervating the tonotopically-organized AP is sensitive to any given frequency. Hence the total number of AP fibers that respond to each stimulus, while unknown, is certainly less than the total number of fibers innervating the AP. Thus, the contribution of fiber number to ABR amplitude for frequencies within the range of the AP and BP in this species is unclear. However, since the intermediate frequencies of 1.5 and 1.75 kHz probably excite fibers from both auditory papillae, the exceptionally large amplitudes measured at these frequencies likely result from summation of responses of fibers from each papilla. The differences in latency between the two frequency ranges were consistent with previous work showing that fibers arising from the AP tend to have slower responses than those innervating the BP (Feng 1982; Hillery and Narins 1984; Stiebler and Narins 1990; Zakon and Capranica 1981). Within the range of the AP, latencies decreased as a function of increasing frequency, a result that is consistent with reports from mammals (Gorga et al., 1988a; Ramsier and Dominy, 2010) and birds (Brittan-Powell et al. 2002; Caras et al. 2010; Henry and Lucas 2008). In humans, it is assumed that this relationship reflects the traveling wave in the cochlea (Hall 2007). The dependence of latency on frequency in the ABRs of gray treefrogs is consistent with previous evidence for a traveling wave within the AP of anurans (Hillery and Narins 1984).

Sex differences in amplitudes and latencies were generally quite small and inconsistent across frequencies in responses to tones and negligible in response to clicks. When there was a sex difference, the trend was for females to have slightly larger ABR amplitudes and slightly shorter latencies. Overall, the effect sizes associated with sex differences in amplitude and latency were much smaller than those associated with stimulus frequency and level. While in the human ABR literature it has long been established that responses in women have larger amplitudes and shorter latencies than those in men (Jerger and Hall 1980), our results are more consistent with the lack of evidence for strong and consistent effects of sex on ABRs in nonhuman animals (Caras et al., 2010; Munro et al., 1997; Zhou et al., 2006).

We found no evidence to suggest body size influenced either the absolute magnitudes of ABR amplitude or latency or how these properties changed in response to different stimuli.

Because sex and body size are correlated in treefrogs, with females being slightly larger than males on average, this lack of a significant size effect suggests any apparent effects of sex on ABR characteristics were independent of sex-dependent size differences.

ABR thresholds

Differences in ABR thresholds were influenced by frequency for tone-evoked responses, but there were no effects of sex or size on ABR thresholds. The ABR audiogram had peaks in sensitivity around 1.2 kHz and 2.6 kHz. These frequencies correspond to the tuning of the AP and BP (Gerhardt 2005; Hillery 1984b), respectively, and are also close to the average frequencies in male advertisement calls (Schrode et al., 2012b). Thresholds increased above and below these frequencies. One might expect increased sensitivity to frequencies between the two peaks noted here, because ABR amplitudes were highest in this frequency region. However, neither of the papillae is tuned to these intermediate frequencies; rather, the large amplitudes observed to occur at intermediate frequencies are likely attributable to summation of the responses of the two auditory papillae at supra-threshold levels. Thus, at lower stimulus levels, intermediate frequencies do not stimulate either papilla, rendering these stimuli undetectable. These results are broadly consistent with predictions of the matched-filter hypothesis (Capranica and Moffat 1983), which suggests that frogs' inner ear organs are maximally sensitive to the frequencies emphasized in conspecific calls. For example, gray treefrogs often communicate in spectrally complex, mixed-species choruses, such as the typical Minnesota chorus depicted in Figure 6a (shaded area). In this example, in addition to the spectral energy in gray treefrog calls (1.25 and 2.5 kHz), leopard frogs calls contribute energy at around 0.6 kHz, American toad (*Bufo americanus*) calls correspond to the spectral peak around 1.8 kHz in the chorus, and boreal chorus frog (*Pseudacris maculata*) calls have a dominant frequency of about 3.5 kHz. Peaks in sensitivity in the ABR audiogram overlap parts of the chorus that correspond to conspecific calls, while somewhat higher thresholds occur at the frequencies emphasized in heterospecific calls (e.g. 1.6-1.8 kHz and ~3.5 kHz). This matched filtering has long been considered a mechanism to improve the detectability of conspecific calls in mixed-species choruses by increasing the signal-to-noise ratio between conspecific and heterospecific signals.

Recordings from the VIIIth nerve of frogs have not generally detected sex or size differences in the thresholds of auditory nerve fibers (Elliott et al., 2007; Frishkopf et al., 1968). Consistent with these results, we found no influence of sex or size on ABR thresholds. Previous

studies of VIIIth nerve fibers found that the BP of female frogs tend to have a best frequency that is lower than the corresponding best frequency of males in many (but not all) species, while AP tuning is not different between the sexes (Narins and Capranica 1976; Wilczynski et al. 1992; Zakon and Wilczynski 1988, but see Elliott et al., 2007). However, we saw little evidence of an effect of sex on tuning in our audiograms, suggesting that *H. chrysoscelis* is not a species in which BP tuning varies strongly between the sexes.

Comparison of ABR and midbrain audiograms

In Fig. 1-6b, we compare our ABR audiogram (based on visual detection) to an audiogram derived from multiunit recordings in the midbrain of gray treefrogs (Hillery 1984b). The most striking difference between the ABR and midbrain audiograms is the overall difference in threshold. ABR thresholds were, on average, 15 to 25 dB higher than thresholds derived from midbrain recordings. It is common for ABR thresholds to be higher than those derived from more invasive recording methods or behavioral methods (Brittan-Powell et al. 2002, 2010a, 2010b; Gorga et al. 1988, but see Henry and Lucas 2009). As an onset response, the ABR is not affected by the ability of the auditory system to integrate sound over time, as are these other methods of threshold determination, which likely accounts for the difference between thresholds (Gorga et al. 1984; Szymanski et al. 1999).

In terms of general shape, the ABR audiogram resembles Hillery's (1984b) midbrain audiogram. That is, frequency tuning (i.e., the differences in thresholds across frequencies) was broadly similar between the ABR audiogram and midbrain audiogram. In both audiograms, sensitivity peaked around the average spectral peaks present in male calls. The low frequency peak in sensitivity for both audiograms was near 1.2 kHz; however, the frequency of the second sensitivity peak was about 400 Hz lower in the midbrain audiogram than the ABR audiogram. While the peaks in the midbrain audiogram had equivalent sensitivity, there was a 2.5 – 4.4 dB difference between thresholds at the two peaks in the ABR audiogram. Thresholds increased similarly for both audiograms in responses to frequencies between the two peaks of greatest sensitivity (Fig. 1-6b). For example, the difference between thresholds at the most sensitive frequencies and the intermediate frequencies was about 5 to 8 dB in the ABR audiogram, compared with approximately 10 dB in the midbrain audiogram. Thresholds in both audiograms increased sharply at frequencies below the low-frequency peak and above the high-frequency peak (Fig. 1-6b). The changes in threshold for frequencies from 0.3 kHz up to the ~1.2 kHz peak

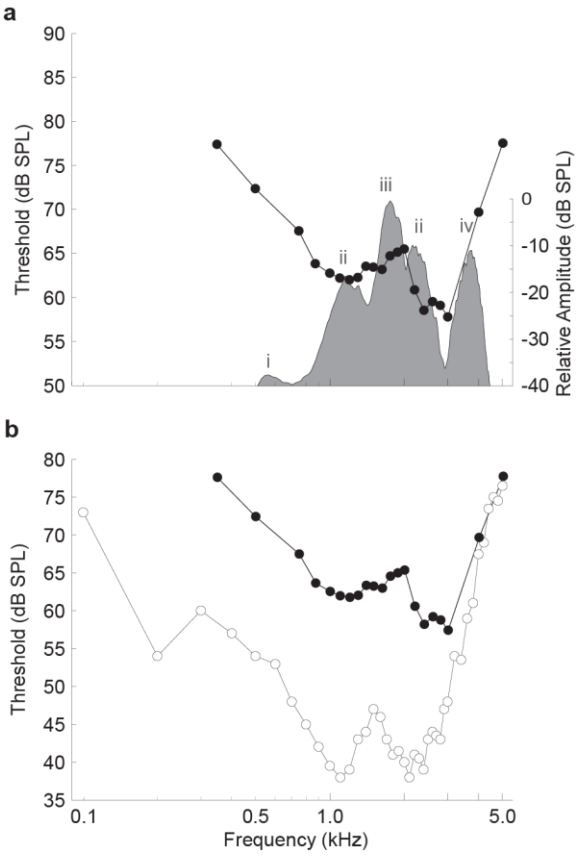


Figure 1-6 Comparison of audiograms
a *Visually detected ABR thresholds from this study (unweighted means averaged across all individuals;* filled circles*) compared with the frequency spectrum of a mixed-species chorus recorded in central Minnesota during the peak of the gray treefrog breeding season (*shaded area*). Peaks of the chorus spectrum are contributed by i) northern leopard frogs (Rana pipiens), ii) Cope's gray treefrogs (Hyla chrysoscelis), iii) American toads (Bufo americanus), and iv) boreal chorus frogs (*Pseudacris maculata). ***b*** *Visually detected ABR thresholds from this study (*filled circles*) compared with average multiunit thresholds from invasive recordings from the midbrain of Cope's gray treefrogs (*open circles*) reported by Hillery (1984b)*

of sensitivity were comparable, with slopes of -10.6 dB/octave in the ABR audiogram and approximately -11.5 dB/octave in the midbrain audiogram (Fig. 1-6b). For frequencies above the second (higher frequency) peak in sensitivity, the slopes differed somewhat more, with rates of 27.6 dB/octave and 35.4 dB/octave in the ABR and midbrain audiograms, respectively (Fig. 1-6b).

We believe the small differences in high-frequency tuning between our ABR audiogram and Hillery's (1984b) midbrain audiogram could reflect evolutionary differences, size differences, or both, between the frogs tested in each study. Regarding evolutionary differences, we note that *H. chrysoscelis* consists of two distinct genetic lineages (Ptacek et al. 1994). The frogs used in the current study were of the western mitochondrial DNA lineage, while those used for midbrain recordings were collected from Tennessee (Hillery 1984b) and belonged to the eastern lineage. Lineage differences in female preferences for spectral properties of advertisement calls have been reported previously (Schrode et al. 2012). Comparative ABR studies of the two lineages might shed considerable light on possible mechanisms underlying the apparent variation in female preferences for spectral call properties. Although we did not see an effect of size on

frequency tuning across the range of sizes in our sample of Minnesota frogs, some previous research has indicated that larger individuals can have BPs tuned to lower frequencies (Zakon and Wilczynski 1988). In the study by Hillery (1984b), subject mass ranged from 4.1 to 11.2 g. This is a wider range shifted to larger body sizes compared to those of the frogs tested in our study (2.8 to 8.3 g). Therefore, we cannot rule out the possibility that population differences in body size contributed to physiological differences in tuning for higher frequencies reflected in the ABR and midbrain audiograms. To determine definitively what differences in the audiograms result from population differences, the best test would be to conduct both types of recording in the same individuals.

Fibers in the anuran auditory nerve tend to cluster into three distinct populations that are sensitive to different frequency ranges (reviewed in Zakon and Wilczynski 1988). The absolute frequency ranges vary by species, but there is generally a group of fibers sensitive to low-frequencies and one sensitive to mid-frequencies, both arising from the AP, and a third group sensitive to high-frequencies, which arises from the BP. Midbrain audiograms from some other hylid treefrogs have peaks near 0.5 kHz that are thought to arise from the low-frequency AP fibers (Hubl and Schneider, 1979; Miranda and Wilczynski, 2009a; Penna et al., 1992; Wilczynski et al., 1993). Although the audiogram described by Hillery (1984b) based on multiunit recordings in the midbrain of Cope's gray treefrogs shows no increased sensitivity near 0.5 kHz, single unit recordings in the midbrain of the closely related eastern gray treefrog (*H. versicolor*) suggest that there is a distinct low-frequency population of neurons sensitive to frequencies around 0.5 kHz in the latter species (Diekamp and Gerhardt, 1995). Our ABR audiogram showed no increased sensitivity at 0.5 kHz relative to nearby frequencies, which is similar to the audiogram of Hillery (1984b). However, we would point out that the use of ABRs often results in overestimation of thresholds to low-frequency tones. This overestimation stems from the use of relatively short tone pips with fast rise/fall times (see discussion in Brittan-Powell et al. 2010b). We chose these temporal stimulus properties to be consistent with several previous studies of the ABR (e.g., Brittan-Powell et al. 2002, 2010b; Katbamna et al. 2006a; Lohr et al. 2013) and invasive studies of evoked potentials in frogs (Katbamna et al. 2006b; Zhang et al. 2012). In addition, longer tone durations and slower rise/fall times can increase ABR latencies (Hecox et al., 1976) and alter waveform morphology (Hall 2007; Popov and Supin 1990). In general, ABRs evoked by short tone pips are probably most useful for assessing sensitivity to

middle and high frequencies in frogs, but may be more limited in assessing low-frequency sensitivity.

Utility of ABRs

Our results indicate that recordings of ABRs via minimally invasive procedures represent a useful method for characterizing and investigating the physiology of hearing in frogs. There are some advantages of using ABRs over more traditional physiological methods. Recording the ABR is quick, allowing for acquisition of data from large sample sizes in a short amount of time (e.g., during a species' breeding season). Because surgery is not required to record subdermal ABRs, animals must be held captive and housed for shorter periods of time and there is dramatically lower risks of infection and death. Many of the more under-studied species are in remote locations, making the potential portability and relatively low cost of the ABR technique ideal for studying these taxa. Additionally, individuals can be used in both behavioral tests and ABR studies, facilitating direct comparison of physiological and behavioral responses in the same animal, as well as within-subject longitudinal studies (e.g., Zhang et al. 2012).

An obvious application for the ABR is in comparative studies of auditory sensitivity across species (e.g., Brittan-Powell et al. 2005, 2010b; Kenyon et al. 1998; Lucas et al. 2002). A particularly interesting comparison would be between closely related species of frogs that exhibit behavioral differences in male calls, female preferences, or both. A considerable focus of previous research in anurans has compared the spectral tuning of the auditory system to the spectral content of advertisements calls and to female frequency preferences (Gerhardt and Schwartz 2001). Recordings of the ABR might provide a useful method for furthering this research using a broader range of species, lineages, or populations and could complement or serve as an alternative to other minimally invasive methods (Meenderink et al., 2010).

Chapter 2 Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*)[2]

Anurans (frogs and toads) are important models for comparative studies of communication, auditory physiology, and neuroethology, but to date, most of our knowledge comes from in-depth studies of a relatively small number of model species. Using the well-studied green treefrog (*Hyla cinerea*), this study sought to develop and evaluate the use of auditory evoked potentials (AEPs) as a minimally invasive tool for investigating auditory sensitivity in a larger diversity of anuran species. The goals of the study were to assess the effects of frequency, signal level, sex, and body size on auditory brainstem response (ABR) amplitudes and latencies, characterize gross ABR morphology, and generate an audiogram that could be compared to several previously published audiograms for green treefrogs. Increasing signal level resulted in larger ABR amplitudes and shorter latencies, and these effects were frequency dependent. There was little evidence for an effect of sex or size on ABRs. Analyses consistently distinguished between responses to stimuli in the frequency ranges of the three previously-described populations of afferents that innervate the two auditory end organs in anurans. The overall shape of the audiogram shared prominent features with previously published audiograms. This study highlights the utility of AEPs as a valuable tool for the study of anuran auditory sensitivity.

[2] This chapter is in press as Buerkle, N. P., Schrode, K. M., & Bee, M. A. (2014) Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*). *Comparative Biochemistry and Physiology*

1. Introduction

Anuran amphibians (frogs and toads) are important model organisms for comparative studies of hearing and sound communication, auditory neurophysiology, and neuroethology (reviewed in Fay and Simmons, 1999; Gerhardt and Huber, 2002; Kelley, 2004; Narins et al., 2007; Wilczynski and Ryan, 2010). However, much of what we know about the anatomy and physiology of the anuran auditory system comes from intensive study of a relatively small number of model species, such as northern leopard frogs (*Rana pipiens*), North American bullfrogs (*Rana catesbeina*), European grass frogs (*Rana temporaria*), African clawed frogs (*Xenopus laevis*), and green treefrogs (*Hyla cinerea*). Among the issues that currently limit neurophysiological investigations to a small number of model species are the expertise and equipment required to perform survival surgeries and single- or multi-unit recordings, as well as the general invasiveness of such procedures. Here, we report results from a minimally invasive study that investigated the anuran auditory system by recording auditory evoked potentials (AEPs) through the intact skull using subcutaneous scalp electrodes. While not a substitute for more invasive neurophysiological studies, AEPs can provide useful neurophysiological measures of auditory function for use in comparative studies (e.g. Brittan-Powell et al., 2010a; Henry and Lucas, 2008; Ladich and Fay, 2013).

The auditory brainstem response (ABR) is one type of AEP that has been widely used to study auditory sensitivity in a diversity of vertebrate animals, including humans (Hall, 2007), other mammals (McFadden et al., 1999; Ramsier and Dominy, 2010; Song et al., 2006; Supin et al., 1993), birds (Brittan-Powell and Dooling, 2004; Brittan-Powell et al., 2002; Brittan-Powell et al., 2005; Gall et al., 2011; Henry and Lucas, 2008; Henry and Lucas, 2009; Lohr et al., 2013), reptiles (Brittan-Powell et al., 2010a; Higgs et al., 2002), and fish (Kenyon et al., 1998; Ladich and Fay, 2013; Wysocki and Ladich, 2001; Wysocki and Ladich, 2003). Though a handful of previous studies have measured brainstem potentials in anurans using methods that require surgery (Bibikov and Elepfandt, 2005; Carey and Zelick, 1993; Corwin et al., 1982; Hillery, 1984a; Katbamna et al., 2006b; Seaman, 1991; Yu et al., 2006), recordings of ABRs in anurans using subcutaneous electrodes have been limited to just three previous studies (Katbamna et al., 2006a; Schrode et al., in press; Zhang et al., 2012).

Greater use of ABRs to investigate the anuran auditory system would potentially confer several benefits. First, using subcutaneous electrodes to collect ABR data can be relatively fast,

allowing for large sample sizes to be tested during relatively short breeding seasons (Schrode et al., 2014). Second, recording ABRs does not require surgery. Thus, animals have lower risk of infection and procedural complications, would not need to remain in long-term captivity, and would have no need for euthanasia after reaching experimental end points. Third, the same individual could be tested repeatedly over long periods, allowing for longitudinal studies into the effects of seasonality (e.g. Zhang et al., 2012), hormone levels, development, and age on auditory sensitivity. Finally, entirely portable yet low-cost and high quality ABR recording and analysis systems are technologically feasible (e.g. Valderrama et al., 2013), meaning that anuran neurophysiology could be studied outside of the laboratory environment using this method. Together, these potential benefits would provide a means to conduct comparative research on the auditory systems of a greater diversity of anuran species.

Our broad aim in this study was to establish the use of ABR recordings for studying anuran auditory sensitivity. To this end, we chose an anuran species, the green treefrog (*Hyla cinerea*), with an exceptionally well-described auditory system and acoustic communication system. Over the last four decades, studies of hearing and sound communication in this species have investigated electrophysiological responses (Ehret and Capranica, 1980; Ehret et al., 1983; Feng and Capranica, 1978; Klump et al., 2004; Lombard and Straughan, 1974; Miranda and Wilczynski, 2009a; Miranda and Wilczynski, 2009b; Mudry and Capranica, 1987b), anatomy (Allison and Wilczynski, 1991; Almli and Wilczynski, 2009; O'Bryant and Wilczynski, 2010), endocrinology (Burmeister and Wilczynski, 2001; Burmeister and Wilczynski, 2005; Burmeister et al., 2001; O'Bryant and Wilczynski, 2010), and sound-mediated behaviors (Ehret and Gerhardt, 1980; Feng et al., 1976; Gerhardt, 1978a; Gerhardt, 1978b; Gerhardt, 1981; Gerhardt, 1987; Gerhardt and Höbel, 2005; Gerhardt et al., 1990; Höbel and Gerhardt, 2003; Megela-Simmons et al., 1985; Moss and Simmons, 1986; O'Bryant and Wilczynski, 2010; Rheinlaender et al., 1979; Simmons et al., 1993b; Vélez and Bee, 2013; Vélez et al., 2012). Focusing our study of the anuran ABR on such a well-described species had the significant advantage of allowing us to directly compare features of the ABR to known features of auditory processing and acoustic signaling in the same species. Such an approach provides a critically important foundation for interpreting future comparative studies using noninvasive ABRs in other frog species with auditory systems that are less well-described.

As with other frogs (Capranica and Moffat, 1983; Gerhardt and Huber, 2002; Narins et al., 2007), the green treefrog auditory system appears well adapted for detecting and processing

conspecific advertisement calls, which consist of a single, short note (120-160 ms) repeated one to two times per second (Gerhardt, 2001a). Each call has a bimodal frequency spectrum with a narrow, low frequency peak near 0.9 kHz and a broader, high-frequency peak in the range of about 2.7−3.0 kHz. Each peak is primarily encoded by a separate sensory papilla in the inner ear. The low frequency peak falls in the range of the amphibian papilla (AP) and the high frequency peak is in the range of the basilar papilla (BP) (Capranica and Moffat, 1983; Ehret and Capranica, 1980). As in other anurans (Capranica, 1976; Feng et al., 1975), the tonotopically-organized AP of green treefrogs can be further subdivided into two populations that have distinct ranges of sensitivity. In the low-frequency population, primary afferents have characteristic frequencies (CFs) lower than about 0.5 kHz; afferents in the mid-frequency population have CFs that range from about 0.5 to 1.2 kHz (Capranica, 1976; Capranica and Moffat, 1983; Ehret and Capranica, 1980). The BP acts as a single acoustic filter, with broadly tuned afferents having similar CFs near 3.2 kHz.

The specific objectives of this study were as follows. First, we sought to assess the effects of frequency, signal level, sex, and body size on ABR waveform amplitudes and latencies and characterize gross ABR morphology for comparison with ABR studies in other animals. Second, we sought to determine the extent to which ABR morphology and dependence on frequency and signal level correspond to the expected tuning of the green treefrog's peripheral auditory system based on results from previous invasive studies (Capranica and Moffat, 1983; Ehret and Capranica, 1980; Lombard and Straughan, 1974). Finally, we sought to generate an ABR audiogram for comparison with previous behavioral (Megela-Simmons et al., 1985; Weiss and Strother, 1965) and neurophysiological (Lombard and Straughan, 1974; Miranda and Wilczynski, 2009b; Penna et al., 1992) audiograms from green treefrogs as well as ABR audiograms from Cope's gray treefrog (*Hyla chrysoscelis*) (Schrode et al., 2014), the African clawed frog (*Xenopus laevis*) (Katbamna et al., 2006a), and the Emei music frog (*Babina daunchina*) (Zhang et al., 2012).

2. Materials and methods

2.1. Subjects

Our subjects were 21 male (mean ± SD mass = 7.8 ± 1.6 g) and 24 female (7.6 ± 1.7 g) green treefrogs collected in amplexus at the East Texas Conservation Center (formerly John D. Parker East Texas State Fish Hatchery) near Jasper, Texas, U.S.A. and transported within 24-48 hours of collection to our laboratory in St. Paul, Minnesota, U.S.A. Frogs were housed on a 12-hour photoperiod in aquaria with damp moss and conditioned tap water and fed a diet of vitamin-dusted crickets. ABR recordings were made between July 10 and August 10, 2011, which is during the species' breeding season. All procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (#1103A97192).

To record ABRs, subjects were immobilized with an intramuscular injection of d-tubocuranine chloride (6.5 – 8.0 μg/g body weight). We allowed animals to regulate their own lung volume as the immobilizing agent took effect over several minutes. Visual inspection of immobilized animals prior to recordings revealed what appeared to us to be normal lung inflation based on lateral extension of the body walls. To facilitate cutaneous respiration, we draped frogs with a single layer of damp surgical gauze. We applied a local anesthetic (2.5% lidocaine HCl) to the scalp just prior to electrode placement. Following all procedures, we allowed the frog to recover in a dish of shallow water and returned it to its home aquarium once full mobility was regained. Experiments typically lasted 2-3 hours and full recovery was usually reached within 4-6 hours of administration of the immobilizing agent. One subject regained mobility prior to presentation of all stimuli; therefore we discarded these interrupted recordings and restarted and completed the experiment with this subject 3 days later.

2.2. Recording the ABR

All ABRs were recorded in a MAC-3 semi-anechoic sound chamber (W × D × H: 81.3 cm × 61 cm × 61 cm; Industrial Acoustics Company, Bronx, NY, U.S.A.) sitting on a vibration isolation table (TMC 68-500, Technical Manufacturing Corporation, Peabody, MA, U.S.A.). The temperature inside the chamber was equivalent to the ambient room temperature (~19° C) and varied less than 1° C across recordings of different subjects. This is a typical temperature at which these frogs breed. The consistency in temperature prevented any of the temperature-induced effects on auditory thresholds known to exist in anurans (Carey and Zelick, 1993; Hubl and Schneider, 1979; Mohneke and Schneider, 1979; Mudry and Capranica, 1987c; Walkowiak, 1980). Each subject was placed in the middle of the chamber on a 2-cm high, acoustically

transparent platform with a natural posture, such that its limbs were tucked next to its body, its mouth was closed, and its head slightly elevated. The subject directly faced a speaker (Orb Mod 1, New York, NY, U.S.A.) and was positioned so that the caudal edges of both tympanic membranes were 30 cm from the front of the speaker. Three platinum alloy subcutaneous needle electrodes (1-3 kΩ, Grass F-E2, West Warwick, RI, U.S.A.) were inserted just under the skin between the eyes (non-inverting) and next to each tympanum (inverting and ground) (Fig. 2-1a). The electrode leads were twisted together to reduce electrical noise and connected to a Tucker Davis Technology (TDT, Gainesville, FL, U.S.A.) RA4LI low impedance headstage and TDT RA4PA preamplifier (20× gain, 25 kHz sampling rate). The signal was then delivered via fiber optic cable to a TDT RZ5 digital processor and stored on a computer for offline analysis. Recordings were notch filtered at 60 Hz and band-pass filtered between 30 Hz and 3.0 kHz.

Acoustic stimuli were created using TDT's SigGenRP software and presented using TDT's BioSigRP software. Signals were output via a TDT RP2.1 processor (50 kHz sampling rate, 16 bit resolution), attenuated by a TDT PA5 programmable attenuator, amplified by a Crown XLS 202 amplifier (Crown Audio, Inc., Elkhart, IN, U.S.A.), and broadcast through the speaker. The stimuli comprised short trains of either rectangular broadband clicks (0.1-ms duration, 25-ms click period) or tones (5-ms duration, 1-ms $\cos^2$ rise/fall, 25-ms tone period). Within a tone train, the frequency of the tone was held constant. Across different tone trains, we tested 23 different frequencies: 0.3 to 1.2 kHz (in 0.15-kHz steps), 1.5 kHz, 1.8 kHz, 2.1 to 3.75 kHz (in 0.15-kHz steps), 4.05 kHz and 5.1 kHz. To ensure the quality of the stimulus presentation through our setup, we digitally recorded all stimuli and measured the percent harmonic distortion (%HD) for tones and the tail-to-signal ratio (TSR) for all stimuli. Example stimulus recordings are depicted in Appendix 2, Fig A2-1. Across stimuli, the median %HD was 0.9 % (interquartile range: 0 to 2.4 %). The median TSR was -16.2 dB (interquartile range: -19.4 to -9.8 dB); that is, echoes were about 10 to 20 dB lower in amplitude than the preceding signal. Given these measurements, we were confident that the neural recordings reflected activity in response to the intended stimuli.

Each recording session began and ended by presenting a train of five equal-amplitude clicks at a suprathreshold sound pressure level (80 dB SPL re 20 μPa, fast root-mean-square [RMS], C-weighted) followed by a 100-ms silent interval (Fig. 2-1b). We used recordings of the response to the five clicks to verify the presence and assess the magnitude of an ABR. Robust click-evoked responses were present at the beginning and end of each recording session and changed little over the course of the session (see Fig. 2-1 legend).

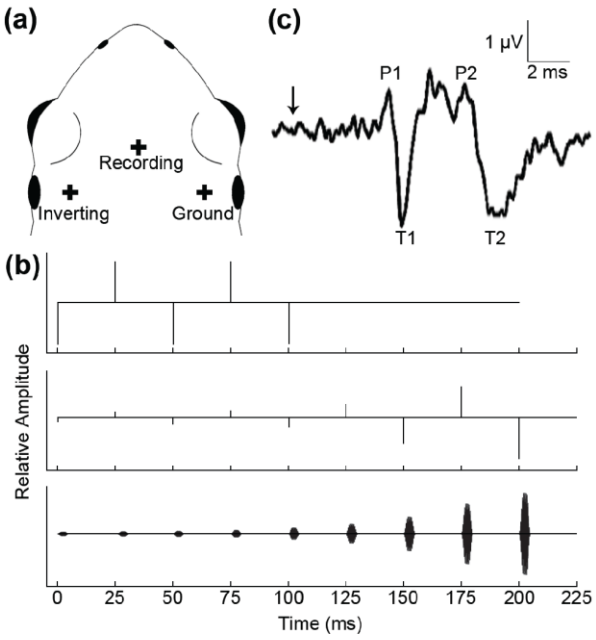


Figure 2-1 ABR methodology
(a) Placements of the three scalp electrodes are indicated on an outline of a green treefrog head. (b) Shown are stimuli that were presented to each subject. The top trace in (b) shows the train of equal-amplitude (80 dB) clicks presented at the beginning and end of each experiment. The middle and bottom traces in (b) show click and tone trains, respectively, in which signal level increased from 40 to 80 dB in 5-dB steps. To obtain each replicate, stimuli were repeated 400 times and click polarity and tone phase alternated between presentations. (c) P1 and P2 amplitudes were measured as the voltage from the top of the respective peak (P1 or P2) to the bottom of the subsequent trough (T1 or T2, respectively). P1 and P2 latencies were measured as the time from arrival of the stimulus at the tympana (vertical arrow) to the top of the respective peak. Click-evoked P1 and P2 amplitudes were similar when measured at the beginning and ending, respectively, of a recording session in response to a click presented at 80 dB SPL (P1: 0.69 ± 0.41 µV and 0.77 ± 0.44 µV; P2: 1.06 ± 0.46 µV and 0.96 ± 0.44 µV).

During a recording session, we presented stimulus trains in which we increased the signal level of successive clicks or tones; henceforth, all signal levels are given in dB SPL. Successive sounds in a train increased in 5-dB increments between 40 and 80 dB (Fig. 2-1b). We presented trains at a rate of 4 trains/s and alternated polarity (for clicks) or phase (for tones) between consecutive sounds in a stimulus train and between consecutive trains to cancel the microphonic. Each stimulus train was presented in two separate replicates of 400 repetitions (800 repetitions total). We collected one replicate for the click train, followed by the two replicates of all tone trains (with frequencies in a different randomized order for each subject within a replicate) and finished with the second replicate of the click train.

Stimuli were calibrated using a Larson Davis model 831 sound level meter (Larson Davis, Depew, NY, U.S.A.) by placing its ½-inch free field microphone (model 377B02) 30 cm from the speaker at the approximate location of the frog's tympana. We note that at this distance there is the potential for responses to tones with a frequency < about 1.1 kHz to be influenced by

near-field particle motion. Levels of the sounds in all stimulus trains were calculated relative to the RMS level of a 1-s tone calibrated to 80 dB. For calibrating clicks, we used a tone frequency of 1.05 kHz; for calibrating tones, we used 1-s tones of equivalent frequency for each tone frequency tested.

2.3. Describing the ABR

Evoked responses were typically composed of a series of 2-3 peaks and troughs occurring between 2 and 12 ms after the stimulus reached the tympana. We used the following analyses to describe the ABR and how its features varied with sound frequency (for tones) and signal level (for clicks and tones), as well as any variation in the ABR due to subject sex and body size.

2.3.1. ABR amplitudes and latencies

An experienced observer measured the amplitude and latency of the first two consistently identifiable peaks of the ABR waveform, as illustrated in Fig. 2-1c, using a custom-written MATLAB program (v2010b, Mathworks, Natick, MA). Waveforms for a given train were aligned vertically (as in Fig. 2-2) and the observer visually identified peaks and the following troughs using an adjustable, sliding cursor. The program then recorded the peak or trough as the data point with the maximum or minimum amplitude, respectively, within ±5 samples (±0.20 ms) of the cursor's position. The recorded peak or trough was displayed and the observer could discard the point and choose again if necessary. Note that for convenience, we refer to the first and second peaks measured in this way as P1 and P2, respectively, without implying knowledge of the generator or generators of each peak within the nervous system. We return to this issue in the Discussion.

P1 was defined as the first positive deflection of the ABR. Following P1, there were sometimes small deflections that varied greatly between the responses of individuals, and often between responses to different signal levels. We defined P2 as the first large, highly consistent deflection observed after P1. Since the shape, amplitude, and latency of P2 differed only slightly between responses, visually comparing evoked responses across signal levels allowed for P2 to be consistently identified. As illustrated in Fig. 2-1c, amplitudes for the two peaks were defined as the voltage difference (in μV) between the peak (P1 or P2) and the subsequent corresponding

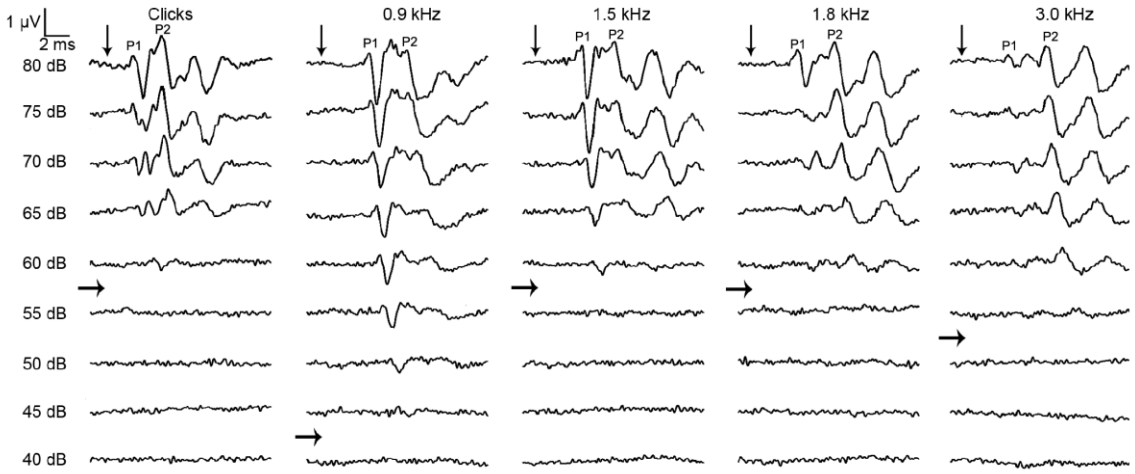


Figure 2-2 Typical evoked responses
Shown are the first 15 ms of ABRs for a typical frog to clicks and to tones presented at four different frequencies. Vertical arrows indicate the time the sound reached the frog's tympana and horizontal arrows indicate visually detected thresholds.

trough (T1 or T2). Latencies for each peak were defined as the time (in ms) from stimulus onset to the respective peak after subtracting the time required for the stimulus to reach the tympana (Fig. 2-1c). We calculated 0.88 ms as the time for sound to travel the 30 cm to the tympana given the average temperature of 19° in the acoustic chamber. Amplitude and latency values were averaged across both replicates of a stimulus for subsequent statistical analysis.

We separately analyzed ABR amplitudes and latencies using factorial analyses of covariance (ANCOVA) to assess the influences of frequency on tone-evoked responses, and the effects of signal level, subject sex, and subject body size on both click-evoked and tone-evoked responses. Before inclusion as a covariate, mass was zero centered by subtracting the mean mass of all individuals from each individual's mass (Delaney and Maxwell, 1981). The inclusion of mass as a covariate in these analyses tested whether body size influenced either the amplitude and latency of the ABR (covariate main effect) or changes in the ABR across levels of the other factors in the models (covariate interactions). Because ABR amplitude and latency measurements required a visible response, subthreshold signal levels necessarily resulted in missing values. We minimized the impact of missing data in our analyses of ABR amplitudes and latencies in the following way. First, we limited our dataset to those frequencies (for tones) and signal levels (for clicks and tones) at which an ABR was present for the majority of individuals. By limiting our dataset to tone frequencies between 0.45 kHz and 3.6 kHz (inclusive) and signal levels between 60 dB and 80 dB (inclusive), we reduced the amount of missing data from 54% to 16% for clicks

and from 50% to 9% for tones. Second, after log-transforming the data to achieve normality, we used multiple imputation to estimate the remaining missing data points (Rubin, 1976; Schafer, 1999). We derived 20 imputed datasets for each of our reduced datasets of amplitude and latency measures. The typical number of imputations recommended is usually less than five (Rubin, 1976; Schafer, 1999); however, there is evidence that increasing the number of imputations beyond five affords greater power to the analysis (Graham et al., 2007). Each imputed dataset for ABR amplitude and latency was analyzed in a separate ANCOVA. Click-evoked responses were analyzed using a 5 signal level (within subjects) × 2 sex (between subject) ANCOVA. Tone-evoked responses were analyzed using a 19 frequency (within subjects) × 5 signal level (within subjects) × 2 sex (between subjects) ANCOVA. For these analyses of imputed datasets, we report the means and ranges for F statistics, P values, and effect sizes (partial η^2). In these and other statistical analyses, which were performed using SPSS v20.0.0 (Armonk, NY, USA), we used a significance criterion of $\alpha = 0.05$ and we report adjusted P values for omnibus tests of within-subjects effects based on the Greenhouse and Geisser (1959) correction. All data are reported as the mean ± SD unless otherwise noted.

2.3.2. ABR gross morphology

We used a cross-correlation analysis to compare ABR morphology across stimulus types and tone frequency. For this analysis, recordings were examined over a 12-ms window that encompassed the entirety of the evoked response. Each response from each individual was aligned in time to the first peak of the ABR (P1) and then windowed between 2 ms preceding and 10 ms following the peak. These windowed responses were subsequently aligned in voltage such that the average amplitude of the first 1 ms (baseline) was 0 μV for all responses. We next averaged the windowed ABR waveform across the two replicates of each stimulus for an individual and then across all individuals to obtain the population mean (and SD) ABR waveform in response to each stimulus. We then separately cross-correlated the mean click-evoked ABR and mean tone-evoked ABRs at frequencies of 0.9, 1.5, 1.8, and 3.0 kHz with the mean tone-evoked ABR at each of the 23 frequencies we tested. These four tone frequencies (0.9, 1.5, 1.8, and 3.0 kHz) were chosen because they were either similar to frequencies contained in male advertisement calls (0.9 and 3.0 kHz) or intermediate between these frequencies. We computed the maximum cross correlation within a maximum time lag of 1 ms. These cross-correlation

analyses were repeated separately for signal levels of 60 to 80 dB. An additional cross correlation analysis examined ABR morphology at an approximately 10 dB sensation level (SL). For this analysis, the population mean ABR for each stimulus was generated by averaging the responses evoked by the stimulus broadcast at a signal level that was 10 dB above each subject's threshold for that stimulus (see section 2.3.3). These cross-correlation analyses were intended to uncover broad patterns of similarities and differences in the gross morphology of the ABR waveform across stimuli. Because there was only one average waveform per stimulus, and because these analyses were not designed to test any particular *a priori* hypotheses, we have not conducted statistical analyses for any specific comparisons.

2.3.3. ABR thresholds

We used two methods to estimate the threshold signal level required to elicit an ABR. In one commonly used method (e.g. Cone-Wesson et al., 1997; Gall et al., 2011; Gorga et al., 1988; Lohr et al., 2013), we determined thresholds visually. We displayed the waveforms of responses to a given stimulus train on a computer monitor and ordered them from high to low signal level, as illustrated in Fig. 2-2. Two experienced observers independently estimated thresholds (Fig. 2-2) as the arithmetic mean between the lowest signal at which a response was present and the highest signal level at which there was no response (Brittan-Powell et al., 2002; Brittan-Powell et al., 2005; Brittan-Powell et al., 2010a; Brittan-Powell et al., 2010b; Higgs et al., 2002; Lohr et al., 2013). Visual threshold detections were made without regard to identifying individual peaks, but rather were estimated based on the gross visual morphology of each response. Thresholds were scored blind with respect to the identity and sex of the frog and the type of stimulus (click or tone). We determined thresholds separately for the two replicates of each stimulus train, and then averaged across replicates. Thresholds were relatively consistent between replicates (mean difference = 0.8 ± 4.08 dB, median = 0.8 dB, mode = 0 dB). We averaged thresholds across observers for further statistical analysis. Differences in threshold estimates between the two observers were very small (across all estimates: mean = 0.05 ± 2.45 dB, median = 0 dB, mode = 0 dB, intraclass correlation = 0.83).

We used ANCOVAs (with mean-centered mass as the covariate) to evaluate the effects of stimulus frequency (for tones) and subject sex and size on visually detected ABR thresholds. Estimates of ABR thresholds for all stimuli were available for all 45 frogs, so it was not necessary

to reduce the dataset and use multiple imputations to analyze this response variable. Residuals were also normally distributed for all but two tone frequencies (Shapiro-Wilk tests: p-values > 0.05), so no transformations were performed. Thresholds for click-evoked responses were analyzed using a univariate ANCOVA with sex as the single between subjects factor. Thresholds for responses to tones were analyzed using a 23 frequency (within subjects) × 2 sex (between subjects) ANCOVA.

As a second, observer-free estimate of ABR thresholds, we used a custom-written MATLAB script to compute thresholds by comparing the RMS amplitude of evoked responses to the RMS amplitude of the biological signal in the absence of an acoustic stimulus. For each subject, the RMS amplitude of the response evoked by each of the nine stimuli in a train was computed over a 10-ms time window between 2 ms and 12 ms following stimulus arrival at the ear and plotted as a function of signal level. We then used MATLAB's *fminsearch* function to minimize the sum-of-squares of a sigmoid curve fit to the nine computed RMS values. We determined the threshold as the minimum signal level at which predicted RMS values along the fitted sigmoid curve first exceeded one of three fixed threshold criteria (see Fig. 2-5a in section 3.4 for an example). These criteria were equal to the mean plus 0.5, 1.0 or 2.0 standard deviations of the RMS amplitude of the biological signal computed over six 10-ms time windows when no acoustic signal was presented. We excluded the automated threshold estimates for five individuals at the frequencies of 0.3 and 5.1 kHz from analysis, because threshold estimates were outside the range of stimulus levels presented. We provide these data for comparison with visually detected thresholds.

3. Results

3.1. ABR amplitudes

3.1.1. Click-evoked responses

In analyses of click-evoked ABRs, there was a significant effect of signal level on both P1 and P2 amplitudes (Table 1; Fig 2). Between 60 dB and 80 dB, P1 amplitudes increased monotonically from 0.33 ± 0.28 μV to 0.85 ± 0.56 μV; mean P2 amplitude increased monotonically from 0.48 ± 0.42 μV to 1.20 ± 0.63 μV over this same range of signal levels. No

other effects in the ANCOVA models for the amplitudes of click-evoked ABRs were significant (Table 1).

3.1.2. Tone-evoked responses

Table 2-1 Results from analyses of covariance (ANCOVA) of ABR amplitudes
The mean and range of F statistics, P values, and effect sizes are shown for the 20 imputed data sets. Significant results (mean P < 0.05) are highlighted in bold.

Stimulus (Peak)	Effect	df	*F*	*P*	Partial η^2
Clicks (P1)	**Level**	**4, 168**	**78.0 (43.8-143.3)**	**< 0.001 (all < 0.001)**	**0.63 (0.51-0.77)**
	Sex	1, 42	1.2 (0.0-3.0)	0.389 (0.088-0.865)	0.03 (0.00-0.07)
	Sex x Level	4, 168	1.8 (0.1-5.7)	0.364 (0.009-0.870)	0.04 (0.00-0.12)
	Mass	1, 42	1.1 (0.0-2.4)	0.376 (0.128-0.966)	0.02 (0.00-0.05)
	Mass x Level	4, 168	1.6 (0.1-5.7)	0.347 (0.004-0.880)	0.04 (0.00-0.12)
Clicks (P2)	**Level**	**4, 168**	**100.1 (48.9-188.2)**	**< 0.001 (all < 0.001)**	**0.69 (0.54-0.82)**
	Sex	1, 42	0.3 (0.0-0.6)	0.650 (0.426-0.973)	0.01 (0.00-0.02)
	Sex x Level	4, 168	2.6 (0.6-9.5)	0.181 (0.001-0.464)	0.06 (0.01-0.18)
	Mass	1, 42	0.6 (0.0-2.4)	0.547 (0.130-0.973)	0.01 (0.00-0.05)
	Mass x Level	4, 168	1.2 (0.0-4.4)	0.432 (0.035-0.907)	0.03 (0.00-0.10)
Tones (P1)	**Frequency**	**18, 756**	**124.0 (117.0-130.8)**	**< 0.001 (all < 0.001)**	**0.75 (0.74-0.76)**
	Level	**4, 168**	**285.2 (238.3-333.9)**	**< 0.001 (all < 0.001)**	**0.87 (0.85-0.89)**
	Frequency x Level	**72, 3024**	**8.4 (7.4-9.7)**	**< 0.001 (all < 0.001)**	**0.17 (0.15-0.19)**
	Sex	1, 42	0.9 (0.7-1.1)	0.348 (0.292-0.419)	0.02 (0.02-0.03)
	Sex x Frequency	18, 756	0.6 (0.3-0.9)	0.642 (0.463-0.810)	0.01 (0.01-0.02)
	Sex x Level	4, 168	1.1 (0.4-2.1)	0.361 (0.142-0.633)	0.03 (0.01-0.05)
	Sex x Frequency x Level	72, 3024	1.0 (0.8-1.5)	0.521 (0.072-0.744)	0.02 (0.02-0.04)
	Mass	1, 42	0.0 (0.0-0.1)	0.900 (0.749-0.999)	0.00 (0.00-0.00)
	Mass x Frequency	18, 756	1.0 (0.5-1.4)	0.421 (0.239-0.655)	0.02 (0.01-0.03)
	Mass x Level	4, 168	0.3 (0.1-0.9)	0.706 (0.415-0.851)	0.01 (0.00-0.02)
	Mass x Frequency x Level	73, 3024	1.1 (0.7-1.6)	0.363 (0.047-0.839)	0.03 (0.02-0.04)
Tones (P2)	**Frequency**	**18, 756**	**11.5 (9.8-12.7)**	**< 0.001 (all < 0.001)**	**0.22 (0.19-0.23)**
	Level	**4, 168**	**236.2 (195.4-283.7)**	**< 0.001 (all < 0.001)**	**0.85 (0.82-0.87)**
	Frequency x Level	**72, 3024**	**4.4 (3.8-5.0)**	**< 0.001 (all < 0.001)**	**0.10 (0.08-0.11)**
	Sex	1, 42	1.2 (1.0-1.3)	0.288 (0.263-0.331)	0.03 (0.02-0.03)
	Sex x Frequency	18, 756	1.4 (0.8-1.9)	0.229 (0.080-0.566)	0.03 (0.02-0.04)
	Sex x Level	4, 168	2.8 (1.9-3.8)	0.079 (0.027-0.155)	0.06 (0.04-0.08)
	Sex x Frequency x Level	72, 3024	1.2 (0.9-1.6)	0.294 (0.077-0.535)	0.03 (0.02-0.04)
	Mass	1, 42	1.8 (1.3-2.1)	0.189 (0.157-0.254)	0.04 (0.03-0.05)
	Mass x Frequency	18, 756	1.6 (1.2-2.0)	0.164 (0.056-0.295)	0.04 (0.03-0.05)
	Mass x Level	4, 168	0.2 (0.0-0.6)	0.867 (0.561-0.969)	0.00 (0.00-0.00)
	Mass x Frequency x Level	73, 3024	1.1 (0.8-1.5)	0.352 (0.111-0.675)	0.03 (0.02-0.03)

In our analyses of tone-evoked responses, the main effects of frequency and signal level and the frequency × signal level interaction were significant for both P1 amplitudes (Table 1; Fig.

2-3a-c) and P2 amplitudes (Table 1; Fig. 2-3d-f). There were no significant effects of subject sex or body size on the amplitude of tone-evoked ABRs (Table 1). As illustrated in Fig. 2-3c,f, sex differences in P1 and P2 amplitudes were quite small compared with the variability observed within each sex.

We consider first the relatively straightforward effects of variation in signal level. Both P1 and P2 amplitudes increased monotonically with increasing signal level (Fig. 2-3b,e; see also Fig. 2-2). These effects of signal level were frequency-dependent and somewhat more pronounced for P1 than P2 (Table 1; cf. Fig. 2-3b and Fig. 2-3e). P1 amplitudes evoked by frequencies of 1.2 kHz and lower (e.g., 0.9 kHz; Fig. 2-3b), as well as frequencies of 2.1 kHz and higher (e.g., 3.0 kHz; Fig. 2-3b), tended to increase linearly with signal level, though the former did so with a steeper slope. At intermediate frequencies (i.e., 1.5 kHz and 1.8 kHz; open symbols in Fig. 2-3b), P1 amplitudes appeared to increase with signal level at two different rates. For example, between 60 dB and 65 dB for 1.5 kHz, and between 60 dB and 70 dB for 1.8 kHz, P1 amplitudes initially increased at rates similar to those observed for 3.0 kHz (Fig. 2-3b). At higher signal levels, however, the rate of level-dependent change in P1 amplitudes evoked by tones of 1.5 kHz and 1.8 kHz increased to rates similar to or higher than those seen at 0.9 kHz (Fig. 2-3b). In contrast to P1 amplitudes, P2 amplitudes at a given signal level, as well as the rate of increase in P2 amplitude across signal level, were more similar across frequencies (cf. Fig. 2-3b,e).

Compared to the effects of signal level, the effects of variation in frequency on ABR amplitudes were more complex. Most notably, P1 and P2 amplitudes did not change monotonically with variation in frequency. This trend is most clearly evident in the contour plots of Fig. 2-3a,d (showing data for all signal levels) and in Fig. 2-3c,f (showing data for just the 80-dB signal level). For P1, amplitude was larger at lower frequencies than at higher frequencies (e.g., $\leq$ 1.2 kHz versus $\geq$ 2.1 kHz; Fig. 2-3a,c). Within the lower frequency range, P1 amplitudes in response to the 0.45 kHz tone were higher than the amplitudes of responses to the adjacent frequencies of 0.3 and 0.6 kHz. P1 amplitudes at intermediate frequencies (1.2 to 2.1 kHz) were sometimes considerably larger than amplitudes at immediately adjacent frequencies (Fig. 2-3c). For example, P1 amplitudes at 1.5 kHz were larger than those at 1.8 kHz and much more similar to amplitudes at lower frequencies such as 0.9 kHz than to those at frequencies > 2.1 kHz (Fig. 2-3b). For P2, on the other hand, there was a tendency for amplitudes to be larger at frequencies near those present in male advertisement calls (0.9 and 3.0 kHz) compared with other frequencies (Fig. 2-3d,f). As a result, the contour plot of P2 amplitude revealed a pattern similar to that of the

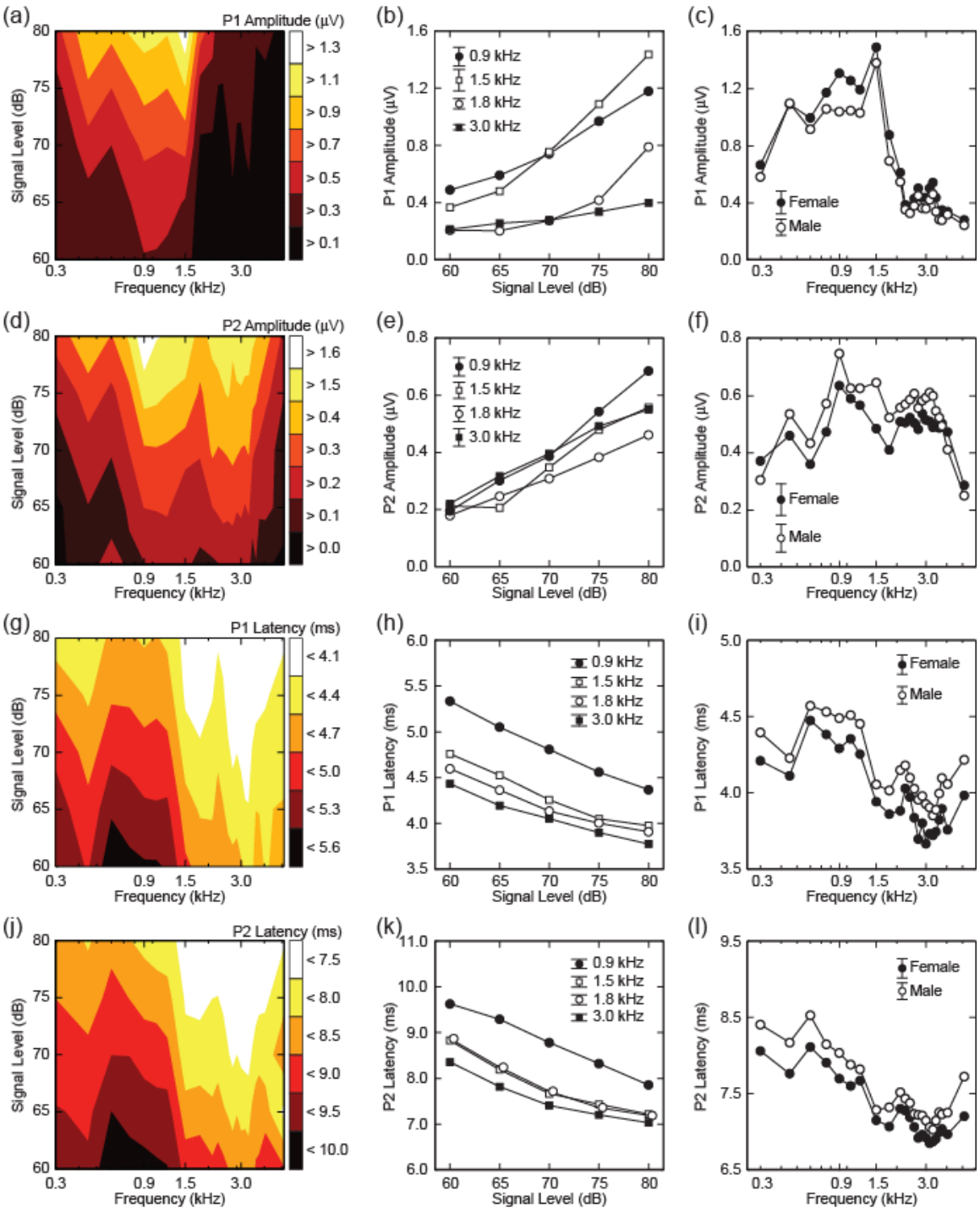


Figure 2-3 ABR amplitudes and latencies of tone-evoked responses

Plotted are (a-c) P1 amplitudes, (d-f) P2 amplitudes, (g-i) P1 latencies, and (j-l) P2 latencies. Contour plots in (a,d,f,j) are plotted as a function of frequency and signal level. In (b,e,h,k) ABR amplitudes and latencies are plotted as a function of signal level for the four representative tone frequencies shown in Fig. 2. Points for the tone frequency of 1.8 kHz are offset in (k) to reveal

underlying points. (c,f,i,l) ABR amplitudes and latencies for 80 dB, plotted as a function of frequency and separated for the two sexes. Error bars in the legends are standard errors averaged across signal levels from 60 to 80 dB and both sexes (b,e,h,k) or all frequencies (c,f,i,l).

ABR audiogram (see below; cf. Fig. 2-3d and 5b,c). There was also a trend for P2 amplitudes in response to the 0.45 kHz tone to be higher than the amplitudes of responses to the adjacent frequencies of 0.3 and 0.6 kHz, similar to the trend in P1 amplitudes.

3.2. ABR latencies

3.2.1. Click-evoked responses

The latency to P1 in click-evoked responses depended on signal level and sex (Table 2). The average latency to P1 decreased monotonically as signal level increased, ranging from 3.46 ± 1.31 ms at 60 dB to 2.77 ± 0.29 ms at 80 dB. Females had P1 latencies that were, on average, 0.23 ± 0.13 ms shorter than those observed in males. While statistically significant, the effect size associated with this sex difference was considerably smaller than that associated with the effects of signal level (Table 2). As with P1 latencies, there was a significant effect of signal level on P2 latencies of click-evoked responses (Table 2). P2 latencies also decreased monotonically with increasing signal level, ranging from 6.15 ± 1.17 ms at 60 dB to 5.26 ± 0.47 ms at 80 dB. Similar to P1 latencies, female P2 latencies were 0.22 ± 0.21 ms shorter than those of males, but the effect of sex was not significant (Table 2). There were no significant effects of body size on click-evoked ABRs for P1 or P2.

3.2.2. Tone-evoked responses

For tone-evoked responses, the ANCOVAs for both P1 and P2 latencies revealed significant effects of frequency, signal level, and their two-way interaction (frequency × signal level), although the effect sizes associated with the main effects were much larger than those for the interactions (Table 2). There was also a significant main effect of subject sex for P1, but not P2 (Table 2). There were no significant effects of body size on the P1 and P2 latencies of tone-evoked ABRs (Table 2). P1 typically occurred with latencies between 3.5 and 6.0 ms after the stimulus reached the tympana (Fig. 2-3g-i). P2 typically occurred a few milliseconds later, between 7.0 and 11.0 ms after the stimulus arrived at the tympana (Fig. 2-3j-l).

At a given frequency, both P1 latencies (Fig. 2-3h) and P2 latencies (Fig. 2-3k) decreased monotonically with increases in signal level. Latencies for both peaks also tended to decrease as a function of increasing frequency. However, these frequency-dependent changes in latency were non-monotonic, and some of the discontinuous changes in latency with frequency were similar to

Table 2-2 Results from analyses of covariance (ANCOVA) of ABR latencies
The mean and range of F statistics, P values, and effect sizes are shown for the 20 imputed data sets. Significant results (mean P < 0.05) are highlighted in bold.

Stimulus (Peak)	Effect	df	*F*	*P*	Partial η^2
Clicks	**Level**	**4, 168**	**47.8 (12.4-70.1)**	**< 0.001 (all < 0.001)**	**0.52 (0.23-0.63)**
(P1)	**Sex**	**1, 42**	**5.5 (2.6-7.8)**	**0.031 (0.008-0.109)**	**0.12 (0.06-0.16)**
	Sex x Level	4, 168	1.6 (0.1-5.2)	0.384 (0.004-0.894)	0.04 (0.00-0.11)
	Mass	1, 42	1.8 (0.3-5.2)	0.237 (0.028-0.604)	0.04 (0.01-0.11)
	Mass x Level	4, 168	1.5 (0.3-5.9)	0.362 (0.010-0.748)	0.03 (0.01-0.12)
Clicks	**Level**	**4, 168**	**85.8 (14.7-122.1)**	**< 0.001 (all < 0.001)**	**0.65 (0.26-0.74)**
(P2)	Sex	1, 42	1.1 (0.0-2.5)	0.357 (0.121-0.960)	0.03 (0.00-0.06)
	Sex x Level	4, 168	1.8 (0.3-8.7)	0.324 (0.001-0.709)	0.04 (0.01-0.17)
	Mass	1, 42	0.3 (0.0-2.1)	0.698 (0.154-0.971)	0.01 (0.00-0.05)
	Mass x Level	4, 168	3.2 (0.3-8.2)	0.177 (0.001-0.692)	0.07 (0.01-0.16)
Tones	**Frequency**	**18, 756**	**123.6 (113.4-134.8)**	**< 0.001 (all < 0.001)**	**0.75 (0.73-0.76)**
(P1)	**Level**	**4, 168**	**574.4 (435.4-704.3)**	**< 0.001 (all < 0.001)**	**0.93 (0.91-0.94)**
	Frequency x Level	**72, 3024**	**2.6 (1.9-4.0)**	**0.005 (all < 0.026)**	**0.06 (0.04-0.09)**
	Sex	**1, 42**	**12.8 (11.7-14.9)**	**0.001 (all < 0.002)**	**0.23 (0.22-0.26)**
	Sex x Frequency	18, 756	1.4 (0.9-2.3)	0.245 (0.030-0.548)	0.03 (0.02-0.05)
	Sex x Level	4, 168	3.7 (1.1-7.3)	0.065 (0.001-0.343)	0.08 (0.03-0.15)
	Sex x Frequency x Level	72, 3024	1.1 (0.7-1.6)	0.392 (0.060-0.832)	0.03 (0.02-0.04)
	Mass	1, 42	0.6 (0.2-0.9)	0.458 (0.350-0.627)	0.01 (0.01-0.02)
	Mass x Frequency	18, 756	1.1 (0.6-1.5)	0.409 (0.157-0.792)	0.03 (0.01-0.04)
	Mass x Level	4, 168	1.5 (0.4-5.8)	0.335 (0.003-0.698)	0.03 (0.01-0.12)
	Mass x Frequency x Level	73, 3024	1.1 (0.7-1.6)	0.382 (0.076-0.788)	0.03 (0.02-0.04)
Tones	**Frequency**	**18, 756**	**115.2 (101.8-127.1)**	**< 0.001 (all < 0.001)**	**0.73 (0.71-0.75)**
(P2)	**Level**	**4, 168**	**589.5 (436.9-761.4)**	**< 0.001 (all < 0.001)**	**0.93 (0.91-0.95)**
	Frequency x Level	**72, 3024**	**3.5 (2.6-4.5)**	**< 0.001 (all < 0.001)**	**0.08 (0.06-0.10)**
	Sex	1, 42	2.6 (2.3-3.3)	0.115 (0.077-0.141)	0.06 (0.05-0.07)
	Sex x Frequency	18, 756	1.4 (0.7-2.0)	0.253 (0.052-0.640)	0.03 (0.02-0.05)
	Sex x Level	4, 168	3.3 (1.4-6.3)	0.082 (0.004-0.247)	0.07 (0.03-0.13)
	Sex x Frequency x Level	72, 3024	1.1 (0.6-1.5)	0.417 (0.105-0.828)	0.02 (0.01-0.03)
	Mass	1, 42	0.3 (0.2-0.4)	0.605 (0.542-0.695)	0.01 (0.00-0.01)
	Mass x Frequency	18, 756	0.9 (0.5-1.3)	0.511 (0.259-0.855)	0.02 (0.01-0.03)
	Mass x Level	4, 168	0.9 (0.1-3.2)	0.471 (0.001-0.800)	0.02 (0.00-0.07)
	Mass x Frequency x Level	73, 3024	1.3 (0.8-2.3)	0.254 (0.005-0.636)	0.03 (0.02-0.05)

those reported above for changes in amplitude with frequency. For example, latencies in response to the 0.45 kHz tone were lower than latencies in response to immediately adjacent frequencies.

Latencies were generally longer at lower frequencies compared with higher frequencies (e.g., ≤ 1.2 kHz versus ≥ 2.1 kHz; Fig. 2-3g, i, j, l). There was a sharp decrease in latencies across the intermediate frequency range between 1.2 and 2.1 kHz. We would additionally note that, across all frequencies, both P1 and P2 latencies decreased at a rate of about 4% (4.1 ± 1.3%) for every 5 dB increase in signal level. Since both P1 and P2 latencies increased with the same *percentage* change per 5 dB, the peak-to-peak latency (i.e., P2 latency minus P1 latency) decreased slightly as signal level increased, with averages of 3.86 ± 0.24 ms at 60 dB and 3.27 ± 0.22 ms at 80 dB.

The mean P1 latency of females was 0.24 ± 0.20 ms shorter than that of males when averaged across all frequencies and signal levels (Fig. 2-3i). This average sex difference in P1 latencies was similar in magnitude to that measured for the P1 latencies of click-evoked responses (0.23 ± 0.13 ms, as reported above). Similar to P1 latencies, P2 latencies for females were 0.23 ± 0.69 ms shorter than male responses averaged across all frequencies and signal levels (Fig. 2-3l).

3.3. ABR gross morphology

Our cross-correlation analyses, which measured similarity in overall waveform shape, revealed several interesting patterns of similarities and differences in the ABRs evoked by clicks and tones of various frequencies. Figure 4 depicts the average ABR for the five stimuli used as references in the cross correlation analysis (clicks and tones at frequencies of 0.9, 1.5, 1.8, and 3.0 kHz) and the resulting correlation coefficients. For clarity, coefficients are shown for only three of the five signal levels analyzed (60, 70, and 80 dB), but trends were similar for the other two signal levels. Click-evoked waveforms (Fig. 2-4a) were most similar (i.e., had the highest cross-correlation coefficients) to responses evoked by tones with frequencies in the range of 0.45-1.2 kHz (Fig. 2-4f). In response to tones, ABR waveforms had consistently different morphologies at relatively low (e.g., 0.9 kHz) and relatively high (e.g., 3.0 kHz) frequencies. This difference in shape can be most easily seen by contrasting Fig. 2-4b and 2-4g with Fig. 2-4e and 2-4j, respectively. Moreover, these frequency-dependent shapes were generally more similar in responses to tones at adjacent frequencies than to tones at more remote frequencies.

The correlation analyses revealed non-monotonic changes across the same frequency ranges observed for ABR amplitudes and latencies (Fig. 2-4f-j). Consider, for example, responses to the 0.9 kHz tone, which were most similar (e.g., r-values > 0.80) to those evoked by other low-frequency tones below about 1.2 kHz (Fig. 2-4g). The similarity between responses to the 0.9

kHz tone and those evoked by tones with frequencies higher than about 1.2 kHz declined sharply (e.g., to *r*-values between 0.4 and 0.65, depending on signal level; Fig. 2-4g). Likewise, responses to the 3.0 kHz tone were most similar to responses to tones at other high frequencies (e.g., ≥ 2.1 kHz) and this similarity declined sharply at lower frequencies (e.g., ≤ 2.1 kHz; Fig. 2-4j).

Correlations computed based on responses at 10 dB SL varied across frequency in ways similar to those computed for absolute signal levels (Fig. 2-4f-j). This result is important, because it confirms that the frequency-dependent patterns of differences observed in both waveforms (Fig 2-4b-e) and correlation coefficients (Fig. 2-4f-j) is not simply a result of differences in auditory sensitivity at different frequencies. In general, differences in signal level had relatively small effects on the magnitudes of correlations across frequency (Fig. 2-4f-j). The largest effects of signal level were observed when the intermediate frequencies of 1.5 and 1.8 kHz served as the reference frequencies for comparison (Fig 4h-i).

3.4. ABR thresholds

Sigmoid curves for the automated method of threshold determination generally fit the RMS amplitude data well (Fig. 2-5a; mean R^2 = 0.88 ± 0.14). Threshold estimates typically increased between 5 and 10 dB from the 0.5 to the 2.0 standard deviations criteria. For responses to clicks, the visually detected threshold of 58.7 dB was most similar to the automatically detected threshold of 59.4 dB obtained using the 1.0 standard deviation criterion. Visually detected thresholds generated an audiogram that most closely matched the automated audiogram generated using the 0.5 standard deviations criterion, with a mean difference of 1.5 ± 1.3 dB between them (Fig. 2-5b). The one frequency where thresholds deviated substantially between these two methods was 1.5 kHz, where the automated threshold for 0.5 standard deviations was 6 dB lower than the visually detected threshold. We could determine no reason for the large difference at this single frequency.

For brevity and consistency with previous studies in frogs (Katbamna et al., 2006a; Schrode et al., 2014; Zhang et al., 2012) and other animals (e.g. Brittan-Powell et al., 2010a; Lohr et al., 2013; Wysocki and Ladich, 2005b), we focused our statistical analysis on the audiogram determined from visually detected thresholds. The thresholds of click-evoked responses were significantly affected by sex ($F_{1,43}$=6.2, P=0.017, partial η^2=0.13), but not the covariate of subject body size ($F_{1,42}$=1.5, P=0.231, partial η^2=0.03). The average click threshold for females (58.4 ±

3.14 dB) was 2.8 dB lower than the average threshold for males (61.2 ± 4.5 dB). Frequency ($F_{22,924}$=244.2, P<0.001, partial η^2=0.85), sex ($F_{1,42}$=12.4, P=0.001, partial η^2=0.23), and the frequency × sex interaction ($F_{22,924}$=5.1, P<0.001, partial η^2=0.11) significantly affected thresholds in response to tones. The covariate of subject body mass ($F_{1,42}$=0.2, P=0.638, partial η^2=0.01) and the mass × frequency interaction ($F_{22,924}$=1.0, P=0.431, partial η^2=0.02) were not

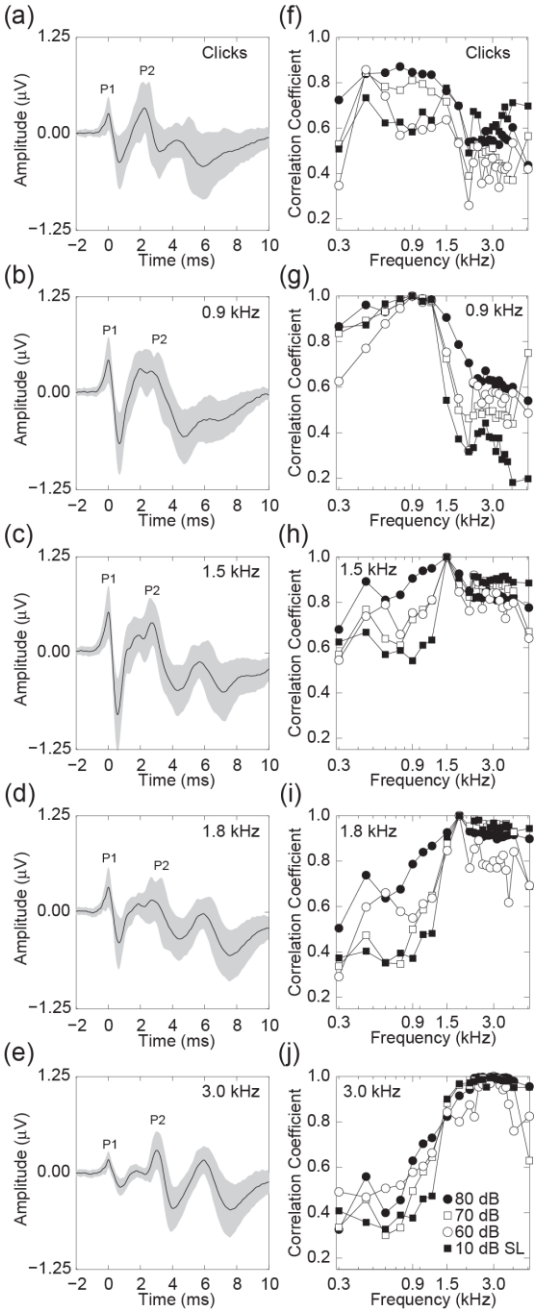


Figure 2-4 ABR gross morphology
(a-e) ABRs were aligned to P1 (t=0 ms) and averaged across replicates and individuals. Shown are responses to stimuli at 80 dB. The shaded region indicates ± 1 SD, across replicates and individuals. (f-j) The average responses in (a-e) were used as reference stimuli and cross-correlated with the average response to every tone frequency tested. This analysis was repeated for signal levels between 60 and 80 dB (data for 65 and 75 dB not shown) and at a 10-dB sensation level (SL).

significant for tones.

The effects of frequency on ABR thresholds were evident in the shape of the audiogram (Fig. 2-5c), which revealed two regions of heightened auditory sensitivity. One region extended from 0.9 to 1.2 kHz and a second, broader region extended from 2.4 to 3.15 kHz. Average ABR thresholds typically ranged between 50 dB and 55 dB within these two frequency ranges, with thresholds being 2-3 dB higher in the second region. At frequencies between these two regions, thresholds were closer to 60 dB. ABR thresholds below 0.9 kHz changed at a rate of -9.1 dB/octave, while those above 3.0 kHz changed at a rate of 20.0 dB/octave. Thresholds for females were slightly lower than those for males (Fig. 2-5c).

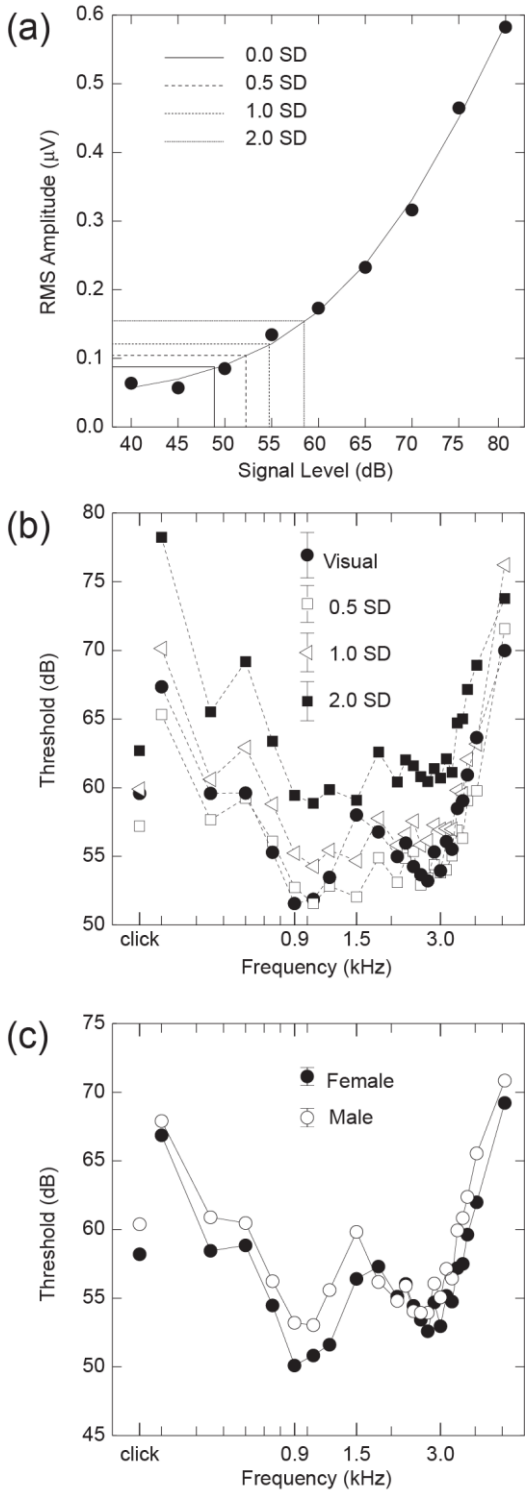
The two regions of enhanced sensitivity just described occurred at frequencies on opposite sides of the sharp differences in ABR amplitudes, latencies, and cross-correlations that occurred between 1.2 kHz and 2.1 kHz (Figs. 3, 4). We would additionally note that sensitivity to tones as a function of frequency in the range 0.3 to 0.9 kHz was also non-monotonic (Fig. 2-5b), mirroring the trends described for amplitudes, latencies, and cross-correlations in this same frequency range.

4. Discussion

4.1. Effects of frequency, signal level, sex, and size

Frequency strongly influenced the amplitudes and latencies of P1 and P2 in responses to tones. Amplitudes of P1 were generally smaller in response to frequencies > 1.5 kHz than to frequencies < 1.5 kHz, consistent with ABR amplitudes in gray treefrogs (Schrode et al., 2014). Amplitudes of P2 were largest for frequencies emphasized in male advertisement calls (0.9 and 3.0 kHz). When considering amplitudes of either peak as a function of frequency, we observed non-monotonicity in the frequency ranges of 0.3 to 0.9 kHz and 1.2 to 2.1 kHz. Latencies to both peaks generally decreased as a function of frequency, which was also similar to results from gray treefrogs (Schrode et al., 2014), as well as recordings from auditory nerve fibers in other species (Feng, 1982; Hillery and Narins, 1984; Stiebler and Narins, 1990; Zakon and Capranica, 1981). However, the frequency-dependent decrease in latency was non-monotonic across the same frequency ranges in which we observed non-monotonic trends in amplitudes.

Figure 2-5 ABR thresholds
(a) Automated threshold detection was accomplished by comparing the RMS amplitude of an evoked response to that of a fixed threshold criterion. Depicted here is an example of threshold detection for the responses to tones of 0.9 kHz in Fig. 2. Filled circles indicate the RMS of responses to each signal level, and the solid line is the best-fit sigmoid curve. The lines indicate calculation of the estimated threshold (vertical) from the predicted amplitude (horizontal), based on the given criterion. The criterion used were 0.5, 1.0, or 2.0 standard deviations (dashed lines) above the mean measured RMS of the neural signal in the absence of a stimulus (indicated by the solid line labeled 0.0 SD). (b) Shown are the mean audiograms for both the visual detection and automated methods of threshold determination. We excluded the automated threshold estimates for five individuals at the frequencies of 0.3 and 5.1 kHz, because threshold estimates were outside the range of stimulus levels presented. (c) Visually detected thresholds are plotted separately for males and females. Error bars in legends for (b) and (c) show the standard error averaged across all frequencies.



Increasing signal level resulted in larger P1 and P2 amplitudes and shorter P1 and P2 latencies in responses to both clicks and tones, as has been reported in previous ABR studies (Brittan-Powell et al., 2002; Brittan-Powell et al., 2005; Kenyon et al., 1998; Nachtigall et al.,

2007b; Zhang et al., 2012). As signal level increased, there was also a decrease in the interval between P1 and P2 latencies. This is in contrast to previous reports of ABRs in humans (Starr and Anchor, 1975; but see Coats, 1978; Stockard et al., 1979), budgerigars (Brittan-Powell et al., 2002), gerbils (Burkard and Voigt, 1989), and cats (Huang and Buchwald, 1978), in which inter-peak intervals were constant across signal levels. There were significant effects of the frequency × level interaction on the amplitudes and latencies of P1 and P2 in tone-evoked responses, although the effects sizes associated with the interaction were consistently smaller than those associated with the main effects of frequency and signal level. The effect of the interaction was most evident in its influence on amplitudes of P1: there was considerable frequency-dependent variation in the slopes associated with the functions relating response amplitude to signal level.

We saw little evidence for an effect of subject sex on amplitudes or latencies of either peak in responses to either clicks or tones. While subject sex did have a significant effect on P1 latencies for both stimulus types, effect sizes suggest that this factor was less important than frequency (for tones) and level in determining latencies. This small effect of subject sex is consistent with previous studies of ABRs in nonhuman animals, including frogs (Caras et al., 2010; Munro et al., 1997; Schrode et al., 2014; Zhou et al., 2006). Similarly, subject size had little effect on either of the peaks in click or tone-evoked responses.

4.2. Gross morphology

Click and tone-evoked ABRs consisted of a series of positive and negative deflections, consistent with the shape of ABRs recorded from a variety of animals (e.g. Brittan-Powell et al., 2010a, 2005; Gall et al., 2011; Higgs et al., 2002; Kenyon et al., 1998; McFadden et al., 1999; Ramsier and Dominy, 2010; Supin et al., 1993), as well as the waveforms of invasive brainstem evoked potentials from other frog species (Carey and Zelick, 1993; Corwin et al., 1982; Katbamna et al., 2006b; Seaman, 1991).

Cross-correlation analyses revealed interesting patterns in the waveforms of responses. Click-evoked ABR waveforms were most similar to those evoked by low and mid-frequencies (0.45-1.2 kHz), in contrast to results from gray treefrogs (Schrode et al., 2014) and humans (Hall, 2007), which have found click-evoked responses to be most similar to responses evoked by tones of higher frequencies (e.g. >1 kHz). Tone-evoked waveforms tended to be most similar to waveforms evoked by other tones of nearby frequencies, which mirrors results from gray

treefrogs (Schrode et al., 2014). Trends in correlation coefficients for tone-evoked responses were non-monotonic in the same frequency ranges in which we observed non-monotonic trends in amplitudes and latencies. Importantly, cross-correlation analyses of waveforms evoked at a common sensation level (10 dB SL) confirmed that the observed effects of frequency did not result from variable sensitivity to different frequencies.

4.3. Relation of the ABR to peripheral physiology

We suggest that the frequency ranges in which we noted non-monotonicity delineate responses to frequencies encoded by the three distinct populations of afferents that innervate the separate sensory papillae in the anuran inner ear, the AP and BP (Capranica, 1976; Capranica and Moffat, 1983; Ehret and Capranica, 1980; Feng et al., 1975). The AP gives rise to two of these populations, which are sensitive to low frequencies (< 0.5 kHz) and middle frequencies (~0.5 – 1.2 kHz). The third population of afferents originates from the BP, and these fibers have higher characteristic frequencies near 3.2 kHz.

The frequency range between 1.2 and 2.1 kHz was characterized by particularly large P1 amplitudes, especially at signal levels > 70 dB. Although there is little evidence that either the AP or BP has populations specifically tuned to frequencies between 1.2 and 2.1 kHz (Ehret and Capranica, 1980), behavioral work with the eastern gray treefrog (*Hyla versicolor*) indicates that at high signal levels, there is overlap in the frequency ranges to which the AP and the BP are sensitive (Gerhardt, 2005). We suggest that the observed patterns in amplitudes within this frequency range result from simultaneous excitation of the two auditory end organs by suprathreshold tones.

While we cannot say with absolute certainty that the evoked potentials we recorded were generated at early stages of the auditory system, we believe this to be the most likely case. One of the most common methods of determining likely generators of ABR peaks lies in comparison of ABR latency data to latencies previously reported from studies using invasive techniques. P1 is generally considered to derive from the VIIIth nerve (Achor and Starr, 1980; Buchwald and Huang, 1975; Seaman, 1991). Consistent with this idea, invasive recordings from the VIIIth nerve of a variety of anuran species have shown latencies to range from 2 to 10 ms (Capranica, 1976; Feng, 1982; Frishkopf and Goldstein Jr., 1963; Hillery and Narins, 1987; Stiebler and Narins, 1990), which encompasses our latency measurements of P1 (2.8 to 3.5 ms for clicks and 3.5 to

6.0 ms for tones). Predicting the P2 generator is more difficult. P2 latencies ranged between 5 and 11 ms, which is consistent with both some of the longer latencies reported for the auditory nerve, as well as the 4-15 ms range of latencies reported from single cell recordings in the first nucleus of the anuran central auditory system, the dorsal medullary nucleus (Fuzessery and Feng, 1983b; Hall and Feng, 1990; van Stokkum, 1987; Yang and Feng, 2007). The overlap in latencies for auditory nerve fibers and units in the dorsal medullary nucleus thus makes the origin of P2 in green treefrogs ambiguous at present. However, the likelihood of P2 being generated at a higher level of the ascending auditory system is unlikely. In single-unit recordings from the superior olivary nucleus, latencies range from 10-50 ms (Condon et al., 1991; Fuzessery and Feng, 1983b), which is rather longer than the latencies we determined for P2.

4.4. Thresholds

ABR threshold estimates revealed two regions of heightened sensitivity near frequencies that correspond to the frequencies emphasized in the advertisement calls of green treefrogs (Gerhardt, 2001a). If, as in many frogs, the auditory papillae of the inner ear are tuned to spectral peaks in the call (Gerhardt and Schwartz, 2001), we would expect that these are the frequencies to which the mid-frequency region of the AP and the BP, respectively, are most sensitive. In the audiogram between 0.3 and 0.9 kHz, the decrease in thresholds was not monotonic, which may reflect responses of afferents from the region of the AP sensitive to low frequencies (Capranica and Moffat, 1983).

We detected no effect of subject size on thresholds, consistent with invasive recordings from the VIIIth nerve of frogs, which have not previously demonstrated an effect of subject size on average thresholds of fibers (Elliott et al., 2007; Frishkopf et al., 1968; Zakon and Wilczynski, 1988). Females generally had lower thresholds than males, an effect that was more pronounced for low and mid-frequencies. However, effect sizes indicated that the effect of sex plays a relatively minor role in determining thresholds relative to the effect of frequency. For many frog species, the frequency to which the BP is most sensitive is lower in females than males (Narins and Capranica, 1976; Wilczynski et al., 1992; Zakon and Wilczynski, 1988; but see Elliott et al., 2007). However, consistent with previous studies in green treefrogs (Miranda and Wilczynski, 2009b; Penna et al., 1992), we did not see any evidence for a difference in the frequencies to which males and females were most sensitive.

In Fig. 2-6, we compare our ABR audiogram to several published green treefrog audiograms generated based on behavioral responses (Fig. 2-6a; Megela-Simmons et al., 1985; Weiss and Strother, 1965) and neurophysiological responses (Fig. 2-6b; Lombard and Straughan, 1974; Miranda and Wilczynski, 2009b; Penna et al., 1992). All of the audiograms showed at least two regions of increased sensitivity, with at least one occurring below about 1.5 kHz and another occurring above about 2.0 kHz. However, the absolute thresholds, the exact frequencies of best sensitivity, and the bandwidth of the sensitive regions vary widely between audiograms. Our ABR thresholds were 10-20 dB higher than those reported for one of the behavioral studies (Fig. 2-6a). Compared with invasive neurophysiological studies, ABR thresholds ranged from 5 - 25 dB higher (Fig. 2-6b), which is not unusual when comparing ABR thresholds to those determined through behavioral tests or invasive neurophysiological studies (e.g. Brittan-Powell et al., 2010a, 2002; Gorga et al., 1988; Ngan and May, 2001; but see Henry and Lucas, 2008, 2009). This difference in thresholds is typically attributed to the fact that the ABR occurs at the onset of

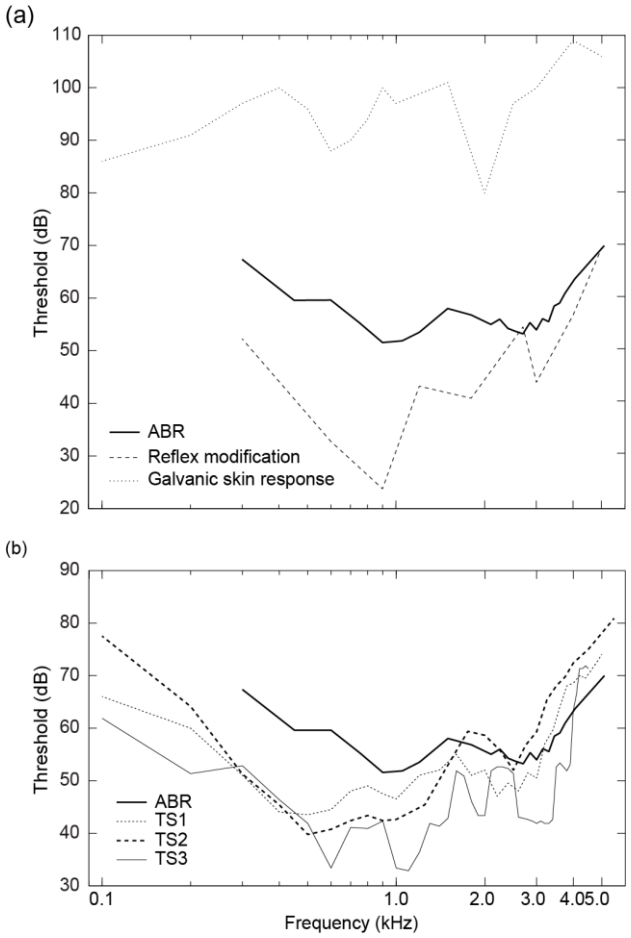


Figure 2-6 Audiogram comparisons
(a) The ABR audiogram constructed from visually detected thresholds is compared to previously published audiograms constructed from behavioral responses in green treefrogs based on reflex modification (Megela-Simmons et al. 1985) or galvanic skin responses (Weiss and Strother 1965). (b) The ABR audiogram is compared to audiograms constructed using multi-unit recordings from the midbrain (torus semicircularis) of green treefrogs (TS1: Miranda and Wilkzynski, 2009b; TS2: Penna et al., 1992; TS3: Lombard and Straughan, 1974).

sound and is not influenced by integration of sound over time (Gorga et al., 1984; Szymanski et al., 1999).

One feature that varied considerably between audiograms was the relative difference in threshold between the two regions of peak sensitivity. This variation is particularly extreme between behavioral audiograms (Fig. 2-6a), where the thresholds for the lower frequency (< 1.5 kHz) region are 20 dB *more* sensitive than those of the higher frequency (> 2.0 kHz) for reflex modification (Megela-Simmons et al., 1985), compared to 10 dB *less* sensitive for the galvanic skin response (Weiss and Strother, 1965). Our ABR audiogram had the smallest relative difference (2.5 - 3 dB) between thresholds at the two regions of peak sensitivity (Fig. 2-6). ABR thresholds tended to diverge more from midbrain thresholds at relatively lower frequencies than higher frequencies (< 1.5 kHz vs. > 1.5 kHz), suggesting that the small relative difference noted above may be due to overestimation of thresholds at low frequencies. It is well-established that methods for recording ABRs tend to overestimate thresholds at low frequencies; however, we chose to use these stimuli for consistency with previous studies using ABRs (e.g. Brittan-Powell et al., 2010a; Schrode et al., 2014; Zhang et al., 2012) and because increasing rise/fall time and tone duration is known to alter ABRs (see review in Hall, 2007).

ABR recordings represent a potentially important, but currently under-utilized, tool in comparative studies of audition in anurans. To illustrate the utility of ABRs for comparative work, we plot our audiogram along with ABR audiograms from three other species of frogs in Fig. 2-7. The audiograms for green treefrogs and Cope's gray treefrogs (*H. chrysoscelis*; Schrode et al., 2014) both have a distinctive "W" shape with peaks in sensitivity at two different

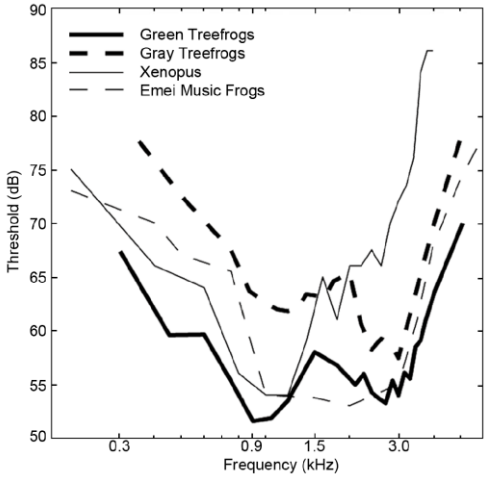


Figure 2-7 Anuran ABR audiograms
ABR audiograms for three other species of frog are compared to our ABR audiogram for green treefrogs. The audiogram for gray treefrogs was reported by Schrode et al. (2014); the audiogram for Emei music frogs was reported by Zhang et al. (2012); and the Xenopus audiogram was reported by Katbamna et al. (2006a).

frequencies. In both species, the peaks in sensitivity occur at the frequencies present in conspecific advertisement calls, and probably also represent the peak sensitivities of the AP and BP. Audiograms for African clawed frogs (*Xenopus laevis*; Katbamna et al., 2006a) and Emei music frogs (*Babina daunchina*; Zhang et al., 2012) both have a more U-like shape. That of the music frog is broad enough to encompass the frequencies containing most of the harmonic energy in male advertisement calls (Chen et al., 2011). The audiogram for *Xenopus*, on the other hand, shows peak sensitivity near 1.0 kHz and no particular sensitivity to the frequencies emphasized in the clicks they use for sexual communication (1.6 and 2.3 kHz; Picker, 1980; Vigny, 1979; Wetzel and Kelley, 1983). There is one caveat to consider in interpreting the ABR audiogram for African clawed frogs, which was generated from responses to airborne sound (Katbamna et al., 2006a). Because these frogs are aquatic, it may be more relevant to consider responses to broadcasts or simulation of waterborne sound, such as those used by to generate behavioral and midbrain neurophysiological audiograms (e.g. Elepfandt et al., 2000; Elliott et al., 2007). Audiograms generated using these methods have distinct peaks in sensitivity at frequencies of 0.6 kHz and in the range of 1.6 to 2.0 kHz, the latter of which does overlap the dominant frequencies in *Xenopus* advertisement calls.

5. Conclusions

Although rarely used in studies of anurans, the ABR is a potentially valuable method for comparative exploration of anuran auditory physiology. This study aimed to evaluate the ABR as a tool for studying the anuran auditory system by comparing the ABR in green treefrogs with that of other animals, as well as evaluating the consistency of ABR measurements with characteristics of the green treefrog auditory system. The similarities of the green treefrog ABR with other ABR studies and the physiology of the green treefrog auditory system validate the use of ABR for further study of anuran auditory sensitivity.

Chapter 3 Evolutionary adaptations for the temporal processing of natural sounds by the anuran auditory system

Sensory systems function most efficiently when processing natural stimuli, and it is thought that this reflects evolutionary adaptation. One of the earliest discoveries of evolutionary adaptation in the auditory system was that the spectral tuning of the anuran auditory system often matches the frequency spectrum of conspecific vocalizations. Matches to the temporal properties of conspecific calls are less well established, but have been documented in the central auditory systems of anurans. There has been little evidence for evolutionary adaptations of peripheral auditory systems. Using auditory evoked potentials, we asked whether there are species-specific or sex-specific adaptations of the auditory systems of gray treefrogs (*Hyla chrysoscelis*) and green treefrogs (*H. cinerea*) to the temporal modulations present in conspecific calls. We constructed modulation rate transfer functions (MRTFs) which revealed that each species was more sensitive than the other to modulation rates typical of conspecific calls. In addition, responses to paired-clicks indicated better temporal resolution in green treefrogs than gray treefrogs, which could represent an adaptation to the faster modulation rates present in their calls. While MRTFs and recovery of responses to paired-clicks were generally similar between the sexes, females of both species showed greater sensitivity than males to modulated tones which had an intermediate (~1.6 kHz) carrier frequency. Together, our results suggest that efficient processing of the temporal properties of behaviorally relevant sounds begins at early stages of the anuran auditory system.

Introduction

A prominent hypothesis in systems neuroscience is that sensory systems are most efficient when processing natural stimuli (Atick, 1992; Barlow, 1961; Hateren, 1992; Laughlin, 1981; Simoncelli and Olshausen, 2001). Because natural stimuli are the most commonly encountered and the most behaviorally relevant, this efficiency reduces energy and resource expenditure associated with sensory processing. Auditory systems appear adapted to process natural sounds such as speech and other communication signals (Rieke et al., 1995; Singh and Theunissen, 2003; Smith and Lewicki, 2006; Suga, 1989; Woolley et al., 2005). Often adaptation manifests as selectivity for behaviorally relevant sounds, which helps increase detectability of signals relative to background noise (Machens et al., 2005; Rieke et al., 1995). For example, the spectro-temporal tuning of neurons in the midbrain and forebrain of songbirds facilitates discrimination between conspecific songs, while limiting interference from modulations inherent in less-behaviorally relevant sounds (Woolley et al., 2005).

Research in anurans, which are important models for the study of hearing and acoustic communication (Gerhardt and Huber, 2002; Narins et al., 2007; Wells, 1977; Wells, 2007), yielded some of the first examples of potential adaptation to natural sounds (Capranica and Moffat, 1975; Frishkopf et al., 1968; Mudry et al., 1977; Narins and Capranica, 1976). In most anuran species, males have repertoires of calls that are used for mate attraction and resource defense. Capranica and Moffat (1983) proposed the "matched filter hypothesis," which suggested that processing in anuran auditory systems should be adapted to match the spectral and temporal properties of conspecific vocalizations. Subsequent work has found support for the spectral matched filter hypothesis in the both the peripheral and central auditory systems of a number of anuran species (Gerhardt and Schwartz, 2001; Hall, 1994; Simmons, 2013). In the periphery, one or both of two inner ear sensory papillae for detecting airborne sound − the amphibian papilla (AP) and the basilar papilla (BP) − and their afferents are predominantly tuned to frequencies emphasized in conspecific calls (Capranica and Moffat, 1983; Frishkopf et al., 1968; Narins and Capranica, 1980; Ryan et al., 1992). Neurons in the central auditory system are also predominantly tuned to frequencies in conspecific calls, with some combination-sensitive neurons firing only when multiple frequencies from conspecific calls are present (Fuzessery and Feng, 1982; Fuzessery and Feng, 1983a; Hall, 1994; Megela, 1983; Mudry and Capranica, 1987b; Mudry and Capranica, 1987c; Mudry et al., 1977). These features of peripheral and central tuning

represent an evolutionary adaptation to the frequency spectra of behaviorally important natural stimuli.

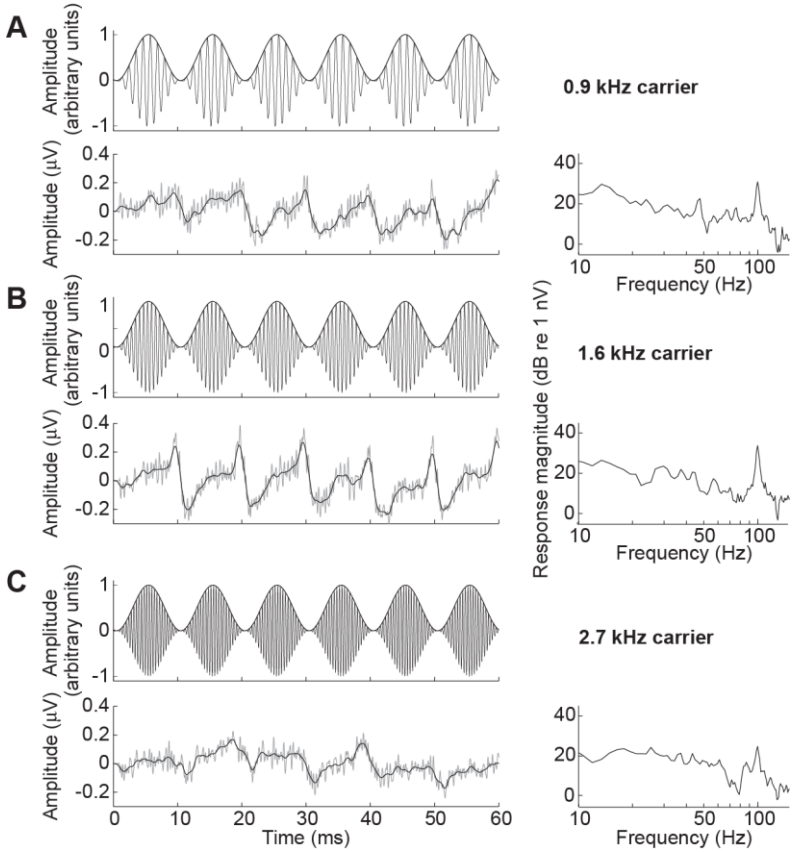


Figure 3-1 Example ASSR stimuli and responses
ASSRs were recorded in response to AM tones of three different carrier frequencies for each of the two species. The example stimuli shown (top panels) were used with green treefrogs and had a modulation frequency of 100 Hz and carrier frequencies of (A) 0.9 kHz, (B) 1.6 kHz, or (C) 2.7 kHz. Examples of neural responses from a green treefrog to each stimulus are plotted in the time (left panel) and frequency (right panel) domains. Responses in the time domain have been high-pass filtered to reveal the periodicity in the trace. Note the peak in the frequency spectrum of the response that matches the 100 Hz modulation rate of the stimulus. The magnitude of this peak is indicative of how well the auditory system followed the AM fluctuation in the envelope of the tone and is used as the response measure in Fig 3-4.

Temporal properties of anuran calls are also critical for species and call recognition and intraspecific discrimination (Castellano and Rosso, 2006; Gerhardt, 1978b; Gerhardt and Doherty, 1988; Rose and Brenowitz, 2002; Schwartz, 1987; Walkowiak and Brzoska, 1982). There is evidence for the operation of temporal matched filters in the central auditory system, but less so in the periphery (Rose and Gooler, 2007; Simmons, 2013). In the central auditory system, neurons exhibit preferences for specific temporal properties of calls such as the rate of pulses or

amplitude modulation (AM) (Diekamp and Gerhardt, 1995; Eggermont, 1990; Gooler and Feng, 1992; Walkowiak, 1984), inter-pulse interval (Alder and Rose, 1998; Edwards et al., 2002), and duration (Condon et al., 1991; Gooler and Feng, 1992; Narins and Capranica, 1980; Penna et al., 1997) using rate codes. In the case of AM, distributions of preferred AM rates are often centered near the pulse rates or modulation rates characteristic of conspecific calls, suggesting specialization for the temporal properties of conspecific signals (Diekamp and Gerhardt, 1995; Penna et al., 2001; Rose and Capranica, 1984; Rose and Capranica, 1985; Rose et al., 1985). In contrast to the rate code common in central auditory neurons, auditory nerve fibers use a periodicity code to encode AM by phase-locking, or discharging at a particular phase of the modulation cycle (Diekamp and Gerhardt, 1995; Eggermont, 1990; Gooler and Feng, 1992; Walkowiak, 1984). The ability of auditory nerve fibers to phase-lock to AM tends to decrease as a function of modulation rate (Dunia and Narins, 1989; Feng et al., 1991; Rose and Capranica, 1985). While many studies have verified the ability of auditory nerve fibers of multiple species to phase-lock to temporal modulations in the amplitude envelopes of conspecific signals (Capranica and Moffat, 1975; Klump et al., 2004; Schwartz and Simmons, 1990; Simmons et al., 1992; Simmons et al., 1993a), there is little evidence for selectivity for the modulations typical of conspecific calls (Frishkopf et al., 1968).

In the present study, we investigated species-specific and sex-specific temporal processing in the auditory systems of two treefrog species that have been well-studied models for hearing and vocal communication, Cope's gray treefrog (*Hyla chrysoscelis*) and the green treefrog (*H. cinerea*) (Bee, 2012; Bee, 2014; Gerhardt, 1982; Gerhardt, 2001b; Gerhardt and Huber, 2002). The advertisement calls that males of each species produce differ in both spectral and temporal properties. The advertisement call of gray treefrogs is comprised of a series of short (10 ms), temporally discrete pulses delivered at species-specific rates of about 40 to 65 pulses/s (Ward et al., 2013). Pulses have energy at frequencies of about 1.25 kHz and 2.5 kHz, with the lower frequency peak attenuated about 11 dB relative to the higher peak (Ward et al., 2013). In contrast, the advertisement call of the green treefrog consists of a single, longer note (120-200 ms; Gerhardt, 1974a) with a waveform periodicity that ranges from about 200 to 500 Hz (Oldham and Gerhardt, 1975). These calls contain energy at around 0.9 kHz and in a band between about 2.5 and 3.6 kHz; on average the relative amplitudes of the spectral peaks differ by about 3 dB (Gerhardt, 1974a). In addition to advertisement calls, males of both species also use aggressive calls in occasional disputes with other males over possession of calling sites. The aggressive calls

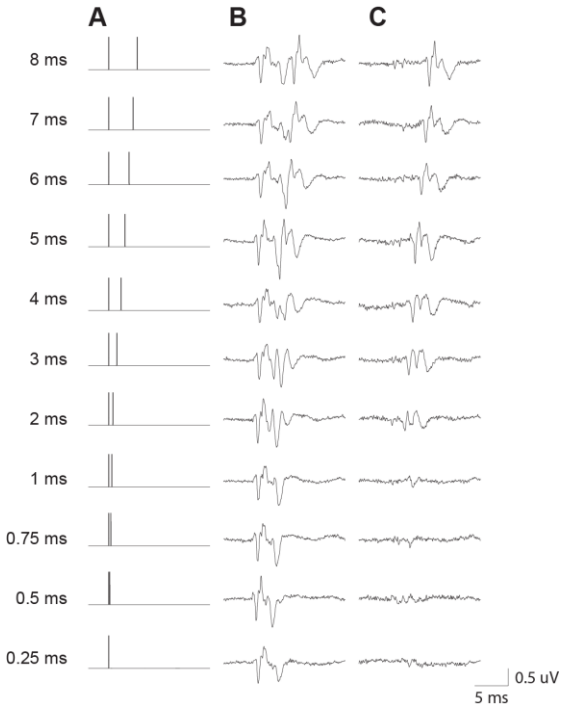
of gray treefrogs typically lack the distinct pulsatile structure of advertisement calls, but do often contain fluctuations in amplitude (Reichert and Gerhardt, 2014); these fluctuations occur with rates ranging from approximately 50 to 100 times/s (MS Reichert, personal communication, June 2014). The aggressive calls of green treefrogs are similar to their advertisement calls, but are pulsed throughout at rates between 40 and 55 pulses/s (Oldham and Gerhardt, 1975). Female treefrogs strongly prefer advertisement calls to aggressive calls (Brenowitz and Rose, 1999; Oldham and Gerhardt, 1975; Schwartz, 1986; Schwartz, 1987; Wells and Bard, 1987), suggesting that aggressive calls may be less salient to females than males.

We tested two main hypotheses. According to the *species-specific adaptation hypothesis*, we predicted that the auditory system of each species would show larger responses to the temporal modulations in conspecific advertisement calls than modulations at other rates. According to the *sex-specific adaptation hypothesis*, we predicted that males would exhibit greater selectivity than females for the modulations in conspecific aggressive calls. We tested these predictions using auditory evoked potentials (AEPs). AEPs measure neural activity from the auditory nerve and brainstem in response to acoustic stimuli, and they are a common tool for studying auditory processing in humans and other animals (Brittan-Powell et al., 2010a; Brittan-Powell et al., 2010b; Gall et al., 2013; Hall, 2007; Henry and Lucas, 2008; Higgs et al., 2002; Katbamna et al., 1992; Kenyon et al., 1998; Ladich and Fay, 2013; Popov and Supin, 1990; Supin et al., 1993).

We chose two well-established AEP techniques that have been used previously to investigate temporal processing, the auditory steady state response (ASSR; alternatively known as the envelope following response or amplitude modulation following response) and the response to paired acoustic clicks (Burkard and Deegan, 1984; Dolphin and Mountain, 1992; Gall et al., 2013; Henry and Lucas, 2008; Mann et al., 2005; Purcell et al., 2004; Wysocki and Ladich, 2005b). We recorded ASSRs in response to tones of three different carrier frequencies, modulated at AM rates between 12.5 Hz and 800 Hz. From the responses, we computed modulation rate transfer functions (MRTFs) that measure the degree of synchronization of the ASSR to AM in the signal (see Fig. 3-1) (Dolphin et al., 1995; Henry and Lucas, 2008; Kuwada et al., 1986; Supin and Popov, 1995b). Our general expectation was that signal modulation rate would have a strong effect on MRTFs, resulting in an overall low-pass shape consistent with phase-locking in the auditory nerve. We recorded responses to paired-clicks in which the amount of time between the clicks (the inter-click interval, ICI), varied between trials (Fig. 3-2). The waveforms of responses

Figure 3-3 Example paired-click stimuli and responses

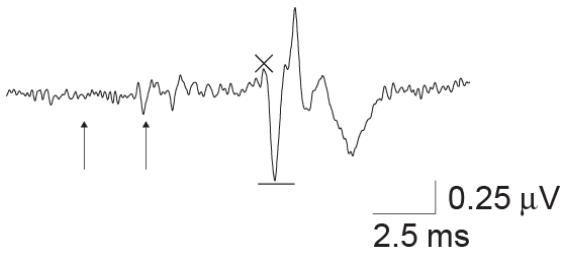
Subjects were tested with pairs of acoustic clicks that varied in ICI. (A) Shown are examples of paired-click stimuli of various ICIs. (B) Plotted are examples of responses from a green treefrog to the stimuli in (A). (C) To disambiguate responses to paired-clicks with short ICIs, residual responses to the second click of each click pair in (A) are derived by point-to-point subtraction of the response to a single click (not shown) from the responses to the paired-clicks in (B).



can include multiple peaks generated by many sources, but we focused analysis on the first peak (P1), which is thought to be generated primarily by the auditory nerve. Percent recovery was calculated as the amplitude (as in Fig. 3-3) of the response to the second click in a pair as a percentage of the amplitude of the response to a single-click. Additionally, we calculated the minimum resolvable ICI at which a response was detected. Both of these measures provide an index of temporal resolution (Burkard and Deegan, 1984; Gall et al., 2012a; Henry and Lucas,

Figure 3-2 Amplitude of response

The amplitude of each residual response to a paired-click stimulus and each response to a single click was measured as the peak-to-peak amplitude from the first positive deflection (x) to the subsequent trough (-). The example shown here is the residual response to a paired-click with an ICI of 8 ms. Arrows indicate times of click presentations.



2008; Popov and Supin, 1990; Supin and Popov, 1995a; Wysocki and Ladich, 2002).

According to the species-specific adaptation hypothesis, we predicted a species × modulation rate interaction would influence MRTFs: gray treefrogs should have larger responses than green treefrogs at the relatively low modulation rates (25 to 100 Hz) more similar to those in gray treefrog advertisement calls (40 to 65 Hz), while green treefrogs should have larger

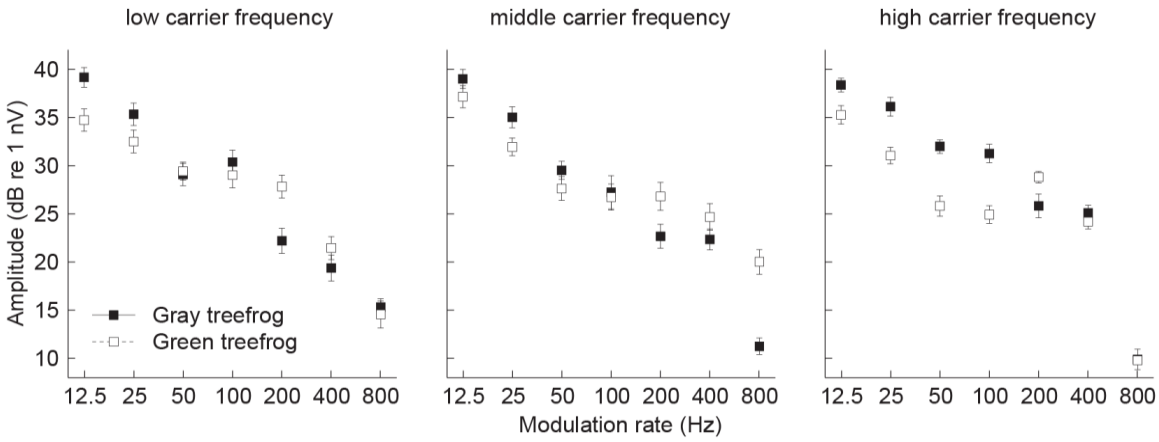


Figure 3-4 MRTFs for each species

An ASSR was measured from each recording as the magnitude of the peak in the frequency domain at the frequency equivalent to the modulation rate of the stimulus. Responses are plotted separately for each species across the three carrier frequencies tested. Carrier frequencies for gray treefrogs were 1.25 kHz, 1.625 kHz, and 2.5 kHz. Carrier frequencies used with green treefrogs were 0.9 kHz, 1.6 kHz, and 2.7 kHz. All error bars are s.e.m

responses than gray treefrogs to stimuli with modulation rates of 200 and 400 Hz, close to those typical of green treefrog advertisement calls (200 to 500 Hz). We also predicted that, in response to paired clicks, green treefrogs would show faster recovery of responses and shorter minimum resolvable ICIs than gray treefrogs because tracking of the faster modulation rates in the green treefrog advertisement call should require greater temporal resolution.

According to the sex-specific adaptation hypothesis, we predicted that a species × sex × modulation rate interaction would influence the shape of MRTFs. Because the modulations in the aggressive calls of gray treefrogs are slightly faster than those in advertisement calls, we predicted the responses of male gray treefrogs would be skewed toward faster modulation rates than those of females. We predicted males of green treefrogs, on the other hand, would have greater responses than females at modulation rates of 25 to 100 Hz, which were the most similar to the rates of pulsation in aggressive calls (40 to 55 Hz). In response to paired clicks, male gray treefrogs would have faster recovery rates and shorter minimum resolvable ICIs than female gray treefrogs, as another indication of better temporal resolution.

Results

We plot MRTFs separately for each species in Fig. 3-4, and for each sex in Fig. 3-5. We examined the effects of species, sex, modulation rate, and carrier frequency, as well as their interactions, on ASSR amplitudes using a repeated-measures linear mixed model (Table 1) and

posthoc contrast tests (Table 2). Percent recovery functions are plotted in Fig. 3-6, separately for each species (Fig. 3-6a) and each sex (Fig. 3-6b). We analyzed the effects of species, sex, and ICI on percent recovery using a repeated measures ANOVA (Table 3) and the effects of species and sex on minimum resolvable ICI with a two-way ANOVA.

Species-specific adaptation hypothesis

Overall, the MRTFs for both species were nearly log-linear, with responses decreasing as a function of increased modulation rate (Fig. 3-4). The effect of modulation rate was significant in the mixed model, and it also had a large effect size compared to the other effects (Table 1). There was no significant main effect of species; however, the species × modulation rate interaction was significant (Table 1). The effect of this interaction can be seen in that each species had larger

Table 3-1 Effects of species and sex on ASSRs

Results of the linear mixed model used to assess effects of species and subject sex on ASSRs. Bold indicates significant terms.

term	df	*F*	*p* value	partial η^2
intercept	**1, 62**	**3857.9**	**<0.001**	**0.98**
species	1, 62	0.3	0.600	0.00
sex	**1, 62**	**5.6**	**0.020**	**0.08**
modulation rate	**6, 776**	**217.9**	**<0.001**	**0.63**
carrier frequency	2, 774	0.2	0.800	0.00
species × modulation rate	**6, 776**	**19.0**	**<0.001**	**0.13**
species × carrier frequency	**2, 774**	**8.7**	**<0.001**	**0.02**
sex × modulation rate	6, 776	1.4	0.220	0.01
sex × carrier frequency	**2, 774**	**18.8**	**<0.001**	**0.05**
species × sex	1, 58	0.5	0.500	0.01
modulation rate × carrier frequency	**12, 772**	**6.4**	**<0.001**	**0.09**
species × modulation rate × carrier frequency	**12, 772**	**2.5**	**0.004**	**0.04**
sex × modulation rate × carrier frequency	**12, 772**	**2.7**	**0.002**	**0.04**

responses than the other at modulation rates typical of conspecific calls. For example, at modulation rates of 25 to 100 Hz, gray treefrogs had significantly larger responses than green treefrogs when stimuli had the highest carrier frequency (Table 2; Fig. 3-4). In contrast, at 200 and 400 Hz, green treefrogs had larger responses than gray treefrogs for most carrier frequencies (Fig. 3-4). The difference was significant for responses to stimuli of all carrier frequencies with modulation rates of 200 Hz (Table 2).

Table 3-2 Results of Tukey posthoc contrasts.
Bold indicates significant terms.

contrast	carrier frequency	modulation rate	estimate	SE	df	*t*	*p* value	Cohen's *d*
species	low	**12.5 Hz**	**5.3**	**2.4**	**764.2**	**2.2**	**0.026**	**0.16**
		25 Hz	3.1	1.7	561.7	1.8	0.070	0.15
		50 Hz	-0.2	1.6	473.4	-0.1	0.892	0.01
		100 Hz	1.3	1.4	353.1	1.0	0.337	0.10
		200 Hz	**-5.5**	**1.5**	**406.5**	**-3.7**	**<0.001**	**0.37**
		400 Hz	-2.7	1.4	373.0	-1.9	0.061	0.19
		800 Hz	1.0	1.6	504.0	0.6	0.533	0.06
	middle	12.5 Hz	3.4	2.4	765.8	1.4	0.153	0.10
		25 Hz	**3.7**	**1.7**	**523.8**	**2.2**	**0.027**	**0.19**
		50 Hz	1.4	1.7	529.1	0.9	0.386	0.08
		100 Hz	-0.4	1.5	412.0	-0.3	0.798	0.03
		200 Hz	**-5.6**	**1.5**	**412.0**	**-3.8**	**0.000**	**0.37**
		400 Hz	-2.3	1.4	359.1	-1.6	0.103	0.17
		800 Hz	**-9.3**	**1.8**	**609.9**	**-5.1**	**<0.001**	**0.41**
	high	12.5 Hz	3.3	2.1	701.3	1.6	0.111	0.12
		25 Hz	**5.2**	**1.6**	**493.3**	**3.2**	**0.001**	**0.29**
		50 Hz	**5.9**	**1.6**	**467.1**	**3.8**	**<0.001**	**0.35**
		100 Hz	**6.2**	**1.4**	**340.1**	**4.5**	**<0.001**	**0.48**
		200 Hz	**-3.4**	**1.4**	**352.3**	**-2.4**	**0.016**	**0.26**
		400 Hz	0.7	1.4	340.1	0.5	0.598	0.06
		800 Hz	-1.7	2.8	796.8	-0.6	0.550	0.04
sex	low	12.5 Hz	0.0	2.4	764.2	0.0	0.989	0.00
		25 Hz	-0.2	1.7	561.7	-0.1	0.916	0.01
		50 Hz	-0.3	1.6	473.4	-0.2	0.837	0.02
		100 Hz	0.2	1.4	353.1	0.2	0.869	0.02
		200 Hz	0.7	1.5	406.5	0.5	0.647	0.05
		400 Hz	**5.3**	**1.4**	**373.0**	**3.7**	**<0.001**	**0.39**
		800 Hz	2.3	1.6	504.0	1.4	0.159	0.13
	middle	12.5 Hz	2.6	2.4	765.8	1.1	0.272	0.08
		25 Hz	2.2	1.7	523.8	1.3	0.186	0.12
		50 Hz	**4.3**	**1.7**	**529.1**	**2.6**	**0.011**	**0.22**
		100 Hz	**8.6**	**1.5**	**412.0**	**5.8**	**<0.001**	**0.57**
		200 Hz	**7.5**	**1.5**	**412.0**	**5.0**	**<0.001**	**0.50**
		400 Hz	**6.4**	**1.4**	**359.1**	**4.5**	**<0.001**	**0.48**
		800 Hz	2.5	1.8	609.9	1.4	0.169	0.11
	high	12.5 Hz	0.1	2.1	701.3	0.0	0.965	0.00
		25 Hz	0.4	1.6	493.3	0.2	0.825	0.02
		50 Hz	-0.2	1.6	467.1	-0.1	0.898	0.01
		100 Hz	-1.4	1.4	340.1	-1.0	0.326	0.11
		200 Hz	0.2	1.4	352.3	0.1	0.883	0.02
		400 Hz	-1.8	1.4	340.1	-1.3	0.194	0.14
		800 Hz	2.9	2.8	796.8	1.1	0.294	0.07

Recovery increased as a function of increasing ICI (Table 3). Recovery functions were overall very similar in shape between the two species (Fig. 3-6a). Consistent with this observation, the ANOVA revealed no significant effect of species on recovery, nor were there significant effects of any of the interactions involving species (Table 3). On average, green treefrogs were able to resolve slightly shorter ICIs than gray treefrogs ($F_{1,61}$ = 5.7, p = 0.020, partial η^2 = 0.09). The average minimum resolvable ICI was ($\bar{X}$ ± s.e.m.) 1.6 ± 0.1 ms in green treefrogs and 2.0 ± 0.1 ms for gray treefrogs.

Sex-specific adaptation hypothesis

Overall, MRTFs were similar between the sexes in both gray treefrogs (Fig. 3-5a) and green treefrogs (Fig. 3-5b). The sex × modulation rate × carrier frequency interaction was significant. However, there was no evidence of larger responses in male gray treefrogs than female gray treefrogs at modulation rates between 50 and 100 Hz, nor did male green treefrogs ever have

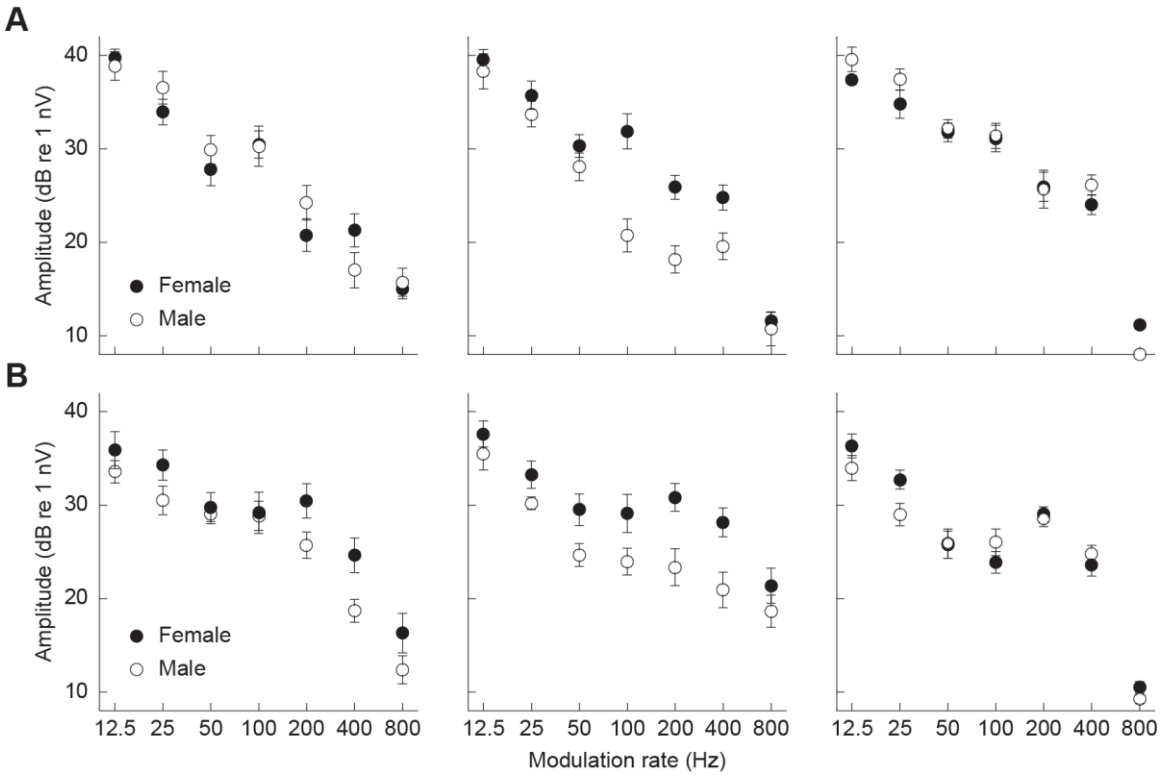


Figure 3-5 MRTFs separated by sex
ASSRs are plotted as a function of modulation rate separately for each sex for (A) gray treefrogs and (B) green treefrogs. Error bars are s.e.m.

larger responses than females. Instead, the interaction is evident in the fact that in response to the middle carrier frequency, females of both species consistently had larger responses than males, a difference that reached significance in response to stimuli with modulation rates of 50 to 400 Hz (Table 2; Fig 5). Responses to stimuli at the middle carrier frequency overall tended to be larger for females and smaller for males than corresponding responses to stimuli with the low or high carrier frequency.

Recovery functions also differed little between the two sexes (Fig. 3-6b). Subject sex did not have a significant effect on percent recovery, and the interaction of sex with ICI was also not significant (Table 3). There was no sex-difference in minimum resolvable ICI ($F_{1,61}$ = 0.5, p = 0.469, partial η^2 = 0.01), and no effect of the species × sex interaction ($F_{1,61}$ = 0.2, p = 0.666, partial η^2 < 0.01).

Table 3-3 Effects of species and subject sex on responses to paired clicks.
Bold indicates significant terms.

term	df	*F*	*p* value	partial η^2
intercept	**1,60**	**1109.8**	**< 0.001**	**0.95**
ICI	**9,540**	**46.1**	**< 0.001**	**0.43**
species	1,60	0.0	0.985	0.00
sex	1,60	0.8	0.377	0.01
species × ICI	9,540	1.3	0.267	0.02
species × sex	1,60	1.3	0.264	0.02
sex × ICI	9,540	0.8	0.490	0.01
species × sex × ICI	9,540	1.7	0.170	0.03

Discussion

While MRTFs between the species and sexes shared an overall similar shape, we found significant differences both between species and between sexes at particular modulation rates. We also found significant differences between the minimum resolvable ICIs of the two species. We discuss our results in the context of our specific hypotheses in the next sections.

Species-specific adaptation hypothesis

Both species showed evidence of adaptations of their auditory systems to the temporal structure of conspecific advertisement calls. We noted differences between the species in response to stimuli with low modulation rates (25 to 100 Hz), where gray treefrogs tended to have larger responses than green treefrogs to stimuli of the highest carrier frequency. This result is consistent with an adaptation of the auditory systems of gray treefrogs to conspecific advertisement calls,

which have pulse rates ranging between 40 and 65 Hz. It is interesting that this difference appeared only when stimuli had the highest carrier frequency. However, this finding is perhaps not surprising, as the amplitude at this frequency in gray treefrog calls is 11 dB greater than the spectral amplitude of the peak at 1.25 kHz (Ward et al., 2013). At high carrier frequencies, stimuli are expected to be encoded predominantly by the BP. Thus, this finding could represent an adaptation specific to this auditory organ.

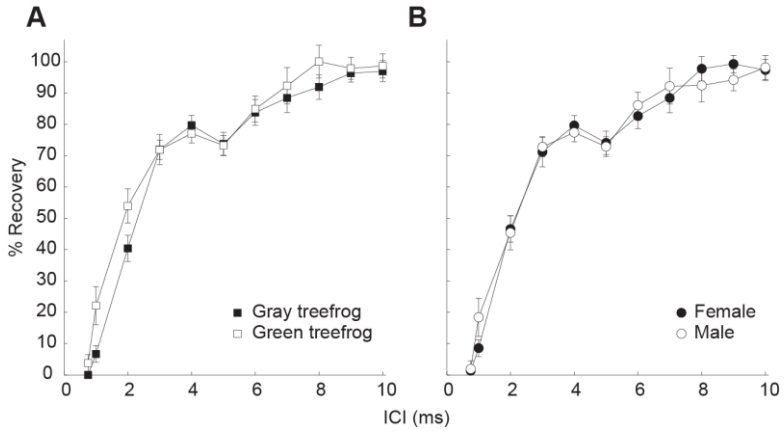


Figure 3-6 Paired-click recovery functions
Recovery was calculated as the ratio of the peak-to-peak amplitude (as calculated in Fig. 3) of the residual response to the peak-to-peak amplitude of the response to a single click. Recovery is plotted separately for (A) each species and (B) each sex, as a function of ICI. All error bars are s.e.m.

Green treefrogs had larger ASSRs than gray treefrogs to stimuli with AM rates of 200 and 400 Hz, which is close to the typical periodicities of 200 to 500 Hz in advertisement calls. Green treefrogs also had slightly shorter minimum resolvable ICIs than gray treefrogs, which is consistent with the species-specific adaptation hypothesis. This increased resolution of the auditory system could facilitate tracking of the relatively faster periodicities in their advertisement calls.

Sex-specific adaptation hypothesis

We saw no evidence that males had better processing than females of the temporal properties inherent in conspecific aggressive calls. Males did not have larger responses than females at modulation rates typical of conspecific aggressive calls, nor was there evidence for a sex-difference in percent recovery functions. Thus, the results did not fit our predictions for the sex-specific adaptation hypothesis.

However, MRTFs differed substantially between sexes in both species when the stimuli had the middle carrier frequency. At this carrier frequency, in response to most modulation rates, females had larger ASSRs than males. The frequencies we selected as middle carrier frequencies are expected to excite both inner ear papillae, so observation of larger responses in females

suggests better recruitment of nerve fibers across papillae in females than males. This result is consistent with previous results from recordings of AEPs in these species (Buerkle et al., 2014; Schrode et al., 2014). In those studies, the amplitudes of responses to tones were larger in females than males when tones had intermediate frequencies (1.5 to 2.0 kHz). At present it is unclear whether this sex-difference in responses is indicative of an evolutionary adaptation

Consideration of previous results

MRTFs in both species were nearly log-linear with respect to modulation rate, with responses decreasing as a function of modulation rate. Given that the ASSR is a measure of synchronization, with a strong component originating in auditory nerve fibers (Henry and Lucas, 2008; Supin and Popov, 1995b), this result is consistent with previous studies of auditory nerve fibers that report decreasing synchronization as a function of modulation rate (Dunia and Narins, 1989; Feng et al., 1991; Rose and Capranica, 1985). The minimum resolvable ICIs of 1.5 – 2.0 ms that we measured were comparable to previous measurements of temporal resolution in anuran auditory nerve fibers (Dunia and Narins, 1989; Feng et al., 1994). In particular, ICIs were similar to the average gap detection times of 1.23-2.16 reported from auditory nerve fibers in leopard frogs (*Rana pipiens*) (Feng et al., 1994). Our range of minimum resolvable ICIs was a little longer than the 0.42 ms resolution time that Dunia and Narins (1989) calculated from responses of auditory nerve fibers in coquí frogs (*Eleutherodactylus coqui*).

Previous comparative work with gray treefrogs and green treefrogs found a difference in their abilities to recognize conspecific advertisement calls in the presence of temporally-fluctuating noise (Vélez and Bee, 2010; Vélez and Bee, 2011; Vélez et al., 2012). When background noise fluctuates in amplitude, human listeners can take advantage of "dips" in noise levels to catch acoustic glimpses of target signals of interest (Bacon et al., 1998; Cooke, 2006; Füllgrabe et al., 2006; Vestergaard et al., 2011), an ability known as "dip listening." A series of studies showed that gray treefrogs, but not green treefrogs, were able to listen in dips to achieve a release from masking in chorus noise modulated with a sinusoidal envelope (Vélez and Bee, 2010; Vélez and Bee, 2011; Vélez et al., 2012). It is thought that dip listening ability is correlated with temporal resolution (Festen, 1993; Qin and Oxenham, 2003), and so we would have predicted that gray treefrogs should have had better temporal resolution than green treefrogs. However, green treefrogs had larger responses at higher modulation rates than gray treefrogs (e.g. 200 Hz) and shorter minimum resolvable ICIs, both of which indicate that green treefrogs should

have better temporal resolution than gray treefrogs. Thus, contrary to our prediction, there do not seem to be species-differences in temporal processing in the early stages of the auditory system that can explain the behavioral species-differences previously reported. At this point we cannot rule out differences in temporal resolution at ascending levels of the auditory system that could account for the species-difference in dip listening.

Materials and methods

Subjects

Subjects were 68 gray treefrogs (35 female) and 59 green treefrogs (30 female). Gray treefrogs were collected from Carver Park Reserve (Carver County, MN, U.S.A.), the Crow-Hassan Park Reserve (Hennepin County, MN, U.S.A.), or Lake Maria State Park (Wright County, MN). Green treefrogs were collected from the East Texas Conservation Center (Jasper County, TX, U.S.A.). All frogs were collected in amplexus during their respective breeding seasons in either 2011 or 2012. Female gray treefrogs ($\bar{X}$ ± s.d.: mass = 5.2 ± 1.0 g; SVL = 39.3 ± 2.7 mm) tended to be larger than male gray treefrogs (4.2 ± 0.8 g; 35.8 ± 1.9 mm). In green treefrogs, females (7.4 ± 1.5 g; 49.4 ± 3.0 mm) were similar in size to males (7.2 ± 1.4 g; 48.0 ± 3.2 mm). After collection, pairs were placed in small containers and transported to the laboratory at the University of Minnesota in St. Paul, where they were maintained on a 12-hour light/dark cycle in terraria at ambient room temperature (~ 20°C). We supplied frogs with fresh water and fed them a regular diet of vitamin-dusted crickets. We tested each subject within three weeks of collection. All animals were collected with permission from the Minnesota Department of Natural Resources (permit #s 17892 & 19061) and Texas Parks and Wildlife (permit # SPR-0410-054) and treated according to protocols approved by the Institutional Animal Care and Use Committee of the University of Minnesota (#1103A97192, last approved 04/16/2013).

General procedures

Equipment and procedures for recording AEPs have been described previously (Buerkle et al., 2014; Schrode et al., 2014). Briefly, we generated all digital stimuli (50 kHz sampling rate, 16-bit) in TDT SigGenRP software (Tucker Davis Technologies, Alachua, FL, USA). TDT BioSigRP software coordinated stimulus output and neural recording through TDT System 3 hardware. Stimuli were broadcast through an Orb Mod 1 speaker (Orb Audio, New York, NY, USA), which was driven by a Crown XLS 202 amplifier (Crown Audio, Inc., Elkhart, IN, USA).

Recordings were made inside a MAC-3 radio-shielded mini-acoustical chamber (W × D × H: 81.3 cm × 61 cm × 61 cm; Industrial Acoustics Company, Bronx, NY, U.S.A.). For recordings, we first immobilized subjects with an intra-muscular injection of d-tubocurarine chloride (3-12 µg/g body weight). Subjects were loosely wrapped in a thin piece of moistened gauze to facilitate cutaneous respiration and seated in a natural position on an acoustically transparent platform, facing the speaker. Temperature was monitored via a Miller & Weber quick-reading thermometer placed against the subject's body wall and ranged between 18.0 and 20.0 °C across recording sessions, which is within the range of temperatures at which both species breed. We placed subjects so that the rostral edges of their tympana were 30 cm from the face of the speaker. We applied a topical anesthetic (2.5% lidocaine HCl) to the scalp of the subject prior to inserting the tips of three subcutaneous electrodes (1-5 kΩ) under the skin. The recording electrode was located between the eyes and the ground and inverting electrodes were placed adjacent to the two tympana. Neural signals were sampled at a rate of 25 kHz, digitized, and amplified before being transmitted via optic fiber cable to a TDT RZ5 processor and stored for offline analysis. On the rare occasion that a recording was contaminated with an obvious artifact (e.g. due to subject movement), that recording was repeated.

Auditory steady-state response

We generated AM tones in SigGenRP by multiplying together two sinusoids, one acting as the modulator, and the second acting as the carrier signal. All stimuli had modulation depths of 100%. Tones were modulated in 1-octave steps at rates of 12.5, 25, 50, 100, 200, 400, and 800 Hz and were of a sufficient duration to ensure that subjects heard at least 10 modulation cycles at each modulation rate. Tones with modulation rates of 12.5 Hz had a duration of 800 ms. All other tones had durations of 400 ms. We used three different carrier frequencies for each species (1.25, 1.625, and 2.5 kHz for gray treefrogs; 0.9, 1.6, and 2.7 kHz for green treefrogs). The low and high frequencies for each species were selected because they correspond to frequencies prominent in conspecific advertisement calls (Gerhardt, 1974a; Gerhardt, 1974b; Schrode et al., 2012b). Additionally, each species tends to be most sensitive to these two frequencies, as determined through recordings from the peripheral and central auditory systems (Buerkle et al., 2014; Hillery, 1984b; Lombard and Straughan, 1974; Miranda and Wilczynski, 2009b; Penna et al., 1992; Schrode et al., 2014). We additionally selected an intermediate frequency for each species which we believe simultaneously excites the AP and BP at high signal levels (Buerkle et al., 2014;

Gerhardt, 2005; Schrode et al., 2014). In Fig. 3-1 we show six cycles of example stimuli used to elicit ASSRs from green treefrogs.

Calibration of signal level was a two-step process. We first calibrated 1-s (unmodulated) tones with frequencies matching the carrier frequencies of the AM tones to 70 dB SPL (re 20 μPa, C-weighted, fast RMS), using the microphone of a Larson Davis System 824 sound level meter (Larson Davis, Depew, NY, USA) placed at the approximate location of the frog's head and facing the speaker. We then matched the peak-to-peak amplitudes of each AM tone to that of the calibrated, unmodulated tone of corresponding frequency. Previous studies indicate 70 dB SPL is approximately 15 to 30 dB above the corresponding frequency-specific auditory thresholds of each species (Hillery, 1984b; Megela-Simmons et al., 1985; Miranda and Wilczynski, 2009b; Penna et al., 1992). The frequency response of the speaker was flat (± 1 dB) across the range of frequencies tested.

We recorded two ASSRs to each stimulus from 30 gray treefrogs (15 females) and 30 green treefrogs (15 females); examples from a green treefrog are shown in Fig. 3-1. Each ASSR consisted of the average of the responses to 400 presentations of the stimulus. We randomized carrier frequencies and modulation rates of 25 – 800 Hz for each subject. Because of their long duration, tones modulated at a rate of 12.5 Hz were presented in a block prior to or following tones modulated at other rates. This had the effect of reducing the overall recording time, thus limiting the potential for changes in the animal's state over the duration of the recordings. The timing of the block before or after the other recordings, and the carrier frequency of tones within the block were randomized for each subject. Recordings of responses to stimuli were notch-filtered at 60 Hz and low-pass filtered at 3 kHz.

The frequency spectrum of each ASSR was extracted using an 8192-point FFT in Matlab v2012b (Mathworks, Natick, MA, USA). We first averaged the two replicates of the ASSR to a given stimulus and then performed FFT analysis on the first 400 ms of the response. The fixed windows selected by others have varied in length from 6 ms (Supin and Popov, 1995b) or around 60 ms (Gall et al., 2012a; Henry and Lucas, 2008) to 16 s (John and Picton, 2000). The length of our analysis window was chosen to achieve a frequency resolution suitable for the modulation rates tested. Additionally, the window length ensured inclusion of an integer number of cycles of the modulation stimulus, which is important for avoiding errors in the calculated frequency spectrum (Herdman and Stapells, 2009; John and Picton, 2000; Nachtigall et al., 2007a; Supin and Popov, 1995b). The frequency spectra of the responses to three example stimuli are

illustrated in Fig. 3-1. Clear peaks corresponding to the 100 Hz modulation rate of the stimuli appear in the spectra of all the examples shown in Fig. 3-1.

To determine whether the evoked response was significantly different from normal fluctuations in the potential due to neural noise, we used a conventional method of calculating an *F* ratio comparing the power at the modulation rate of the stimulus to an estimate of the background noise (Cone-Wesson et al., 2002; Dobie and Wilson, 1996; Gorga et al., 2004; Hall, 2007; Herdman and Stapells, 2009; Korczak et al., 2012; Picton et al., 2003; Purcell et al., 2004; Valdes et al., 1997; van der Reijden et al., 2005). We estimated the background noise from the average power in the 16 FFT bins adjacent to the modulation rate of the stimulus. Bins were approximately 3 Hz in width, so the background noise was estimated for a range of about 48 Hz surrounding the modulation rate of the stimulus. Responses were considered significant if the corresponding *F* ratio exceeded the critical value of $F_{2,32}$ (where the degrees of freedom in the denominator are 2 times the number of frequency bins used to estimate background noise). While increasing the number of adjacent frequency bins increases the statistical power of the analysis, increasing the number of bins in this case did not noticeably change the results.

We only considered responses that were significantly different from background noise; because not all subjects exhibited ASSRs at all modulation rates for all carrier frequencies, this resulted in some missing values. We investigated the effects of species, sex, modulation rate, and carrier frequency on the responses using a linear mixed model in R (R Development Core Team, 2014) fit using the lme4 (Bates et al., 2014) and afex (Singmann, 2014) packages. Linear mixed models are advantageous with such datasets because they take advantage of all of the available data and handle missing values well. We included species, sex, modulation rate, and carrier frequency as fixed factors, all two-way interactions, and the three-way interactions of modulation rate × carrier frequency with species and sex. We performed Tukey post-hoc contrasts using the lsmeans package (Lenth, 2014) to compare between groups. A significance criterion of $\alpha = 0.05$ was used for all analyses.

Responses to paired-clicks

Click stimuli (0.1-ms duration) were generated in SigGenRP. Clicks output through our setup had a broadband spectrum, with a center frequency of approximately 1.6 kHz and 6-dB down points of approximately 0.345 and 2.8 kHz. Paired-clicks consisted of two acoustic clicks, separated by a specified ICI. Examples are illustrated in Fig. 3-2a. We tested ICIs of 0.5 ms, 0.75 ms, and 1 to

10 ms, in 1-ms steps, with order randomized between subjects. Each presentation of a paired-click stimulus was followed by at least 40 ms of silence and then a single-click stimulus. We recorded two replicate responses to the paired-click and single-click stimuli, with each replicate consisting of the average responses to 1200 presentations of the stimulus. There was a silent interval of at least 40 ms between the single click and the onset of the next stimulus presentation. Click polarity was constant for all three clicks within a presentation, but alternated between each presentation to reduce the microphonic potential. Clicks were calibrated to 80 dB by matching the peak-to-peak amplitude of each click to that of a calibrated 1-s tone with a frequency of 1000 Hz. From our previous studies of click-evoked potentials in these species, we have determined that 80 dB is well above auditory threshold (Buerkle et al., 2014; Schrode et al., 2014).

We recorded responses to paired-clicks from 38 gray treefrogs (20 females) and 29 green treefrogs (15 females). Example responses from a green treefrog are given in Fig. 3-2b. At relatively long ICIs (e.g. 8 ms), distinct responses to each of the clicks in the paired-click stimuli are evident. However, at shorter ICIs, the responses to the first and second click overlap. To disambiguate these responses, we derived the response to the second click by aligning the responses to the single and paired clicks at stimulus onset and then subtracting, point-by-point, the first 25 ms of the average response to the single-click from the average response to the paired-click. This subtraction effectively removed any response elicited by the first click of the pair, leaving only the residual response to the second click (Fig. 3-2c). Using a custom-written, cursor-based program in Matlab, we measured the amplitude of all residual responses and responses to single clicks as the peak-to-peak amplitude from the top of the first positive deflection to the bottom of the subsequent trough (see Fig. 3-3) (Buerkle et al., 2014; Schrode et al., 2014). If a peak was not visible, we considered the amplitude to be 0 μV. These values were used to calculate the percent recovery as the ratio of the amplitude of a residual paired-click-evoked response to the amplitude of the corresponding single-click-evoked response, multiplied by 100%. For each subject, we also measured the shortest resolvable ICI. After plotting evoked responses in order of ICI (as in Fig. 3-2a), we selected the minimum resolvable ICI as the shortest ICI for which a response was visually detectable.

Because we had a full dataset with no missing values, we used a repeated-measures ANOVA to investigate the effects of species and sex on percent recovery. ANOVA allowed us to calculate partial η^2 values as a measure of effect size in addition to determining significance. We tested for significant differences in minimum resolvable ICI using a two-way ANOVA. Species

and subject sex were included as fixed factors. We used a significance criterion of $\alpha = 0.05$ for both analyses and report p-values corrected based on the Greenhouse-Geisser method (1959) where applicable.

Chapter 4 A test of the channeling hypothesis in a non-mammalian auditory system

Auditory systems must parse and sort sound elements into perceptual "streams," a task known as stream segregation. One of the traditional ideas about the formation of streams, "the channeling hypothesis," is that separate streams are created when separate "channels" in the peripheral auditory system are excited. These channels refer to frequency channels (resulting from tonotopy) or lateral channels (resulting from left and right ears). However, the organization of channels often differs between the auditory systems of different animals in several important ways. For example, some animals have sensory organs in the inner ear that are not tonotopically organized, and most non-mammalian vertebrates have internally coupled ears. In the present study, the channeling hypothesis was tested in a treefrog, an animal that has internally coupled ears and two physically independent auditory papillae that encode distinct frequency ranges, only one of which is tonotopically organized. Subjects heard stimuli that were primarily encoded by one or both of the papillae, facilitating a test of segregation both between and within channels. Additionally, frogs were tested in co-localized and spatially separated conditions to evaluate the channeling hypothesis for internally coupled ears. We find that the channeling hypothesis can explain some instances of segregation, but that it fails in a number of cases.

Introduction

Acoustic communication in social aggregations can be difficult when the signals generated by multiple individuals overlap in frequency and time (Brumm & Slabbekoorn 2005; Brumm 2013). The sensory input transduced by the ear is a composite sound, which must be parsed into individual sound elements, such as the syllables in speech or tones in a melody. Auditory stream segregation refers to the sorting of these sound elements into coherent "streams" (Bregman, 1990). Integration of sounds is generally favored when sounds share similarity in one or more features. Similarity in frequency, for example is a strong cue for integration of sounds, while *differences* in frequency (ΔF) are strong cues for segregation. For example, the tendency to segregate sounds (e.g. speech or vowels) that differ in fundamental frequency (F_0) into separate streams increases as a function of the difference between the F_0s (Assmann, 1999; Assmann and Summerfield, 1994; Bird and Darwin, 1998; Brokx and Nooteboom, 1982). Another example is the now classic psychophysical task of segregating sequences of two interleaved tones differing in frequency (e.g. ABAB…) (van Noorden, 1975). Listeners are more likely to segregate the A and B tones into two separate streams (e.g. A-A- and -B-B) as ΔF increases (Bregman, 1990; Carlyon, 2004; Moore and Gockel, 2002). Stream segregation has been most intensively studied in humans and other mammals (Christison-Lagay and Cohen, 2014; Izumi, 2002; Ma et al., 2010; Moss and Surlykke, 2001), but there is also evidence for stream segregation in other animals including birds (MacDougall-Shackleton et al., 1998), frogs (Nityananda and Bee, 2011), fish (Fay, 1998; Fay, 2000) and even insects (Schul and Sheridan, 2006).

Despite extensive research into the neural correlates of auditory stream segregation (Bee and Klump, 2004; Bee and Klump, 2005; Bee et al., 2010; Elhilali and Shamma, 2008; Elhilali et al., 2009; Fishman et al., 2001; Fishman et al., 2004; Kanwal et al., 2003; Micheyl et al., 2007; Pressnitzer et al., 2008; Shamma and Micheyl, 2010; Snyder and Alain, 2007), the neural mechanisms that underlie the formation of streams are still not fully understood. In particular, the role of the mammalian auditory cortex (or its evolutionary homolog) in stream segregation has been widely studied (Bee and Klump, 2004; Bee and Klump, 2005; Bee et al., 2010; Deike et al., 2010; Elhilali et al., 2009; Fishman et al., 2001; Fishman et al., 2004; Micheyl et al., 2005; Micheyl et al., 2007; Snyder et al., 2006). Recent work has shown that formation of streams may begin in subcortical regions of the central auditory system, as early as the cochlear nucleus (Pressnitzer et al., 2008). However, some have suggested that streams actually form in the periphery (Beauvois and Meddis, 1994; Beauvois and Meddis, 1996; Hartmann and Johnson,

1991; van Noorden, 1975). Indeed, many of the processes identified at cortical and subcortical levels may have their origins in the periphery. In the current study, we investigated stream segregation in an animal that lacks an auditory cortex and has a peripheral auditory system that differs in several interesting ways from those of birds and mammals.

One prevailing hypothesis in favor of a strong role for peripheral mechanisms is the "channeling hypothesis." Two types of peripheral channels are thought to exist in the auditory system. *Frequency channels* are based on the filtering that occurs in the sensory organs of the vertebrate inner ear (Allen, 1994; Fletcher, 1940). In terms of physiology, a frequency channel could be defined as a hair cell and the auditory nerve fibers that innervate it (Eggermont, 2000). *Lateral channels* correspond to the two ears, which filter and process sounds independently through the two auditory nerve bundles (Hartmann and Johnson, 1991). The channeling hypothesis posits that sounds are integrated when processed through the same peripheral channel and segregated when they excite different peripheral channels (Hartmann and Johnson, 1991; van Noorden, 1975). Much of the auditory stream segregation data can be accounted for by the channeling hypothesis (Cusack and Roberts, 2000; Grimault et al., 2002; Micheyl et al., 2007; Roberts et al., 2002; Shamma and Micheyl, 2010; Vliegen and Oxenham, 1999). However, there are also many instances in which processing in the periphery is insufficient to explain the perceptual segregation of auditory streams. For example, sequential sounds that are processed through the same peripheral channels can nevertheless be segregated if they differ in properties such as timbre or temporal modulation patterns (Cusack and Roberts, 2000; Grimault et al., 2002; Moore and Gockel, 2002).

Most studies of the channeling hypothesis have been in mammals (Elhilali et al., 2009; Fishman et al., 2001; Fishman et al., 2004; Snyder et al., 2006; Stainsby et al., 2004), and so the results of these studies necessarily reflect the physiology of frequency and lateral channels in the mammalian auditory system. Our goal in this study was to test the channeling hypothesis in a frog, an animal in which channels are organized somewhat differently than in the systems typically studied. Frogs are well-studied models for acoustic communication (Gerhardt and Huber, 2002; Narins et al., 2007; Ryan, 2001). A few studies have investigated integration and segregation of sounds by frogs, which provide a solid foundation for the current study (Bee, 2010; Bee and Riemersma, 2008; Farris and Ryan, 2011; Farris et al., 2002; Farris et al., 2005; Nityananda and Bee, 2011; Schwartz and Gerhardt, 1995).

Frequency channels in frog ears are distributed across two sensory organs. While mammals have a single organ in the inner ear, the tonotopically-organized basilar membrane, which encodes airborne sounds of across a wide range of frequencies, frog inner ears instead have two primary sensory papillae (Fig. 4-1a). The tuning of these organs generally corresponds to frequencies emphasized in the species-specific advertisement calls (Capranica and Moffat, 1983; Gerhardt and Schwartz, 2001). The amphibian papilla (AP) encodes frequencies typically between about 0.3 and 1.5 kHz and is tonotopically organized (Lewis et al., 1982), allowing frequency discrimination to be achieved independent of level. Afferents originating in the basilar papilla (BP) are homogenously tuned to a frequency above 1.8 kHz (Feng et al., 1975; Frishkopf and Goldstein Jr., 1963). The sensitivity of the BP varies across frequency, with the result that nerve fibers arising from the BP respond with decreasing firing rates as a function of decreasing sensitivity (Schwartz and Gerhardt, 1998). Thus, a change in the frequency of a stimulus that excites the BP cannot be distinguished from a change in its level. Thus, not only do the two papillae represent two physically distinct frequency channels, one of these channels (the AP) is further subdivided into multiple frequency channels (similar to the mammalian condition), while the other (the BP) functions as a single channel (Fig. 4-1a). This two-organ auditory periphery, thus, offers the opportunity to test the channeling hypothesis both between organs (i.e. across the AP and BP channels) and within organs (i.e. across tonotopic channels in the AP).

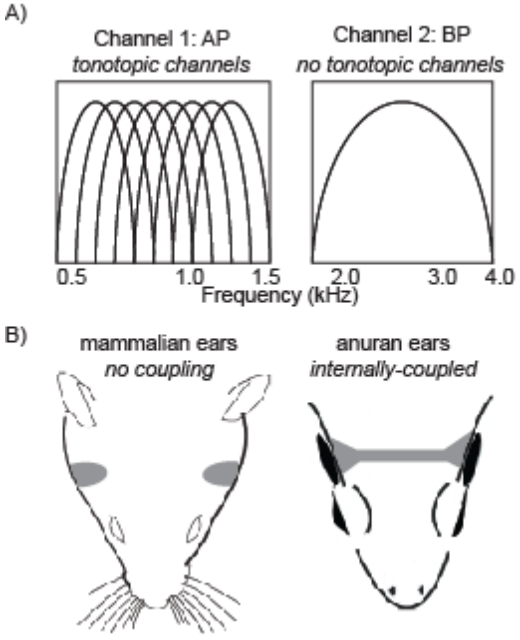


Figure 4-1 Peripheral channels
(A) Schematics depicting the organization of peripheral channels in anuran ears. The two sensory papillae, the AP and BP constitute two physically distinct tonotopic channels. The tonotopic AP is further divided into multiple channels (left), while the BP consists of a single channel (right). (B) The middle ears of mammals are physically independent (left), while those of anurans are coupled internally (right).

Lateral channels in the frog auditory system also differ somewhat from those of mammals. The ears of most frogs are internally coupled through the mouth and wide Eustachian tubes. This coupling has the effect of making the ears function as inherently-directional pressure difference receivers, but also has the effect of making the input to each ear dependent on input to the other (Fig. 4-1b; reviewed in Christensen-Dalsgaard 2005). In contrast, in the mammalian auditory system, the inputs to the two ears are independent, and directionality is achieved through comparisons in the central auditory system of the inputs to the ears. Thus, while the frog auditory system incorporates two independent lateral channels, the ears are not independent at the level of the periphery.

We tested the channeling hypothesis in Cope's gray treefrog (*Hyla chrysoscelis*). Like many frogs, males of this species gather in dense aggregations (choruses) in which they communicate using acoustic advertisement calls. Choruses may consist of hundreds of males, sometimes of multiple species, resulting in considerable overlap of advertisement calls and high noise levels (Schwartz et al., 2002). The advertisement calls of this species are composed of around 30 pulses (Fig. 4-2a), produced at a species-specific rate of approximately 50 pulses/s (Ward et al., 2013). These pulses have a bimodal frequency spectrum, with a fundamental frequency between 1100 and 1500 Hz and a second harmonic between about 2100 and 2900 Hz (Schrode et al., 2012). Female treefrogs choose mates based on the advertisement calls of males, and exhibit strong preferences based on the pulse structure of the calls (Bush et al., 2002; Schul and Bush, 2002). Female treefrogs exhibit a stereotyped approach behavior (phonotaxis) toward preferred calls and will perform the behavior in the laboratory in response to synthetic versions (e.g. Fig. 4-2b) of these calls (Gerhardt, 1995).

The approach we used to test the channeling hypothesis mimicked the classic ABAB paradigm described earlier. For the purposes of the current study, we use the letters "A" and "B" to denote groups of five pulses in synthetic advertisement calls. Subjects were tested in two-alternative choice tests that paired a relatively long call in which groups of pulses differed in one or more acoustic or spatial features (*Alt-Long*: ABABABA) and a shorter call in which all pulses were identical (*Alt-Short*: AAAAA), as illustrated in Fig. 4-2c,d.

The design of our two-alternative choice tests exploited two known preferences of female Cope's gray treefrogs. Females prefer longer calls over shorter calls (e.g. AAAAAA > AAAA) (Bee, 2008), and they strongly discriminate against calls containing silent gaps in favor of calls with consecutive pulses (e.g. AAAA > A-A-A-A), even if the total number of pulses is the same

(Seeba et al., 2010). Therefore, we reasoned that, if subjects integrated the A and B pulses into a single coherent stream (as in Fig. 4-2d, left), they would prefer *Alt-Long* (ABABABA) over *Alt-Short* (AAAAA). If, instead, they segregated the A and B pulses into two separate streams (e.g. A-A-A-A and -B-B-B-, as in Fig. 4-2d, middle and right), we predicted preferences would shift toward the alternative containing consecutive pulses, *Alt-Short*. Thus a high proportion of subjects choosing *Alt-Long* would indicate integration, with increasing proportions of subjects choosing *Alt-Long* over *Alt-Short* indicating greater tendency toward segregation.

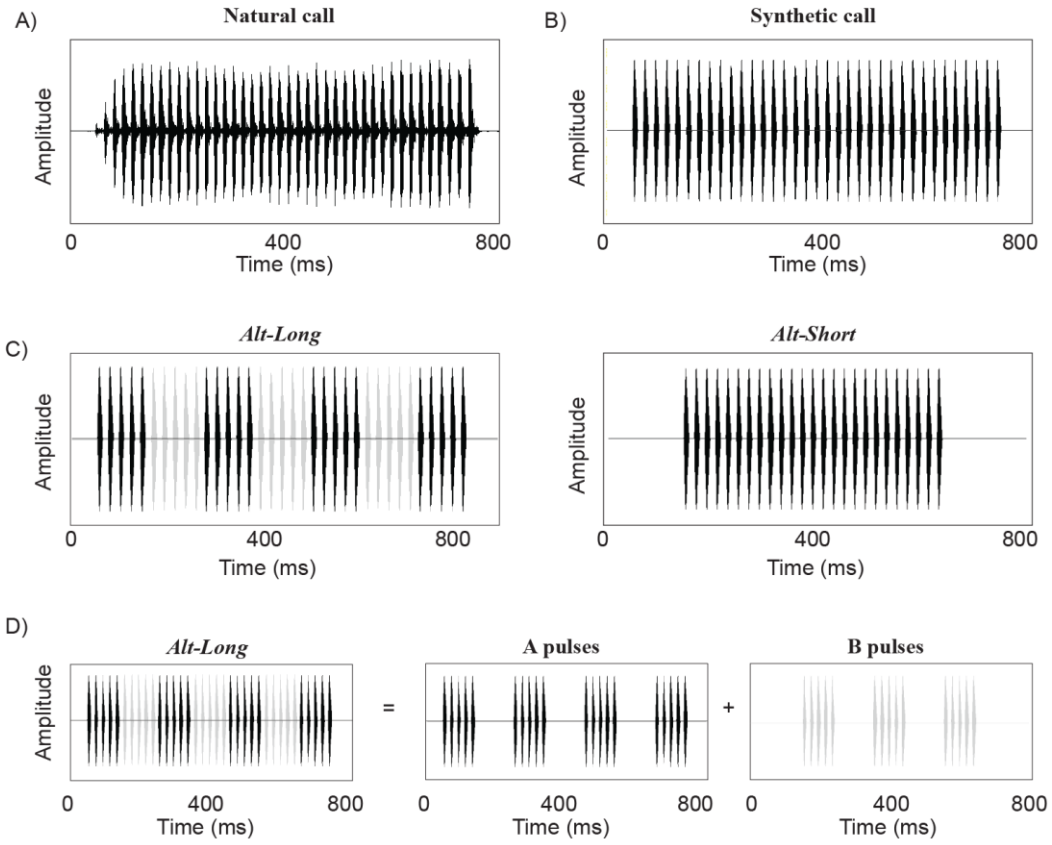


Figure 4-2 Natural and synthetic advertisement calls
Shown are examples of a typical advertisement call (A) and a synthetic version of an advertisement call (B). (C) In two-alternative choice tests, subjects chose between a 35-pulse Alt-Long *and a 25-pulse* Alt-Short. *All pulses in* Alt-Short *were identical (AAAAA). (D) Alt-Long was constructed by interleaving groups of A and B pulses that differed in some acoustic feature (e.g. ABABABA). If subjects integrated the A and B pulses, they were expected to prefer* Alt-Long *over* Alt-Short. *If subjects segregated the A and B pulses into separate streams, their preferences were expected to shift to* Alt-Short.

We varied differences in frequency (ΔF) through manipulations of the B pulses in *Alt-Long* (Fig. 4-2d). All pulses had a single spectral peak. Females respond readily to calls with a single spectral peak (Bee, 2010). The A pulses always had a frequency equal to one of the frequencies in natural calls (either 1.3 or 2.6 kHz), which is hereafter denoted the "carrier frequency." This aspect of the experimental design ensured that the A pulses were primarily encoded by either the AP or the BP. The B pulses were selected so that they were either primarily encoded by the same papilla as the A pulses (e.g. $0.7 \leq B \leq 1.3$ kHz; A = 1.3 kHz) or by the opposite papilla (i.e. $1.3 < B \leq 2.6$ kHz; A = 1.3 kHz).

Based on the channeling hypothesis, we should expect segregation of sounds that differentially excite the two papillae, as the papillae represent distinct peripheral channels. Thus we predicted the proportions selecting *Alt-Long* should be low when *Alt-Long* sequentially excited both papillae ($1.3 < B < 2.6$). In tests in which both A and B pulses had frequencies within the range of the tonotopic AP (e.g. A = 1.3 kHz; $0.7 \leq B \leq 1.3$ kHz), we predicted most subjects would choose *Alt-Long* when ΔF was small, and that the proportions would decrease as a function of increasing ΔF. When considering the BP, it is important to recall that while it functions as a single channel, it is not uniformly sensitive to all of the frequencies that stimulate it. The BP thus encodes frequency through level, a cue that could be used for segregation. Therefore, in tests in which *Alt-Long* excited only the BP (e.g. A = 2.6 kHz; $2.6 \leq B \leq 4.1$), we predicted decreasing proportions to select *Alt-Long* as a function of increasing ΔF.

Because our predictions when *Alt-Long* stimulated the BP were based on perceived level differences, we also directly tested the effects of differences in level (ΔL) on stream segregation. We varied ΔL by manipulating the level of the B pulses. Just as two sounds that only excite the BP excite the same peripheral channel, two sounds that differ only in sound level will also excite the same peripheral channel. However, sounds with different sound levels will elicit different response patterns in the peripheral auditory system, a cue that can be used by the auditory system to segregate sounds differing in level. Thus, we predicted lower proportions of subjects to choose *Alt-Long* as a function of ΔL.

We manipulated differences in location (Δθ) by changing the angle between the positions from which the A and B pulses in *Alt-Long* were broadcast. Due to the coupling of frog ears, information about the location of a sound is represented at the level of the periphery, a cue which could be used to segregate the sounds. We therefore predicted lower proportions should select *Alt-Long* as a function of increasing Δθ. The ears of gray treefrogs show the strongest

directionality at high (e.g. >2 kHz) frequencies (Caldwell et al., 2014). Given that better directionality should facilitate segregation of sounds, we expected the effect of Δθ to be greater when the A pulses have a frequency of 2.6 kHz than when they are 1.3 kHz.

General Methods

Subjects

Subjects were 650 female gray treefrogs collected in the years 2011-2014. Collections took place between mid-May and early-July from the Carver Park Reserve (Carver County, MN, U.S.A.), the Crow-Hassan Park Reserve (Hennepin County, MN, U.S.A.), or Lake Maria State Park (Wright County, MN). Frogs were collected in amplexus, placed in small containers and brought to the laboratory in St. Paul, Minnesota. At the laboratory, aged tap water was added to the containers and the pairs were maintained at 2° C until behavioral testing. At least thirty minutes prior to testing, frogs were placed in an incubator set to 20° C (± 1° C), and they were kept in the incubator between tests. Subjects were tested and returned to their respective points of collection within 3 days. All animals were collected with permission from the Minnesota Department of Natural Resources (permit #s 17031, 17892 & 19061) and treated according to protocols approved by the Institutional Animal Care and Use Committee of the University of Minnesota (#1401-31258A, last approved March 3, 2014).

Phonotaxis procedures

We performed three phonotaxis experiments, outlined in Table 4-1. Experiment 1, we investigated the effects of ΔF, Δθ, and ΔL on integration of sounds. Experiments 2 and 3 were control experiments.

Tests took place under infrared (IR) lighting in a temperature-controlled, semi-anechoic sound chamber (W × D × H: 300 cm × 280 cm × 216 cm; Industrial Acoustics Company, Bronx, NY, U.S.A.). Temperature inside the chamber was maintained at 20° C (± 1° C). To begin a test, we placed a subject at the center of a circular arena, 2 m in diameter, inside the sound chamber. The arena walls were visually opaque but acoustically transparent, and the subject was initially restrained using an acoustically transparent cage. Sound presentation began after one minute of silence. Speakers were located outside the arena walls and faced into the center of the arena. We changed the location of the speakers between sets of two to four subjects to eliminate directional

biases in responses. All tests were scored in real time by two observers located outside the chamber. A response was scored when the subject touched the arena wall in a 15° arc in front of an active speaker. The test ended when the subject responded or after five minutes had elapsed. Response latency was calculated as the time from release to the subject's response, or 300 seconds if the subject failed to respond. Each subject had a time-out of at least five minutes between tests. In Experiments 1 and 3, a subject that failed to make a choice in a test was re-tested in the same condition in the next test, up to a total of four times (incidences of 3rd and 4th attempts were rare). Only subjects that completed all tests were included in the dataset.

In most experiments, *Alt-Long* consisted of 35 pulses, and *Alt-Short* was composed of 25. The pulses in both alternatives were 10 ms in duration. The sequence of A and B pulses in *Alt-Long* differed in each experiment, as indicated in Table 4-1. Both *Alt-Long* and *Alt-Short* repeated with a period of 5 s and alternated such that an equal amount of silence preceded and succeeded each presentation. The subject heard four presentations of each alternative before it was released remotely. Female treefrogs will sometimes show preferences for a signal that temporally leads others (Klump and Gerhardt, 1992; Whitney and Krebs, 1975). To avoid biasing responses toward *Alt-Long,* we began every test with *Alt-Short*. In Experiments 1 and 3, any variable that was tested between subjects included 30 subjects per level. Variables tested within subject were randomized across tests in all experiments.

Table 4-1 Experimental design

Experiment	Variables Manipulated	*Alt-Long*	*Alt-Short*
Experiment 1	ΔF, Δθ, and ΔL	ABABABA	AAAAA
Experiment 2*	ΔF	BBBBBBB	
Experiment 3a	ΔF	BBBAAAAA or AAAAABBB	AAAAA
Experiment 3b	ΔF	BBBBBBB	AAAAA

* Note: this was a no-choice experiment in which there was no second alternative.

All acoustic stimuli (44.1 kHz sampling rate, 16-bit) were generated in Matlab v2008b (Mathworks, Natick, MA, USA) using custom-written scripts. Stimulus presentation was controlled with Adobe Audition 1.5 (Adobe Systems Incorporated, San Jose, CA, U.S.A.). Sounds were broadcast using an M-Audio FireWire 410 multichannel soundcard (M-Audio USA, Irwindale, CA) and an amplifier driving Orb Mod 1 speakers (Orb Audio, New York, NY, USA). Stimuli were calibrated (re 20 μPa, C-weighted, max fast RMS) separately for each speaker by placing the microphone of a Larson-Davis System 834 sound level meter (Larson Davis, Depew, NY, USA) or Brüel & Kjær Type 2250L sound level meter (Brüel & Kjær, Norcross, GA, U.S.A.) pointed at the speaker, with the tip of the microphone at the approximate location of a

subject's head at the start of the test. The frequency spectra of the speakers were flat (± 1.5 dB) across all the frequencies tested.

Data analysis

We used R v3.0.3 (R Development Core Team, 2014) for all statistical analyses and a significance criterion of $\alpha = 0.05$ throughout the manuscript.

Experiment 1: Effect of Differences in Frequency (ΔF), Angle (Δθ), and Level (ΔL)

Method

We varied ΔF, ΔL, or Δθ through manipulations of the A and B pulses. In tests of ΔF, the frequency of B pulses was selected to achieve ΔFs of 0, ±2, ±4, ±6, ±8, or ±12 semitones (ST) relative to the frequency of the A pulses. An example is shown in Fig. 4-3a. Semitones are a commonly used measure of frequency separation and are based on a ratio of $2^{1/12}$ between the frequencies of interest. Positive and negative values of ΔF indicate the frequency of the B pulses was higher or lower, respectively, than that of the A pulses. We did not test a separation of +12 ST when the frequency of the A pulses was equal to 2.6 kHz, because we could not avoid strong harmonic distortion at this frequency at all sounds levels. We tested ΔF within subject, and carrier frequency between subjects. In tests of ΔL, we attenuated the level of the B pulses by 0, 3, 6, 12, and 24 dB relative to the level of the A pulses (Fig. 4-3c). We tested ΔL and carrier frequency within subject. We investigated the effect of Δθ in conjunction with that of ΔF, using two different spatial configurations (Fig. 4-3b). In the co-localized condition, the A and B pulses of *Alt-Long* were broadcast from a single speaker placed 180° from the speaker broadcasting *Alt-Short* (Fig. 4-3b, left). In the separated condition, the A and B pulses constituting *Alt-Long* were broadcast from speakers placed 90° apart from each other, and 135° from the speaker broadcasting *Alt-Short*, as illustrated in Fig. 4-3b, right. In this condition, we scored a response to *Alt-Long* if a subject touched the arena wall in front of either speaker broadcasting components of this alternative. Again ΔF was tested within subject, but Δθ and carrier frequency were tested between subjects. Several studies of communication in anurans have shown that overall sound pressure level (SPL) can influence responses (Bee et al., 2012; Christensen-Dalsgaard et al., 2002; Ehret and Gerhardt, 1980; Gerhardt, 1987; Gerhardt, 2008; Schwartz and Gerhardt, 1998). To investigate this possibility, we replicated the experiment at multiple overall signal levels. We tested the effects of ΔF, ΔL, and Δθ at 85 and 73dB SPL. The effects of ΔF and ΔL were

additionally tested at overall levels of 61 and 49 dB SPL. Overall level was always tested between subjects.

To ensure that the pulses in *Alt-Long*, which did not occur consecutively, were calibrated consistently with those of *Alt-Short*, we created special calibration stimuli. For each type of B pulse, we constructed a 25-pulse stimulus consisting of consecutive B pulses, which we calibrated to the specified overall signal level. We then matched the peak-to-peak amplitude of the B pulses

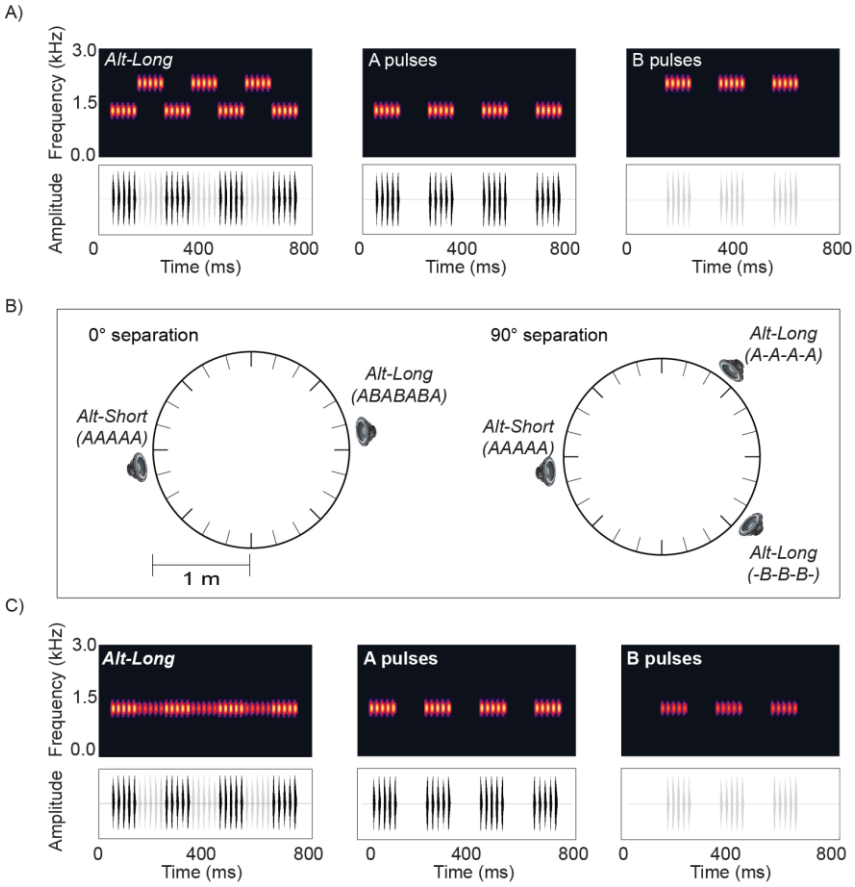


Figure 4-3 Experiment 1: Design
A pulses had a frequency, denoted the carrier frequency, equal to one of the natural frequencies in advertisement calls (1.3 kHz or 2.6 kHz). (A) We manipulated ΔF by varying the frequency of the B pulses in Alt-Long*. In the example here, the frequency of the A pulses is 1.3 kHz, and ΔF is +8 ST. We used two spatial configurations. (B) In the co-localized condition (left),* Alt-Short *and* Alt-Long *were broadcast from speakers on opposite sides of the arena. In the separated condition (right), the A and B pulses composing* Alt-Long *were broadcast separately from two speakers positioned 90° apart and opposite the speaker broadcasting* Alt-Short*. (C) We manipulated ΔL by attenuating the level of the B pulses relative to that of the A pulses. In the example illustrated, ΔL was 6 dB.*

in *Alt-Long* to that of the corresponding calibrated 25-pulse stimulus. The A pulses in *Alt-Long* were calibrated similarly.

To statistically analyze the effects of ΔF, ΔL, and Δθ on the proportions of subjects preferring *Alt-Long* to *Alt-Short*, we constructed separate generalized linear mixed models (GLMMs) using the *glmmPQL* function in the MASS package (Venables and Ripley, 2002). In each model we specified the distribution of the response variable as binomial and used logit as the corresponding link function. We also used linear mixed models (LMM) to analyze the response latencies, using the *lmerTest* package (Kuznetsova et al., 2014). Latency was log-transformed prior to analysis to achieve normality. In the GLMM and LMM models, carrier frequency and Δθ were always considered categorical predictors, and ΔF and ΔL were considered continuous variables. Overall level was centered by subtracting the mean from each value of the variable before including it as a continuous variable in the model. We included subject in every model as a random effect to account for repeated measures, and we also included order of stimulus presentation as a covariate. For each analysis, we report the partial correlation coefficient, *r*, for each term as a measure of effect size (Nakagawa and Cuthill, 2007).

Results and Discussion

Effects of ΔF

In Fig. 4-4a,b, we plot proportions choosing *Alt-Long* as a function of ΔF, averaged across overall level. These same data are plotted separated by overall level in Fig. 4-4c,d. Similarly, latencies are plotted as a function of ΔF, averaged across overall level in Fig. 4-4e,f, and separately for each level in Fig. 4-4g,h. We also indicate three "zones" in Figs 4a-h. These zones 1, 2, and 3, correspond to the frequencies ranges in which *Alt-Long* was expected to excite primarily the AP ($0.7 \leq B \leq 1.3$ kHz; A = 1.3 kHz), primarily the BP ($2.6 \leq B \leq 4.1$ kHz; A = 2.6 kHz), or both ($1.3 < B < 2.6$ kHz), respectively.

The GLMM revealed a significant effect of ΔF on the proportions selecting *Alt-Long* (Table 4-2). Of particular interest is zone 2, in which *Alt-long* was expected to excite both papillae. Due to this excitation of multiple channels, the channeling hypothesis predicted segregation of A and B pulses, and thus low proportions selecting *Alt-Long*. Instead, within zone 2, the proportions responding were constant or increased over their values at ΔFs of 0 ST (Fig. 4-4a-b). These high proportions indicate that subjects integrated pulses across papillae rather than segregating them as predicted by the channeling hypothesis. Overall level entered the model both

as a significant main effect and in interactions with ΔF and carrier frequency. Decreases in overall level generally had the effect of decreasing the proportions responding to *Alt-Long* (Fig. 4-4c,d). This change in the relative attractiveness of *Alt-Long* and *Alt-Short* likely stemmed from decreased sensitivity to the frequencies of the B pulses at these lower sound levels, an issue we consider further in Experiment 3. Considered across overall level, the shapes of the response curves were broadly similar, such that proportions were elevated in zone 2 relative to zones 1 and 3 for all overall levels (Fig. 4-4c,d). It has been proposed that there is overlap in the frequency ranges of the two papillae, and support for this hypothesis comes from a behavioral study with gray treefrogs (Gerhardt, 2005), as well as recent studies of auditory brainstem responses in gray and green treefrogs (Buerkle et al., 2014; Schrode et al., 2014). It could therefore be argued that the frequencies of the B pulses within zone 2 simultaneously stimulated both sensory papillae, providing an additional cue for integration. However, simultaneous excitation of the two papillae is only expected at high signal levels. The fact that the proportions selecting *Alt-Long* remained high even when overall level was attenuated by up to 36 dB suggests that simultaneous excitation of the two papillae cannot explain this result.

Based on the channeling hypothesis, we predicted that decreasing proportions would select *Alt-Long* as a function of increasing ΔF in zone 1, when *Alt-Long* was primarily encoded by the AP. Consistent with this prediction, proportions decreased as a function of ΔF within zone 1 (Fig. 4-4a). When *Alt-Long* was primarily encoded by the BP, we again predicted decreasing proportions to select *Alt-Long* as a function of increasing ΔF, but this prediction was based on variation in sensitivity of the papilla across the frequencies in zone 3 (2.6 to 4.7 kHz). As predicted, proportions within zone 3 decreased as a function of increasing ΔF (Fig. 4-4b). There was a significant effect of the ΔF × carrier frequency interaction in the GLMM, which is most apparent when comparing the response curves in zones 1 and 3. The decrease in proportions in zone 1 had a steeper slope as a function of ΔF than the decrease in zone 3. The shapes of the response curves in both zones 1 and 3 were again broadly similar across overall level (Fig. 4-4c,d).

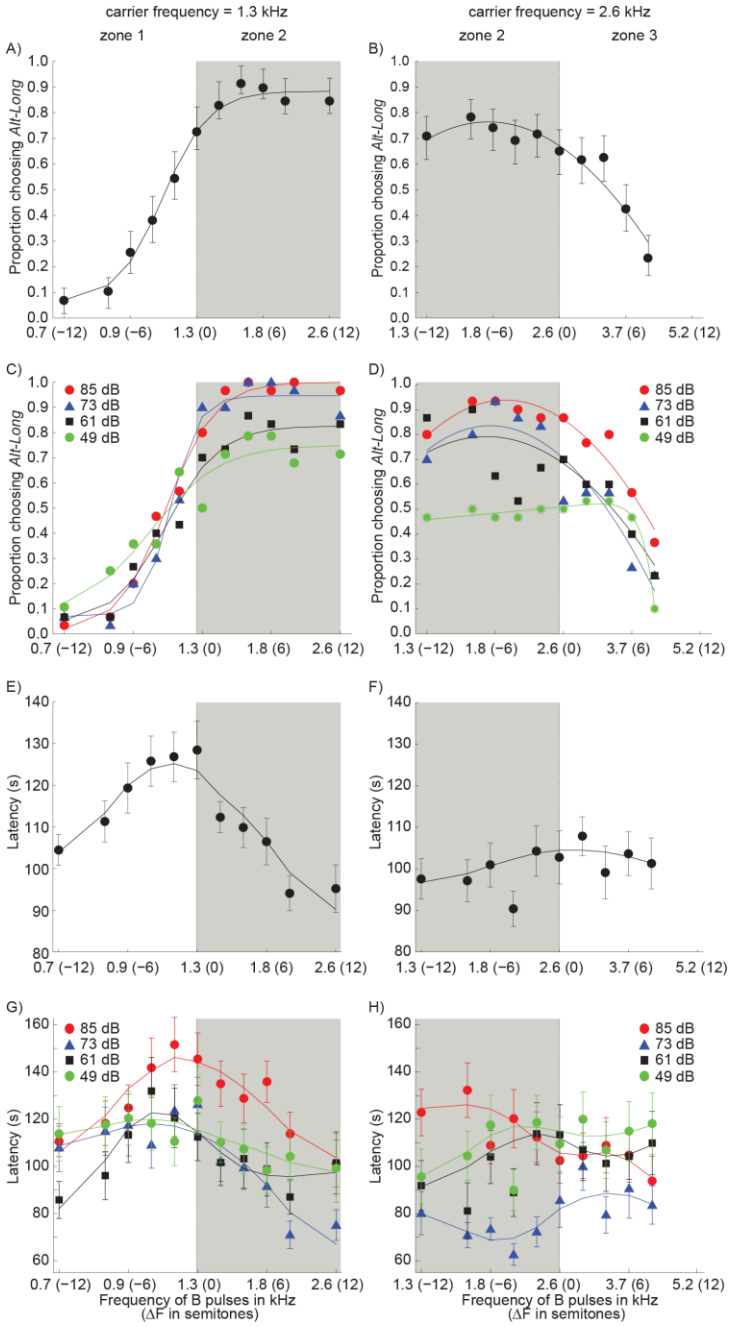


Figure 4-4 Experiment 1: Effects of ΔF

Proportions selecting Alt-Long over Alt-Short *are plotted as a function of ΔF averaged across overall level (A, B) and at each individual overall level (C, D), for tests in which the carrier frequency was 1.3 kHz (A, C) and 2.6 kHz (B, D). Error bars in (A, B) are 95% exact binomial confidence intervals, based on the plotted proportions. Error bars are omitted in (C, D) for clarity. Latencies to responses are plotted as a function of ΔF averaged across overall level (E, F) and at each individual overall level (G, H), for tests in which the carrier frequency was 1.3 kHz (E, G) and 2.6 kHz (F, H). Error bars are s.e.m. In all plots, the hatched area identifies zone 2, in which the frequency of the B pulses was such that* Alt-Long *was expected to excite both sensory papillae. We also label zones 1 and 3, in which Alt-Long was expected to excite only the AP or the BP, respectively. The curves plotted are either sinusoids fitted by least squares or a smooth curve calculated using a moving average*

Latencies depended significantly on ΔF as well as carrier frequency (Table 4-3). In tests in which the frequency of the A pulses was 1.3 kHz, latencies followed a consistent pattern both within zones 1 and zones 2 (Fig. 4-4e). Latencies were longest at a ΔF of -2 and decreased as a function of increasing ΔF (Fig. 4-4e). The signals that evoked the longest latencies corresponded to those for which subjects showed no preference (proportions of 0.5), suggesting subjects found these choices to be particularly difficult. Latencies likely decreased as a function of ΔF as the

choice in favor of one of the stimuli became more definitive. In tests in which the frequency of the A pulses was 2.6 kHz, latencies were relatively constant as a function of ΔF (Fig. 4-4f). Latencies also tended overall to be shorter when A pulses had a frequency of 2.6 kHz compared to 1.3 kHz (cf. Fig. 4-4e,f), which is consistent with previous findings that females are more attracted to unimodal signals with frequencies near the higher spectral peak of conspecific calls compared with those having frequencies near the lower peak (Bee, 2010). Overall level had a significant effect of latencies, as did its interaction with carrier frequency. The latency by ΔF functions were relatively similar across overall level when the carrier frequency was 1.3 kHz (Fig. 4-4g), but varied as a function of overall level when the A pulses had a frequency of 2.6 kHz (Fig. 4-4h). Order of stimulus presentation also had a significant effect on latencies (Table 4-3). However, this effect was driven by particularly long responses in the first test, as removing data from the first test for each frog resulted in the loss of the effect, while preserving the remaining effects (data not shown). In any case, the effect reflected a small difference of about 6 s across presentations.

Table 4-2 Effects of ΔF on proportions

term	DF	*t*	*p*	*r*
intercept	**3415**	**3.3**	**0.001**	**0.06**
ΔF	**3415**	**19.8**	**<0.001**	**0.32**
carrier frequency	356	-1.7	0.097	0.09
overall level	**356**	**4.4**	**<0.001**	**0.23**
order	3415	1.8	0.070	0.03
ΔF × carrier frequency	**3415**	**-22.2**	**<0.001**	**0.35**
ΔF × overall level	**3415**	**7.3**	**<0.001**	**0.12**
carrier frequency × overall level	356	-0.9	0.347	0.05
ΔF × carrier frequency × overall level	**3415**	**-7.0**	**<0.001**	**0.12**

Nityananda and Bee (2011) found that females of Cope's gray treefrog were able to use ΔF as a cue for segregation of consecutive sounds. In their study, subjects were presented with an attractive target signal, based on a communication call. The authors simultaneously broadcast a train of "distractor" pulses interleaved with the pulses of the target. The frequency of the distractor pulses differed from that of the target pulses, and varied across tests. The subjects would perceive the target signal only if they perceptually segregated it from the train of distractor pulses. Subjects were more likely to segregate the target as an increasing function of ΔF, consistent with the results of the current study. However, while the target had a frequency of 1.3 or 2.6 kHz, the distractor pulses usually had frequencies within what we have defined as zone 2,

suggesting that subjects were capable of segregating pulses that differentially stimulated the two papillae. This result contrasts with the result in the current study in which subjects integrated pulses across papillae. Taken together, these results suggest that for stimuli within zone 2 neither integration nor segregation is obligatory, and that the tendency toward one over the other depends on stimulus context.

Table 4-3 Effects of ΔF on latencies

term	DF	*t*	*p*	*r*
intercept	**743**	**169.9**	**<0.001**	**0.99**
ΔF	**3413**	**-2.7**	**0.008**	**0.05**
carrier frequency	**360**	**-5.3**	**<0.001**	**0.27**
overall level	**347**	**2.8**	**0.005**	**0.15**
order	**3413**	**-3.3**	**0.001**	**0.06**
ΔF × carrier frequency	3413	-0.8	0.428	0.01
ΔF × overall level	**3413**	**2.3**	**0.020**	**0.04**
carrier frequency × overall level	**359**	**-2.0**	**0.048**	**0.10**
ΔF × carrier frequency × overall level	**3413**	**-6.3**	**<0.001**	**0.11**

Effects of ΔL

The proportion of subjects selecting *Alt-Long* decreased linearly and significantly as a function of ΔL (Fig. 4-5a,b; Table 4-4). There was also a significant effect of overall level on proportions (Table 4-4); the proportions selecting *Alt-Long* were higher for signals at 85 dB SPL than at the other signal levels (Fig. 4-5a,b). The effects of carrier frequency and its interaction with ΔL were also significant (Table 4-4). This effect is evident in the slightly steeper slopes of the proportion functions when the frequency of the A pulses was 1.3 kHz relative to 2.6 kHz.

Table 4-4 Effects of ΔL on proportions

term	DF	*t*	*p*	*r*
intercept	**1073**	**4.6**	**<0.001**	**0.14**
ΔL	**1073**	**9.8**	**<0.001**	**0.29**
carrier frequency	**1073**	**-2.2**	**0.029**	**0.07**
overall level	**118**	**5.5**	**<0.001**	**0.45**
order	1073	0.2	0.860	0.01
ΔL × carrier frequency	**1073**	**-2.7**	**0.007**	**0.08**
ΔL × overall level	1073	1.8	0.065	0.06
carrier frequency × overall level	1073	-1.3	0.189	0.04
ΔL × carrier frequency × overall level	1073	0.1	0.958	<0.00

There were significant effects of ΔL, carrier frequency and overall level on latencies (Table 4-5). The effect size associated with ΔL, however, was much smaller than those associated

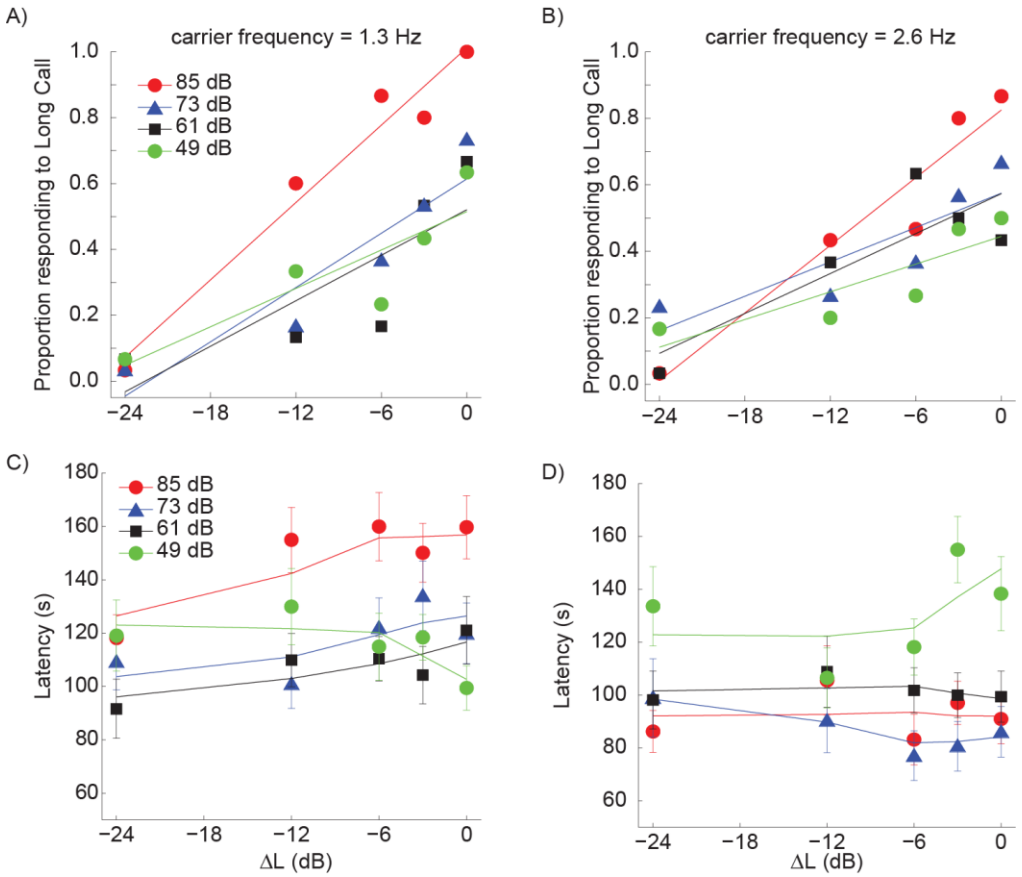


Figure 4-5 Experiment 1: Effects of ΔL

Proportions of subjects selecting Alt-Long *over* Alt-Short *as a function of ΔL when the carrier frequency was (A) 1.3 kHz and (B) 2.6 kHz. Data are plotted for four overall levels. Error bars are omitted for clarity. Linear regressions were fit to the data for each carrier frequency and overall level, yielding transfer functions used in Experiment 2. Latencies of responses as a function of ΔL when the carrier frequency was (C) 1.3 kHz and (D) 2.6 kHz. Data are plotted for four overall signal levels. Error bars are s.e.m.*

with the other significant effects (Table 4-5), indicating that ΔL is not a good predictor of latency. There were also significant effects of the carrier frequency × overall level and ΔL × carrier frequency × overall level interactions (Table 4-5). For overall levels of 69, 73, and 85 dB SPL, latencies increased as a function of decreasing ΔL when the frequency of the A pulses was 1.3 kHz, and decreased as a function of decreasing ΔL when the frequency of the A pulses was 2.6 kHz (Fig. 4-5c,d). On average, latencies were slightly shorter and the slopes of the latency by ΔL functions were steeper when the frequency of the A pulses was 1.3 kHz than when it was 2.6 kHz.

The results of our manipulation of ΔL are consist with those of Seeba et al (2010). In the study by Seeba, females of Cope's gray treefrog were given a choice between a Standard Call

with consecutive pulses and a Gap Call in which some pulses were replaced with silent gaps. The frequency spectra of both alternatives contained two peaks at the frequencies typical of natural calls. This represents a difference between the design of their study and the current study, as the alternatives in the current study had a single spectral peak. Seeba et al (2010) found that subjects strongly preferred the Standard Call over the Gap Call. At large ΔLs, subjects were expected to segregate *Alt-Long* into two streams, each resembling a Gap Call. Subjects in our study strongly preferred *Alt-Short*, despite its shorter duration, over *Alt-Long* at large ΔLs, consistent with Seeba et al (2010).

Table 4-5 Effects of ΔL on latencies

term	DF	*t*	*p*	*r*
intercept	**756**	**101.4**	**<0.001**	**0.97**
ΔL	**1073**	**3.0**	**0.003**	**0.09**
carrier frequency	**1073**	**-6.4**	**<0.001**	**0.19**
overall level	**376**	**4.0**	**<0.001**	**0.20**
order	1073	-0.9	0.349	0.03
ΔL × carrier frequency	1073	-1.5	0.139	0.05
ΔL × overall level	1073	1.8	0.074	0.05
carrier frequency × overall level	**1073**	**-7.6**	**<0.001**	**0.23**
ΔL × carrier frequency × overall level	**1073**	**-2.1**	**0.040**	**0.06**

Effects of Δθ

The effects of Δθ can be seen in Fig. 4-6. The proportions of subjects selecting *Alt-Long* were generally reduced in the spatially separated condition compared to the co-localized condition, as predicted by the channeling hypothesis (Fig. 4-6a-b); however, the effect of Δθ on proportions choosing *Alt-Long* was not significant (Table 4-6). The effects of Δθ were dependent on carrier frequency, as predicted. At an overall level of 85 dB SPL, the effect of Δθ was much stronger when the frequency of A pulses was 2.6 kHz relative to when it was 1.3 kHz (cf Fig. 4-6a,b), resulting in significant carrier frequency × Δθ and ΔF × carrier frequency × Δθ interactions (Table 4-6). This interaction between Δθ and carrier frequency was not as apparent when the overall level was 73 dB SPL. The level-dependence of the effects of Δθ and carrier frequency are reflected in the significant interaction of overall level with carrier frequency and Δθ (Table 4-6). It is worth noting that although the proportions selecting *Alt-Long* were decreased in the spatially separated condition, for the majority of frequencies, the spatial effect did not result in the proportions of subjects selecting *Alt-Long* dropping below the level of chance (0.5).

The main effect of Δθ on latencies was not significant (Table 4-7). However, Δθ did influence the effects of carrier frequency and ΔF through carrier frequency × Δθ and ΔF × carrier frequency × Δθ interactions (Table 4-7). In zone 2, latencies were increased in the spatially

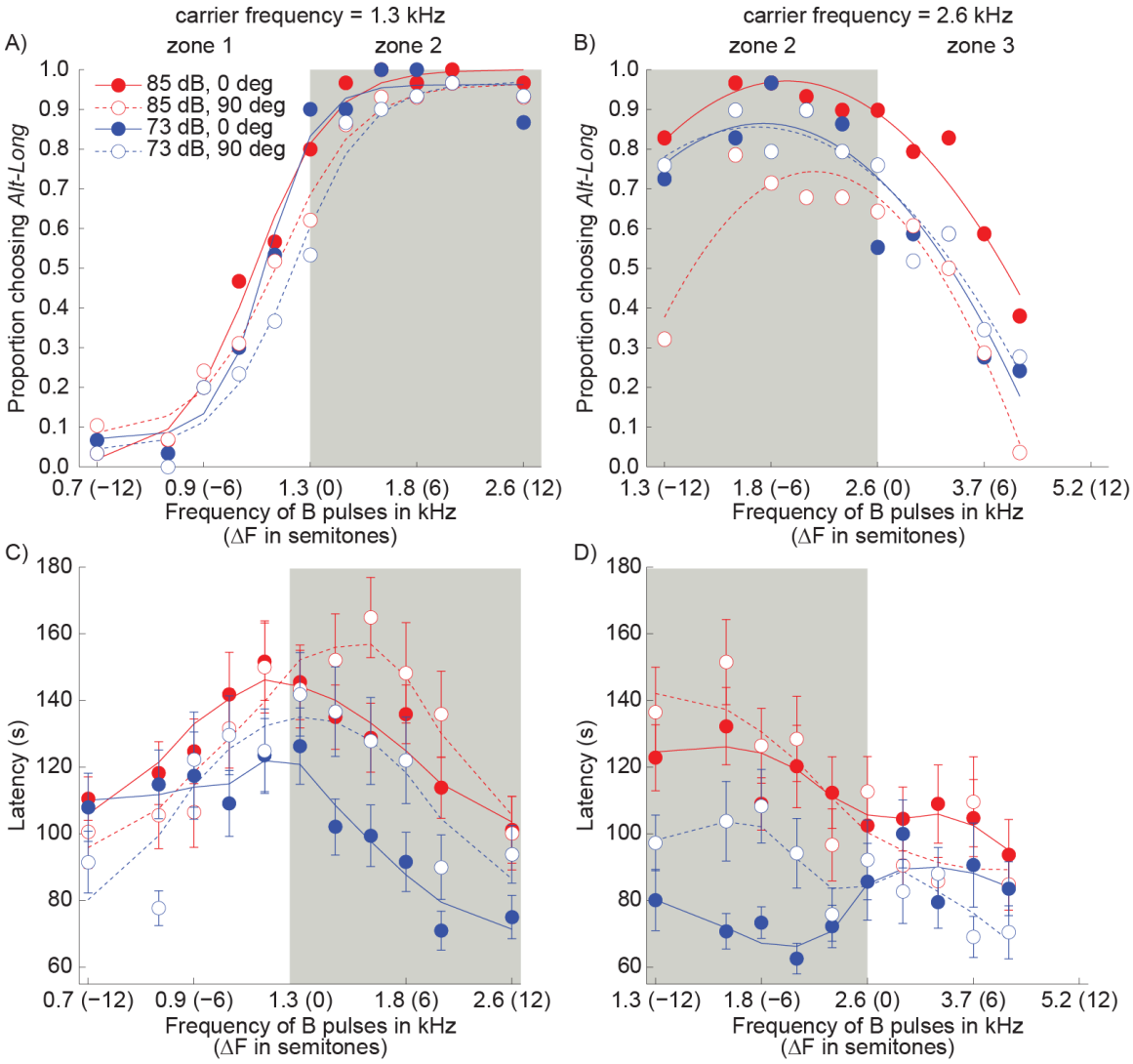


Figure 4-6 Experiment 1: Effects of Δθ
Proportions selecting Alt-Long *over* Alt-Short *are plotted as a function of ΔF for co-localized and separated conditions at 85 and 73 dB SPL for tests in which the carrier frequency was 1.3 kHz (A) and 2.6 kHz (B). Responses to stimuli at 85 dB SPL are re-plotted from Fig. 4-4c,d. Error bars are omitted for clarity. Latencies to responses are plotted as a function of ΔF for co-localized and separated conditions at 85 and 73 dB SPL for tests in which the carrier frequency was 1.3 kHz (C) and 2.6 kHz (D). Error bars are s.e.m. In all plots, the hatched area identifies zone 2, in which the frequency of the B pulses was such that* Alt-Long *was expected to excite both sensory papillae. We also label zones 1 and 3, in which* Alt-Long *was expected to excite only the AP or the BP, respectively.*

separated condition relative to the co-localized condition (Fig. 4-6c,d). This increase in latencies suggests that *Alt-Long* was less attractive in the spatially-separated condition. This result was consistent with the predictions of the channeling hypothesis, as the decrease in attractiveness could be due to the increased tendency to segregate the A and B pulses into an unattractive percept. Previous work has shown that the middle ears of gray treefrog are particularly directional in the frequency range corresponding to zone 2 (Caldwell et al., 2014; Jørgensen and Gerhardt, 1991), which could facilitate segregation of spatially separated signals. Alternatively, the increase in latencies could reflect difficulty in localizing the signal to a particular speaker; subjects often visited the speakers broadcasting the A and B pulses several times before making a response (KMS, personal observation). In zones 1 and 3, latencies were shorter in the spatially separated condition than in the co-localized condition. Given the relatively low proportions that selected *Alt-Long* in these zones, these short latencies indicate that the spatial separation made the choice between *Alt-Long* and *Alt-Short* particularly straightforward.

Table 4-6 Effects of Δθ on proportions

term	DF	*t*	*p*	*r*
intercept	**2272**	**2.6**	**0.008**	**0.06**
ΔF	**2272**	**7.0**	**<0.001**	**0.14**
carrier frequency	232	-1.9	0.058	0.12
Δθ	232	-1.9	0.053	0.13
overall level	232	0.5	0.611	0.03
order	2272	1.3	0.204	0.03
ΔF × carrier frequency	**2272**	**-9.3**	**<0.001**	**0.19**
ΔF × Δθ	2272	-1.2	0.225	0.03
carrier frequency × Δθ	**232**	**2.1**	**0.033**	**0.14**
ΔF × overall level	2272	-0.3	0.750	0.01
carrier frequency × overall level	232	1.4	0.171	0.09
Δθ× overall level	232	0.0	0.995	0.00
ΔF × carrier frequency × Δθ	**2272**	**2.0**	**0.045**	**0.04**
ΔF × carrier frequency × overall level	2272	1.1	0.280	0.02
ΔF × Δθ× overall level	2272	-0.2	0.861	0.00
carrier frequency × Δθ× overall level	**232**	**-2.5**	**0.013**	**0.16**

The results reported here are consistent with those of several previous studies of the use of spatial cues by anurans (Bee and Riemersma, 2008; Farris et al., 2002; Farris et al., 2005; Schwartz and Gerhardt, 1995). In particular, the subjects in each of these studies were relatively tolerant of spatial separations between components of conspecific advertisement calls, with most

subjects responding to stimuli separated by up to 180° in some cases (Bee and Riemersma, 2008; Farris et al., 2002; Farris et al., 2005; Schwartz and Gerhardt, 1995). Bee and Riemersma (2008) tested females of *H. chrysoscelis* with stimuli consisting of interleaved pulses that were spatially separated. They observed that subjects often approached the speakers broadcasting components of the separated stimuli several times before responding, similar to the behavior observed here.

Table 4-7 Effects of Δθ on latencies

term	DF	*t*	*p*	*r*
intercept	**265**	**69.2**	**<0.001**	**0.97**
ΔF	**2271**	**-4.4**	**<0.001**	**0.09**
carrier frequency	**233**	**-3.0**	**0.003**	**0.19**
Δθ	226	1.1	0.295	0.07
overall level	**226**	**3.3**	**0.001**	**0.22**
order	**2271**	**-4.4**	**<0.001**	**0.09**
ΔF × carrier frequency	**2271**	**4.2**	**<0.001**	**0.09**
ΔF × Δθ	**2271**	**2.7**	**0.006**	**0.06**
carrier frequency × Δθ	233	-0.2	0.860	0.01
ΔF × overall level	**2271**	**2.1**	**0.035**	**0.04**
carrier frequency × overall level	233	0.5	0.594	0.03
Δθ× overall level	226	-1.0	0.305	0.07
ΔF × carrier frequency × Δθ	**2271**	**-5.9**	**<0.001**	**0.12**
ΔF × carrier frequency × overall level	**2271**	**-4.5**	**<0.001**	**0.09**
ΔF × Δθ× overall level	2271	0.5	0.640	0.01
carrier frequency × Δθ× overall level	232.3	0.1	0.907	0.01

Experiment 2: Can effects of ΔF be explained by sensitivity?

The results of Experiment 1 suggested that increasing ΔF facilitated segregation of the A and B pulses in *Alt-Long*. However, for *Alt-Long* that stimulated only the BP (zone 2), we had expected segregation of pulses to be mediated by level cues resulting from variation in sensitivity to the frequency of the B pulses. Experiment 2 was designed to test the hypothesis that variation in sensitivity could explain the effects of ΔF. We first used single-stimulus no-choice tests to determine whether alternatives with frequencies equal to those of the B pulses in Experiment 1 (e.g. BBBBBBB) were sufficiently audible to elicit responses from subjects. If variation in sensitivity was responsible for the effects of ΔF, we predicted the proportions of subjects responding would decrease as a function of ΔF (relative to the frequencies of the A pulses in Experiment 1). We next compared the results from Experiment 1 with auditory sensitivity as

assessed previously by auditory brainstem responses (Schrode et al., 2014). We predicted that the slopes of the response curves from Experiment 1 and the sensitivity curve should be very similar, if variation in sensitivity was driving the effects of ΔF.

Method

To investigate audibility, we broadcast a single stimulus consisting of 35 consecutive pulses (BBBBBBB) in no-choice tests. *Alt-Long* had a frequency equal to one of a subset of the frequencies of B pulses used in Experiment 1. We also tested subjects in a sham condition in which no signal was presented to assess the likelihood of scoring a response by chance. In this condition, a response was counted if the subject touched the wall in front of the speaker that broadcast stimuli in the other tests. Subjects were also tested in a reference condition in which the stimulus broadcast was a "standard call" with a bimodal frequency spectrum and all parameters based on population means. Responses in the reference condition verified that the subject was motivated, thus confirming that a lack of motivation was not the cause of a failure to respond in other tests. Subjects were tested in the reference condition at the beginning and end of the session; the order of all other tests was randomized. We noted both whether a subject responded and the latency to a response. If a subject did not respond in a given test, we recorded a latency of five minutes (300 seconds) and moved to the next test. The experiment was replicated with different sets of 20 subjects at 85, 73, 61, and 49 dB SPL. We used exact binomial tests to determine when the proportion of responses to a given signal was significantly greater than the probability of responding by chance (as determined from the sham condition). To analyze latencies, we used an LMM as in Experiment 1 and planned contrasts in which latencies in each frequency condition were compared with those of the sham condition.

To facilitate the comparison between the effects of ΔF and auditory sensitivity, it was necessary that the results of the manipulation of ΔF in Experiment 1 be on the same scale as auditory sensitivity. We used the results of the manipulation of ΔL as transfer functions to convert the proportions choosing *Alt-Long* into a measure of response strength in dB. For each combination of carrier frequency and overall level, we fit a separate regression to the proportions of subjects selecting *Alt-Long* as a function of ΔL (Fig. 4-6.a,b). Fits were quite good, with a mean (± s.d.) R^2 of 0.8 ± 0.1. The proportions responding to *Alt-Long* (from Fig. 4-4c,d) were converted to response strengths using the slopes of the transfer functions. To maintain the relative relationships between the response functions at different overall levels, we aligned all of the

response functions relative to the maximum response exhibited in Experiment 1 for the given carrier frequency. The auditory sensitivity curve was derived from the thresholds determined from auditory brainstem responses (Schrode et al., 2014).

Results and Discussion

Subjects readily responded to alternatives with frequencies ≥ 0.7 kHz. The proportions of frogs responding to alternatives of all frequencies were significantly greater than predicted from the sham condition when the signal level was 85 dB SPL (Fig. 4-7a). The proportions responding decreased with decreasing overall level, particularly in response to the lowest (0.7 and 0.9 kHz) and highest (3.7 and 4.1 kHz) frequencies, but in the majority of cases remained significantly different from those in the sham condition. The LMM revealed significant effects of frequency (t_{638} = -8.9, $p < 0.001$, $r = 0.34$), overall level (t_{78} = -6.1, $p < 0.001$, $r = 0.57$), and their interaction (t_{638} = -4.2, $p < 0.001$, $r = 0.16$) on latencies. At 85 dB SPL, latencies were significantly shorter than those in the sham condition for all frequencies except 0.7 kHz (Fig. 4-7b). Latencies generally increased with decreasing signal level, and were especially long in response to alternatives which elicited low proportions of responses (e.g. frequency of 0.7 kHz and signal levels of 49 dB). This pattern held, although the differences in latencies were less extreme, if we ignored tests in which a subject did not respond rather than assigning a latency of 300 seconds (data not shown). The patterns in proportions and latencies reported here indicate that responses were goal-directed, rather than the result of random meandering, and that most frequencies at most of the signal levels broadcast were clearly audible and attractive to the subjects. However, the lowest frequency (0.7 kHz) and the highest two frequencies (3.7 and 4.1 kHz) were noticeably poorer than the others at eliciting responses from subjects, particularly at low overall levels. These low responses suggest that subjects were particularly insensitive to these extreme frequencies, which may have reduced the responses of subjects to stimuli containing these same frequencies in Experiment 1.

The "M-shaped" sensitivity curve had two peaks at frequencies corresponding approximately to the carrier frequencies of 1.3 and 2.6 kHz (Fig. 4-7c,d). Sensitivity was reduced

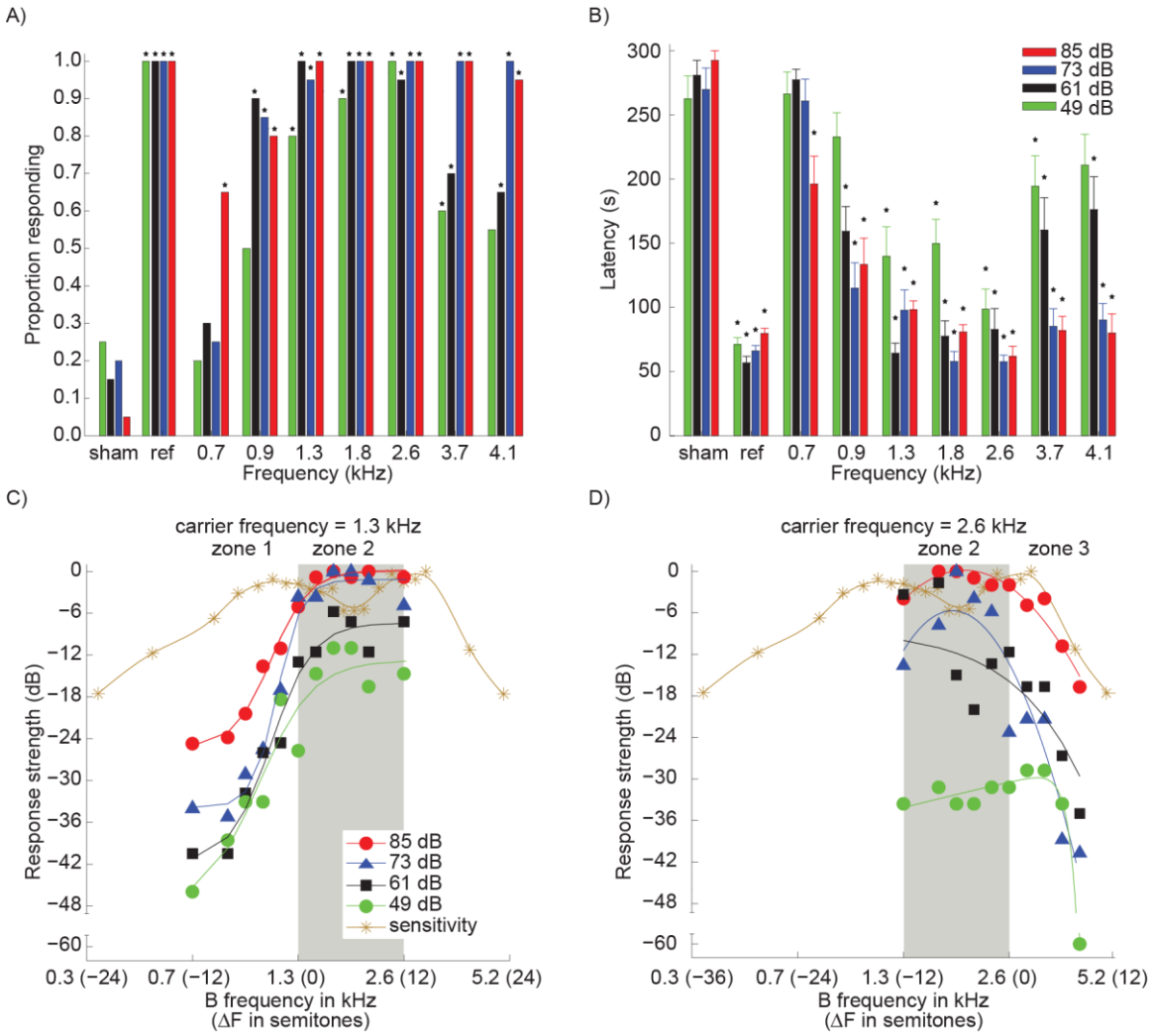


Figure 4-7 Experiment 2: Sensitivity

(A) Proportions of subjects responding in no-choice tests to Alt-Long*, which varied in frequency and overall level. Error bars are 95% binomial confidence intervals. Asterisks indicate proportions that are significantly different in exact binomial tests from the proportions responding by random chance (as determined in sham condition). (B) Latencies to responses. A latency of five minutes was assigned when a subject failed to respond in a trial. Error bars are s.e.m. Asterisks indicate latencies significantly different than those in the sham condition (planned contrasts ps < 0.05). (C, D) Proportions of subjects selecting* Alt-Long *in Experiment 1 were converted to response strengths using the transfer functions in Fig 6c,d. Response strength is plotted as a function of ΔF at each of the overall levels tested when the carrier frequency was (C) 1.3 kHz and (D) 2.6 kHz. Also plotted in both (C, D) is an auditory sensitivity curve. This sensitivity curve is an inverted version of an audiogram derived from auditory brainstem responses (Schrode et al., 2014).*

somewhat between these two frequencies. Sensitivity also decreased along with response strength when the signals were expected to excite the AP or BP exclusively (zone 1 or 3, respectively). When *Alt-Long* was entirely with the range of the AP (zone 1), response strengths decreased at rates of about 2.3 to 3.6 dB/ST, while sensitivity decreased at a rate of only 0.8 dB/ST (Fig. 4-7c). Within the range of the BP (zone 3), response strengths decreased with slopes of approximately 1.7 to 4 dB/ST, and sensitivity decreased at a rate of around 1.8 dB/ST (Fig. 4-7d). The slopes of the response strength and auditory sensitivity curves were very closely aligned within the range of the BP for responses at overall levels of 69 and 85 dB SPL, suggesting that variation in sensitivity could explain the effects of ΔF within the range of the BP. Within the range of the AP, responses strengths always decreased at faster rates than sensitivity. Note for example, that within the frequency range of about 0.9 to 1.6 kHz, sensitivity changed less than 3 dB, while response strength decreased by nearly 24 dB (Fig. 4-7c). This large difference between the sensitivity and response strength suggests that the effects of ΔF when the AP was excited were not driven by variation in sensitivity. Together, these results suggested that the segregation apparent in Experiment 1 within the range of the BP was level-dependent, but that within the range of the AP, segregation was level-*in*dependent. This conclusion is corroborated by the fact responsiveness, as assessed by proportions responding and latency in the audibility test, was lower in response to the highest frequencies (e.g. 3.7 and 4.1 kHz) than to most other frequencies when overall level was < 73 dB SPL (Fig. 4-7a,b).

While the comparison of response curves to sensitivity suggested that responses within zone 1 were level-independent, we did observe reduced proportions responding and longer latencies in response to the lowest frequency tested in the audibility test (0.7 kHz). Taken together, these results suggest that it was a lack of attraction rather than insensitivity that resulted in the small number of responses to signals of 0.7 kHz. We consider the issue of attraction further in Experiment 3.

Experiment 3: Can effects of ΔF be explained by reduced attractiveness of B pulses?

The results of Experiments 1 and 2 suggested that subjects were increasingly likely to segregate A and B pulses as a function of increasing ΔF, and that they used a level-dependent mechanism when *Alt-long* stimulated the BP (zone 3), but not the AP (zone 1). However, the results of Experiment 2, as well as previous work, suggested that the preferences of females vary by frequency, with females discriminating particularly against lower-than average frequencies

(Schrode et al., 2012). These findings suggest that the decreased response to *Alt-Long* in zone 1 at negative ΔFs could have been the result of reduced attraction to the frequency of the B pulses. We tested the hypothesis that reduced attraction to the frequency of B pulses could account for the effects of ΔF in Experiment 1. Subjects were tested in two-alternative choice tests in which *Alt-Long* consisted either of consecutive groups of A and B pulses (e.g. AAAAABBB) or entirely of B pulses (e.g. BBBBBBB). As in Experiment 1, we varied ΔF by manipulating the frequency of the B pulses. In each of these cases, attraction to *Alt-Long* did not depend on integration of the pulses across frequency. If attractiveness of the frequency of B pulses was driving the effects of ΔF, we predicted the proportions of subjects selecting *Alt-Long* to be significantly reduced in tests in which the B pulses had low frequencies.

Method

Experiment 3 had two components. In Experiment 3a, *Alt-Long* consisted of 25 A pulses and 15 B pulses. The number of A pulses was equal to that in *Alt-Short*, and the B pulses occurred either before or after the A pulses, in a pre (Fig. 4-8a; BBBAAAAA) or post (Fig. 4-8b; AAAAABBB) condition, respectively. We selected 15 as the number of B pulses to match the number of B pulses *Alt-Long* in Experiment 1. B pulses had a frequency of -12, -6, 0, 6, or 12 semitones when the frequency of the A pulses was 1.3 kHz, and -12, -6, 0, 6, or 8 ST when the frequency of the A pulses was 2.6 kHz. All pulses were calibrated to 85 dB SPL, and all conditions were tested within-subject.

We expected that the responses of subjects would depend on two factors: whether subjects integrated the A and B pulses of *Alt-Long*, and whether the B pulses were repulsive. If subjects integrated the A and B pulses (AAAAABBB or BBBAAAAA) and were not repelled by the frequency of the B pulses, the proportions choosing *Alt-Long* should be higher than chance. If, however, subjects found the frequency of the B pulses repulsive, we expected the proportions selecting *Alt-Long* to be lower than chance. On the other hand, if subjects segregated the A and B pulses into two streams (AAAAA and BBB), their choice between *Alt*-Long and *Alt*-Short would resemble a choice between two identical stimuli (AAAAA), so they should show no preference for either alternative. We analyzed responses using two-tailed exact binomial tests to determine whether subjects' choices were significantly non-random. In this analysis, we considered a proportion of 0.5 to be the proportion responding by chance. We did not correct for multiple

comparisons, because doing so would have reduced the likelihood of finding significant results in favor of this alternative hypothesis.

In Experiment 3b, *Alt-Long* consisted of 35 consecutive B pulses (BBBBBBB), in which we manipulated ΔF relative to the frequency of pulses in *Alt-Short* (AAAAA). Thus, subjects were faced with a trade-off between preferences for longer durations and spectral preferences. All pulses were calibrated to 85 dB SPL, and we tested carrier frequency between subjects. To determine if the preferences in Experiment 1 could be explained by the attractiveness of the frequency of B pulses, we tested whether the proportion responding to each *Alt-Long* in Experiment 3b was significantly different than the proportion responding to *Alt-Long* when the frequency of the B pulses did not differ from that of the A pulses (data from Experiment 1). We again did not perform any correction for multiple comparisons to avoid biasing the results against this alternative hypothesis.

Results and Discussion

In most cases, the proportions favoring the *Alt-Long* over the *Alt-Short* in Experiment 3a were greater than those expected by chance (Fig. 4-8c-d). These responses in favor of *Alt-Long* suggest that subjects integrated A and B pulses across frequency. Exceptions to this trend occurred in response to signals in which the absolute frequency of the B pulses was one of the two lowest frequencies tested (Fig. 4-8c; but only in the Post condition) or one of the two highest frequencies tested (Fig. 4-8d), in which cases, the proportions responding were not significantly different than expected by chance. The lack of difference from chance in these conditions suggests that subjects segregated the A and B pulses and responded to the choice between two 25-pulses calls. The proportions selecting *Alt-Long* were never significantly lower than expected by chance, indicating that subjects were not repelled by the presence of any frequencies.

In comparing the proportions responding in Experiment 3a with those responding in the corresponding condition of Experiment 1 (illustrated in Fig. 4-8), some interesting observations emerge. The proportions were broadly consistent between the two experiments in both zones 2 and 3, with a difference of no more than 0.2. Of particular importance, however, is zone 1. Within this zone, the proportions selecting *Alt-Long* in Experiment 1 were much lower than those in Experiment 3b. The high proportions selecting *Alt-Long* in the Pre condition indicate that subject integrated pulses across this large ΔF into an attractive percept. This result indicates that the

effect of ΔF in Experiment 1 was not due to the presence of lower-than-average frequencies, as *Alt-Long* in both experiments contained these same frequencies. Rather, the low proportions in

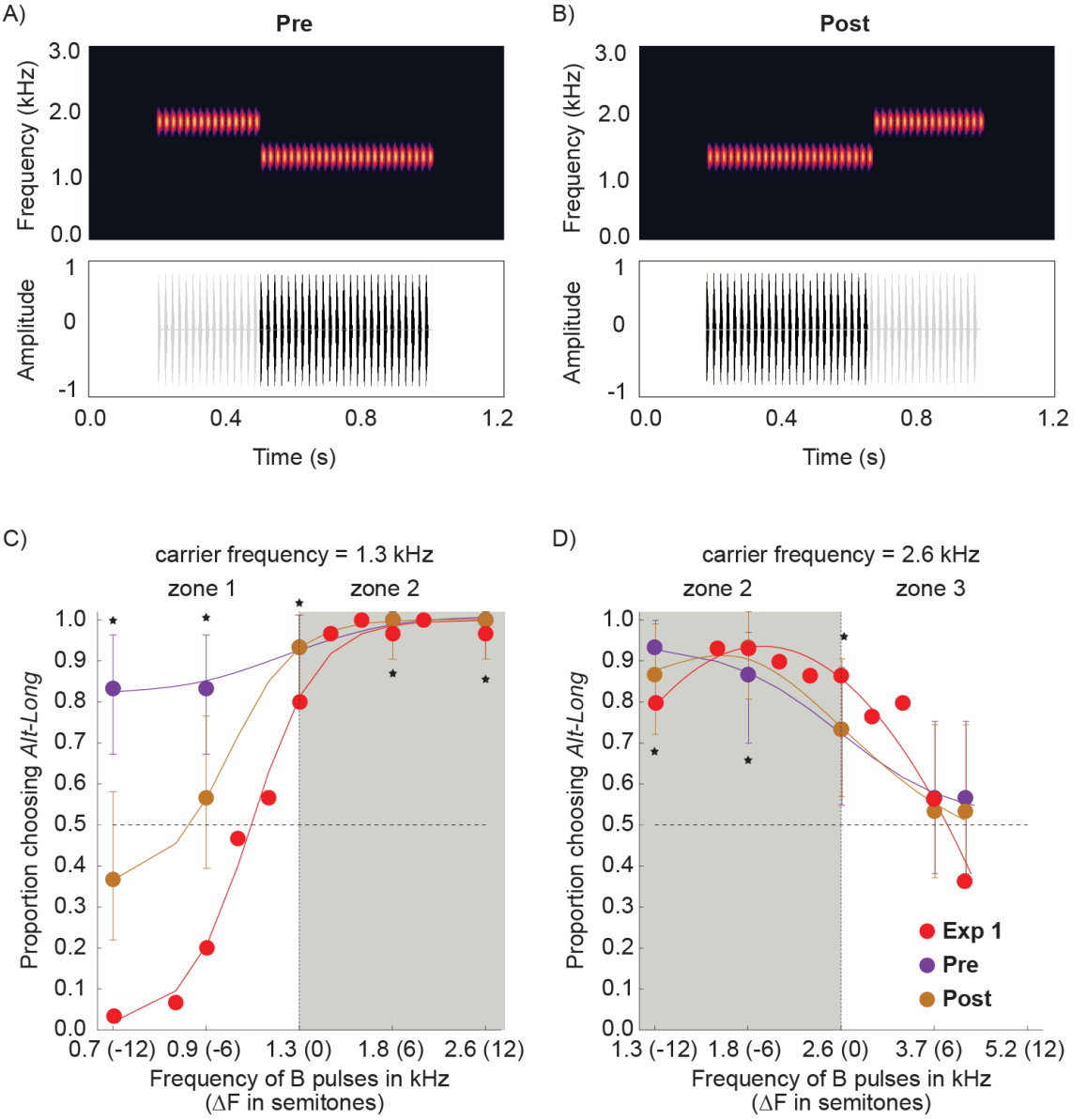


Figure 4-8 Experiment 3a: Frequency avoidance
Alt-Long *consisted of 15 consecutive B pulses that came either before (A; pre) or after (B; post) 25 consecutive A pulses. We varied the frequency of the B pulses relative to that of the A pulses. Proportions of subjects selecting* Alt-Long *are plotted for the pre and post conditions as a function of ΔF when the carrier frequency was (C) 1.3 kHz and (D) 2.6 kHz. Asterisks denote proportions determined through exact binomial tests to be significantly different from chance (0.5). Proportions choosing* Alt-Long *in Experiment 1 in response to signals of 85 dB SPL are also re-plotted from Fig. 4-4c,d for comparison.*

Experiment 1 must have been in response to the percepts emerging from segregation of the A and B pulses in *Alt-Long*. In addition to the difference in proportions between the two experiments, there was also a difference between the Pre and Post conditions of Experiment 3. These differences between the Pre and Post conditions suggest that the temporal ordering of the B pulses within *Alt-Long* affected whether subjects integrated the pulses of *Alt-Long* or not. The importance of temporal ordering has been studied in gray treefrogs previously. Our result is consistent with results of Seeba et al (2010), who found that adding a noise burst before, but not after, a 20-pulse call made it more attractive to gray treefrogs. However, Gerhardt et al. (2007) reported that adding an appendage after a signal, but not before, increased its attractiveness to female gray treefrogs. While the effect was strongest in the eastern gray treefrog (*H. versicolor*), a weak effect was also found in Cope's gray treefrogs (Gerhardt et al., 2007).

In Experiment 3b, we found that proportions selecting *Alt-Long* decreased within zone 1 as a function of ΔF, and consequently, as a function of absolute frequency of the B pulses (Fig. 4-9a). In this zone, the proportions selecting *Alt-Long* of all frequencies were significantly lower than those selecting *Alt-Long* when it did not differ in frequency from *Alt-Short*. This pattern indicates that decreasing the frequency of *Alt-Long* decreased its attractiveness, consistent with previous findings (Schrode et al., 2012). Within zone 2 when the frequency of A pulses was 1.3 kHz, the proportions selecting *Alt-Long* remained high (Fig. 4-9a). These proportions were statistically higher than those selecting *Alt-Long* when it had the same frequency as *Alt-Short*. The remarkable similarity between the shape of this curve in the Experiment 3 and the corresponding curve from Experiment 1 (Fig. 4-9a) provides support for the hypothesis that attractiveness of the B pulses was driving the effects of ΔF in Experiment 1. However, the results of Experiment 3a, suggest that the presence of low frequencies in a stimulus is not inherently repulsive. A more likely possibility then is that at low frequencies, subjects tended to segregate A and B pulses as expected, resulting in a percept of two Gap Calls that had an attractiveness approximately equal to that of a call entirely composed of low-frequency pulses.

In response to signals in which the A pulses had a frequency of 2.6 kHz, proportions selecting *Alt-Long* decreased as a function of ΔF in both zones 2 and 3 (Fig. 4-9b). For all $\Delta F \neq 0$, the proportions selecting *Alt-Long* were statistically lower compared to when ΔF was 0 ST. This pattern of responses indicates that subjects had a strong preference for the natural frequency of 2.6 kHz, which was not overcome by lengthening the alternative. In comparison with the corresponding curve from the Experiment 1, proportions were consistently lower, suggesting that the presence of A pulses increased the attractiveness of *Alt-Long*. In particular, the difference between the proportions selecting *Alt-Long* within zone 2 provides support for the idea that alternatives containing these frequencies were only attractive when they sequentially stimulated both of the two papillae, as in Experiment 1. This result, therefore, runs counter to the predictions of the channeling hypothesis.

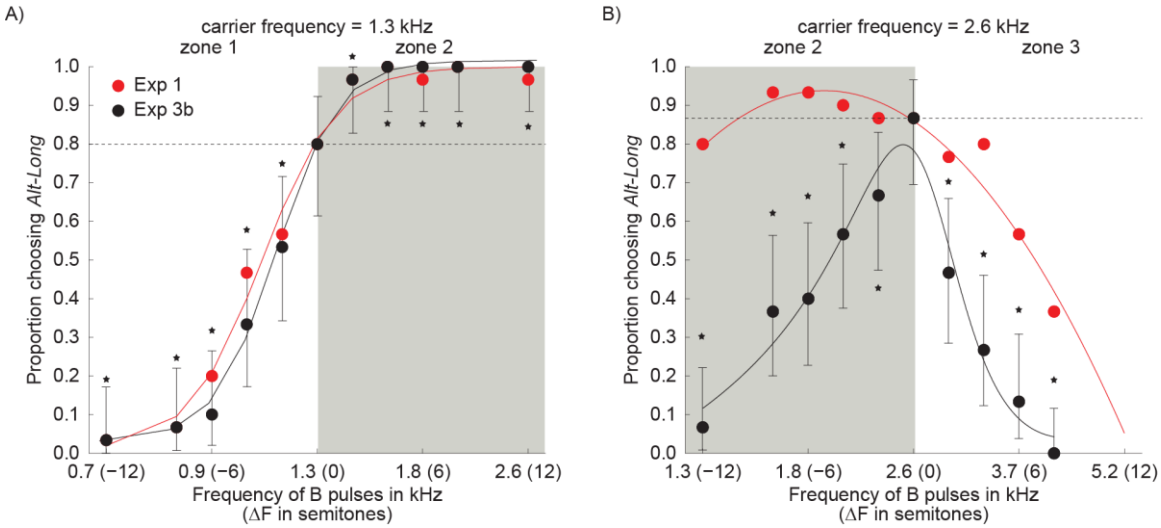


Figure 4-9 Experiment 3b: Spectral preferences
Alt-Long *consisted entirely of B pulses, which had a frequency that differed from that of the A pulses in* Alt-Short *(ΔF). Plotted are the proportions selecting* Alt-Long *as a function of ΔF for carrier frequencies of (A) 1.3 kHz and (B) 2.6 kHz. Proportions choosing* Alt-Long *in Experiment 1 in response to signals of 85 dB SPL are also re-plotted from Fig. 4-4c,d for comparison.*

General Discussion

We found that the channeling hypothesis cannot completely explain segregation of sounds by treefrogs. In particular, when *Alt-Long* sequentially stimulated both the AP and the BP (zone 2), the channeling hypothesis predicted segregation of A and B pulses into two streams. Instead, subjects in Experiment 1 strongly preferred *Alt-Long* when it stimulated both auditory papillae, providing evidence in favor of across-frequency integration of temporally separated pulses into a

single stream. This result could not be explained by the B pulses simultaneously exciting both of the papillae, because decreasing overall level did not eliminate the effect. Furthermore, in Experiment 3b, when *Alt-Long* consisted of only B pulses it was unable to elicit a strong preference from subjects. If simultaneous stimulation of the two organs was sufficient to make a stimulus attractive, subjects should have strongly preferred the alternatives that consisted entirely of B pulses with frequencies within zone 2 in Experiment 3b.

Given that the channeling hypothesis could not account for the integration of pulses across papillae into a single stream, we must consider alternative mechanisms for the formation of this stream. According to the channeling hypothesis, stream formation begins in the peripheral channels and is maintained at ascending levels of the auditory system (Hartmann and Johnson, 1991). The most likely explanation for the formation of a stream across papillae is that two streams are formed in the auditory peripheral channels, but that the responses of the channels are integrated through processing at some ascending level of the auditory system. This type of mechanism has been hypothesized to exist in the auditory cortex of mammals (Elhilali et al., 2009; Shamma et al., 2011; Snyder and Alain, 2007). As anurans do not have an auditory cortex, if such a mechanism exists, it must occur at a subcortical level in the anuran brain. Likely candidates for the location of such processing are the inferior colliculus or the thalamus, both of which contain neurons that integrate ascending responses from the two papillae (Fuzessery and Feng, 1982; Fuzessery and Feng, 1983; Hall, 1999; Megela, 1983).

Our results were more consistent with the predictions of the channeling hypothesis when the sounds stimulated a single sensory papilla. Within the range of the tonotopic AP (zone 1), responses in Experiment 1 decreased as a function of ΔF, consistent with the channeling hypothesis. The results of Experiment 2 suggested that this trend was consistent with a level-*independent* mechanism of segregation. We considered the alternative possibility that the effect of ΔF was driven by spectral preferences, as there is some evidence that female gray treefrogs discriminate against lower-than-average frequencies. However, the results of Experiment 3 indicated that the presence of low frequencies in a call was not sufficient to prevent subjects from choosing it (Experiment 3a), so long as the call contained pulses with frequencies of 1.3 or 2.6 kHz (Experiment 3b). Taken together, these results suggested it was in fact segregation of A and B pulses of *Alt-Long* that resulted in the effects of ΔF on preferences.

In the central auditory systems of birds and mammals, tonotopic mechanisms may promote the integration and segregation of sounds into streams (Bee and Klump, 2004; Bee and

Klump, 2005; Elhilali et al., 2009; Fishman et al., 2001; Fishman et al., 2004; Micheyl et al., 2005). In these studies single or multi-unit activity was recorded in response to a series of tones alternating in frequency. The A tones were presented at the characteristic frequency of the neuron or neurons being recorded from, and the B tones varied in frequency. In each of these studies, the difference between the responses to the tones increased as a function of increasing ΔF (Bee and Klump, 2004; Bee and Klump, 2005; Elhilali et al., 2009; Fishman et al., 2001; Fishman et al., 2004; Micheyl et al., 2005). While the recordings in these studies were made in the central nervous system, similar responses are present at subcortical levels, even at the level of the cochlear nucleus (Pressnitzer et al., 2008). Given that the tonotopy that exists throughout the auditory system arises in the periphery, similar correlates may already be present in the periphery. The AP in frogs is similar to the basilar membranes of birds and mammals in that it is tonotopically organized. This tonotopy is what gives rise to the peripheral frequency channels, and it is maintained through the central auditory systems of frogs (Mohneke, 1983; Pettigrew et al., 1981), as well as birds (Carr and Code, 2000) and mammals (Winer and Schreiner, 2005). Thus, the present data highlight the importance of tonotopy at peripheral levels of the auditory system for stream segregation, and suggest that such peripheral processing mechanisms might complement previously described central mechanisms.

Within the range of the BP (zone 3), we expected segregation of A and B pulses to be driven by level cues resulting from variation in sensitivity to the frequency of the B pulses. In Experiment 1, we observed decreasing proportions selecting *Alt-Long* as a function of increasing ΔF. The comparison of the response curves with a curve of auditory sensitivity in Experiment 2 suggested that the effect of ΔF was consistent with a level-*dependent* mechanism when *Alt-Long* excited only the BP (zone 3). Furthermore, the results of the manipulation of ΔL in Experiment 1 were consistent with our predictions. Preferences for *Alt-Long* decreased as a function of increasing ΔL, regardless of the carrier frequency. These results suggest that level differences can function as segregation cues, and provide further support for the hypothesis that a level-dependent mechanism facilitated segregation of *Alt-Long* when it stimulated the BP in manipulations of ΔF. Differences in sensation level have been implicated in frequency discrimination (Nelson et al., 1983; Wier et al., 1977), and there is evidence that they may affect stream segregation by frequency (Rose and Moore, 2000). However, the formation of multiple streams from sounds processed through a single channel is not trivial. Sound level is encoded in the auditory nerve through variation in firing rate (Feng, 1982), and frequency can similarly be encoded through

level by fibers arising from the BP (Schwartz and Gerhardt, 1998). Neurons at an ascending level of the auditory system must extract information from this rate code to create a representation of the auditory stream(s) in the brain. Thus segregation of *Alt-Long* into streams in any condition in which it excited a single channel was likely achieved in the central auditory system.

In Experiment 1, preferences for *Alt-Long* were decreased in the spatially-separated condition relative to the co-localized condition, indicating that Δθ increased segregation of A and B pulses, consistent with the channeling hypothesis. The strongest effect of Δθ occurred when the A pulses had a frequency of 2.6 kHz, and the effect was level-dependent. One possible explanation for the interaction of Δθ with carrier frequency may be that the spatial separation induced additional level cues that facilitated segregation when *Alt-Long* stimulated the BP. Alternatively, the effect may have been related to frequency-dependent differences in localization ability. Using laser-Doppler vibrometry, Caldwell et al (2014) determined that the tympanic membranes of gray treefrogs are most directional in response to high (> 2kHz) frequencies. This strong directionality may have made it difficult to integrate pulses across the large spatial separation when pulses had high frequencies (e.g. when A pulses had a frequency of 2.6 kHz).

In summary, this study finds that the channeling hypothesis cannot account for stream segregation of two sounds that differentially excite the sensory organs in the auditory periphery of frogs. Peripheral processing can account for stream segregation when sounds are encoded by the tonotopically organized sensory organ (AP). In the organ that is not tonotopically organized (BP), a level-dependent mechanism can account for stream segregation. Spatial separation of sounds facilitated stream segregation, and the strength of the effect of Δθ was consistent with the frequency-dependent directionality of the peripheral auditory system. Together, our findings suggest that the organization of the periphery may play a strong role in the formation of auditory streams.

Chapter 5 Perceptual channels and neural codes

Channels in the brain

Color vision

The role of the sensory systems is to convey information about the world to the brain. Sensory systems use a variety of coding strategies to transmit this information. This chapter focuses on neural coding in sensory systems, with a particular focus on perceptual channels.

An early hypothesis of color vision was developed by Thomas Young at the beginning of the 19th century. A prevailing idea at the time was that nerve fibers in the periphery could respond to only one particular kind of external stimulus (e.g. a particular wavelength of light) and thus only transmit that kind of sensory information to the brain. This idea necessitated that an infinite number of nerve fibers subserve color vision. Young argued that an infinite number of nerve fibers seemed unreasonable. He based his hypothesis of color vision in the results of Newton's prism experiments, which showed that light of certain colors could be deconstructed into two or more component colors. In a lecture that was later published (Young, 1802), Young posited "that particles" (what we would now call receptors) in the retina and their corresponding groups of nerve fibers primarily respond to one of three colors. He speculated that the three colors the receptors would respond to should be primary colors, combinations of which would yield all of the additional known colors. Initially, Young's choice of colors was based on the primary colors of the artist (red, blue, and yellow) as it was known that by mixing pigments of these colors it was possible to acquire additional colors (Young, 1802). Young later revised his choice of colors to the primary colors of the visible light spectrum: red, green, and violet (Young, 1807).

According to Young's hypothesis, visible light would excite the three types of particles to varying degrees, depending on the sensitivities of each class of particles. While light of a primary color would predominantly excite only one type of particle, the sensation of a non-primary color could be achieved through strong excitation of at least two types of particles. For example, red light would strongly activate the particles sensitive to red and the others only slightly, while the sensation of yellow light would be achieved through the stimulation of both the particles sensitive to red and to green (Young, 1807). By varying the ratio of excitation of the three different kinds of particles, the sensation of any hue in the visible spectrum could be acquired (Young, 1807).

Young's hypothesis went largely ignored until Hermann von Helmholtz dismissed it in one of his first papers on color (Helmholtz, 1852). However, based on evidence from his own,

and later Clerk Maxwell's, "color-matching" experiments (Helmholtz, 1852; Helmholtz, 1867; Maxwell, 1855; Maxwell, 1857), Helmholtz began to revise his opinion. In Helmholtz's color-matching experiments, human subjects are asked to compare a mixture of different colored lights to a target light of a given wavelength. The quantities of the component lights in the mixture are adjusted until the mixture is perceived as identical in color to the target. These experiments showed that any color in the visible spectrum could be reproduced through a combination of no more than three primary colors (Helmholtz, 1867; Maxwell, 1857), lending credence to the idea that human color perception could by accounted for by neural elements differentially responsive to three primary colors.

Further support for Young's hypothesis came from studies of color-blind individuals. Both Maxwell and Helmholtz determined that the colors visible to color-blind subjects were limited to those that could be acquired through mixtures of two colors. Most of the subjects they studied seemed to be insensitive red, while a smaller number seemed insensitive to green. The color-specific impairments of these individuals suggested that both red and green were colors to which normal eyes were sensitive, as had been suggested by Young (Helmholtz, 1867; Maxwell, 1855). Helmholtz soon adopted Young's hypothesis, developing it further and putting it into a more quantitative form (Helmholtz, 1867). Despite Helmholtz's initial dismissal of Young's hypothesis, he is now considered one of its earliest champions, and the hypothesis has become known as the Young-Helmholtz theory or the trichromatic theory of vision. While Young and Helmholtz both thought of visual processing in terms of optic nerve fibers, later revisions of the trichromatic theory described three independent channels that corresponded to the three types of cone cells eventually identified in the retina (Boothe, 2002; Bowmaker, 1983).

Almost a century after Young's hypothesis, a rival hypothesis of color vision was put forth by Ewald Hering (Hering, 1874). Hering noted that some aspects of color vision, such as mixtures of complementary colors and afterimages could not be explained by the trichromatic theory of vision. Complementary colors are pairs of colors that, when mixed in particular ratios, are perceived as white or gray. Mixing the complementary colors red and green, for example, might be expected to yield a reddish-green hue, but instead appears as a neutral color. Helmholtz had discovered these complementary colors in his color-mixing experiments (Helmholtz, 1867), but largely ignored them. Hering, however, considered the existence of complimentary colors a violation of the trichromatic theory, because it suggested that the human visual system could not respond to both red and green at the same time. Complementary colors are also observed in

retinal afterimages. Afterimages appear when the retina has been steadily stimulated with an image of a given color for a prolonged duration. When the observer's gaze shifts to a neutral background, the resulting percept matches the previously attended image, except that all of the colors are converted to their complements.

Helmholtz had been well-aware of the afterimage phenomenon and had explained it as a combination of persistent stimulation of the retina and "fatigue" of the nerve fibers (Helmholtz, 1867). He postulated that the fibers that responded most to the stimulus would fatigue making them less sensitive to light, and thus the color of the persistent afterimage resulted from stimulation of the unfatigued fibers (Helmholtz, 1867). Hering thought that the phenomenon of afterimages could be better explained as the result of "opponent processes" in the eye or brain (Hering, 1874). According to Hering's hypothesis, there are three independent physiological processes, each associated with a pair of opponent (or complementary) colors: red-green, blue-yellow, and white-black. Each process has two opposed or mutually exclusive responses, one in favor of each color of the associated pair (Hurvich and Jameson, 1957). So, for example, the eye can respond only to red or green, but not both simultaneously. This hypothesis was consistent with the observations of Helmholtz that mixtures of complementary colors result in the percept of a neutral color. In terms of afterimages, Hering thought that the visual system preferred to maintain a neutral equilibrium state; thus continued stimulation of one color would cause a gradual decrease in the response to that color and increased response to its opponent (Hurvich and Jameson, 1957). When stimulation ceased, as by shifting one's gaze, the effect of the response to the opponent color can be seen as the afterimage, with the image fading away as the process returns to the neutral state. The opponent process hypothesis also better explained common types of colorblindness, in that colorblind individuals are not simply insensitive to one color, but typically confuse two or more colors that form an opponent pair. While Hering's hypothesis initially had several supporters, it eventually lost popularity because no one could conceive of a physiological basis for the idea. Several decades later, with increased neurophysiological research and the discovery of inhibition in the nervous system, Hering's hypothesis regained traction and his opponent "processes" became more commonly described as color-opponent channels.

Evidence for channels in color vision

Helmholtz and Hering were bitter adversaries and their theories of color vision were thought to be irreconcilable for many years, with researchers typically interpreting data as

supporting one theory over the other. Both theories, however, depended on finding evidence for three independent channels, although the term "channel" would not become popular until much later. In the case of the trichromatic theory, these channels would encode the colors blue, green, and red. In the case of the opponent processes theory, these channels included two chromatic channels, a red-green and a blue-yellow channel, and one achromatic white-black channel. In both cases, these channels are essentially feature detectors or filters (Graham, 1980; Regan, 1982). The methods that scientists used to test the trichromatic theory and the opponent-processes theory (i.e. adaptation and masking studies) would eventually establish the existence of trichromatic and color-opponent channels. They would also be the same methods used to establish the existence of channels for the processing of other stimulus features, as well.

Early work in support of the trichromatic theory was mostly psychophysical. Wright, for instance, in testing the trichromatic theory, performed a color-matching experiment that involved adaptation (Wright, 1934). The observer viewed a mixture of three colors with the left eye and a test stimulus with the right eye. The subject then adjusted the mixture in the left eye until it matched the perceived test stimulus. The test was repeated before and after the right eye had been adapted to a stimulus of a particular color. Adaptation would change the relative sensitivities of the receptors, and the extent the adaptation would depend how close the color of the adapting stimulus was to the test stimulus. Because perception of most colors requires excitation of two or three types of receptors, adaptation would shift the perceived color of the test stimulus, resulting in a corresponding shift in the composition of the mixture that the subject selected. However, when the test stimulus was selected to only stimulate receptors of one type, adaptation would not cause a shift in the perceived color of the test stimulus, but only change its perceived intensity. Through these experiments, Wright was able to determine three "fundamental colors," blue, green, and red, which corresponded to the colors to which the receptors in the retina were presumed to be tuned.

Another approach of testing the trichromatic theory, used by Stiles and Wald (Stiles, 1949; Stiles, 1959; Wald, 1964), was an "increment-threshold" method. In this method, a subject was first fully adapted to a stimulus which ideally covered most of the visual field (known as a field or adapting stimulus). A small patch of light serving as the test stimulus was superimposed and its intensity was adjusted to determine a threshold at which the test stimulus became visible. By varying the test stimulus in some additional feature dimension, such as wavelength, one could determine a sensitivity function in terms of that feature. Wald (1964) used a specific variation of

the increment-threshold method in which he took advantage of opponent colors to isolate the responses of the hypothesized receptors. The adapting stimulus was made to have a wavelength complementary to the wavelength to which the receptor of interest was sensitive. For example, by stimulating with a bright yellow light, it was expected that the "blue" receptors would be isolated. Wald then measured the sensitivity function of each psychophysically isolated receptor type across a range of wavelengths. Using these methods, he determined there were three kinds of receptors, roughly sensitive to blue, green, and red wavelengths (Wald, 1964).

Although the psychophysical data were suggestive of the validity of the trichromatic theory, it was thought that the most direct support for the trichromatic theory would come from discovery of three components of the retina that were sensitive to different wavelengths of light. Rods and cones were identified in the retina during Helmholtz's time (Schultze, 1866). Based on the differences in the responses of these cells to light, it was quickly hypothesized that cones subserved vision in light environments and were consequently responsible for color vision. Scientists began working on the problem of showing that the retina perceived different colors independently.

In a series of studies, Ragnar Granit used electrophysiology to study color sensitivity in the retinas of a variety of animals (Granit, 1945). Inserting a microelectrode into the retina, he recorded the discharges of a small number of cells in the vicinity of the electrode tip (Granit, 1942). Under photopic conditions, the impulses were presumed to originate in cone cells (Granit, 1942). Granit presented light at a variety of wavelengths to determine the tuning of the cells. The recordings revealed a variety of tuning functions, suggesting that tuning differed between cells (Granit, 1943). The most common sensitivities across animals were to wavelengths that could be classified as blue, green, and red. Precise tuning differed, however, and some animals lacked one or more kinds of cells. Extending these results to the human retina, Granit's experiments provided evidence in favor of the trichromatic theory. However, it was not for a several more decades that it was definitively shown that there were indeed three different kinds of cones in the human retina, each sensitive to a different wavelength of light (Brown and Wald, 1964; Marks et al., 1964).

When Hering's opponent processes hypothesis re-emerged in the 20th century, it was generally accepted (although without complete evidence as yet) that the human retina contained three types of cone cells with sensitivity to different wavelengths. However, many scientists felt that the trichromatic theory could not fully explain color vision and found the opponent processes

hypothesis compelling. Experiments that focused on the apparent natural pairing of colors by the visual system provided evidence of Hering's hypothesis. Hurvich and Jameson performed a series of psychophysical experiments known as hue cancellation experiments (Hurvich and Jameson, 1957). Here, the hypothesis was that if the processes driven by two opponent colors were antagonistic, then stimulation with one color should be canceled by equal stimulation with the opponent color. The procedure involved simply presenting a test light of a given color, and then asking the participant to add light of a "cancellation color" until the combination no longer had the original hue, but also had not taken on the hue of the cancellation color. Using the four colors central to the opponent processes hypothesis as test colors (i.e. blue, green, yellow, and red), Hurvich and Jameson (1957) created psychometric curves that provided one of the first quantifications of the hypothesized opponent processes.

Some of the first neurophysiological evidence for a neural substrate for the proposed opponent channels in the visual system came from recordings in the retina of teleost fish (Svaetichin and MacNichol, 1958). Svaetichin and MacNichol recorded graded potentials which differed in direction based on the wavelength of light presented. For example, in some cases the potential would decrease when presented with red light and increase when presented with green light. Changes in luminance resulted in hyperpolarization, regardless of the wavelength of light (Svaetichin and MacNichol, 1958). Svaetichin and MacNichol (1958) performed an additional manipulation in which a background colored light was presented before and after the test light. They observed adaptation of the neural response to the background light, which had the effect of decreasing subsequent responses to similar wavelengths and enhancing responses to the wavelengths of opposing colors, although at the time they simply interpreted the result as subtraction of potentials of opposite signs (Svaetichin and MacNichol, 1958).

Around the same time that Svaetichin and MacNichol recorded graded potentials from the retina, similar patterns were observed in the responses of some cells in the lateral geniculate nucleus (LGN) of primates. Valois et al (1958) discovered that some of these cells showed on-off responses not unlike those described by Hartline from recordings in optic nerve fibers (Hartline, 1938). While Hartline found effects based on the presence or absence of light (Hartline, 1938), the cells that Valois recorded from changed their responses based on the wavelength of the light presented. They found, for example, cells that exhibited red-on, green-off responses (Valois et al., 1958). Similarly, cells were identified which exhibited blue-on, greenish/yellow-off responses.

These on-off cells are thought to underlie the opponent channels hypothesized by the opponent processes theory.

Evidence for channels in the visual and auditory systems

Experiments on color vision gave rise to the idea of channels and introduced the methods used to show that they existed. And while trichomatic and color-opponent channels were some of the first described, channels involved in other aspects of sensory processing have also been discovered. Early definitions of channels tended to be abstract, but as neurophysiology progressed and neural pathways that seemed related to psychophysical channels were discovered, channels came to refer to arrays of neurons with similar receptive fields or neural pathways that shared similar tuning properties (Graham, 1980; Regan, 1982). The neural units that comprise a channel need not be identical in their response to all stimuli, but must respond in a similar manner to at least one type of stimulus (Braddick et al., 1978). Channels can exist at any stage of processing, and the organization of channels between successive processing stages is not necessarily conserved (Regan, 1982).

A number of methods have been employed to test for the existence of channels, including both psychophysical and neurophysiological experiments. Methods using adaptation or masking are common to both psychophysical and neurophysiological approaches. The general idea is to look for interactions between the psychophysical or physiological effects of several stimuli. Interactions can include a suppression of the effect of one stimulus by another or summation of the effects of the stimuli. Interactions between the effects of two stimuli imply that the stimuli are processed through the same channel, while a lack of interaction between the effects of the stimuli suggests they are processed through distinct channels. It is worth noting, however, that the strict independence of channels is somewhat overstated. While it is true that channels excited by very different stimuli are indeed independent, in many channel systems, there is evidence that channels with overlapping sensitivity show some level of interaction. For instance, models that include lateral inhibition suggests that channels receive inhibitory input from other channels with moderately similar, but not identical, tuning characteristics (Houtgast, 1972; Polat and Sagi, 1993; Sagi and Hochstein, 1985; Tolhurst and Barfield, 1978). Lateral inhibition may serve to sharpen the output of the channels or improve contrast between similar stimulus representations.

While the study of color first introduced the ideas associated with channels, other features of visual objects are thought to be processed through channels as well. Evidence for these

channels has come from studies similar to the kinds used to reveal the existence of color channels: adaptation and masking in psychophysical and electrophysiological studies. For example, Campbell and Kulikowski (1966) used a masking experiment to investigate processing of orientation by humans. Subjects were presented with overlapping gratings, one of which served as the test, and a second which served as a masker. The contrast of the masking grating was fixed, and the subject adjusted the contrast of the test grating until it was just visible. The contrast required to detect the test grating decreased as a function of increasing difference between the orientations of the gratings (Campbell and Kulikowski, 1966). The fact that greater interaction (in this case masking) occurred between the effects of the gratings when they had similar orientations (e.g. a difference of 0° or 15°) suggested that stimuli of similar orientations were processed by a single channel. There was little masking when the two gratings had larger differences in orientation (e.g. 35°), suggesting that gratings with such large differences in orientation are processed through distinct channels. The discovery of Hubel and Wiesel (1959; 1962) that neurons in the visual cortex of cats were sensitive to specific orientations provided a possible neural substrate for orientation channels.

Spatial frequency is another feature of visual stimuli thought to be processed through channels. Blakemore et al (1970) found that when an observer gazed at a grating for an extended period of time, adaptation to the frequency of the grating occurred. This adaptation resulted in a shift of the perceived frequency of gratings viewed subsequently, as determined by matching the grating seen through an unadapted eye to that seen through the adapted eye. The effect was frequency-specific, so that the greatest shift in perceived frequency occured when subsequently viewing gratings of frequency similar to the adapting frequency (Blakemore et al., 1970). Using a different paradigm in which observers were presented with gratings that had been modulated simultaneously with two different frequencies, Sachs et al (1971) found additional support for independent frequency channels. Later studies found that neurons in the primate's visual cortex were tuned to spatial frequency (De Valois et al., 1982), providing a neural correlate for spatial frequency channels.

The auditory system is generally accepted to have two kinds of channels: frequency channels and lateral channels. The evidence for frequency channels begins with the place theory or resonance theory, put forth by von Helmholtz (1895). This theory stated that sounds of different frequencies were represented by resonance at different parts of the basilar membrane in the inner ear. Several years later after the introduction of the resonance theory, studying cochleae

dissected out of the ears of animals and cadavers, Georg von Békésy measured the movement of the basilar membrane in response to sounds of different frequencies and discovered the existence of a traveling wave along the membrane (Békésy, 1960). His studies confirmed the tonotopic organization of the cochlea, as hypothesized by Helmholtz, but disproved Helmholtz's theory that resonance was the mechanism underlying the tonotopy. An earlier study had shown that presentation of a tone had a masking effect on the detection of a second tone, which was greatest when the tones were of similar frequencies (Wegel and Lane, 1924). Combining this psychophysical result and Békésy's biomechanical results, auditory researchers began to think of the auditory system as an array of filters, or a filter bank (Fletcher, 1940; Green, 1958; Huggins and Licklider, 1951; Korn, 1969).

One of the earliest formulations of the filter bank idea came from Fletcher's notion of a critical bandwidth, which he developed through the use of masking experiments (Fletcher, 1938a; Fletcher, 1938b). Using narrowband noises to mask a tone with the same center frequency, he determined the signal-to-noise ratio required for the tone to be detectable. This signal-to-noise ratio served as a measure of the effectiveness of the masking. Varying the bandwidth of the masking noise, Fletcher determined that the masking increased as a function of increasing bandwidth, up to a certain "critical bandwidth," after which there was no change (Fletcher, 1938a; Fletcher, 1938b). This result suggested to Fletcher that the auditory system functioned as a bank of overlapping bandpass filters, where each critical bandwidth was equal to the bandwidth of a filter. As the bandwidth of the noise had increased, the energy in the filter likewise increased, which caused an increase in masking. However, once the bandwidth increased beyond the bandwidth of the filter, additional energy was not processed through the filter containing the tone, and thus did not affect its detectability.

The filters identified through these masking experiments fit the notion of a channel that was introduced earlier. That is, when the stimuli are processed through the same filter, they can have interacting effects as in the masking reported here. When stimuli are processed through independent filters, there are no interactions in their effects. As the idea of an auditory filterbank developed, scientists began to refer to individual filters as channels (Chistovich et al., 1974; Gibson and Hirsch, 1975; Hill et al., 1968; Huggins and Licklider, 1951; Schwent and Hillyard, 1975). In terms of the physiology of the auditory system, a frequency channel may refer to the tuning of an inner hair cell (Huggins and Licklider, 1951), or more commonly a single nerve fiber (Bregman and Campbell, 1971; Carney, 1993; Kiang and Moxon, 1974).

Evidence that the two ears acted as independent input channels likewise arose before they became labeled as "channels." While Wegel and Lane's tone-on-tone masking experiments showed that a tone could be masked by a second tone of similar frequency, they also found that this effect was only present when both tones were presented to the same ear. If the second tone was presented to the opposite ear, there was no masking unless the intensity of the second tone was greatly increased (Wegel and Lane, 1924). These results are suggestive of independent ear-based channels. However, it was probably the discovery that localization of sounds relied on comparisons of the inputs at the ears that drove the notion that the ears functioned independently (Jeffress, 1948). Investigators soon adopted the channel concept to describe processing between the two ears (Bregman and Campbell, 1971; Hillyard et al., 1973; Jeffress et al., 1956; Moray, 1960).

Using adaptation paradigms, several studies have also found support for the existence of amplitude-modulation channels in the auditory system (Dau, 1999; Kay and Matthews, 1972; Regan and Tansley, 1979; Yost et al., 1989). In these studies, a subject listened first to a conditioning tone that was amplitude-modulated at a given rate. The subject then heard a set of test stimuli varying in rate of amplitude-modulation. For each stimulus, a threshold was determined by having the subject adjust the depth of modulation in the test stimulus until it was just detectable. It was found that subjects' thresholds were elevated most in response to test stimuli with modulation rates similar to the rate in the conditioning stimulus. The elevation in threshold decreased as a function of distance between the modulation rates of the test stimulus and the conditioning stimulus. More recently, there have been reports of neurons in the central auditory systems of mammals (Langner and Schreiner, 1988; Nelson and Carney, 2007), birds (Woolley and Casseday, 2005), and frogs (Rose and Capranica, 1983), which are thought to be the underlying mechanism for amplitude-modulation channels. Researchers are increasingly supportive of the idea of amplitude-modulation channels (Joris et al., 2004; Plack, 2013), but the idea has not been universally accepted. Using similar adaptation studies, early work also supported the existence of frequency-modulation channels in the auditory system (Kay and Matthews, 1972; Regan and Tansley, 1979). A later study, however, suggested that the adaptation effects initially thought to be temporary were more likely associated with learning of the detection paradigm (Moody et al., 1984). This finding of Moody et al (1984) highlights the importance of repeating adaptation experiments multiple times and across days to avoid conflating the effects of adaptation with those of learning.

Channels in other sensory systems

The concept of channels exists to varying degrees in the other sensory modalities as well, but the properties of these channels differ somewhat from those in the visual and auditory systems. Often, the organization of the channels in other modalities is not as easily classified as an array of feature detectors tuned along a one-dimensional axis, a reflection of the more complex nature of the responses of receptors in other modalities. However, boundaries can be identified within which stimuli have interacting effects.

For example, there are considered to be four channels used in the processing of tactile sensation. These channels are based on the four types of mechanoreceptive fibers in skin (Vallbo and Johansson, 1984). In a series of experiments, researchers tested the hypothesis that these four mechanoreceptors underlay four tactile channels (Bolanowski Jr et al., 1988; Capraro et al., 1979; Gescheider et al., 1982; Gescheider et al., 1983; Hamer et al., 1983). The basic method involved presenting a test stimulus either simultaneously with or shortly following a masking stimulus. Generally, one of the two vibrotactile stimuli was selected to excite one kind of mechanoreceptor, while the properties of the second stimulus varied across trials and were primarily expected to excite a different type of mechanoreceptor. The threshold for detection of the test stimulus was then determined by adjusting the intensity of the masking stimulus. For example, Gescheider et al (1982) selected a narrow-band noise with a center frequency of 275 Hz as the masking stimulus, which was expected to optimally excite Pacinian corpuscles. The test stimuli were bursts with frequencies that varied in their ability to excite Pacinian corpuscles. Gescheider et al (1982) found that the masking stimulus elevated thresholds for detection of a 300-Hz test stimulus as a linear function of masker intensity, consistent with both the test and masking stimuli exciting the same channel. The masking stimulus only elevated thresholds for detection of a 15-Hz test stimulus when the masker intensity was very high, consistent with these two stimulus types being processed by independent channels. The pattern of threshold elevation was more complicated for a test stimulus with a frequency of 80 Hz, which is moderately well-detected by Pacinian corpuscles, but also detected by non-Pacinian receptors. For this stimulus, thresholds increased as a function of masker intensity only when both the test and masker stimuli were detected by both channels. At intermediate masker intensities, when the test stimulus and masker were expected to excite opposite channels, there was no increase in threshold.

Researchers have begun considering the olfactory system in terms of channels as well (Laurent, 1999; Schlief and Wilson, 2007; Vassar et al., 1994). The most common use of the word "channel" in this literature refers to the neural pathway that conveys the responses of a single olfactory receptor type (Bhandawat et al., 2007; Liang and Luo, 2010; Olsen and Wilson, 2008; Olsen et al., 2007; Schlief and Wilson, 2007). This pathway includes the specific set of olfactory receptor neurons in the periphery (all of which express the same olfactory receptor gene) and the glomerulus innervated by those neurons. It is difficult to classify the channels described in this way as an array of feature detectors, as the parameters that define feature detection by olfactory neurons is still an open question (Wilson and Mainen, 2006). While it is clear that the olfactory receptor neurons and glomeruli respond differentially to distinct odorants, the relationships between odorants are, in many cases, ambiguous and difficult to order along any one dimension (Wilson and Mainen, 2006). Furthermore, although there is evidence for interactions between the effects of odors processed through a glomerular channel (Payne and Dickens, 1976; Silbering and Galizia, 2007), as would generally be expected in a channel processing organization, it is unclear the extent to which this is a general property of olfactory channels.

Types of neural codes

Channels or population codes

The role of sensory systems is to provide information about the world to the brain of an organism so that the individual can modify its behavior in a manner appropriate to its environment. An interesting question, then, is whether and how a channel-like arrangement of neural elements subserves this goal. The organization of neural processing through channels is often known simply as population coding, or occasionally coarse coding (Dayan and Abbott, 2005; Kandel et al., 2012); the term channel is generally not used.

Descriptions of population coding begin with a population of sensory neurons that are sensitive to overlapping ranges of a stimulus (e.g. color or frequency). A code is considered a population code if representation of the stimulus requires the combined input of the population of neurons (Kandel et al., 2012). The tuning properties of neurons that comprise the population play an important role in determining how information can be read out from the collective activity of the population. If the sensory cells are narrowly tuned, only a small number of cells will respond to any stimulus, greatly reducing uncertainty about the identity of the stimulus. However, narrow

tuning is not the only way to encode information with great precision; high resolution can be achieved with broadly tuned filters, given sufficient numbers of cells in the population. While more narrowly tuned filters must be evenly and closely spaced along the stimulus space for the system to accurately identify stimulus identify over a wide range of values, having cells with broader tuning allows for more tolerance in the spacing of the filters (Heiligenberg, 1987; Zhang and Sejnowski, 1999). When the cells in the population are broadly tuned, the output can often be decoded from the vector sum of the responses across the population (Seung and Sompolinsky, 1993). There is also evidence that similar population codes function in motor processing (Georgopoulos et al., 1986; Lee et al., 1988; Levi and Camhi, 2000). The fact that an organization using channels, explicitly stated or otherwise, is the most commonly cited example of a population code implies the ubiquity of this kind of arrangement of neural processing.

As illustrated in the previous section, using channels to organize neural activity is one way of processing information in a population of neurons, but it is certainly not the only way. There is evidence for several other coding strategies in the brain. Perhaps the best way to evaluate the usefulness of channels is to consider alternative ways that the nervous system can be organized.

Local codes

One alternative to the channel scheme is a case in which each possible stimulus is selectively coded for by a different cell (or group of cells). In reality, this type of coding can be thought of as an extreme version of channels, where the bandwidth of the tuning curves is narrow enough to pass only one kind of stimulus. This strategy is known in some literature as a "localist" representation (Roy, 2012). For instance, the peripheral olfactory system, where each receptor neuron expresses one (or rarely two or three) receptor types, each of which binds a limited number of odor molecules, could be considered an example of this highly specific coding strategy. It should be noted, however, that even the receptor neurons in this olfactory example are not quite one-to-one, because receptors can sometimes bind a few different molecules, with varying affinities.

A more typical example of a local coding strategy uses so-called grandmother cells which are thought to respond selectively to a single, specific stimulus (such as one's grandmother). Quiroga and coworkers have indeed found cells in the human brain that respond selectively and invariantly to a certain location or person (Quian Quiroga and Kreiman, 2010; Quiroga et al.,

2005). Similarly, Gross and colleagues have found neurons in cortical regions that respond selectively to hands or faces (Gross, 2008).

A coding strategy with such high selectivity has both advantages and disadvantages. Given the response of a cell with highly selective tuning, information transfer is essentially noiseless and so there is little chance of error (Cover and Thomas, 2012); it is thus trivial to ascertain the identity of the original stimulus. However, strict one-to-one coding is inefficient as it requires a large number of differentially-tuned neurons to cover the stimulus space. Even coding the vast array of possible stimuli with cells that are as selective as olfactory receptors mentioned earlier poses a challenge, as illustrated by the fact that olfactory receptors in humans are coded for by some 300+ genes (Malnic et al., 2004) compared to the four genes that code the opsins used in human vision.

A highly specialized tuning system is also more susceptible to damage, as loss of the cells that code for a given stimulus will greatly reduce or eliminate the ability to perceive that stimulus. One can consider, for example, tactile receptors, which innervate and are consequently tuned precisely to specific locations on the body (Johansson, 1978; Johansson and Vallbo, 1979). If damage occurs to the receptors in a portion of the hand, for example, all vibro-tactile sensation in that location is eliminated (Novak et al., 1993).

There is little evidence for a true localist coding strategy in the strictest sense anywhere in the vertebrate brain. Even in the examples of cells responsive to hands and faces, these cells are not so selective that they will only respond to one kind of face (Gross, 2008). For this reason, the idea of grandmother cells in cortex has largely fallen out of favor. If we consider, instead, highly selective sensory channels, the best examples are seen at early stages of sensory systems, as in the spatial tuning in the periphery of the somatosensory system or the specificity of olfactory receptors. One could argue that localist coding is more common in invertebrates, where single neurons are often identifiable and exhibit stereotyped responses across individuals (Gabbiani et al., 2002; Kristan Jr. et al., 2005; Rankin, 2002). However, even the identified neurons in these animals often have multiple roles and exhibit plasticity and learning (Burrell et al., 2001; Hedwig, 2000; Jarriault et al., 2009; Kristan Jr. et al., 2005).

Distributed codes

Another non-channel coding strategy is one in which a stimulus is represented by multiple neurons and the neurons respond to multiple stimuli. This kind of representation that

relies on the integration of the responses of populations of cells is known as a distributed code. In contrast to channel coding, tuning curves of cells are typically very broad and overlapping, and the tuning of cells may change with learning or under different contexts.

An example of a distributed code is seen in the encoding of gustatory information by neurons in the nucleus of the solitary tract (NST). Recordings from neurons in the nucleus of rates revealed that NST neurons are broadly tuned with varying preferences for sucrose, NaCl, or HCl (Lemon and Smith, 2006). Lemon and Smith found that classification of the stimulus was poor when considering either the firing of single neurons or by averaging responses across neurons. However, when taking into account known relationships between the spiking rates of different neurons in response to the stimulus, it was possible to decode the identity of the stimulus from the responses of the population of NST neurons (Lemon and Smith, 2006).

One apparent disadvantage of a distributed code is that it is relatively noise and can be prone to error. However, accuracy can be improved by recruiting additional neurons into the population. In a study of decoding of hippocampal pyramidal cells, Zhang et al. (1998) found that the accuracy of the prediction of a rat's location in space increased as the number of cells included in the analysis increased. Similar findings have also been reported for other brain structures (van der Meer et al., 2010). The requirement of large numbers of neurons was cited as a disadvantage for the one-to-one code discussed above, but a key point is that the neurons that represent a distributed code are not necessarily permanently tuned in a particular way. Because tuning of these neurons can change with context or learning the same neurons can be used in multiple representations. The repurposing of neurons for different representations reduces the overall number of neurons that are required to encode multiple stimuli.

Using a large number of neurons to encode a representation as in a distributed code also has an advantage over other types of representations in that the network is more resistant to damage. Even if some part of the brain is damaged, enough of the network may be intact to maintain the representation. Alternatively, if the representation has changed, the brain should be able to learn the new representation. For example, in the NST neurons described earlier, if a large number of the neurons most sensitive to sucrose died, the full range of gustatory stimuli could still be represented by the remaining populations of NST neurons. Even if the brain did not immediately recognize the representation for a stimulus absent the sucrose-sensitive neurons, a new representation involving the remaining neurons could be learned through Hebbian processes (Hebb, 1952). This resistance to loss of representation is known as "graceful degradation," and

has been illustrated analytically. For instance, Ghazanfar et al. (2000) recorded ensembles of neurons from the barrel cortex of rats as individual whiskers were deflected. The authors used a neural network to classify the identity of the stimulated whisker based on the recorded neural activity. Evaluating performance of the network as neurons were removed stepwise from the ensemble, Ghazanfar et al. (2000) found that performance of the network decayed slowly as a function of the number of neurons removed. This smooth decay contrasted with the sharp drop in performance that would be expected if whisker deflection was selectively encoded by individual neurons.

Another interesting example of a distributed representation and its resistance to damage is found in the nervous system of the octopus. While there are no reports of the actual neural activity that can confirm, it is thought that tactile information is incorporated into the brain through a distributed representation (Young, 1983). Support for this hypothesis comes from the fact that tactile information from the nerves in the arms of these animals is immediately distributed throughout multiple regions of the brain (Budelmann, 1995; Budelmann and Young, 1985). Additionally, experience or learning changes the synaptic connections between large numbers of neurons (Young, 1983). Lesions in one part of brain of an octopus will alter its ability to complete, for example, a tactile discrimination task. However, the octopus can continue to learn new discriminations even if large parts of the brain have been removed (Young, 1983), illustrating how a distributed representation can reduce the impact of damage to a localized part of the brain.

One disadvantage of a distributed code lies in its complexity. It is not immediately intuitive how such a code could be used by the brain. Scientists use complicated decoding algorithms and network analysis techniques to "readout" a discrimination or classification from the neural activity in the brain, and it is often assumed or implied that downstream neurons perform a similar function (Andersen, 1997; Hung et al., 2005; Laurent, 2002). These "observer" or "decorder" neurons would receive projections from the neurons holding the representation and provide a readout of the encoded stimulus. In the olfactory system of the locust, for example, Laurent argues that the distributed code represented by projection neurons is decoded by Kenyon cells that act as coincidence detectors (Laurent, 2002). The Kenyon cells must receive strong synchronous input from projection neurons before they will fire an action potential, resulting in sparseness of the representation at the level of the Kenyon cells and high selectivity of individual neurons (Laurent, 2002). However, in general few examples of such observer neurons have been

discovered in the brain. While decoding studies provide evidence that there is information in the neural activity of neuron populations that can be decoded, this doesn't necessarily mean that the brain actually does such computations. An alternative to the notion of observer neurons is that an explicit neural readout is not even necessary, as the brain can continue to transform between different population codes (Abbott and Sejnowski, 1999). According to this theory, a final neural readout in the brain may only be necessary to generate a motor output, and it could therefore be achieved by inputs to motor cortex (Abbott and Sejnowski, 1999). It may be that the complexity of a distributed representation presents more of a problem for researchers trying to decode the representation than for the brain that is using it.

Organization of neural codes in the brain: from peripheral to central processing

Some generalities can be made about the neural processing as information moves through the brain from peripheral structures to the central nervous system. The primary function of peripheral parts of sensory systems is to transform the energy of a stimulus into a neural signal. As the signal progresses through processing stages in the central nervous system, it is maintained as a pattern of electrical energy, but there are often transformations from one pattern to another. The dimensions of stimuli represented by neurons in the central parts of the nervous system are typically greater relative than those represented by neurons in the periphery.

Ascending the vertebrate auditory system

These general principles can be observed by considering changes in representations at ascending levels of the vertebrate auditory system. The sensory receptors in the peripheral auditory system are hair cells, which are typically located on some kind of sensory organ, such as the cochlea in mammals or the amphibian and basilar papillae in anurans. The hair cells function as frequency filters, forming the initial channel organization in the auditory system. The sensory organs are typically bilateral, so that individuals have one organ that corresponds to each ear. Hair cells are innervated by ganglion cells, which send their axons through the auditory nerve to synapse onto the cochlear nucleus in the brainstem. Each ganglion cell innervates several hair cells sensitive to similar frequencies. This convergence of inputs to ganglion cells maintains frequency selectivity at the level of the auditory nerve so that frequency channels remain intact through this neural layer. In addition to frequency selectivity at the level of the auditory nerve,

there is also separation of the auditory nerve fibers between the two ears, which serves as the basis for the two lateral channels.

At successive stages of the auditory system, typically there is little loss of the information encoded at previous stages, but there are often transformations in the neural representations of stimulus properties that include sharpening and increased complexity of tuning to specific features. Frequency channels are maintained through the ascending levels of central auditory system, in the cochlear nucleus, olivary nucleus, and inferior colliculus (Escabí and Read, 2003). However, evidence from cats and bats suggests that there is a tendency for frequency tuning to sharpen at ascending stages of the auditory system (Katsuki et al., 1958; Katsuki et al., 1959; Suga, 1995). This sharpening is thought to be the result of lateral inhibition between channels and is hypothesized to provide greater contrast between the representations of similar stimulus properties (Katsuki et al., 1958; Katsuki et al., 1959; Suga, 1995).

A transformation is also evident in the encoding of temporal modulation at ascending levels of the auditory system. The auditory nerve and early stages of the central auditory system encode other stimulus properties as well, such as temporal modulations and intensity. These additional properties are encoded by the firing rates and timing of spikes in the active nerve fibers, rather than by the identities of the fibers. For example, the auditory nerve and early stages of the central auditory system encode temporal modulations by aligning spikes to a certain phase of the modulation of a stimulus. At progressive stages of the auditory system, neurons are more likely to exhibit selectivity for particular modulation rates through a rate code than through spike timing. These neurons that show tuning for modulation rates thus exhibit feature selectivity in two dimensions: both frequency and modulation rate. As mentioned in the "Perceptual channels and neural codes" section, some consider modulation rate to be encoded in the form of channels in the central auditory system. The broad tuning for modulation rates that neurons in the central auditory system exhibit could provide the neural basis for these proposed modulation channels.

At progressive stages of the auditory system, neurons also exhibit sensitivity to stimulus features that are derived from neural computations. These features emerge as a result of integration of the information coded across neurons at earlier stages of the auditory system, rather than through explicit coding at these early stages. Neurons in the inferior colliculus of mammals, for example, are sensitive to the direction of changes in frequency, a feature known as frequency modulation (Fuzessery, 1994; Fuzessery et al., 2006; Gordon and O'Neill, 1998; Nelson et al., 1966). This feature is represented through a typical population code at the level of the inferior

colliculus. While individual neurons at earlier stages of the auditory system do not exhibit selectivity for frequency-modulation, the information about this feature must be present at these stages in order for neurons in the inferior colliculus to respond to it. Evidence suggests that the selectivity for frequency modulation arises as a result of the precisely timed integration of inputs from neurons at earlier stages of the auditory system (Fuzessery et al., 2006). Thus, a representation of frequency-modulation in the stimulus is encoded diffusely across the population of neurons providing input to neurons in the inferior colliculus. This diffuse representation at stages of the auditory system prior to the inferior colliculus is similar to a distributed code in that no single group of neurons responds selectively to the feature of interest; instead, the feature can only be extracted by considering the outputs of a population of neurons with diverse responses.

The complexity of neural representations of sounds increases further at the level of the auditory cortex. Neurons in auditory cortex retain many features of earlier stages of the central auditory system, such as frequency selectivity and sensitivity to modulation rate. However, cortical neurons often respond best to complex sounds and show remarkable flexibility in their responses to stimuli (Schulte et al., 2002; Tian et al., 2001). In addition to input from earlier stages of the auditory system, auditory cortex also receives projections from other parts of the brain. As a consequence of the variety of input, many neurons in the auditory cortex respond to stimuli in other modalities (Fu et al., 2003; Lakatos et al., 2007; Schroeder and Foxe, 2002). In addition, the auditory cortex is typically associated with cognitive processes like attention and auditory scene analysis (Fishman et al., 2001; Fishman et al., 2004; Näätänen et al., 2001; Snyder and Alain, 2007b). Much of the neural coding in auditory cortex seems to rely on distributed representations. For example, there is evidence for the use of distributed codes in representations of spatial location, speech sounds, and repetition rates of sounds in the auditory cortex (Kilgard and Merzenich, 1999; Stecker and Middlebrooks, 2003; Wong and Schreiner, 2003).

Sensory hierarchies

There are some advantages to the nearly-hierarchical processing that occurs in the auditory system and other sensory systems. In the early stages of a sensory system, particularly in the periphery, the main function of the sensory system is to obtain all possible information about a stimulus. Once past the periphery, there is no way for more information about the outside world to enter the system, so everything the brain might want to know about the stimulus must be encoded in the periphery. Information must then be encoded in a way that will make it easily

accessible to multiple parts of the brain. The channels of the auditory system are an excellent strategy for this purpose, because they transmit information efficiently and accurately and organize information neatly by frequency and ear of input. Frequency and ear of input are physical properties of a sound, so their extraction can be "hard-wired" into the auditory system. As information passes through ascending stages of a sensory system, each part of the brain extracts whatever information it finds useful. At some levels of the sensory system, during processing and computing information may be discarded, but this same information could well be retained and used by other parts of the system. Computations often yield new, explicit representations of features, such as the emergence of frequency modulation in the auditory system.

At ascending levels of a sensory system, there tends to be a shift toward the representation of more complex and abstract stimuli. It is commonly argued that the cortex represents perceptual objects, corresponding to distinct stimuli in the environment. The channels that tend to occur at lower levels of sensory systems organize representations of stimulus features in such a way as to facilitate the grouping or integration of these features into perceptual objects (Alain and Arnott, 2000; Beck et al., 1987; Hartmann and Johnson, 1991; Le Meur et al., 2005). Concomitant with the increase in complexity of the stimuli represented at more central levels of a sensory system is a heavier reliance on distributed codes. Distributed codes afford cortical regions the ability to respond to complex stimuli without using tremendous numbers of neurons, as a one-to-one coding scheme would require. The use of distributed representations also underlies the ability of cortical regions to learn and modulate responses based on attention, context, or motivation (Kilgard et al., 2001; Nudo et al., 2001; Serences and Yantis, 2006).

Significance and future directions

Significance

The processing of sensory information by sensory systems has long been a topic of interest in the field of neuroscience. Information from sensory systems is incorporated in the brain to affect all aspects of behavior, from locomotion and foraging to decision making and mate choice. Thus understanding how sensory information is processed and stored in the brain will impact the understanding of many other aspects of an animal's biology. An advantage of studying sensory systems is that the stimulus can be explicitly controlled so that the input to the circuit is well-known. Having this same control is non-trivial when the input to a circuit is something abstract or

an internal state, as might be the case in studies of decision-making or psychiatric disorders. However, processing of sensory information is done by neurons, just like all other processing in the brain. Thus it is likely that some of the same processes and mechanisms that exist in sensory systems are present in other parts of the brain, and elucidating the general mechanisms by which sensory representations are encoded and transformed can improve our understanding of information processing throughout the brain, regardless of the function it subserves.

One reason that my research is important is because it exploited a relatively unique model system for the study of auditory neuroscience, the treefrog. Treefrogs are good models in which to study the auditory system because they communicate acoustically and are strongly dependent on their auditory systems for reproductive success. As frogs communicate mostly in noisy and multi-signaler environments, they are a particularly good model in which to address questions about signal detection and processing in the real-world. Additional attributes that make treefrogs good model systems are that they exhibit robust behavioral responses to biologically relevant sounds and are highly tractable to neurophysiological recordings.

As illustrated in chapters 1 and 2, my work furthered the development of a technique for investigating auditory sensitivity in anurans, the auditory brainstem response (ABR). This is an important first step that will facilitate the wider study of auditory sensitivity and processing and its relation to species-specific behaviors in anurans. A better understanding of these processes in anurans can also extend comparative work across taxa. Most of our knowledge about processing in the auditory system, and sensory systems in general, is based on work in a few species of mammalian animals. By limiting our research to a select few species, we run the risk of making unwarranted assumptions about what principals are general across sensory systems and never discovering other ways that nervous systems operate. An additional benefit of studying the systems of other animals is that we may discover novel mechanisms of neural processing, which could inspire improvements in prosthetic devices for the impaired.

Another reason the work in this dissertation is important is because it elucidates mechanisms associated with the processing of naturalistic stimuli. Although we have a good understanding of how simple stimulus are encoded by most sensory systems (e.g. a single point of light or a pure-tone of a single frequency), sensory input in natural settings is far more complex. While the importance of studying how complex and natural stimuli are encoded has been increasingly recognized, we still do not fully understand the mechanisms involved. This is

particularly true of senses like gustation and olfaction, but also in the auditory system, where research has seemed to lag slightly behind that of the visual system.

In chapter 3, I demonstrated a strong connection between processing in the early stages of the auditory systems of treefrogs and the temporal properties of natural sounds. This result contributes to a growing literature on processing of natural stimuli. Importantly, most studies of how natural stimuli are processed focus on cortical regions in mammals, but my finding provides evidence that selective processing occurs at subcortical stages, suggesting that special processing for natural stimuli emerges at an earlier stage of the auditory system than generally assumed.

In chapter 4, I show that treefrogs integrate and segregate sounds into "auditory objects" using frequency cues and level cues, and reject the traditional hypothesis (the "channeling hypothesis") that processing in the auditory peripheral channels is sufficient to achieve appropriate integration and segregation. Current views hold that the integration of sounds across frequency into an abstract auditory object occurs in the auditory cortex. Thus, the fact that frogs, which do not have a cortex, can integrate sounds across frequency suggests that these processes can in fact occur at subcortical levels, at least in frogs. The rejection of the channeling hypothesis suggests that there must be cross-channel processing at some sub-cortical stage of the auditory system. These data raise an interesting question about the extent to which subcortical processing contributes to auditory object formation in mammals. While it is possible that divergent evolutionary pressures have resulted in sensory systems that solve the problem of auditory object formation in different ways, it might be the case that subcortical influences play a greater role in mammals than is commonly appreciated. Furthermore, this study demonstrates the use of sensation level as a mechanism for segregation of frequency cues, which has previously been noted to contribute to discrimination between frequencies (Nelson et al., 1983; Wier et al., 1977), but not in the context of sound source segregation.

Future directions

The ability to record ABRs in anurans suggests a number of interesting future studies. For example, several studies, including the two detailed in chapters 1 and 2 here, have suggested a link between the spectral sensitivity of a species' auditory system and its communication calls (Gerhardt and Schwartz, 2001). However, frequency sensitivity has been studied in a relatively small number of species of anurans, compared to the 6000+ species that exist. It is well known that the morphology of the ear varies considerably across different ears, from "earless" frogs

which can hear, despite lacking a tympanic membrane (Boistel et al., 2013; Lindquist et al., 1998) to frogs that show sensitivity to ultrasonic frequencies (Feng and Narins, 2008; Feng et al., 2006; Shen et al., 2008). There is also a considerable diversity in the spectral properties of the communication signals used by different species (Penna and Veloso, 1990; Wells and Schwartz, 2006), and even within the repertoire of a single species (Christensen-Dalsgaard et al., 2002; Feng et al., 2002; Feng et al., 2009). Thus a more extensive analysis of auditory sensitivity in relation to the frequency spectrum of calls, particularly in regard to some of the species with less "typical" auditory systems or calls, would greatly inform our current understanding of how evolutionary forces have shaped communication systems.

The ability to repeatedly record the ABR from the same animal facilitates longitudinal studies as well. For example, there has long been an interest in seasonal and hormonal effects on the auditory system. There is some evidence that auditory thresholds are elevated outside the breeding season (Miranda and Wilczynski, 2009a; Miranda and Wilczynski, 2009b; Zhang et al., 2012), but this effect is not well studied, and the mechanism underlying the change is unknown. In addition, females show a waning interest in the calls of males during egg deposition, but it is again unknown whether this is entirely due to hormone-inducted changes in motivation or whether there are immediate changes in the auditory system that reduce the attractiveness of the males' calls. Because behavioral experiments require the corporation of an animal and chronic, invasive recordings are non-trivial, it has been difficult to properly study longitudinal effects. However, with repeated ABRs, one could take repeated measurements over the course of a season, or in conjunction with hormone treatments.

While ABRs are a useful advance that will hopefully increase in the breadth of neurophysiological research in frogs, other advances would further the productivity of neurophysiological research in these animals. One advantage that some animals, such as rats, monkeys, and even crickets, have over frogs is that there are established methods for recording from the brains of these animals while the animal is behaving. To date, all neurophysiological data from the auditory systems of anurans has been in immobilized animals. However, in a series of classic papers, Jörg-Peter Ewert and colleagues recorded from neurons in the toad's visual system during prey capture (Ewert and Borchers, 1974; Megela et al., 1983; Schürg-Pfeiffer et al., 1993), suggesting that recordings from the auditory system during communication-related behaviors could be feasible. The ability to record from awake, behaving frogs during phonotaxis

or evoked-calling behaviors would greatly increase our insight into the neural processes involved in auditory processing and perception, decision making, and directed behavior.

An additional limitation of using frogs is that the established behavioral paradigms are somewhat limiting in the types of stimuli that can be tested. The stimuli used must be inherently motivational to the subject in order to observe the behavior. Several attempts have been made to condition anurans, with varying degrees of success. For example, toads were taught to associate a human hand or an odor with a food stimulus, and would exhibit prey-catching behaviors (orienting, snapping) upon appearance of a hand (Ewert et al., 2001). Another attempt at conditioning was a reflex modification paradigm used to investigate auditory sensitivity (Megela-Simmons et al., 1985; Moss and Simmons, 1986; Simmons and Moss, 1995). However, none of the methods used have become widely-established. A behavior that could be trained and flexibly associated with a variety of stimuli and ideally incorporated with neurophysiological recordings would greatly expand our current ability to investigate sensory perception in anurans.

The results of the studies involving temporal processing suggest a number of future directions. While I showed that the auditory systems of two species of treefrogs were selective for particular temporal properties, it is an open question how this information is actually encoded in the early stages of the auditory system. Is the entire population of auditory nerve fibers better at encoding the relevant temporal feature or is the feature encoded by a small group of fibers that dominate the response? There was an interesting species-difference that was not clearly related to frequencies in the communication calls. The difference occurred only at intermediate frequencies, suggesting that it may be due to excitation of populations arising from both sensory papillae. This hypothesis could be investigated by obtaining similar recordings after cutting the nerve fibers arising from one papilla. Another approach would be to methodically characterize the auditory nerve populations and their individual responses in both sexes, and then see if a model based on these measures would predict the observed sex differences.

I found that my results did not explain previously-observed differences between the two treefrog species in exploitation of temporal fluctuations in noise. One point worth noting, however, is that I did not test the full range of temporal modulations that are present in natural chorus noise. Further work could test these additional modulation rates. My results also do not preclude the possibility that through additional processing at ascending levels of the auditory system, a difference in responses might emerge that reflects the difference seen in behavior. This possibility should also be tested. Current work, however, is focused on the mechanism that

underlies that ability to exploit temporal fluctuations in noise. By manipulation the timing of communication signals relative to the temporal fluctuations in noise, we are testing the hypothesis that frogs use a dip listening mechanism, rather than stochastic resonance, to exploit temporal fluctuations. Additionally, we are assessing whether this information is represented through a rate or temporal code in the auditory midbrain, which could provide an explanation for the difference in the abilities of the two species to exploit temporal fluctuations.

The results of the study on the integration and segregation of sounds in chapter 4 strongly suggest that frogs integrate sounds that are processed in the periphery across distinct frequency channels. In order for integration of these sounds to occur, there must be either an interaction between the channels or integration of the sounds at some high stage of processing. The large ABRs recorded in response to tones of intermediate frequencies suggest that integration occurs at early stages of the auditory system, possibly at the level of the auditory nerve. By recording from neurons in response to stimuli similar to those used in the behavioral experiments, it should be possible to determine at what stage of the auditory system these interactions occur.

Of course, the situation presented to subjects in my study was necessarily artificial, and it is always important to consider how applicable the results are to more natural conditions. To this end, it would be of interest to run similar experiments with stimuli that included both of the spectral peaks normally present in a call. Another relevant manipulation would be to broadcast additional pulses, either in the form of additional calls or as "distractors" and determine whether segregation is still possible. This study was also limited in that it focused only on sequential integration and segregation. Integration and segregation of simultaneous sounds based on properties such as harmonicity and common onset are also important mechanisms used in sound source segregation. Given that the two spectral peaks in the calls of gray treefrogs are harmonically related, it would be straightforward to test how manipulation of the onset of the spectral peaks affects their integration. Results from a similar study in a different species of treefrog suggest that frogs are quite sensitive to small variations in timing of call components.

Some interesting results came out of the control experiments as well. In one experiment, whether the subjects would respond to a call depended on whether an unattractive frequency occurred at the beginning or end of a call. This result raises the possibility that the frogs responded based on some kind of precedence effect. Further investigation would be necessary to determine if frogs use this sort of context-based decision making. Furthermore, it would be interesting to determine the mechanism underlying the effect. One strong contender is that the

first sounds heard could initiate forward masking of the later tones, so that the neural representation of the earlier tones was stronger than that of the later tones.

A second interesting finding that arose from the control experiments was that the strength of the response to a given stimulus could be change depending on the identity of the alternative stimulus, suggesting that at least some stimuli did not induce obligatory integration or segregation. Stimuli that can be either integrated or segregated lie at the boundary between stimuli in which integration or segregation is mandatory. Future studies could map these boundaries and compare them to results in other animals to determine how general these boundaries are across auditory systems.

Bibliography

Abbott, L. and Sejnowski, T. J. (1999). *Neural Codes and Distributed Representations: Foundations of Neural Computation*. MIT Press.

Achor, L. J. and Starr, A. (1980). Auditory brain stem responses in the cat. I. Intracranial and extracranial recordings. *Electroencephalography and Clinical Neurophysiology* **48**, 154–173.

Aitkin, L., Nelson, J. and Shepherd, R. (1996). Development of hearing and vocalization in a marsupial, the Northern Quoll, Dasyurus hallucatus. *Journal of Experimental Zoology* **276**, 394–402.

Alain, C. and Arnott, S. R. (2000). Selectively attending to auditory objects. *Frontiers in Bioscience* **5**, d202–212.

Alder, T. B. and Rose, G. J. (1998). Long-term temporal integration in the anuran auditory system. *Nat Neurosci* **1**, 519–523.

Allison, J. D. and Wilczynski, W. (1991). Thalamic and Midbrain Auditory Projections to the Preoptic Area and Ventral Hypothalamus in the Green Treefrog (*Hyla Cinerea*). *Brain, Behavior and Evolution* **38**, 322–331.

Almli, L. M. and Wilczynski, W. (2009). Sex-Specific Modulation of Cell Proliferation by Socially Relevant Stimuli in the Adult Green Treefrog Brain (*Hyla cinerea*). *Brain, Behavior and Evolution* **74**, 143–154.

Amoser, S. and Ladich, F. (2005). Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? *J Exp Biol* **208**, 3533–3542.

Andersen, R. A. (1997). Multimodal integration for the representation of space in the posterior parietal cortex. *Phil. Trans. R. Soc. Lond. B* **352**, 1421–1428.

Assmann, P. F. (1999). FUNDAMENTAL FREQUENCY AND THE INTELLIGIBILITY OF COMPETING VOICES. In *Proceedings of the 14th International Congress of Phonetic Sciences*, pp. 179–182. CiteSeerX.

Assmann, P. F. and Summerfield, Q. (1994). The contribution of waveform interactions to the perception of concurrent vowels. *The Journal of the Acoustical Society of America* **95**, 471–484.

Atick, J. J. (1992). Could information theory provide an ecological theory of sensory processing? *Network: Computation in neural systems* **3**, 213–251.

Bacon, S. P., Opie, J. M. and Montoya, D. Y. (1998). The effects of hearing loss and noise masking on the masking release for speech in temporally complex backgrounds. *J. Speech Lang. Hear. Res.* **41**, 549–563.

Barlow, H. B. (1961). Possible principles underlying the transformation of sensory messages. In *Sensory communication* (ed. Rosenblith, W. A.), pp. 217–234. Cambridge, MA: MIT Press.

Bartol, S. M., Musick, J. A. and Lenhardt, M. L. (1999). Auditory Evoked Potentials of the Loggerhead Sea Turtle (Caretta caretta). *Copeia* **1999**, 836–840.

Bates, D., Maechler, M., Bolker, B. and Walker, Steven (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-5.

Beck, J., Sutter, A. and Ivry, R. (1987). Spatial frequency channels and perceptual grouping in texture segregation. *Computer Vision, Graphics, and Image Processing* **37**, 299–325.

Bee, M. A. (2008). Parallel female preferences for call duration in a diploid ancestor of an allotetraploid treefrog. *Animal Behaviour* **76**, 845–853.

Bee, M. A. (2010). Spectral preferences and the role of spatial coherence in simultaneous integration in gray treefrogs (Hyla chrysoscelis). *Journal of Comparative Psychology* **124**, 412–424.

Bee, M. A. (2012). Sound source perception in anuran amphibians. *Current Opinion in Neurobiology* **22**, 301–310.

Bee, M. A. (2014). Treefrogs as animal models for research on auditory scene analysis and the cocktail party problem. *International Journal of Psychophysiology*.

Bee, M. A. and Klump, G. M. (2004). Primitive Auditory Stream Segregation: A Neurophysiological Study in the Songbird Forebrain. *Journal of Neurophysiology* **92**, 1088–1104.

Bee, M. A. and Klump, G. M. (2005). Auditory Stream Segregation in the Songbird Forebrain: Effects of Time Intervals on Responses to Interleaved Tone Sequences. *Brain, Behavior and Evolution* **66**, 197.

Bee, M. A. and Micheyl, C. (2008). The cocktail party problem: What is it? How can it be solved? And why should animal behaviorists study it? *Journal of Comparative Psychology* **122**, 235–251.

Bee, M. A. and Riemersma, K. K. (2008). Does common spatial origin promote the auditory grouping of temporally separated signal elements in grey treefrogs? *Animal Behaviour* **76**, 831–843.

Bee, M. A., Micheyl, C., Oxenham, A. J. and Klump, G. M. (2010). Neural adaptation to tone sequences in the songbird forebrain: patterns, determinants, and relation to the build-up of auditory streaming. *J Comp Physiol A* **196**, 543–557.

Bee, M. A., Vélez, A. and Forester, J. D. (2012). Sound level discrimination by gray treefrogs in the presence and absence of chorus-shaped noise. *The Journal of the Acoustical Society of America* **131**, 4188–4195.

Békésy, G. V. (1960). *Experiments in hearing*. New York: McGraw-Hill.

Bhandawat, V., Olsen, S. R., Gouwens, N. W., Schlief, M. L. and Wilson, R. I. (2007). Sensory processing in the Drosophila antennal lobe increases reliability and separability of ensemble odor representations. *Nat Neurosci* **10**, 1474–1482.

Bibikov, N. G. (2002). Addition of noise enhances neural synchrony to amplitude-modulated sounds in the frog's midbrain. *Hearing Research* **173**, 21–28.

Bibikov, N. G. and Elepfandt, A. (2005). Auditory evoked potentials from medulla and midbrain in the clawed frog, *Xenopus laevis laevis*. *Hearing Research* **204**, 29–36.

Bird, J. and Darwin, C. (1998). Effects of a difference in fundamental frequency in separating two sentences. In *Psychophysical and physiological advances in hearing* (ed. Palmer, A. R., Rees, A., Summersfield, Q., and Meddis, R.), pp. 263–269. London: Whurr Publishers.

Blakemore, C., Nachmias, J. and Sutton, P. (1970). The perceived spatial frequency shift: evidence for frequency-selective neurones in the human brain. *J Physiol* **210**, 727–750.

Boettcher, F. A., Mills, J. H., Norton, B. L. and Schmiedt, R. A. (1993). Age-related changes in auditory evoked potentials of gerbils. II. Response latencies. *Hearing Research* **71**, 146–156.

Boistel, R., Aubin, T., Cloetens, P., Peyrin, F., Scotti, T., Herzog, P., Gerlach, J., Pollet, N. and Aubry, J.-F. (2013). How minute sooglossid frogs hear without a middle ear. *PNAS* **110**, 15360–15364.

Bolanowski Jr, S. J., Gescheider, G. A., Verrillo, R. T. and Checkosky, C. M. (1988). Four channels mediate the mechanical aspects of touch. *The Journal of the Acoustical Society of America* **84**, 1680–1694.

Boothe, R. G. (2002). *Perception of the Visual Environment*. Springer Science & Business Media.

Bowmaker, J. K. (1983). Trichromatic colour vision: why only three receptor channels? *Trends in Neurosciences* **6**, 41–43.

Braddick, O., Campbell, F. W. and Atkinson, J. (1978). Channels in Vision: Basic Aspects. In *Perception* (ed. Held, R., Leibowitz, H. W., and Teuber, H.-L.), pp. 3–38. Springer Berlin Heidelberg.

Bregman, A. S. (1990). *Auditory Scene Analysis: The Perceptual Organization of Sound*. Cambridge, MA: MIT Press.

Bregman, A. S. and Campbell, J. (1971). Primary auditory stream segregation and perception of order in rapid sequences of tones. *Journal of Experimental Psychology* **89**, 244–249.

Brenowitz, E. A. and Rose, G. J. (1999). Female choice and plasticity of male calling behaviour in the Pacific treefrog. *Animal Behaviour* **57**, 1337–1342.

Brittan-Powell, E. F. and Dooling, R. J. (2004). Development of auditory sensitivity in budgerigars (*Melopsittacus undulatus*). *The Journal of the Acoustical Society of America* **115**, 3092–3102.

Brittan-Powell, E. F., Dooling, R. J. and Gleich, O. (2002). Auditory brainstem responses in adult budgerigars (*Melopsittacus undulatus*). *J. Acoust. Soc. Am.* **112**, 999–1008.

Brittan-Powell, E. F., Lohr, B., Hahn, D. C. and Dooling, R. J. (2005). Auditory brainstem responses in the Eastern Screech Owl: An estimate of auditory thresholds. *J. Acoust. Soc. Am.* **118**, 314–321.

Brittan-Powell, E. F., Christensen-Dalsgaard, J., Tang, Y., Carr, C. and Dooling, R. J. (2010a). The auditory brainstem response in two lizard species. *J. Acoust. Soc. Am.* **128**, 787–794.

Brittan-Powell, E. F., Dooling, R. J., Ryals, B. and Gleich, O. (2010b). Electrophysiological and morphological development of the inner ear in Belgian Waterslager canaries. *Hearing Research* **269**, 56–69.

Brokx, J. and Nooteboom, S. (1982). Intonation and the perceptual separation of simultaneous voices. *Journal of Phonetics* **10**, 23–36.

Brown, P. K. and Wald, G. (1964). Visual Pigments in Single Rods and Cones of the Human Retina. *Science* **144**, 45–52.

Brumm, H. ed. (2013). *Animal Communication and Noise*. New York: Springer.

Brumm, H. and Slabbekoorn, H. (2005). Acoustic Communication in Noise. In *Advances in the Study of Behavior* (ed. Peter J. B. Slater, C. T. S.), pp. 151–209. Academic Press.

Buchwald, J. S. and Huang, C. (1975). Far-field acoustic response: origins in the cat. *Science* **189**, 382–384.

Budelmann, B. U. (1995). The cephalopod nervous system: What evolution has made of the molluscan design. In *The Nervous Systems of Invertebrates: An Evolutionary and Comparative Approach* (ed. Breidbach, P. D. D. O. and Kutsch, P. D. W.), pp. 115–138. Birkhäuser Basel.

Budelmann, B. U. and Young, J. Z. (1985). Central Pathways of the Nerves of the Arms and Mantle of Octopus. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **310**, 109–122.

Buerkle, N. P., Schrode, K. M. and Bee, M. A. (2014). Assessing stimulus and subject influences on auditory evoked potentials and their relation to peripheral physiology in green treefrogs (*Hyla cinerea*). *Comparative Biochemistry and Physiology*.

Burkard, R. and Deegan, D. (1984). Brainstem evoked responses to paired-click stimuli: the use of digital response subtraction. *International Journal of Audiology* **23**, 85–98.

Burkard, R. and Voigt, H. F. (1989). Stimulus dependencies of the gerbil brain-stem auditory-evoked response (BAER). I: Effects of click level, rate, and polarity. *The Journal of the Acoustical Society of America* **85**, 2514–2525.

Burmeister, S. S. and Wilczynski, W. (2001). Social Context Influences Androgenic Effects on Calling in the Green Treefrog (*Hyla cinerea*). *Hormones and Behavior* **40**, 550–558.

Burmeister, S. S. and Wilczynski, W. (2005). Social Signals Regulate Gonadotropin-Releasing Hormone Neurons in the Green Treefrog. *Brain, Behavior and Evolution* **65**, 26–32.

Burmeister, S., Somes, C. and Wilczynski, W. (2001). Behavioral and Hormonal Effects of Exogenous Vasotocin and Corticosterone in the Green Treefrog. *General and Comparative Endocrinology* **122**, 189–197.

Burrell, B. D., Sahley, C. L. and Muller, K. J. (2001). Non-Associative Learning and Serotonin Induce Similar Bi-Directional Changes in Excitability of a Neuron Critical for Learning in the Medicinal Leech. *J. Neurosci.* **21**, 1401–1412.

Caldwell, M. S., Lee, N., Schrode, K. M., Johns, A. R., Christensen-Dalsgaard, J. and Bee, M. A. (2014). Spatial hearing in Cope's gray treefrog: II. Frequency-dependent directionality in the amplitude and phase of tympanum vibrations. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **200**, 285–304.

Campbell, F. W. and Kulikowski, J. J. (1966). Orientational selectivity of the human visual system. *J Physiol* **187**, 437–445.

Capranica, R. R. (1976). Morphology and physiology of the auditory system. In *Frog Neurobiology: A handbook* (ed. Llinas, R. and Precht, W.), pp. 551–575. New York: Springer.

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

Capranica, R. R. and Moffat, A. J. M. (1983). Neurobehavioral correlates of sound communication in anurans. In *Advances in Vertebrate Neuroethology* (ed. Ewert, J. P., Capranica, R. R., and Ingle, D. J.), pp. 701–730. New York: Plenum Press.

Capraro, A. J., Verrillo, R. T. and Zwislocki, J. J. (1979). Psychophysical evidence for a triplex system of cutaneous mechanoreception. *Sensory Processes* **3**, 334–352.

Caras, M. L., Brenowitz, E. and Rubel, E. W. (2010). Peripheral auditory processing changes seasonally in Gambel's white-crowned sparrow. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **196**, 581–599.

Carey, M. B. and Zelick, R. (1993). The effect of sound level, temperature and dehydration on the brainstem auditory evoked potential in anuran amphibians. *Hearing Research* **70**, 216–228.

Carlyon, R. P. (2004). How the brain separates sounds. *TICS* **8**, 465.

Carney, L. H. (1993). A model for the responses of low-frequency auditory-nerve fibers in cat. *The Journal of the Acoustical Society of America* **93**, 401–417.

Castellano, S. and Rosso, A. (2006). Variation in call temporal properties and female preferences in *Hyla intermedia*. *Behaviour* **143**, 405–424.

Chen, Q., Cui, J., Fang, G., Brauth, S. E. and Tang, Y. (2011). Acoustic Analysis of the Advertisement Calls of the Music Frog, *Babina daunchina*. *Journal of Herpetology* **45**, 406–416.

Cherry, E. C. (1953). Some Experiments on the Recognition of Speech, with One and with Two Ears. *The Journal of the Acoustical Society of America* **25**, 975–979.

Chistovich, I. A., Granstrem, M. P., Kozhevnikov, V. A., Lesogor, L. W., Shupljakov, V. S., Taljasin, P. A. and Tjulkov, W. A. (1974). A Functional Model of Signal Processing in the Peripheral Auditory System. *Acta Acustica united with Acustica* **31**, 349–353.

Chiu, C., Xian, W. and Moss, C. F. (2009). Adaptive echolocation behavior in bats for the analysis of auditory scenes. *J Exp Biol* **212**, 1392–1404.

Christensen-Dalsgaard, J. (2005). Directional Hearing in Nonmammalian Tetrapods. In *Sound Source Localization* (ed. Popper, A. N. and Fay, R. R.), pp. 67–123. Springer New York.

Christensen-Dalsgaard, J. (2011). Vertebrate pressure-gradient receivers. *Hearing Research* **273**, 37–45.

Christensen-Dalsgaard, J. and Carr, C. E. (2008). Evolution of a sensory novelty: Tympanic ears and the associated neural processing. *Brain Research Bulletin* **75**, 365–370.

Christensen-Dalsgaard, J. and Jorgensen, M. B. (1996). One-tone suppression in the frog auditory nerve. *The Journal of the Acoustical Society of America* **100**, 451–457.

Christensen-Dalsgaard, J. and Walkowiak, W. (1999). In Vitro and In Vivo Responses of Saccular and Caudal Nucleus Neurons in the Grassfrog (*Rana temporaria*). *European Journal of Morphology* **37**, 145–149.

Christensen-Dalsgaard, J., Breithaupt, T. and Elepfandt, A. (1990). Underwater hearing in the clawed frog,Xenopus laevis. *Naturwissenschaften* **77**, 135–137.

Christensen-Dalsgaard, J., Jørgensen, M. B. and Kanneworff, M. (1998). Basic response characteristics of auditory nerve fibers in the grassfrog (Rana temporaria). *Hearing Research* **119**, 155–163.

Christensen-Dalsgaard, J., Ludwig, T. A. and Narins, P. M. (2002). Call Diversity in an Old World Treefrog: Level Dependence and Latency of Acoustic Responses. *Bioacoustics* **13**, 21–35.

Church, M. ., Willaims, H. . and Holloway, J. . (1984). Brain-stem auditory evoked potentials in the rat: Effects of gender, stimulus characteristics and ethanol sedation. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section* **59**, 328–339.

Coats, A. (1978). Human auditory nerve action potentials and brain stem evoked responses: Latency-intensity functions in detection of cochlear and retrocochlear abnormality. *Arch Otolaryngol* **104**, 709–717.

Condon, C. J., Chang, S.-H. and Feng, A. S. (1991). Processing of behaviorally relevant temporal parameters of acoustic stimuli by single neurons in the superior olivary nucleus of the leopard frog. *J Comp Physiol A* **168**, 709–725.

Cone-Wesson, B. K., Hill, K. G. and Liu, G.-B. (1997). Auditory brainstem response in tammar wallaby (*Macropus eugenii*). *Hearing Research* **105**, 119–129.

Cone-Wesson, B., Dowel, R. C., Tomlin, D., Rance, G. and Wu Jia Ming (2002). The auditory steady-state response: Comparisons with the auditory brainstem response. *Journal of the American Academy of Audiology* **13**, 173.

Cooke, M. (2006). A glimpsing model of speech perception in noise. *J. Acoust. Soc. Am.* **119**, 1562–1573.

Corwin, J. T., Bullock, T. H. and Schweitzer, J. (1982). The auditory brain stem response in five vertebrate classes. *Electroencephalography and Clinical Neurophysiology* **54**, 629–641.

Cover, T. M. and Thomas, J. A. (2012). *Elements of Information Theory*. John Wiley & Sons.

Cusack, R. and Roberts, B. (2000). Effects of differences in timbre on sequential grouping. *Perception & Psychophysics* **62**, 1112–1120.

D'angelo, G. J., De Chicchis, A. R., Osborn, D. A., Gallagher, G. R., Warren, R. J. and Miller, K. V. (2007). Hearing Range of White-Tailed Deer as Determined by Auditory Brainstem Response. *The Journal of Wildlife Management* **71**, 1238–1242.

Dau, T. (1999). *Modeling auditory processing of amplitude modulation*. BIS Verlag.

Dayan, P. and Abbott, L. F. (2005). *Theoretical Neuroscience: Computational and Mathematical Modeling of Neural Systems*. MIT Press.

Delaney, H. D. and Maxwell, S. E. (1981). On Using Analysis Of Covariance In Repeated Measures Designs. *Multivariate Behavioral Research* **16**, 105–123.

De Valois, R. L., Albrecht, D. G. and Thorell, L. G. (1982). Spatial frequency selectivity of cells in macaque visual cortex. *Vision Research* **22**, 545–559.

Diekamp, B. and Gerhardt, H. C. (1995). Selective phonotaxis to advertisement calls in the gray treefrog *Hyla versicolor*: behavioral experiments and neurophysiological correlates. *J Comp Physiol A* **177**, 173–190.

Dobie, R. A. and Wilson, M. J. (1996). A comparison of t test, F test, and coherence methods of detecting steady-state auditory-evoked potentials, distortion-product otoacoustic emissions, or other sinusoids. *The Journal of the Acoustical Society of America* **100**, 2236–2246.

Dolphin, W. F. and Mountain, D. C. (1992). The envelope following response: Scalp potentials elicited in the mongolian gerbil using sinusoidally AM acoustic signals. *Hearing Research* **58**, 70–78.

Dolphin, W. F., Au, W. W. L., Nachtigall, P. E. and Pawloski, J. (1995). Modulation rate transfer functions to low-frequency carriers in three species of cetaceans. *J Comp Physiol A* **177**, 235–245.

Dunia, R. and Narins, P. M. (1989). Temporal resolution in frog auditory-nerve fibers. *The Journal of the Acoustical Society of America* **85**, 1630–1638.

Edwards, C. J. and Kelley, D. B. (2001). Auditory and Lateral Line Inputs to the Midbrain of an Aquatic Anuran; Neuroanatomic Studies in Xenopus laevis. *J Comp Neurol* **438**, 148–162.

Edwards, C. J., Alder, T. B. and Rose, G. J. (2002). Auditory midbrain neurons that count. *Nat Neurosci* **5**, 934–936.

Eggermont, J. J. (1990). Temporal modulation transfer functions for single neurons in the auditory midbrain of the leopard frog. Intensity and carrier-frequency dependence. *Hearing Research* **43**, 181–198.

Ehret, G. and Capranica, R. R. (1980). Masking patterns and filter characteristics of auditory nerve fibers in the green treefrog (*Hyla cinerea*). *J. Comp. Physiol.* **141**, 1–12.

Ehret, G. and Gerhardt, H. C. (1980). Auditory masking and effects of noise on responses of the green treefrog (*Hyla cinerea*) to synthetic mating calls. *J. Comp. Physiol.* **141**, 13–18.

Ehret, G., Moffat, A. J. M. and Capranica, R. R. (1983). Two-tone suppression in auditory nerve fibers of the green treefrog (*Hyla cinerea*). *The Journal of the Acoustical Society of America* **73**, 2093–2095.

Elepfandt, A., Eistetter, I., Fleig, A., Gunther, E., Hainich, M., Hepperle, S. and Traub, B. (2000). Hearing threshold and frequency discrimination in the purely aquatic frog *Xenopus laevis* (Pipidae): measurement by means of conditioning. *J Exp Biol* **203**, 3621–3629.

Elliott, T. M., Christensen-Dalsgaard, J. and Kelley, D. B. (2007). Tone and call responses of units in the auditory nerve and dorsal medullary nucleus of *Xenopus laevis*. *J Comp Physiol A* **193**, 1243–1257.

Elliott, T. M., Christensen-Dalsgaard, J. and Kelley, D. B. (2011). Temporally selective processing of communication signals by auditory midbrain neurons. *J Neurophysiol* **105**, 1620–1632.

Escabí, M. A. and Read, H. L. (2003). Representation of spectrotemporal sound information in the ascending auditory pathway. *Biol. Cybern.* **89**, 350–362.

Ewert, J.-P. and Borchers, H.-W. (1974). Antwort von retinalen Ganglienzellen bei freibeweglichen Kröten (Bufo bufo L.). *J. Comp. Physiol.* **92**, 117–130.

Ewert, J.-P., Buxbaum-Conradi, H., Dreisvogt, F., Glagow, M., Merkel-Harff, C., Röttgen, A., Schürg-Pfeiffer, E. and Schwippert, W. W. (2001). Neural modulation of visuomotor functions underlying prey-catching behaviour in anurans: perception, attention, motor performance, learning. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **128**, 417–460.

Farris, H. E. and Ryan, M. J. (2011). Relative comparisons of call parameters enable auditory grouping in frogs. *Nat Commun* **2**, 410.

Farris, H. E., Rand, A. S. and Ryan, M. J. (2002). The Effects of Spatially Separated Call Components on Phonotaxis in Tungara Frogs: Evidence for Auditory Grouping. *Brain Behav Evol* **60**, 181–188.

Farris, H. E., Rand, A. S. and Ryan, M. J. (2005). The effects of time, space and spectrum on auditory grouping in túngara frogs. *J Comp Physiol A* **191**, 1173–1183.

Fay, R. R. and Popper, A. N. (2000). Evolution of hearing in vertebrates: the inner ears and processing. *Hearing Research* **149**, 1–10.

Fay, R. R. and Simmons, A. M. (1999). The Sense of Hearing in Fishes and Amphibians. In *Comparative Hearing: Fish and Amphibians* (ed. Fay, R. R. and Popper, A. N.), pp. 269–318. Springer New York.

Feng, A. S. (1982). Quantitative analysis of intensity-rate and intensity-latency functions in peripheral auditory nerve fibers of northern leopard frogs (*Rana p. pipiens*). *Hearing Research* **6**, 241–246.

Feng, A. S. and Capranica, R. R. (1976). Sound localization in anurans. I. Evidence of binaural interaction in dorsal medullary nucleus of bullfrogs (Rana catesbeiana). *Journal of Neurophysiology* **39**, 871–881.

Feng, A. S. and Capranica, R. R. (1978). Sound localization in anurans. II. Binaural interaction in superior olivary nucleus of the green tree frog (*Hyla cinerea*). *J Neurophysiol* **41**, 43–54.

Feng, A. S. and Narins, P. M. (2008). Ultrasonic communication in concave-eared torrent frogs (Amolops tormotus). *J Comp Physiol A* **194**, 159–167.

Feng, A. S., Narins, P. M. and Capranica, R. R. (1975). Three populations of primary auditory fibers in the bullfrog (*Rana catesbeiana*): Their peripheral origins and frequency sensitivities. *J Comp Physiol A* **100**, 221–229.

Feng, A. S., Gerhardt, H. C. and Capranica, R. R. (1976). Sound localization behavior of the green treefrog (*Hyla cinerea*) and the barking treefrog (*H. gratiosa*). *J. Comp. Physiol.* **107**, 241–252.

Feng, A. S., Hall, J. C. and Siddique, S. (1991). Coding of temporal parameters of complex sounds by frog auditory nerve fibers. *J Neurophysiol* **65**, 424–445.

Feng, A. S., Lin, W.-Y. and Sun, L. (1994). Detection of gaps in sinusoids by frog auditory nerve fibers: importance in AM coding. *J Comp Physiol A* **175**, 531–546.

Feng, A. S., Narins, P. M. and Xu, C.-H. (2002). Vocal acrobatics in a Chinese frog, Amolops tormotus. *Naturwissenschaften* **89**, 352–356.

Feng, A. S., Narins, P. M., Xu, C.-H., Lin, W.-Y., Yu, Z.-L., Qiu, Q., Xu, Z.-M. and Shen, J.-X. (2006). Ultrasonic communication in frogs. *Nature* **440**, 333–336.

Feng, A. S., Riede, T., Arch, V. S., Yu, Z., Xu, Z.-M., Yu, X.-J. and Shen, J.-X. (2009). Diversity of the Vocal Signals of Concave-Eared Torrent Frogs (Odorrana tormota): Evidence for Individual Signatures. *Ethology* **115**, 1015–1028.

Festen, J. M. (1993). Contributions of comodulation masking release and temporal resolution to the speech-reception threshold masked by an interfering voice. *The Journal of the Acoustical Society of America* **94**, 1295–1300.

Fishman, Y. I., Reser, D. H., Arezzo, J. C. and Steinschneider, M. (2001). Neural correlates of auditory stream segregation in primary auditory cortex of the awake monkey. *Hearing Research* **151**, 167–187.

Fishman, Y. I., Arezzo, J. C. and Steinschneider, M. (2004). Auditory stream segregation in monkey auditory cortex: effects of frequency separation, presentation rate, and tone duration. *J. Acoust. Soc. Am.* **116**, 1656–1670.

Fletcher, H. (1938a). Loudness, Masking and Their Relation to the Hearing Process and the Problem of Noise Measurement. *The Journal of the Acoustical Society of America* **9**, 275–293.

Fletcher, H. (1938b). The Mechanism of Hearing as Revealed Through Experiment on the Masking Effect of Thermal Noise. *Proc Natl Acad Sci U S A* **24**, 265–274.

Fletcher, H. (1940). Auditory Patterns. *Rev. Mod. Phys.* **12**, 47–65.

Frishkopf, L. S. and Goldstein Jr., M. H. (1963). Responses to Acoustic Stimuli from Single Units in the Eighth Nerve of the Bullfrog. *The Journal of the Acoustical Society of America* **35**, 1219–1228.

Frishkopf, L. S., Capranica, R. R. and Goldstein, M.H., J. (1968). Neural coding in the bullfrog's auditory system a teleological approach. *Proceedings of the IEEE* **56**, 969 – 980.

Fu, K.-M. G., Johnston, T. A., Shah, A. S., Arnold, L., Smiley, J., Hackett, T. A., Garraghty, P. E. and Schroeder, C. E. (2003). Auditory Cortical Neurons Respond to Somatosensory Stimulation. *J. Neurosci.* **23**, 7510–7515.

Füllgrabe, C., Berthommier, F. and Lorenzi, C. (2006). Masking release for consonant features in temporally fluctuating background noise. *Hearing Research* **211**, 74–84.

Fuzessery, Z. M. (1994). Response selectivity for multiple dimensions of frequency sweeps in the pallid bat inferior colliculus. *Journal of Neurophysiology* **72**, 1061–1079.

Fuzessery, Z. M. and Feng, A. S. (1981). Frequency representation in the dorsal medullary nucleus of the leopard frog, *Rana p. pipiens*. *J Comp Physiol A* **143**, 339–347.

Fuzessery, Z. M. and Feng, A. S. (1982). Frequency selectivity in the anuran auditory midbrain: Single unit responses to single and multiple tone stimulation. *J. Comp. Physiol.* **146**, 471–484.

Fuzessery, Z. M. and Feng, A. S. (1983a). Mating call selectivity in the thalamus and midbrain of the leopard frog (*Rana p. pipiens*): Single and multiunit analyses. *J. Comp. Physiol.* **150**, 333–344.

Fuzessery, Z. M. and Feng, A. S. (1983b). Frequency selectivity in the anuran medulla: Excitatory and inhibitory tuning properties of single neurons in the dorsal medullary and superior olivary nuclei. *J. Comp. Physiol.* **150**, 107–119.

Fuzessery, Z. M., Richardson, M. D. and Coburn, M. S. (2006). Neural Mechanisms Underlying Selectivity for the Rate and Direction of Frequency-Modulated Sweeps in the Inferior Colliculus of the Pallid Bat. *Journal of Neurophysiology* **96**, 1320–1336.

Gabbiani, F., Krapp, H. G., Koch, C. and Laurent, G. (2002). Multiplicative computation in a visual neuron sensitive to looming. *Nature* **420**, 320–324.

Gall, M. D., Brierley, L. E. and Lucas, J. R. (2011). Species and sex effects on auditory processing in brown-headed cowbirds and red-winged blackbirds. *Animal Behaviour* **81**, 973–982.

Gall, M. D., Henry, K. S. and Lucas, J. R. (2012a). Two measures of temporal resolution in brown-headed cowbirds (*Molothrus ater*). *J Comp Physiol A* **198**, 61–68.

Gall, M. D., Brierley, L. E. and Lucas, J. R. (2012b). The sender-receiver matching hypothesis: support from the peripheral coding of acoustic features in songbirds. *J Exp Biol*.

Gall, M. D., Salameh, T. S. and Lucas, J. R. (2013). Songbird frequency selectivity and temporal resolution vary with sex and season. *Proc. R. Soc. B* **280**, 20122296.

Geisler, C. D., van Bergeijk, W. A. and Frishkopf, L. S. (1964). The inner ear of the bullfrog. *Journal of Morphology* **114**, 43–57.

Georgopoulos, A. P., Schwartz, A. B. and Kettner, R. E. (1986). Neuronal population coding of movement direction. *Science* **233**, 1416–1419.

Gerhardt, H. C. (1974a). Behavioral isolation of the tree frogs, *Hyla cinerea* and *Hyla andersonii*. *American Midland Naturalist* **91**, 424–433.

Gerhardt, H. C. (1974b). Mating call differences between eastern and western populations of the treefrog *Hyla chrysoscelis*. *Copeia* **1974**, 534–536.

Gerhardt, H. C. (1974c). The Significance of Some Spectral Features in Mating Call Recognition in the Green Treefrog (*Hyla Cinerea*). *J Exp Biol* **61**, 229–241.

Gerhardt, H. C. (1975). Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. *J. Comp. Physiol.* **102**, 1–12.

Gerhardt, H. C. (1978a). Discrimination of intermediate sounds in a synthetic call continuum by female green tree frogs. *Science* **199**, 1089–1091.

Gerhardt, H. C. (1978b). Mating call recognition in the green treefrog (*Hyla Cinerea*): The significance of some fine-temporal properties. *J Exp Biol* **74**, 59–73.

Gerhardt, H. C. (1981). Mating call recognition in the green treefrog (*Hyla cinerea*): Importance of two frequency bands as a function of sound pressure level. *J. Comp. Physiol.* **144**, 9–16.

Gerhardt, H. C. (1982). Sound pattern recognition in some North American treefrogs (Anura: Hylidae): Implications for mate choice. *Amer. Zool.* **22**, 581–595.

Gerhardt, H. C. (1987). Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. *Animal Behaviour* **35**, 1479–1489.

Gerhardt, H. C. (1995). Phonotaxis in females frogs and toads: execution and design of experiments. In *Methods in comparative psychoacoustics* (ed. Klump, G. M., Dooling, R., Fay, R. R., and Stebbins, W.), pp. 209–220. Birkhäuser Verlag.

Gerhardt, H. C. (2001a). Acoustic communication in two groups of closely related treefrogs.pp. 99–167. Academic Press.

Gerhardt, H. C. (2001b). Acoustic communication in two groups of closely related treefrogs. In *Advances in the Study of Behavior* (ed. Peter J. B. Slater, J. S. R., Charles T. Snowdon and Timothy J. Roper), pp. 99–167. Academic Press.

Gerhardt, H. C. (2005). Acoustic spectral preferences in two cryptic species of grey treefrogs: implications for mate choice and sensory mechanisms. *Animal Behaviour* **70**, 39–48.

Gerhardt, H. C. (2008). Phonotactic selectivity in two cryptic species of gray treefrogs: effects of differences in pulse rate, carrier frequency and playback level. *J Exp Biol* **211**, 2609–2616.

Gerhardt, H. C. and Bee, M. A. (2007). Recognition and localization of acoustic signals. In *Hearing and sound communication in amphibians* (ed. Narins, P. M., Feng, A., Fay, R. R., and Popper, A. N.), pp. 113–146. Springer.

Gerhardt, H. C. and Doherty, J. A. (1988). Acoustic communication in the gray treefrog, *Hyla versicolor*: evolutionary and neurobiological implications. *J. Comp. Physiol.* **162**, 261–278.

Gerhardt, H. C. and Höbel, G. (2005). Mid-frequency suppression in the green treefrog (*Hyla cinerea*): mechanisms and implications for the evolution of acoustic communication. *J Comp Physiol A* **191**, 707–714.

Gerhardt, H. C. and Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. 1st ed. University Of Chicago Press.

Gerhardt, H. C. and Schwartz, J. J. (2001). Auditory tuning and frequency preferences in anurans. In *Anuran Communication* (ed. Ryan, M. J.), pp. 73–85. Washington D.C.: Smithstonian Institution Press.

Gerhardt, H. C., Allan, S. and Schwartz, J. J. (1990). Female green treefrogs (*Hyla cinerea*) do not selectively respond to signals with a harmonic structure in noise. *J Comp Physiol A* **166**, 791–794.

Gerhardt, H. C., Humfeld, S. C. and Marshall, V. T. (2007). Temporal order and the evolution of complex acoustic signals. *Proc. R. Soc. B* **274**, 1789–1794.

Gescheider, G. A., Verrillo, R. T. and Doren, C. L. V. (1982). Prediction of vibrotactile masking functions. *The Journal of the Acoustical Society of America* **72**, 1421–1426.

Gescheider, G. A., O'Malley, M. J. and Verrillo, R. T. (1983). Vibrotactile forward masking: Evidence for channel independence. *The Journal of the Acoustical Society of America* **74**, 474–485.

Ghazanfar, A. A., Stambaugh, C. R. and Nicolelis, M. A. L. (2000). Encoding of Tactile Stimulus Location by Somatosensory Thalamocortical Ensembles. *J. Neurosci.* **20**, 3761–3775.

Gibson, J. M. and Hirsch, H. R. (1975). Psychoneural models of the auditory masking process. *Journal of Theoretical Biology* **51**, 135–147.

Gooler, D. M. and Feng, A. S. (1992). Temporal coding in the frog auditory midbrain: the influence of duration and rise-fall time on the processing of complex amplitude-modulated stimuli. *J Neurophysiol* **67**, 1–22.

Gordon, M. and O'Neill, W. E. (1998). Temporal processing across frequency channels by FM selective auditory neurons can account for FM rate selectivity. *Hearing Research* **122**, 97–108.

Gorga, M. P., Beauchaine, K. A., Reiland, J. K., Worthington, D. W. and Javel, E. (1984). The effects of stimulus duration on ABR and behavioral thresholds. *The Journal of the Acoustical Society of America* **76**, 616–619.

Gorga, M. P., Kaminski, J. R., Beauchaine, K. A. and Jesteadt, W. (1988a). Auditory brainstem responses to tone bursts in normally hearing subjects. *J Speech Hear Res* **31**, 87–97.

Gorga, M. P., Kaminski, J. R., Beauchaine, K. A. and Jesteadt, W. (1988b). Auditory Brainstem Responses to Tone Bursts in Normally Hearing Subjects. *J Speech Hear Res* **31**, 87–97.

Gorga, M. P., Neely, S. T., Hoover, B. M., Dierking, D. M., Beauchaine, K. L. and Manning, C. (2004). Determining the upper limits of stimulation for auditory steady-state response measurements. *Ear Hear* **25**, 302–307.

Graham, N. (1980). Spatial-frequency channels in human vision: detecting edges without edge detectors. In *Visual Coding and Adaptability* (ed. Harris, C. S.), pp. 215–262. Psychology Press.

Graham, J. W., Olchowski, A. E. and Gilreath, T. D. (2007). How many imputations are really needed? Some practical clarifications of multiple imputation theory. *Prev Sci* **8**, 206–213.

Granit, R. (1942). Colour Receptors of the Frog's Retina. *Acta Physiologica Scandinavica* **3**, 137–151.

Granit, R. (1943). A PHYSIOLOGICAL THEORY OF COLOUR PERCEPTION : Abstract : Nature. *Nature* **151**, 11–14.

Granit, R. (1945). The colour receptors of the mammalian retina. *Journal of Neurophysiology* **8**, 195–210.

Green, D. M. (1958). Detection of Multiple Component Signals in Noise. *The Journal of the Acoustical Society of America* **30**, 904–911.

Greenhouse, S. W. and Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika* **24**, 95–112.

Grimault, N., Bacon, S. P. and Micheyl, C. (2002). Auditory stream segregation on the basis of amplitude-modulation rate. *The Journal of the Acoustical Society of America* **111**, 1340–1348.

Gross, C. G. (2008). Single neuron studies of inferior temporal cortex. *Neuropsychologia* **46**, 841–852.

Hall, J. C. (1994). Central processing of communication sounds in the anuran auditory system. *Amer. Zool.* **34**, 670–684.

Hall, J. W. (2007). *New Handbook for Auditory Evoked Responses*. 1st ed. Allyn & Bacon.

Hall, J. C. and Feng, A. S. (1990). Classification of the temporal discharge patterns of single auditory neurons in the dorsal medullary nucleus of the northern leopard frog. *J Neurophysiol* **64**, 1460–1473.

Hamer, R. D., Verrillo, R. T. and Zwislocki, J. J. (1983). Vibrotacile masking of Pacinian and non-Pacinian channels. *The Journal of the Acoustical Society of America* **73**, 1293–1303.

Hartline, H. K. (1938). The Response of Single Optic Nerve Fibers of the Vertebrate Eye to Illumination of the Retina. *American Journal of Physiology -- Legacy Content* **121**, 400–415.

Hartmann, W. M. and Johnson, D. (1991). Stream Segregation and Peripheral Channeling. *Music Perception: An Interdisciplinary Journal* **9**, 155–183.

Hateren, J. H. van (1992). A theory of maximizing sensory information. *Biol. Cybern.* **68**, 23–29.

Hebb, D. O. (1952). *The organisation of behaviour: a neuropsychological theory*. New York: Wiley.

Hecox, K., Squires, N. and Galambos, R. (1976). Brainstem auditory evoked responses in man. I. Effect of stimulus rise–fall time and duration. *The Journal of the Acoustical Society of America* **60**, 1187–1192.

Hedwig, B. (2000). Control of Cricket Stridulation by a Command Neuron: Efficacy Depends on the Behavioral State. *Journal of Neurophysiology* **83**, 712–722.

Heiligenberg, W. (1987). Central processing of sensory information in electric fish. *J. Comp. Physiol.* **161**, 621–631.

Helmholtz, H. (1852). LXXXI. On the theory of compound colours. *Philosophical Magazine Series 4* **4**, 519–534.

Helmholtz, H. von (1867). Handbuch der physiologischen Optik. In *Allgemeine Encyklopädie der Physik*, Leipzig, Germany: Voss.

Helmholtz, H. von (1895). *On the sensations of tone as a physiological basis for the theory of music*. London, New York : Longmans, Green, and Co.

Henry, K. S. and Lucas, J. R. (2008). Coevolution of auditory sensitivity and temporal resolution with acoustic signal space in three songbirds. *Animal Behaviour* **76**, 1659–1671.

Henry, K. S. and Lucas, J. R. (2009). Vocally correlated seasonal auditory variation in the house sparrow (*Passer domesticus*). *J Exp Biol* **212**, 3817–3822.

Henry, K. S. and Lucas, J. R. (2010a). Habitat-related differences in the frequency selectivity of auditory filters in songbirds. *Functional Ecology* **24**, 614–624.

Henry, K. S. and Lucas, J. R. (2010b). Auditory sensitivity and the frequency selectivity of auditory filters in the Carolina chickadee, Poecile carolinensis. *Animal Behaviour* **80**, 497–507.

Herdman, A. T. and Stapells, D. R. (2009). Thresholds determined using the monotic and dichotic multiple auditory steady-state response technique in normal-hearing subjects.

Hering, E. (1874). Grundzüge einer Theorie des Farbensinnes. In *Zur Lehre vom Lichtsinn*, pp. 107–141. Vienna: Akad. Wiss. Wien.

Higgs, D., Brittan-Powell, E. F., Soares, D., Souza, M., Carr, C. E., Dooling, R. and Popper, A. N. (2002). Amphibious auditory responses of the American alligator (*Alligator mississipiensis*). *J Comp Physiol A* **188**, 217–223.

Hill, F. J., McRae, L. P. and McClellan, R. P. (1968). Speech Recognition as a Function of Channel Capacity in a Discrete Set of Channels. *The Journal of the Acoustical Society of America* **44**, 13–18.

Hillery, C. M. (1984a). Detection of amplitude-modulated tones by frogs: Implications for temporal processing mechanisms. *Hearing Research* **14**, 129–143.

Hillery, C. M. (1984b). Seasonality of two midbrain auditory responses in the treefrog, *Hyla chrysoscelis*. *Copeia* **1984**, 844–852.

Hillery, C. M. and Narins, P. M. (1984). Neurophysiological Evidence for a Traveling Wave in the Amphibian Inner Ear. *Science* **225**, 1037–1039.

Hillery, C. M. and Narins, P. M. (1987). Frequency and time domain comparison of low-frequency auditory fiber responses in two anuran amphibians. *Hearing Research* **25**, 233–248.

Hillyard, S. A., Hink, R. F., Schwent, V. L. and Picton, T. W. (1973). Electrical Signs of Selective Attention in the Human Brain. *Science* **182**, 177–180.

Ho, C. C. K. and Narins, P. M. (2006). Directionality of the pressure-difference receiver ears in the northern leopard frog, Rana pipiens pipiens. *J Comp Physiol A* **192**, 417–429.

Höbel, G. and Gerhardt, H. C. (2003). Reproductive Character Displacement in the Acoustic Communication System of Green Tree Frogs (*Hyla Cinerea*). *Evolution* **57**, 894–904.

Horodysky, A. Z., Brill, R. W., Fine, M. L., Musick, J. A. and Latour, R. J. (2008). Acoustic pressure and particle motion thresholds in six sciaenid fishes. *J Exp Biol* **211**, 1504–1511.

Houtgast, T. (1972). Psychophysical Evidence for Lateral Inhibition in Hearing. *The Journal of the Acoustical Society of America* **51**, 1885–1894.

Hu, M. Y., Yan, H. Y., Chung, W.-S., Shiao, J.-C. and Hwang, P.-P. (2009). Acoustically evoked potentials in two cephalopods inferred using the auditory brainstem response (ABR) approach. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **153**, 278–283.

Huang, C.-M. and Buchwald, J. S. (1978). Factors that affect the amplitudes and latencies of the vertex short latency acoustic responses in the cat. *Electroencephalography and Clinical Neurophysiology* **44**, 179–186.

Hubel, D. H. and Wiesel, T. N. (1959). Receptive fields of single neurones in the cat's striate cortex. *J Physiol* **148**, 574–591.

Hubel, D. H. and Wiesel, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J Physiol* **160**, 106–154.2.

Hubl, L. and Schneider, H. (1979). Temperature and auditory thresholds: Bioacoustic studies of the frogsRana r. ridibunda, Hyla a. arborea andHyla a. savignyi (Anura, amphibia). *J. Comp. Physiol.* **130**, 17–27.

Huggins, W. H. and Licklider, J. C. R. (1951). Place Mechanisms of Auditory Frequency Analysis. *The Journal of the Acoustical Society of America* **23**, 290–299.

Hulse, S. H. (2002). Auditory scene analysis in animal communication. In *Advances in the Study of Behavior* (ed. Peter J. B. Slater, J. S. R.), pp. 163–200. Academic Press.

Hung, C. P., Kreiman, G., Poggio, T. and DiCarlo, J. J. (2005). Fast Readout of Object Identity from Macaque Inferior Temporal Cortex. *Science* **310**, 863–866.

Hurvich, L. M. and Jameson, D. (1957). An opponent-process theory of color vision. *Psychological Review* **64**, 384–404.

Itatani, N. and Klump, G. M. (2009). Auditory Streaming of Amplitude-Modulated Sounds in the Songbird Forebrain. *J Neurophysiol* **101**, 3212–3225.

Izumi, A. (2002). Auditory stream segregation in Japanese monkeys. *Cognition* **82**, B113–B122.

Jarriault, D., Gadenne, C., Rospars, J.-P. and Anton, S. (2009). Quantitative analysis of sex-pheromone coding in the antennal lobe of the moth Agrotis ipsilon: a tool to study network plasticity. *J Exp Biol* **212**, 1191–1201.

Jeffress, L. A. (1948). A place theory of sound localization. *Journal of Comparative and Physiological Psychology* **41**, 35–39.

Jeffress, L. A., Blodgett, H. C., Sandel, T. T. and Iii, C. L. W. (1956). Masking of Tonal Signals. *The Journal of the Acoustical Society of America* **28**, 416–426.

Jerger, J. and Hall, J. (1980). Effects of Age and Sex on Auditory Brainstem Response. *Arch Otolaryngol Head Neck Surg* **106**, 387–391.

Johansson, R. S. (1978). Tactile sensibility in the human hand: receptive field characteristics of mechanoreceptive units in the glabrous skin area. *J Physiol* **281**, 101–125.

Johansson, R. S. and Vallbo, A. B. (1979). Tactile sensibility in the human hand: relative and absolute densities of four types of mechanoreceptive units in glabrous skin. *J Physiol* **286**, 283–300.

John, M. S. and Picton, T. W. (2000). MASTER: a Windows program for recording multiple auditory steady-state responses. *Computer Methods and Programs in Biomedicine* **61**, 125–150.

Jørgensen, M. B. and Gerhardt, H. C. (1991). Directional hearing in the gray tree frog Hyla versicolor: Eardrum vibrations and phonotaxis. *J Comp Physiol A* **169**, 177–183.

Joris, P. X., Schreiner, C. E. and Rees, A. (2004). Neural Processing of Amplitude-Modulated Sounds. *Physiological Reviews* **84**, 541–577.

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A. and Hudspeth, A. J. (2012). *Principles of Neural Science, Fifth Edition*. 5th edition. New York: McGraw-Hill Professional.

Katbamna, B., Thodi, C., Senturia, J. B. and Metz, D. A. (1992). Auditory-evoked brainstem responses in the hibernating woodchuck *Marmota monax*. *Comparative Biochemistry and Physiology Part A: Physiology* **102**, 513–517.

Katbamna, B., Brown, J. A., Collard, M. and Ide, C. F. (2006a). Auditory brainstem responses to airborne sounds in the aquatic frog *Xenopus laevis*: correlation with middle ear characteristics. *Journal of Comparative Physiology A* **192**, 381–387.

Katbamna, B., Langerveld, A. J. and Ide, C. F. (2006b). Aroclor 1254 impairs the hearing ability of *Xenopus laevis*. *Journal of Comparative Physiology A* **192**, 971–983.

Katsuki, Y., Sumi, T., Uchiyama, H. and Watanabe, T. (1958). Electric Responses of Auditory Neurons in Cat to Sound Stimulation. *Journal of Neurophysiology* **21**, 569–588.

Katsuki, Y., Watanabe, T. and Maruyama, N. (1959). Activity of Auditory Neurons in Upper Levels of Brain of Cat. *Journal of Neurophysiology* **22**, 343–359.

Kay, R. H. and Matthews, D. R. (1972). On the existence in human auditory pathways of channels selectively tuned to the modulation present in frequency-modulated tones. *J Physiol* **225**, 657–677.

Keddy-Hector, A. C., Wilczynski, W. and Ryan, M. J. (1992). Call Patterns and Basilar Papilla Tuning in Cricket Frogs. II. Intrapopulation Variation and Allometry. *Brain, Behavior and Evolution* **39**, 238–246.

Kelley, D. B. (2004). Vocal communication in frogs. *Current Opinion in Neurobiology* **14**, 751–757.

Kenyon, T. N., Ladich, F. and Yan, H. Y. (1998). A comparative study of hearing ability in fishes: the auditory brainstem response approach. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **182**, 307–318.

Kiang, N. Y. S. and Moxon, E. C. (1974). Tails of tuning curves of auditory-nerve fibers. *The Journal of the Acoustical Society of America* **55**, 620–630.

Kilgard, M. P. and Merzenich, M. M. (1999). Distributed representation of spectral and temporal information in rat primary auditory cortex. *Hearing Research* **134**, 16–28.

Kilgard, M. P., Pandya, P. K., Vazquez, J., Gehi, A., Schreiner, C. E. and Merzenich, M. M. (2001). Sensory Input Directs Spatial and Temporal Plasticity in Primary Auditory Cortex. *Journal of Neurophysiology* **86**, 326–338.

Klishin, V. O., Diaz, R. P., Popov, V. V. and Supin, A. Y. (1990). Some characteristics of hearing of the Brazilian manatee, Trichechus inunguis. *Aquatic Mammals* **16**, 139–144.

Klump, G. M. and Gerhardt, H. C. (1992). Mechanisms and Function of Call-Timing in Male-Male Interactions in Frogs. In *Playback and Studies of Animal Communication* (ed. McGregor, P. K.), pp. 153–174. Springer US.

Klump, G. M., Benedix Jr., J. H., Gerhardt, H. C. and Narins, P. M. (2004). AM representation in green treefrog auditory nerve fibers: neuroethological implications for pattern recognition and sound localization. *J Comp Physiol A* **190**, 1011–1021.

Korczak, P., Smart, J., Delgado, R., M. Strobel, T. and Bradford, C. (2012). Auditory steady-state responses. *Journal of the American Academy of Audiology* **23**, 146–170.

Korn, T. S. (1969). Theory of Audio Information. *Acta Acustica united with Acustica* **22**, 336–344.

Kristan Jr., W. B., Calabrese, R. L. and Friesen, W. O. (2005). Neuronal control of leech behavior. *Progress in Neurobiology* **76**, 279–327.

Kuwada, S., Batra, R. and Maher, V. L. (1986). Scalp potentials of normal and hearing-impaired subjects in response to sinusoidally amplitude-modulated tones. *Hearing Research* **21**, 179–192.

Kuznetsova, A., Brockhoff, P. B. and Bojesen, R. H. (2014). lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package).

Ladich, F. and Fay, R. R. (2013). Auditory evoked potential audiometry in fish. *Rev Fish Biol Fisheries* **23**, 317–364.

Ladich, F. and Yan, H. Y. (1998). Correlation between auditory sensitivity and vocalization in anabantoid fishes. *J Comp Physiol A* **182**, 737–746.

Lakatos, P., Chen, C.-M., O'Connell, M. N., Mills, A. and Schroeder, C. E. (2007). Neuronal Oscillations and Multisensory Interaction in Primary Auditory Cortex. *Neuron* **53**, 279–292.

Langner, G. and Schreiner, C. E. (1988). Periodicity coding in the inferior colliculus of the cat. I. Neuronal mechanisms. *Journal of Neurophysiology* **60**, 1799–1822.

Laughlin, S. B. (1981). A simple coding procedure enhances a neuron's information capacity. *Z. Naturforsch* **36**, 910–912.

Laurent, G. (1999). A Systems Perspective on Early Olfactory Coding. *Science* **286**, 723–728.

Laurent, G. (2002). Olfactory network dynamics and the coding of multidimensional signals. *Nat Rev Neurosci* **3**, 884–895.

Lee, C., Rohrer, W. H. and Sparks, D. L. (1988). Population coding of saccadic eye movements by neurons in the superior colliculus. *Nature* **332**, 357–360.

Le Meur, O., Thoreau, D., Le Callet, P. and Barba, D. (2005). A spatio-temporal model of the selective human visual attention. In *IEEE International Conference on Image Processing, 2005. ICIP 2005*, pp. III–1188–91.

Lemon, C. H. and Smith, D. V. (2006). Influence of Response Variability on the Coding Performance of Central Gustatory Neurons. *J. Neurosci.* **26**, 7433–7443.

Lenth, R. V. (2014). lsmeans: Least-Squares Means. R package version 2.00-4.

Levi, R. and Camhi, J. M. (2000). Population Vector Coding by the Giant Interneurons of the Cockroach. *J. Neurosci.* **20**, 3822–3829.

Lewis, E. R. and Lombard, R. E. (1988). The amphibian inner ear. In *The Evolution of the amphibian auditory system* (ed. Fritzsch, B., Ryan, M. J., Wilczynski, W., Hetheringon, T., and Walkowiak, W.), pp. 93–123. New York: Wiley.

Lewis, E. R., Leverenz, E. L. and Koyama, H. (1982). The tonotopic organization of the bullfrog amphibian papilla, an auditory organ lacking a basilar membrane. *J. Comp. Physiol.* **145**, 437–445.

Liang, L. and Luo, L. (2010). The olfactory circuit of the fruit fly Drosophila melanogaster. *Sci. China Life Sci.* **53**, 472–484.

Lindquist, E. D., Hetherington, T. E. and Volman, S. F. (1998). Biomechanical and neurophysiological studies on audition in eared and earless harlequin frogs (Atelopus). *J Comp Physiol A* **183**, 265–271.

Lohr, B., Brittan-Powell, E. F. and Dooling, R. J. (2013). Auditory brainstem responses and auditory thresholds in woodpeckers. *The Journal of the Acoustical Society of America* **133**, 337–342.

Lombard, R. E. and Straughan, I. R. (1974). Functional aspects of anuran middle ear structures. *J Exp Biol* **61**, 71–93.

Lovell, J. M., Findlay, M. M., Moate, R. M. and Yan, H. Y. (2005). The hearing abilities of the prawn Palaemon serratus. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **140**, 89–100.

Lucas, J., Freeberg, T., Krishnan, A. and Long, G. (2002). A comparative study of avian auditory brainstem responses: correlations with phylogeny and vocal complexity, and seasonal effects. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* **188**, 981–992.

Lugli, M., Yan, H. Y. and Fine, M. L. (2003). Acoustic communication in two freshwater gobies: the relationship between ambient noise, hearing thresholds and sound spectrum. *J Comp Physiol A* **189**, 309–320.

Ma, L., Micheyl, C., Yin, P., Oxenham, A. J. and Shamma, S. A. (2010). Behavioral measures of auditory streaming in ferrets (Mustela putorius). *Journal of Comparative Psychology* **124**, 317–330.

MacDougall-Shackleton, S. A., Hulse, S. H., Gentner, T. Q. and White, W. (1998). Auditory scene analysis by European starlings (Sturnus vulgaris): Perceptual segregation of tone sequences. *J. Acoust. Soc. Am.* **103**, 3581–3587.

Machens, C. K., Gollisch, T., Kolesnikova, O. and Herz, A. V. M. (2005). Testing the efficiency of sensory coding with optimal stimulus ensembles. *Neuron* **47**, 447–456.

Malnic, B., Godfrey, P. A. and Buck, L. B. (2004). The human olfactory receptor gene family. *PNAS* **101**, 2584–2589.

Manley, G. A. (2001). Evidence for an Active Process and a Cochlear Amplifier in Nonmammals. *J Neurophysiol* **86**, 541–549.

Manley, G. A., Popper, A. N. and Fay, R. R. (2004). *Evolution of the Vertebrate Auditory System*. Springer.

Mann, D. A., Colbert, D. E., Gaspard, J. C., Casper, B. M., Cook, M. L. H., Reep, R. L. and Bauer, G. B. (2005). Temporal resolution of the Florida manatee (*Trichechus manatus latirostris*) auditory system. *J Comp Physiol A* **191**, 903–908.

Marks, W. B., Dobelle, W. H. and MacNichol, E. F., Jr. (1964). Visual Pigments of Single Primate Cones. *Science* **143**, 1181–1183.

Martin, K. J., Alessi, S. C., Gaspard, J. C., Tucker, A. D., Bauer, G. B. and Mann, D. A. (2012). Underwater hearing in the loggerhead turtle (Caretta caretta): a comparison of behavioral and auditory evoked potential audiograms. *J Exp Biol* **215**, 3001–3009.

Mason, M. J. (2007). Pathways for Sound Transmission to the Inner Ear in Amphibians. In *Hearing and Sound Communication in Amphibians* (ed. Narins, P. M., Feng, A. S., Fay, R. R., and Popper, A. N.), pp. 147–183. Springer New York.

Maxwell, J. C. (1855). Experiments on Colour, as perceived by the Eye, with Remarks on Colour-Blindness. *Transactions of the Royal Society of Edinburgh* **21**, 275–298.

Maxwell, J. C. (1857). IV. Account of experiments on the perception of colour. *Philosophical Magazine Series 4* **14**, 40–47.

McFadden, S., Henselman, L. and Zheng, X.-Y. (1999). Sex Differences in Auditory Sensitivity of Chinchillas Before and After Exposure to Impulse Noise. *Ear & Hearing April 1999* **20**, 164–174.

Meenderink, S. W. F., Kits, M. and Narins, P. M. (2010). Frequency matching of vocalizations to inner-ear sensitivity along an altitudinal gradient in the coqui frog. *Biol. Lett.* **6**, 278–281.

Megela, A. L. (1983). Auditory response properties of the anuran thalamus: nonlinear facilitation. In *Advances in Vertebrate Neuroethology* (ed. Ewert, J.-P., Capranica, R. R., and Ingle, D. J.), pp. 895–899. Springer US.

Megela, A. L., Borchers, H.-W. and Ewert, J.-P. (1983). Relation between activity of tectal neurons and prey-catching behavior in toads Bufo bufo. *Naturwissenschaften* **70**, 100–102.

Megela-Simmons, A., Moss, C. F. and Daniel, K. M. (1985). Behavioral audiograms of the bullfrog (*Rana catesbeiana*) and the green tree frog (*Hyla cinerea*). *The Journal of the Acoustical Society of America* **78**, 1236–1244.

Micheyl, C., Tian, B., Carlyon, R. P. and Rauschecker, J. P. (2005). Perceptual Organization of Tone Sequences in the Auditory Cortex of Awake Macaques. *Neuron* **48**, 139–148.

Micheyl, C., Carlyon, R. P., Gutschalk, A., Melcher, J. R., Oxenham, A. J., Rauschecker, J. P., Tian, B. and Courtenay Wilson, E. (2007). The role of auditory cortex in the formation of auditory streams. *Hearing Research* **229**, 116–131.

Miranda, J. A. and Wilczynski, W. (2009a). Female reproductive state influences the auditory midbrain response. *J Comp Physiol A* **195**, 341–349.

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

Mohneke, R. and Schneider, H. (1979). Effect of temperature upon auditory thresholds in two anuran species,Bombina v. variegata andAlytes o. obstetricans (Amphibia, Discoglossidae). *J. Comp. Physiol.* **130**, 9–16.

Moody, D. B., Cole, D., Davidson, L. M. and Stebbins, W. C. (1984). Evidence for a reappraisal of the psychophysical selective adaptation paradigm. *The Journal of the Acoustical Society of America* **76**, 1076–1079.

Mooney, T. A., Hanlon, R. T., Christensen-Dalsgaard, J., Madsen, P. T., Ketten, D. R. and Nachtigall, P. E. (2010). Sound detection by the longfin squid (Loligo pealeii) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. *The Journal of Experimental Biology* **213**, 3748 –3759.

Moore, B. C. J. and Gockel, H. (2002). Factors influencing sequential stream segregation. *Acta Acustica united with Acustica* **88**, 320–333.

Moray, N. (1960). Broadbent's filter theory: Postulate H and the problem of switching time. *Quarterly Journal of Experimental Psychology* **12**, 214–220.

Moss, C. F. and Simmons, A. M. (1986). Frequency selectivity of hearing in the green treefrog, *Hyla cinerea*. *J. Comp. Physiol.* **159**, 257–266.

Moss, C. F. and Surlykke, A. (2001). Auditory scene analysis by echolocation in bats. *The Journal of the Acoustical Society of America* **110**, 2207–2226.

Mudry, K. M. and Capranica, R. R. (1987a). Correlation between auditory thalamic area evoked responses and species-specific call characteristics. *J. Comp. Physiol.* **161**, 407–416.

Mudry, K. M. and Capranica, R. R. (1987b). Correlation between auditory thalamic area evoked responses and species-specific call characteristics. *J. Comp. Physiol.* **161**, 407–416.

Mudry, K. M. and Capranica, R. R. (1987c). Correlation between auditory evoked responses in the thalamus and species-specific call characteristics. *J. Comp. Physiol.* **160**, 477–489.

Mudry, K. M., Constantine-Paton, M. and Capranica, R. R. (1977). Auditory sensitivity of the diencephalon of the leopard frog *Rana p. pipiens*. *J. Comp. Physiol.* **114**, 1–13.

Munro, K. J., Shiu, J. N. and Cox, C. L. (1997). The Effect of Head Size on the Auditory Brainstem Response for Two Breeds of Dog. *British Journal of Audiology* **31**, 309–314.

Näätänen, R., Tervaniemi, M., Sussman, E., Paavilainen, P. and Winkler, I. (2001). "Primitive intelligence" in the auditory cortex. *Trends in Neurosciences* **24**, 283–288.

Nachtigall, P. E., Mooney, T. A., Taylor, K. A. and Yuen, M. M. L. (2007a). Hearing and auditory evoked potential methods applied to odontocete cetaceans. *Aquatic Mammals* **33**, 6–13.

Nachtigall, P. E., Supin, A. Y., Amundin, M., Röken, B., Møller, T., Mooney, T. A., Taylor, K. A. and Yuen, M. (2007b). Polar bear *Ursus maritimus* hearing measured with auditory evoked potentials. *J Exp Biol* **210**, 1116–1122.

Nakagawa, S. and Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews* **82**, 591–605.

Narins, P. M., Feng, A., Fay, R. R. and Popper, A. N. eds. (2007). *Hearing and sound communication in amphibians*. Springer.

Narins, P. M. and Capranica, R. R. (1976). Sexual differences in the auditory system of the tree frog *Eleutherodactylus coqui*. *Science* **192**, 378–380.

Narins, P. M. and Capranica, R. R. (1980). Neural adaptations for processing the two-note call of the Puerto Rican treefrog, *Eleutherodactylus coqui*. *Brain, Behavior and Evolution* **17**, 48–66.

Nelson, P. C. and Carney, L. H. (2007). Neural Rate and Timing Cues for Detection and Discrimination of Amplitude-Modulated Tones in the Awake Rabbit Inferior Colliculus. *Journal of Neurophysiology* **97**, 522–539.

Nelson, P. G., Erulkar, S. D. and Bryan, J. S. (1966). Responses of units of the inferior colliculus to time-varying acoustic stimuli. *Journal of Neurophysiology* **29**, 834–860.

Nelson, D. A., Stanton, M. E. and Freyman, R. L. (1983). A general equation describing frequency discrimination as a function of frequency and sensation level. *The Journal of the Acoustical Society of America* **73**, 2117–2123.

Ngan, E. M. and May, B. J. (2001). Relationship between the auditory brainstem response and auditory nerve thresholds in cats with hearing loss. *Hearing Research* **156**, 44–52.

Nityananda, V. and Bee, M. A. (2011). Finding Your Mate at a Cocktail Party: Frequency Separation Promotes Auditory Stream Segregation of Concurrent Voices in Multi-Species Frog Choruses. *PLoS ONE* **6**, e21191.

Noirot, I. C., Brittan-Powell, E. F. and Dooling, R. J. (2011). Masked auditory thresholds in three species of birds, as measured by the auditory brainstem response (L). *The Journal of the Acoustical Society of America* **129**, 3445–3448.

Novak, C. B., Mackinnon, S. E. and Kelly, L. (1993). Correlation of two-point discrimination and hand function following median nerve injury. *Ann Plast Surg* **31**, 495–498.

Nudo, R. J., Plautz, E. J. and Frost, S. B. (2001). Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* **24**, 1000–1019.

O'Bryant, E. L. and Wilczynski, W. (2010). Changes in Plasma Testosterone Levels and Brain AVT Cell Number during the Breeding Season in the Green Treefrog. *Brain, Behavior and Evolution* **75**, 271–281.

Oldham, R. S. and Gerhardt, H. C. (1975). Behavioral isolating mechanisms of the treefrogs *Hyla cinerea* and *H. gratiosa*. *Copeia* **1975**, 223–231.

Olsen, S. R. and Wilson, R. I. (2008). Lateral presynaptic inhibition mediates gain control in an olfactory circuit. *Nature* **452**, 956–960.

Olsen, S. R., Bhandawat, V. and Wilson, R. I. (2007). Excitatory Interactions between Olfactory Processing Channels in the Drosophila Antennal Lobe. *Neuron* **54**, 89–103.

Payne, T. L. and Dickens, J. C. (1976). Adaptation to determine receptor system specificity in insect olfactory communication. *Journal of Insect Physiology* **22**, 1569–1572.

Penna, M. and Veloso, A. (1990). Vocal Diversity in Frogs of the South American Temperate Forest. *Journal of Herpetology* **24**, 23–33.

Penna, M., Capranica, R. R. and Somers, J. (1992). Hormone-induced vocal behavior and midbrain auditory sensitivity in the green treefrog, *Hyla cinerea*. *J Comp Physiol A* **170**, 73–82.

Penna, M., Lin, W.-Y. and Feng, A. S. (1997). Temporal selectivity for complex signals by single neurons in the torus semicircularis of *Pleurodema thaul* (Amphibia: Leptodactylidae). *J Comp Physiol A* **180**, 313–328.

Penna, M., Lin, W.-Y. and Feng, A. S. (2001). Temporal selectivity by single neurons in the torus semicircularis of *Batrachyla antartandica* (Amphibia: Leptodactylidae). *J Comp Physiol A* **187**, 901–912.

Picker, M. D. (1980). *Xenopus laevis* (Anura: Pipidae) mating systems - A preliminary synthesis with some data on the female phonoresponse. *S. Afr. J. Zool.* **15**, 150–158.

Picton, T., John, M., Dimitrijevic, A. and Purcell, D. (2003). Human auditory steady-state responses. *International Journal of Audiology* **42**, 177–219.

Plack, C. J. (2013). *The Sense of Hearing: Second Edition*. Psychology Press.

Polat, U. and Sagi, D. (1993). Lateral interactions between spatial channels: Suppression and facilitation revealed by lateral masking experiments. *Vision Research* **33**, 993–999.

Popov, V. V. and Supin, A. Y. (1990). Auditory brain stem responses in characterization of dolphin hearing. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **166**, 385–393.

Pressnitzer, D., Sayles, M., Micheyl, C. and Winter, I. M. (2008). Perceptual Organization of Sound Begins in the Auditory Periphery. *Current Biology* **18**, 1124–1128.

Ptacek, M. B., Gerhardt, H. C. and Sage, R. D. (1994). Speciation by Polyploidy in Treefrogs: Multiple Origins of the Tetraploid, Hyla versicolor. *Evolution* **48**, 898–908.

Purcell, D. W., John, S. M., Schneider, B. A. and Picton, T. W. (2004). Human temporal auditory acuity as assessed by envelope following responses. *The Journal of the Acoustical Society of America* **116**, 3581–3593.

Qin, M. K. and Oxenham, A. J. (2003). Effects of simulated cochlear-implant processing on speech reception in fluctuating maskers. *The Journal of the Acoustical Society of America* **114**, 446–454.

Quian Quiroga, R. and Kreiman, G. (2010). Postscript: About grandmother cells and Jennifer Aniston neurons. *Psychological Review* **117**, 297–299.

Quiroga, R. Q., Reddy, L., Kreiman, G., Koch, C. and Fried, I. (2005). Invariant visual representation by single neurons in the human brain. *Nature* **435**, 1102–1107.

Ramsier, M. A. and Dominy, N. J. (2010). A comparison of auditory brainstem responses and behavioral estimates of hearing sensitivity in *Lemur catta* and *Nycticebus coucang*. *American Journal of Primatology* **72**, 217–233.

Rankin, C. H. (2002). From gene to identified neuron to behaviour in Caenorhabditis elegans. *Nat Rev Genet* **3**, 622–630.

Ratnam, R. and Feng, A. S. (1998). Detection of Auditory Signals by Frog Inferior Collicular Neurons in the Presence of Spatially Separated Noise. *Journal of Neurophysiology* **80**, 2848–2859.

R Development Core Team (2014). R: A language and environment for statistical computing. *R foundation for statistical computing*.

Regan, D. (1982). Visual information channeling in normal and disordered vision. *Psychological Review* **89**, 407–444.

Regan, D. and Tansley, B. W. (1979). Selective adaptation to frequency-modulated tones: Evidence for an information-processing channel selectively sensitive to frequency changes. *The Journal of the Acoustical Society of America* **65**, 1249–1257.

Reichert, M. S. and Gerhardt, H. C. (2014). Behavioral strategies and signaling in interspecific aggressive interactions in gray tree frogs. *Behavioral Ecology* **25**, 520–530.

Rheinlaender, J., Gerhardt, H. C., Yager, D. D. and Capranica, R. R. (1979). Accuracy of phonotaxis by the green treefrog (*Hyla cinerea*). *J. Comp. Physiol.* **133**, 247–255.

Rieke, F., Bodnar, D. A. and Bialek, W. (1995). Naturalistic stimuli increase the rate and efficiency of information transmission by primary auditory afferents. *Proc. R. Soc. Lond. B* **262**, 259–265.

Roberts, B., Glasberg, B. R. and Moore, B. C. J. (2002). Primitive stream segregation of tone sequences without differences in fundamental frequency or passband. *The Journal of the Acoustical Society of America* **112**, 2074–2085.

Rose, G. J. and Brenowitz, E. A. (2002). Pacific treefrogs use temporal integration to differentiate advertisement from encounter calls. *Animal Behaviour* **63**, 1183–1190.

Rose, G. and Capranica, R. R. (1983). Temporal selectivity in the central auditory system of the leopard frog. *Science* **219**, 1087–1089.

Rose, G. J. and Capranica, R. R. (1984). Processing amplitude-modulated sounds by the auditory midbrain of two species of toads: matched temporal filters. *J. Comp. Physiol.* **154**, 211–219.

Rose, G. J. and Capranica, R. R. (1985). Sensitivity to amplitude modulated sounds in the anuran auditory nervous system. *Journal of Neurophysiology* **53**, 446–465.

Rose, G. J. and Gooler, D. M. (2007). Function of the amphibian central auditory system. In *Hearing and Sound Communication in Amphibians* (ed. Narins, P. M., Feng, A. S., Fay, R. R., and Popper, A. N.), pp. 250–290. Springer New York.

Rose, G. J., Brenowitz, E. A. and Capranica, R. R. (1985). Species specificity and temperature dependency of temporal processing by the auditory midbrain of two species of treefrogs. *J. Comp. Physiol. A* **157**, 763–769.

Roy, A. (2012). A theory of the brain: localist representation is used widely in the brain. *Front Psychol* **3**,.

Rubin, D. B. (1976). Inference and missing data. *Biometrika* **63**, 581–592.

Ryan, M. J. (2001). *ANURAN COMMUNICATION*. Smithsonian.

Ryan, M. J., Perrill, S. A. and Wilczynski, W. (1992). Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. *The American Naturalist* **139**, 1370–1383.

SACHS, M. B., NACHMIAS, J. and ROBSON, J. G. (1971). Spatial-Frequency Channels in Human Vision. *J. Opt. Soc. Am.* **61**, 1176–1186.

Sagi, D. and Hochstein, S. (1985). Lateral inhibition between spatially adjacent spatial-frequency channels? *Perception & Psychophysics* **37**, 315–322.

Schafer, J. L. (1999). Multiple imputation: a primer. *Stat Methods Med Res* **8**, 3–15.

Schafer, J. L. and Olsen, M. K. (1998). Multiple Imputation for Multivariate Missing-Data Problems: A Data Analyst's Perspective. *Multivariate Behavioral Research* **33**, 545–571.

Schlief, M. L. and Wilson, R. I. (2007). Olfactory processing and behavior downstream from highly selective receptor neurons. *Nat Neurosci* **10**, 623–630.

Schnupp, J. W. H. and Carr, C. E. (2009). On hearing with more than one ear: lessons from evolution. *Nat Neurosci* **12**, 692–697.

Schrode, K. M., Buerkle, N. P., Brittan-Powell, E. F. and Bee, M. A. (in press). Auditory brainstem responses in Cope's gray treefrog (*Hyla chrysoscelis*): effects of frequency, level, sex and size. *J Comp Physiol A*.

Schrode, K., Ward, J., Vélez, A. and Bee, M. (2012a). Female preferences for spectral call properties in the western genetic lineage of Cope's gray treefrog (<i>Hyla chrysoscelis</i>). *Behavioral Ecology and Sociobiology* 1–12.

Schrode, K. M., Ward, J. L., Vélez, A. and Bee, M. A. (2012b). Female preferences for spectral call properties in the western genetic lineage of Cope's gray treefrog (*Hyla chrysoscelis*). *Behav Ecol Sociobiol* **66**, 1595–1606.

Schrode, K. M., Buerkle, N. P., Brittan-Powell, E. F. and Bee, M. A. (2014). Auditory brainstem responses in Cope's gray treefrog (*Hyla chrysoscelis*): effects of frequency, level, sex and size. *J Comp Physiol A* **200**, 221–238.

Schroeder, C. E. and Foxe, J. J. (2002). The timing and laminar profile of converging inputs to multisensory areas of the macaque neocortex. *Cognitive Brain Research* **14**, 187–198.

Schulte, M., Knief, A., Seither-Preisler, A. and Pantev, C. (2002). Different Modes of Pitch Perception and Learning-Induced Neuronal Plasticity of the Human Auditory Cortex. *Neural Plasticity* **9**, 161–175.

Schultze, M. (1866). Zur Anatomie und Physiologie der Retina. *Archiv f. mikrosk. Anatomie* **2**, 175–286.

Schürg-Pfeiffer, E., Spreckelsen, C. and Ewert, J.-P. (1993). Temporal discharge patterns of tectal and medullary neurons chronically recorded during snapping toward prey in toads Bufo bufo spinosus. *J Comp Physiol A* **173**, 363–376.

Schwartz, J. J. (1986). Male calling behavior and female choice in the neotropical treefrog *Hyla microcephala*. *Ethology* **73**, 116–127.

Schwartz, J. J. (1987). The importance of spectral and temporal properties in species and call recognition in a neotropical treefrog with a complex vocal repertoire. *Animal Behaviour* **35**, 340–347.

Schwartz, J. J. and Gerhardt, H. C. (1995). Directionality of the auditory system and call pattern recognition during acoustic interference in the gray treefrog, Hyla versicolor. *Auditory Neuroscience* **1**, 195–206.

Schwartz, J. J. and Gerhardt, H. C. (1998). The neuroethology of frequency preferences in the spring peeper. *Animal Behaviour* **56**, 55–69.

Schwartz, J. J. and Simmons, A. M. (1990). Encoding of a spectrally-complex communication sound in the bullfrog's auditory nerve. *J Comp Physiol A* **166**, 489–499.

Schwent, V. L. and Hillyard, S. A. (1975). Evoked potential correlates of selective attention with multi-channel auditory inputs. *Electroencephalography and Clinical Neurophysiology* **38**, 131–138.

Seaman, R. L. (1991). Method to record evoked potentials from the frog eighth nerve. *Hearing Research* **51**, 301–305.

Seeba, F., Schwartz, J. J. and Bee, M. A. (2010). Testing an auditory illusion in frogs: perceptual restoration or sensory bias? *Animal Behaviour* **79**, 1317–1328.

Serences, J. T. and Yantis, S. (2006). Selective visual attention and perceptual coherence. *Trends in Cognitive Sciences* **10**, 38–45.

Seung, H. S. and Sompolinsky, H. (1993). Simple models for reading neuronal population codes. *PNAS* **90**, 10749–10753.

Shamma, S. A. and Micheyl, C. (2010). Behind the Scenes of Auditory Perception. *Curr Opin Neurobiol* **20**, 361–366.

Shen, J.-X., Feng, A. S., Xu, Z.-M., Yu, Z.-L., Arch, V. S., Yu, X.-J. and Narins, P. M. (2008). Ultrasonic frogs show hyperacute phonotaxis to female courtship calls. *Nature* **453**, 914–916.

Shofner, W. P. and Feng, A. S. (1981). Post-Metamorphic Development of the Frequency Selectivities and Sensitivities of the Peripheral Auditory System of the Bullfrog, Rana Catesbeiana. *J Exp Biol* **93**, 181–196.

Silbering, A. F. and Galizia, C. G. (2007). Processing of Odor Mixtures in the Drosophila Antennal Lobe Reveals both Global Inhibition and Glomerulus-Specific Interactions. *J. Neurosci.* **27**, 11966–11977.

Simmons, A. M. (2013). "To ear is human, to frogive is divine": Bob Capranica's legacy to auditory neuroethology. *Journal of Comparative Physiology A* **199**, 169–182.

Simmons, A. M. and Ferragamo, M. (1993). Periodicity extraction in the anuran auditory nerve. *J Comp Physiol A* **172**, 57–69.

Simmons, A. M. and Moss, C. F. (1995). Reflex Modification: A Tool for Assessing Basic Auditory Function in Anuran Amphibians. In *Methods in Comparative Psychoacoustics* (ed. Klump, P. D. G. M., Dooling, P. R. J., Fay, P. R. R., and Stebbins, P. W. C.), pp. 197–208. Birkhäuser Basel.

Simmons, A. M., Schwartz, J. J. and Ferragamo, M. (1992). Auditory nerve representation of a complex communication sound in background noise. *The Journal of the Acoustical Society of America* **91**, 2831–2844.

Simmons, A. M., Reese, G. and Ferragamo, M. (1993a). Periodicity extraction in the anuran auditory nerve. II: Phase and temporal fine structure. *The Journal of the Acoustical Society of America* **93**, 3374–3389.

Simmons, A. M., Buxbaum, R. C. and Mirin, M. P. (1993b). Perception of complex sounds by the green treefrog, *Hyla cinerea*: envelope and fine-structure cues. *J Comp Physiol A* **173**, 321–327.

Simmons, A. M., Sanderson, M. I. and Garabedian, C. E. (2000). Representation of Waveform Periodicity in the Auditory Midbrain of the Bullfrog, Rana catesbeiana. *JARO* **1**, 2–24.

Simmons, D. D., Meenderink, S. W. F. and Vassilakis, P. N. (2007). Anatomy, Physiology, and Function of Auditory End-Organs in the Frog Inner Ear. In *Hearing and Sound Communication in Amphibians* (ed. Narins, P. M., Feng, A. S., Fay, R. R., and Popper, A. N.), pp. 184–220. Springer New York.

Simoncelli, E. P. and Olshausen, B. A. (2001). Natural Image Statistics and Neural Representation. *Annual Review of Neuroscience* **24**, 1193–1216.

Singh, N. C. and Theunissen, F. E. (2003). Modulation spectra of natural sounds and ethological theories of auditory processing. *J. Acoust. Soc. Am.* **114**, 3394–3411.

Singmann, H. (2014). afex: Analysis of Factorial Experiments. R package version 0.8-94.

Smith, E. C. and Lewicki, M. S. (2006). Efficient auditory coding. *Nature* **439**, 978–982.

Smith, M. E., Kane, A. S. and Popper, A. N. (2004). Noise-induced stress response and hearing loss in goldfish (Carassius auratus). *J Exp Biol* **207**, 427–435.

Snyder, J. S. and Alain, C. (2007a). Toward a neurophysiological theory of auditory stream segregation. *Psychological Bulletin* **133**, 780–799.

Snyder, J. S. and Alain, C. (2007b). Toward a neurophysiological theory of auditory stream segregation. *Psychol Bull* **133**, 780–799.

Song, L., McGee, J. and Walsh, E. J. (2006). Frequency- and level-dependent changes in auditory brainstem responses (ABRs) in developing mice. *The Journal of the Acoustical Society of America* **119**, 2242–2257.

Starr, A. and Achor, L. (1975). Auditory brain stem responses in neurological disease. *Arch Neurol* **32**, 761–768.

Stecker, G. C. and Middlebrooks, J. C. (2003). Distributed coding of sound locations in the auditory cortex. *Biol. Cybern.* **89**, 341–349.

Stiebler, I. B. and Narins, P. M. (1990). Temperature-dependence of auditory nerve response properties in the frog. *Hearing Research* **46**, 63–81.

Stiles, W. S. (1949). Increment thresholds & the mechanisms of colour vision. *Doc Ophthalmol* **3**, 138–165.

Stiles, W. S. (1959). COLOR VISION: THE APPROACH THROUGH INCREMENT-THRESHOLD SENSITIVITY. *Proc Natl Acad Sci U S A* **45**, 100–114.

Stockard, J., Stockard, J., BF Westmoreland and JL Corfits (1979). Brainstem auditory-evoked responses: Normal variation as a function of stimulus and subject characteristics. *Arch Neurol* **36**, 823–831.

Suga, N. (1989). Principles of auditory information-processing derived from neuroethology. *J Exp Biol* **146**, 277–286.

Suga, N. (1995). Sharpening of frequency tuning by inhibition in the central auditory system: tribute to Yasuji Katsuki. *Neuroscience Research* **21**, 287–299.

Supin, A. Y. and Popov, V. V. (1995a). Temporal resolution in the dolphin's auditory system revealed by double-click evoked potential study. *The Journal of the Acoustical Society of America* **97**, 2586–2593.

Supin, A. Y. and Popov, V. V. (1995b). Envelope-following response and modulation transfer function in the dolphin's auditory system. *Hearing Research* **92**, 38–46.

Supin, A. Y., Popov, V. V. and Klishin, V. O. (1993). ABR frequency tuning curves in dolphins. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **173**, 649–656.

Svaetichin, G. and MacNichol, E. F. (1958). Retinal Mechanisms for Chromatic and Achromatic Vision. *Annals of the New York Academy of Sciences* **74**, 385–404.

Szymanski, M. D., Bain, D. E., Kiehl, K., Pennington, S., Wong, S. and Henry, K. R. (1999). Killer whale (*Orcinus orca*) hearing: Auditory brainstem response and behavioral audiograms. *The Journal of the Acoustical Society of America* **106**, 1134–1141.

Tian, B., Reser, D., Durham, A., Kustov, A. and Rauschecker, J. P. (2001). Functional Specialization in Rhesus Monkey Auditory Cortex. *Science* **292**, 290–293.

Tolhurst, D. J. and Barfield, L. P. (1978). Interactions between spatial frequency channels. *Vision Research* **18**, 951–958.

Uetake, K., Yayou, K. and Okamoto, T. (1996). Auditory brainstem response and objective assessment of hearing thresholds in cowshed calves. *J. Ethol.* **14**, 73–75.

Uzuka, Y., Fukaki, M., Hara, Y. and Matsumoto, H. (1998). Brainstem Auditory Evoked Responses Elicited by Tone-Burst Stimuli in Clinically Normal Dogs. *Journal of Veterinary Internal Medicine* **12**, 22–25.

Valderrama, J. T., Alvarez, I., De la Torre, A., Segura, J. C., Sainz, M. and Vargas, J. L. (2013). A portable, modular, and low cost auditory brainstem response recording system including an algorithm for automatic identification of responses suitable for hearing screening. In *2013 IEEE Point-of-Care Healthcare Technologies (PHT)*, pp. 180–183.

Valdes, J., Perez-Abalo, M., Martin, V., Savio, G., Sierra, C., Rodriguez, E. and Lins, O. (1997). Comparison of statistical indicators for the automatic detection of 80 Hz auditory steady state responses. *Ear & Hearing October 1997* **18**, 420–429.

Vallbo, A. B. and Johansson, R. S. (1984). Properties of cutaneous mechanoreceptors in the human hand related to touch sensation. *Hum Neurobiol* **3**, 3–14.

Valois, R. L. D., Smith, C. J., Kitai, S. T. and Karoly, A. J. (1958). Response of Single Cells in Monkey Lateral Geniculate Nucleus to Monochromatic Light. *Science* **127**, 238–239.

Van der Meer, M. A. A., Johnson, A., Schmitzer-Torbert, N. C. and Redish, A. D. (2010). Triple Dissociation of Information Processing in Dorsal Striatum, Ventral Striatum, and Hippocampus on a Learned Spatial Decision Task. *Neuron* **67**, 25–32.

Van der Reijden, C., Mens, L. H. and Snik, A. F. (2005). EEG derivations providing auditory steady-state responses with high signal-to-noise ratios in infants. *Ear & Hearing June 2005* **26**, 299–309.

Van Noorden, L. P. A. S. (1975). Temporal coherence in the perception of tone sequences.

Van Stokkum, I. H. M. (1987). Sensitivity of neurons in the dorsal medullary nucleus of the grassfrog to spectral and temporal characteristics of sound. *Hearing Research* **29**, 223–235.

Vassar, R., Chao, S. K., Sitcheran, R., Nun˜ez, J. M., Vosshall, L. B. and Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. *Cell* **79**, 981–991.

Vélez, A. and Bee, M. A. (2010). Signal recognition by frogs in the presence of temporally fluctuating chorus-shaped noise. *Behav Ecol Sociobiol* **64**, 1695–1709.

Vélez, A. and Bee, M. A. (2011). Dip listening and the cocktail party problem in grey treefrogs: signal recognition in temporally fluctuating noise. *Animal Behaviour* **82**, 1319–1327.

Vélez, A. and Bee, M. A. (2013). Signal recognition by green treefrogs (*Hyla cinerea*) and Cope's gray treefrogs (*Hyla chrysoscelis*) in naturally fluctuating noise. *Journal of Comparative Psychology* **127**, 166–178.

Vélez, A., Höbel, G., Gordon, N. M. and Bee, M. A. (2012). Dip listening or modulation masking? Call recognition by green treefrogs (*Hyla cinerea*) in temporally fluctuating noise. *J Comp Physiol A* **198**, 891–904.

Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*. Springer.

Vestergaard, M. D., Fyson, N. R. C. and Patterson, R. D. (2011). The mutual roles of temporal glimpsing and vocal characteristics in cocktail-party listening. *J. Acoust. Soc. Am.* **130**, 429–439.

Vigny, C. (1979). The mating calls of 12 species and sub-species of the genus Xenopus (Amphibia : Anura). *Journal of Zoology* **188**, 103–122.

Vliegen, J. and Oxenham, A. J. (1999). Sequential stream segregation in the absence of spectral cues. *The Journal of the Acoustical Society of America* **105**, 339–346.

Wald, G. (1964). The Receptors of Human Color Vision. *Science* **145**, 1007–1016.

Walkowiak, W. (1980). Sensitivity, range and temperature dependence of hearing in the grass frog and fire-bellied toad. *Behavioural Processes* **5**, 363–372.

Walkowiak, W. (1984). Neuronal correlates of the recognition of pulsed sound signals in the grass frog. *J. Comp. Physiol.* **155**, 57–66.

Walkowiak, W. and Brzoska, J. (1982). Significance of spectral and temporal call parameters in the auditory communication of male grass frogs. *Behav Ecol Sociobiol* **11**, 247–252.

Walsh, E. J., McGee, J. and Javel, E. (1986). Development of auditory-evoked potentials in the cat. II. Wave latencies. *The Journal of the Acoustical Society of America* **79**, 725–744.

Ward, J. L., Love, E. K., Vélez, A., Buerkle, N. P., O'Bryan, L. R. and Bee, M. A. (2013). Multitasking males and multiplicative females: dynamic signalling and receiver preferences in Cope's grey treefrog. *Animal Behaviour* **86**, 231–243.

Wegel, R. L. and Lane, C. E. (1924). The Auditory Masking of One Pure Tone by Another and its Probable Relation to the Dynamics of the Inner Ear. *Phys. Rev.* **23**, 266–285.

Weiss, B. and Strother, W. (1965). Hearing in the green treefrog (*Hyla cinerea cinerea*). *J Audit Res* **5**, 297–305.

Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* **25**, 666–693.

Wells, K. D. (2007). *The ecology & behavior of amphibians*. Chicago: University of Chicago Press.

Wells, K. D. and Bard, K. M. (1987). Vocal communication in a neotropical treefrog, *Hyla Ebraccata*: responses of females to advertisement and aggressive calls. *Behaviour* **101**, 200–210.

Wells, K. D. and Schwartz, J. J. (2006). The Behavioral Ecology of Anuran Communication. In *Hearing and Sound Communication in Amphibians* (ed. Narins, P. M., Feng, A. S., Fay, R. R., and Popper, A. N.), pp. 44–86. Springer New York.

Wetzel, D. M. and Kelley, D. B. (1983). Androgen and gonadotropin effects on male mate calls in South African clawed frogs, *Xenopus laevis*. *Hormones and Behavior* **17**, 388–404.

Whitney, C. L. and Krebs, J. R. (1975). Mate selection in Pacific tree frogs. *Nature* **255**, 325–326.

Wier, C. C., Jesteadt, W. and Green, D. M. (1977). Frequency discrimination as a function of frequency and sensation level. *The Journal of the Acoustical Society of America* **61**, 178–184.

Wilczynski, W. and Capranica, R. R. (1984). The auditory system of anuran amphibians. *Prog. Neurobiol.* **22**, 1–38.

Wilczynski, W. and Ryan, M. J. (2010). The behavioral neuroscience of anuran social signal processing. *Current Opinion in Neurobiology* **20**, 754–763.

Wilczynski, W., Keddy-Hector, A. C. and Ryan, M. J. (1992). Call patterns and basilar papilla tuning in cricket frogs. I. Differences among populations and between sexes. *Brain Behav. Evol* **39**, 229–237.

Wilczynski, W., McClelland, B. E. and Rand, A. S. (1993). Acoustic, auditory, and morphological divergence in three species of neotropical frog. *J Comp Physiol A* **172**, 425–438.

Will, U. and Fritzsch, B. (1988). The eighth nerve of amphibians: peripheral and central distribution. In *The Evolution of the amphibian auditory system* (ed. Fritzsch, B., Wolkowiak, W., Ryan, M., Wilczynski, W., and Hetheringon, T.), pp. 159–183. New York: Wiley.

Wilson, R. I. and Mainen, Z. F. (2006). Early Events in Olfactory Processing. *Annual Review of Neuroscience* **29**, 163–201.

Wong, S. W. and Schreiner, C. E. (2003). Representation of CV-sounds in cat primary auditory cortex: intensity dependence. *Speech Communication* **41**, 93–106.

Woolley, S. M. N. and Casseday, J. H. (2005). Processing of Modulated Sounds in the Zebra Finch Auditory Midbrain: Responses to Noise, Frequency Sweeps, and Sinusoidal Amplitude Modulations. *Journal of Neurophysiology* **94**, 1143–1157.

Woolley, S. M. N., Fremouw, T. E., Hsu, A. and Theunissen, F. E. (2005). Tuning for spectro-temporal modulations as a mechanism for auditory discrimination of natural sounds. *Nat Neurosci* **8**, 1371–1379.

Wright, W. D. (1934). The Measurement and Analysis of Colour Adaptation Phenomena. *Proc. R. Soc. Lond. B* **115**, 49–87.

Wysocki, L. E. and Ladich, F. (2001). The ontogenetic development of auditory sensitivity, vocalization and acoustic communication in the labyrinth fish *Trichopsis vittata*. *J Comp Physiol A* **187**, 177–187.

Wysocki, L. E. and Ladich, F. (2002). Can fishes resolve temporal characteristics of sounds? New insights using auditory brainstem responses. *Hearing Research* **169**, 36–46.

Wysocki, L. E. and Ladich, F. (2003). The representation of conspecific sounds in the auditory brainstem of teleost fishes. *J Exp Biol* **206**, 2229–2240.

Wysocki, L. E. and Ladich, F. (2005a). Hearing in Fishes under Noise Conditions. *J Assoc Res Otolaryngol* **6**, 28–36.

Wysocki, L. E. and Ladich, F. (2005b). Effects of noise exposure on click detection and the temporal resolution ability of the goldfish auditory system. *Hearing Research* **201**, 27–36.

Yang, S. and Feng, A. S. (2007). Heterogeneous Biophysical Properties of Frog Dorsal Medullary Nucleus (Cochlear Nucleus) Neurons. *J Neurophysiol* **98**, 1953–1964.

Yost, W. A., Sheft, S. and Opie, J. (1989). Modulation interference in detection and discrimination of amplitude modulation. *The Journal of the Acoustical Society of America* **86**, 2138–2147.

Young, T. (1802). The Bakerian Lecture: On the Theory of Light and Colours. *Phil. Trans. R. Soc. Lond.* **92**, 12–48.

Young, T. (1807). *A course of lectures on natural philosophy and the mechanical arts*. London: Johnson.

Young, J. Z. (1983). The Distributed Tactile Memory System of Octopus. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **218**, 135–176.

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

Zakon, H. and Capranica, R. R. (1981). An anatomical and physiological study of regeneration of the eighth nerve in the leopard frog. *Brain Research* **209**, 325–338.

Zakon, H. and Wilczynski, W. (1988). The physiology of the anuran eighth nerve. In *The Evolution of the amphibian auditory system* (ed. Fritzsch, B., Wolkowiak, W., Ryan, M., Wilczynski, W., and Hetheringon, T.), pp. 125–155. New York: Wiley.

Zhang, K. and Sejnowski, T. J. (1999). Neuronal Tuning: To Sharpen or Broaden? *Neural Computation* **11**, 75–84.

Zhang, K., Ginzburg, I., McNaughton, B. L. and Sejnowski, T. J. (1998). Interpreting Neuronal Population Activity by Reconstruction: Unified Framework With Application to Hippocampal Place Cells. *Journal of Neurophysiology* **79**, 1017–1044.

Zhang, D., Cui, J. and Tang, Y. (2012). Plasticity of Peripheral Auditory Frequency Sensitivity in Emei Music Frog. *PLoS ONE* **7**, e45792.

Zhou, X., Jen, P. H.-S., Seburn, K. L., Frankel, W. N. and Zheng, Q. Y. (2006). Auditory brainstem responses in 10 inbred strains of mice. *Brain Research* **1091**, 16–26.

Appendix 1 Audio recordings of stimuli for ABRs in *H. chrysoscelis*

We made digital recordings (44.1 kHz sampling rate, 16-bit resolution) of the acoustic stimuli used in this study presented through our experimental setup and recorded in the sound chamber. Recordings were made using a Marantz PMD 670 solid-state digital recorder (D&M Professional, Itasca, IL) with a handheld Sennheiser ME62 microphone (Sennheiser USA, Old Lyme, CT) facing the speaker. The tip of the microphone was positioned 30 cm from the speaker, at the approximate location of the frog's head during neural recordings. Stimulus trains were broadcast at the highest range of levels used in the experiment (55-95 dB SPL for tones of 0.35, 0.5, 4.0, and 5.0 kHz; 50-90 dB pSPL for clicks and 50-90 dB SPL for all other tones). The highest-amplitude tone for six of the recorded stimuli are depicted in Online Resource 1 Fig. 4-3-1. These include the four stimuli used for much of our interpretation in the manuscript (clicks and tones at 1.3, 1.625, and 2.6 kHz), as well as the tones with the lowest and highest frequencies used (0.35 kHz and 5.0 kHz). These latter two stimuli were selected as the most likely to have distorted waveforms or spectra.

To evaluate the quality of our acoustic stimuli, we calculated the percent harmonic distortion (%HD; Shmilovitz, 2005) for each tone stimulus, and the tail-to-signal ratio (TSR; Holland et al., 2001) for all stimuli. Analysis of recordings was done in Adobe Audition 3.0 (Adobe Systems Inc., San Jose, CA) and MATLAB. We measured the power (FFT size= 1,024, Hanning window) of each of the first four spectral peaks for each stimulus. We then calculated the %HD as the ratio of the sum of the powers of the first three harmonic frequencies to the power of the fundamental frequency, %HD = $\sum_{i=2}^{4} \mathrm{Pi}$ $\sqrt{(/P_1)} \times 100\%$, where P_i is the power of the *i*th harmonic and $i = 1$ is corresponds to the fundamental frequency. The %HD was quite low for the vast majority of the stimuli we presented. The median %HD across frequencies and levels was 0.78 % (interquartile range 0.39 – 1.50 %). Stimuli for all but three frequencies had %HDs ≤ 1%, and measurements were quite consistent across levels. Tones of frequencies 0.35 kHz and 0.5 kHz had the highest distortion, with %HDs of ~4% and ~15%, respectively, at all stimulus levels. To quantify the amount of reverberation in the recorded signal, we calculated the TSR as the ratio of the energy of the recorded signal up to the beginning of the tail (Ey) and the energy in the tail (Et), TSR = 10 log (Et/Ey), where mean background noise energy has been subtracted from both energy measurements. All echoes were, on average, about 10 to 20 dB lower relative to the preceding signal. The mean (± s.d.) TSR across frequencies decreased monotonically from -9.9 ±

3.7 dB SPL at the lowest signal level to -19.4 ± 4.2 dB SPL at the highest signal level. Except at the highest signal level, the echo levels were lower than the visually detected threshold for the respective frequency.

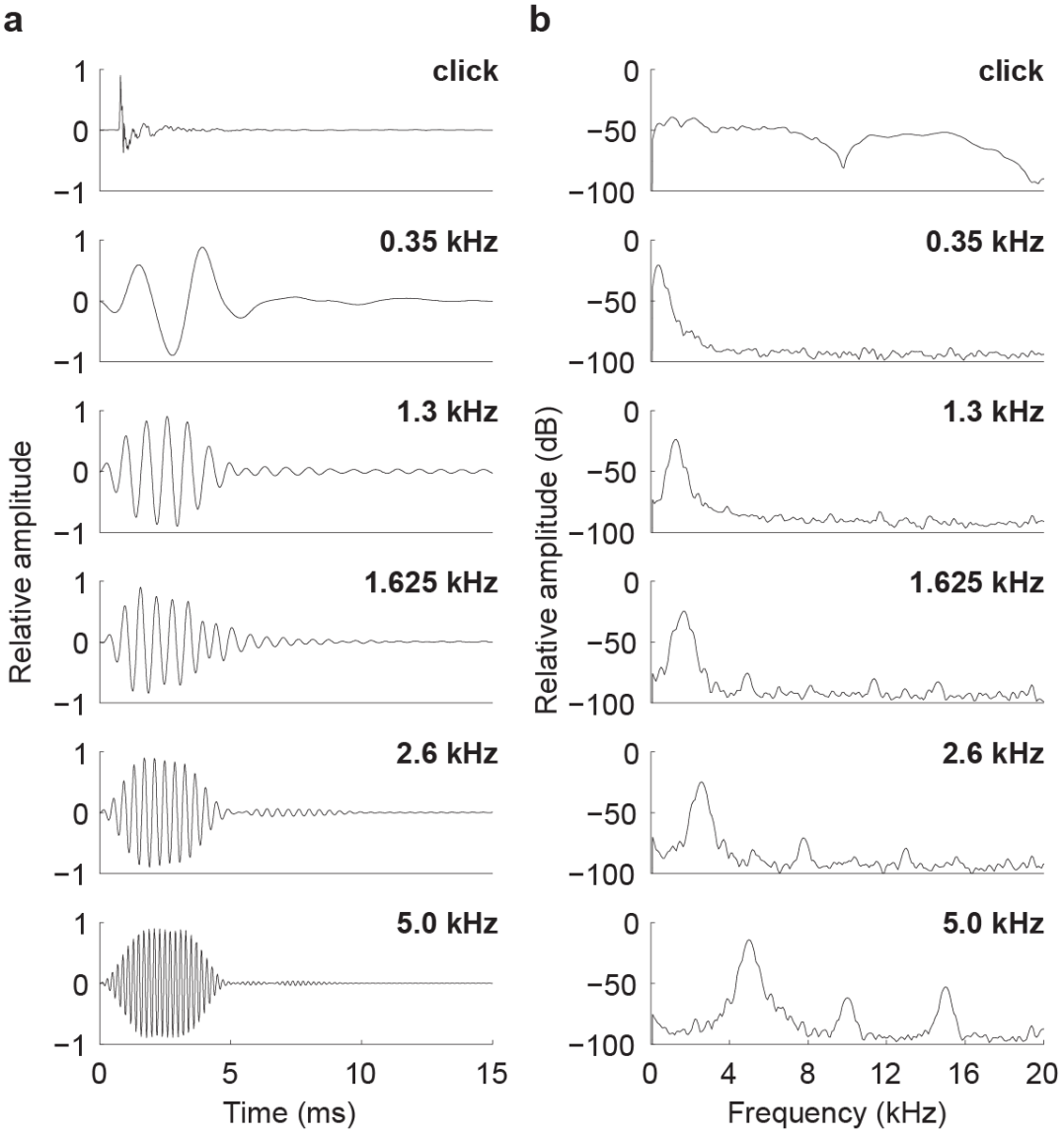


Figure A1-1 Recordings of acoustic stimuli for *Hyla chrysoscelis*
*Depicted are **a** time-amplitude waveforms and **b** spectral plots for recordings of the highest-amplitude tone from a stimulus train of clicks and tones with frequencies of 0.35 kHz, 1.3 kHz, 1.625 kHz, 2.6 kHz, and 5.0 kHz. The tones depicted here were broadcast at 90 dB pSPL for clicks, 95 dB SPL for tones of 0.35 kHz and 5.0 kHz, and 90 dB SPL for all other tones*

Appendix 2 Audio recordings of stimuli for ABRs in *Hyla cinerea*

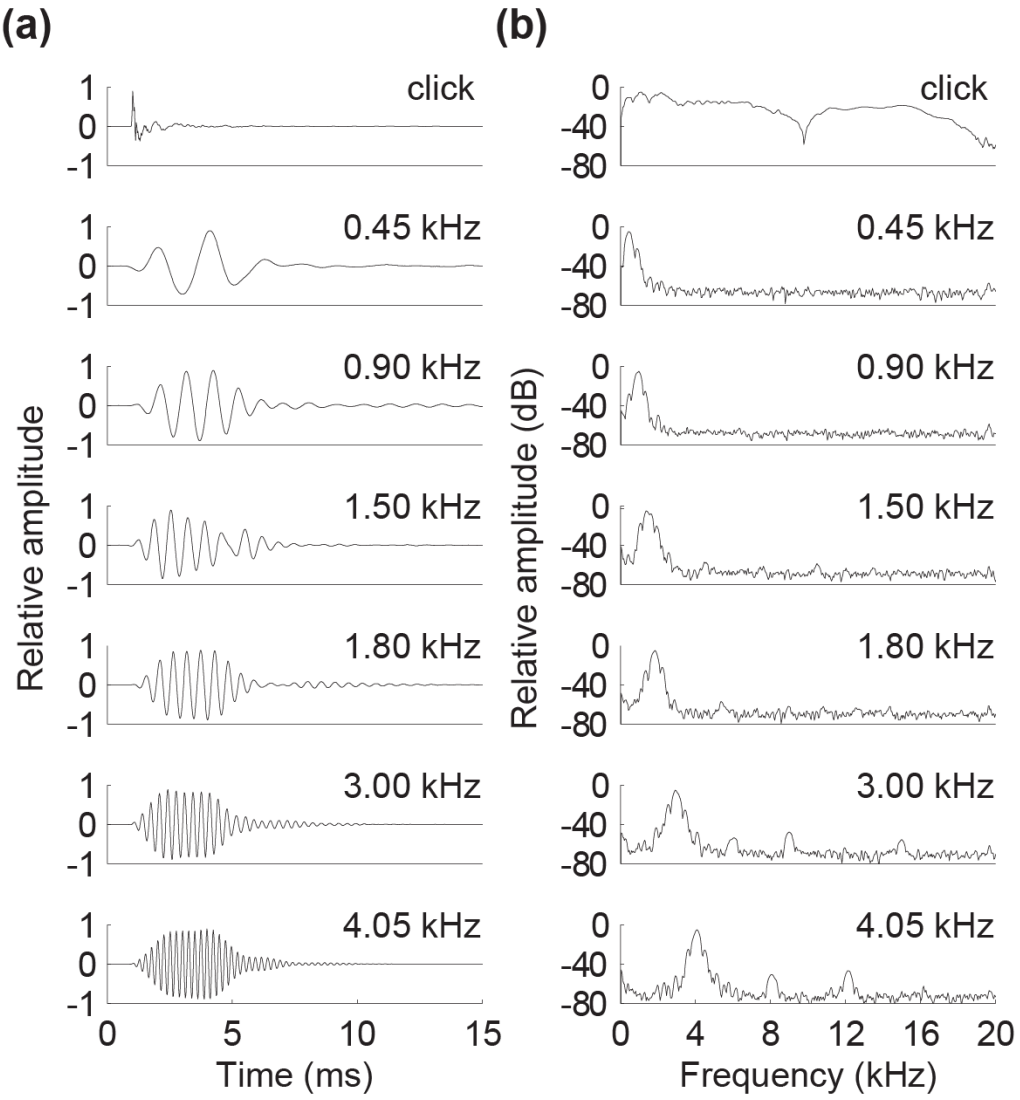


Figure A2-1 Recordings of acoustic stimuli for *Hyla cinerea*
We made digital acoustic recordings (44.1 kHz sampling rate, 16-bit resolution) of all stimuli using a Marantz PMD 670 solid-state digital recorder (D&M Professional, Itasca, IL) with a handheld Sennheiser ME62 microphone (Sennheiser USA, Old Lyme, CT) placed 30 cm from the speaker. Depicted here are recordings of the highest amplitude (80 dB) sounds from several stimulus trains shown in the (a) time and (b) frequency domains. The stimuli include a click train and tone trains with frequencies that span the range of frequencies used in the study. To verify the quality of our broadcasts, we calculated the percent harmonic distortion (%HD) and tail-to-signal ratio (TSR) for all stimuli, which are reported in the main article text.