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A comparative analysis of avian auditory responses to complex stimuli: using auditory evoked potentials to reverse engineer avian vocal communication

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Abstract

We tested the relationship between auditory processing and vocal-repertoire properties using auditory evoked potentials (AEP's) in response to frequency modulated (FM) tones, 2- and 3-tone chords, and Schroeder-phase waveforms. Auditory brainstem responses (ABR's) were measured for FM tones, and phase-locking intensity was measured for all input stimuli. Comparisons of AEP's from tufted titmice, white-breasted nuthatches, and house sparrows indicate that auditory processing enhances species-specific properties of vocal signals. Nuthatches had poor processing of rapid FM tones and a long ABR latency. However, they were the only species where higher frequency tones in chords increased phase-locking to lower frequency tones; they had strong harmonic peaks in response to these chords; and they excelled at processing Schroeder-phase complexes. Sparrows performed best at processing rapid FM signals, but surprisingly showed no phase locking in the frequency range of their song (3.5-5 kHz). Titmice had the shortest latency ABR peaks, but were relatively poor at processing FM tones and multi-tone chords. We found no sex differences for FM tones, but sexes differed in some aspects of the response to 2- and 3-tone chords and to Schroeder-phase complexes. We discuss these results in light of species-specific vocal patterns, habitat use, and social behavior.

Keywords: Auditory evoked potential (AEP), frequency following response (FFR), bird hearing, vocal complexity, auditory phase-locking

Abbreviations *AEP* auditory evoked potential – *CM* cochlear microphonic – *FFR* frequency following response – *EEG* electroencephalogram – *FM* frequency modulation – *AM* amplitude modulation

Introduction

The reigning paradigm for understanding communication systems is a focus on the sender and receiver of a signal (Bradbury and Vehrencamp 1998) or, more broadly, on a network of senders and receivers (McGregor 2005). A striking aspect of the literature describing this approach is that it often focuses on the sender and the signal (e.g., Espmark et al. 2000; Marler and Slabbekoorn 2004). We know much less about the receiver of signals, despite the fact that a signal conveys no information if the receiver is anatomically or physiologically incapable of processing the signal. Indeed, B. Lohr (2006) recently estimated that we have information about bird song for over 1000 species, but we have information about auditory capabilities for only about 50 bird species.

One potential reason for the disparity between the volume of work on sender versus receiver is the relative simplicity of analyzing sound compared with the relatively complex nature of analyzing auditory performance. This problem is exacerbated by the fact that vocal signals possess a myriad of properties (Nelson and Marler 1990), including frequency modulation (FM), amplitude modulation (AM), harmonic structure (e.g., number and frequency of overtones), and timing (e.g., trill or click rates). It has become trivial for us to describe these properties in sound, but it is an open question as to which spectral or timing properties of a complex signal the receiver extracts to decode information (Dooling et al. 2000). Nonetheless, several results suggest that hearing capacity is an integral part of the evolution of vocal communication. For example, animals are often better able to detect species-specific vocalizations (Dooling et al. 1996); upper limits to the highest perceived frequencies correlate with the maximal frequencies in vocal signals (Dooling 1982; Feng et al. 2006); and seasonal variation in response to tones of

different frequencies correlates with the timing of reproduction and song production (Lucas et al. 2002, 2007; also see Sisneros et al. 2004, Feng et al. 2006).

We show here that auditory evoked potentials (AEP's) using a battery of input stimuli can help bridge the gap between our depth of understanding of sender and receiver coding. AEP's are voltage changes resulting from hair cell (i.e., cochlear) or neural (i.e., auditory nerve, brainstem, and possibly midbrain) activity caused by auditory input. Voltage changes are measured with surface electrodes on the scalp (Hall 1992). The EEG trace caused by a sound has several characteristics that reflect electrical activity of populations of cells in different parts of the auditory system. The two most basic characteristics are an onset response (called the Auditory Brainstem Response, or ABR, for activity in the brainstem) and a sustained (or phase-locking) response to tonal inputs where a population of hair cells or auditory neurons fire at a rate that approximates the frequency of the tone (e.g. Møller 2006; Fig. 1). The *onset response* is characterized by a series of positive and negative peaks that represent neural activity at specific sites in the auditory system. The first peak represents activity in the auditory nerve (Brown-Borg et al. 1987). The *sustained response* consists of two components, a cochlear component (the Cochlear Microphonic or CM) and a neural component (the Frequency Following Response or FFR, referred to here simply as phase-locking). The CM is strongest at signal intensities 90 dB SPL or greater for our birds (Lucas et al. 2007), whereas the FFR dominates at lower signal intensities (all test stimuli used in our experiments were 80 dB SPL or less, so FFR should dominate).

The value of AEP's for studies of communication was nicely illustrated by Kraus and Nicol (2005) who showed that consonants in human speech are encoded with the onset response whereas vowels are encoded with phase-locking. Indeed, qualities of a stimulus involving 'what

it is', 'where it is coming from', and 'who or what is producing it' are processed separately, yet simultaneously, by different neural mechanisms in the brainstem *before* the information is passed to the cortex (Kraus and Nicol 2005, as first described by Ananthanarayan 1999). This fact makes AEP's extraordinarily powerful indices of the processing of auditory signals. We will focus primarily on phase-locking here, because phase-locking is the primary mechanism whereby the brainstem encodes fine-scale information about frequency in the frequency range where neural phase-locking is possible (e.g. below 5kHz in humans; see Cunningham et al. 2002; Köppl 1997; Kraus and Nicol 2005).

In addition to human speech (Cunningham et al. 2002; Krishnan 2002), AEP's have been used to characterize audiograms in birds (Brittan-Powell et al. 2002, 2005), temporal resolution (e.g. modulation rate transfer functions) in dolphins (Mooney et al. 2006), manatee (Mann et al. 2005), humans (Rees et al. 1986; Kuwada et al. 2002), and gerbils (Dolphin and Mountain 1993), auditory filter shape in porpoises (Popov et al. 2006), detection of amplitude envelopes (Henry 2002), processing of clicks on tonal backgrounds in humans (Junius and Dau 2005), and the influence of auditory training on brainstem function in humans (Russo et al. 2005).

Here we report a study of the auditory system using AEP's from three species: tufted titmice (*Baeolophus bicolor*), white-breasted nuthatches (*Sitta carolinensis*), and house sparrows (*Passer domesticus*). Four kinds of input stimuli (sinusoidal frequency modulated tones, linear frequency modulated tones, 2- and 3-tone chords, and 49-tone Schroeder-phase complexes) were used to characterize the auditory system of each species. [Note: we use 'chords' here to refer to harmonic stacks of tones]. The rationale for the input stimuli is listed below. We tested the prediction that spectral components of species-specific vocalizations should correlate with species-specific auditory responses to our input stimuli. In particular, the nuthatch auditory

system should be particularly responsive to complex sounds with overtones; the house sparrow auditory system should be more responsive than the other two species to rapid frequency modulation; and titmice should be poor at most aspects of the extraction of complex information from input sounds (see Lucas et al. 2007; also see "The System" below).

The System

Tufted titmice are members of the family Paridae, and nuthatches are Sittidae. Sibly and Ahlquist (1990) placed both families in the superfamily Sylvioidea, although more recently Jønsson and Fjeldså (2006) placed the Sittidae in a separate superfamily, the Muscicapoidea. House sparrows belong to the family Passeridae in the superfamily Passeroidea (Sibly and Ahlquist 1990). The implication is that these species are not strongly taxonomically related.

Vocal complexity differs markedly between these species. Nuthatches have a small repertoire that typically consists of a single, repeated note with no rapid transitions (Fig. 2A). The notes have little frequency modulation but are rich in harmonics (Ritchison, 1983). Both song and the contact ("quank") call have similar properties. The vocal repertoire of titmice is more diverse than that of nuthatches (Offutt, 1965; Schroeder and Wiley, 1983), with a chick-a-dee-like vocalization that lacks the note complexity characteristic of chickadees (Owens and Freeberg 2007; Fig. 2B) and a pure-tone "peter-peter" song with relatively slow frequency modulation rates (Fig. 2C). House sparrows have complex (chirrup) song notes characterized by high frequencies (>3.5kHz) with very rapid frequency modulation and rapid modulation of harmonic overtones (Fig. 2D; Lowther and Cink 1992; Henry and Lucas, submitted). They also use several different contact calls, one of which incorporates fairly rapid trills.

AEP responses to clicks in all three species were reported in Lucas et al. (2002). AEP responses to pure tones in titmice and nuthatches were described by Lucas et al. (2007). Audiograms and modulation rate transfer functions (i.e., phase-locking to AM signals at different AM rates) in all three species are described in Henry and Lucas (submitted).

Methods

General methodology

All birds were caught in the morning with treadle traps baited with mixed seed at two wildlife areas west of West Lafayette, IN and at two private residences in Lafayette, IN. We showed in earlier papers that there is seasonal variation in the auditory physiology of these species (Lucas et al. 2002, 2007). We therefore limited the data collected for the experiments described here to the interval from July through January (for FM signals) or July through December (for chords and Schroeder phase complexes).

Sex was determined using plumage patterns in nuthatches and house sparrows, and using wing chord length in titmice. The cutoffs (< 80 mm are female titmice), originally determined by Thirakhupt (1985), have been verified using laparotomy (Lucas et al. 1993). Only adults were tested; adult status (late summer) was determined using outer retrix (tail feather) shape in titmice, plumage color in house sparrows, and mouth color in house sparrows, titmice and nuthatches (Pyle 1997). After capture, the birds were immediately transported to an indoor aviary at Purdue University where they were kept in 1-m^3 stainless steel mesh cages (one bird per cage), and given ad lib water, sunflower seeds, mealworms, and grit. The light-dark cycle of the aviary was set to local conditions. The afternoon of capture, birds were weighed then anesthetized with 50-60 mg ketamine/kg and 10-12 mg xylazine/kg. They typically are

anesthetized within 5 min of injection. If a bird was not down after 5 min (e.g., eyes open or wings flapping), it was given another 5 min with lights off. If it was still not down then, the bird was not tested. The data from a bird was also not used if the bird woke up before testing finished. Of the birds caught (see Results for sample sizes), a total of three house sparrows and three nuthatches were caught but not included in the analyses for these reasons. After about 30 min, the birds were given one or two supplemental injections of ketamine (15-20 mg/kg) and xylazine (2-3 mg/kg) in order to complete the entire set of auditory tests (in approximately 80 minutes).

Subdermal needle electrodes (Nicolet Biomedical, Fitchburg, WI) were placed just under the skin. The positive electrode was placed at the crown directly above and midway between the eyes. The negative electrode was placed just behind the ear closest to speaker. The ground was placed on the back (nape) of the neck. The bird was then placed on a pre-warmed heating pad ('Snuggle-Safe' pad at 52° C) covered with towels. A thermister was placed between the bird and the substrate. Internal body temperature was not measured directly during the experiments, but we maintained the temperature between the subject's body and heating pad at 38 ± 2°C by adding and removing layers of towel.

The test chamber consisted of a 1.2 ×1.4 ×1.2 m box lined with acoustic tile and 3"-thick Sonex foam (Acoustic Solutions; Richmond, VA). Subjects were positioned centrally on the floor of the chamber with the lights off and their right ear facing upwards. Stimulus presentation, AEP acquisition, and data storage were coordinated by a Tucker Davis Technologies system II modular rack-mount system (TDT; Gainesville, FL) and Dell PC running TDT SigGen32/BioSig32 software in an adjacent room. Digitally generated stimuli passed through a TDT DA1 digital-to-analogue converter and Crown D75 power amplifier before presentation

through a downward projecting, electromagnetically shielded loudspeaker suspended 30 cm above the subject (RCA model 40-5000; 140-20,000 Hz frequency response). Responses were recorded through needle electrodes feeding into a TDT HS4 headstage and amplified with a TDT DB4 biological amplifier before passing through an AD1 analogue-to-digital converter to the computer for storage. The placement and integrity of the electrodes was checked by measuring impedance between each of the electrodes: impedance had to be less than 7 K for the test to proceed. If the impedance was too high, the electrodes were repositioned to ensure impedances below threshold.

Stimuli were calibrated to within ± 2 dB SPL at all relevant frequencies using a Bruel and Kjaer model 1613 Precision Sound Level Meter and model 4131 1" condenser microphone placed at the approximate location of the bird's ear. We tested the frequency output of the system using a Sennheiser ME62 microphone run through a Marantz PMD690 digital recorder.

Before and after each auditory test (see below), we ran a standard 90 dB SPL click to ensure that the birds' auditory system did not change over the course of the trial. The click standards also help identify birds with damaged auditory systems and provide an additional check for electrode placement. This is because the onset response to a click is quite stereotyped (see Lucas et al. 2002), and it is easy to tell an aberrant ABR by eye. One house sparrow with deficient hearing was identified using the click stimuli.

Test stimuli

We tested the birds with two sets of frequency modulated tones and two sets of stacked overtones. Sound files were constructed in Praat (ver 4.6; Boersma 2001) using the "create sound from formula" option. Sounds were filtered with Cool Edit Pro (ver 2.0) graphic equalizer

to ensure that the signal was 80 dB SPL at all frequencies. All input stimuli had 3 ms $\cos^2$ rise/fall times. The FM signals and Schroeder-phase complexes were presented at 11.13 stimuli/sec; the 2- and 3-tone chords were presented at 13.13 stimuli/sec. AEP's were sampled at 40 kHz with a response amplification of 200k, high-pass filtered at 100Hz, and low-pass filtered at 10kHz with a notch filter at 60 Hz. AEP waveforms used in our analyses were based on averages of 500 stimuli presentations, and two waveforms (replicates) were collected for each stimulus. The test stimuli used in this study were as follows:

1) We used two types of frequency-modulated (FM) tones to indicate the ability of each species to process frequency modulation. One is represented by four different variants of **linear tone sweeps**. Two of these were 50 msec sweeps ("slow"), and two were 30 msec sweeps ("fast"). One fast and one slow sweep increased in frequency (1 to 6 kHz) and the others decreased in frequency (6 to 1 kHz). This frequency range is seen in the introductory whistled note of titmice (Owens and Freeberg 2007). The slow sweeps match properties of frequency modulation in house sparrow notes and some titmice notes. The fast sweeps are faster than is typically found in these species.

2) The **sinusoidal FM tones** all ranged from 1.7 to 2.3 kHz but varied in modulation rate. Pure tones in this frequency range elicit strong phase-locking in our birds (Lucas et al. 2007). Four FM rates were used: 20, 40, 70, and 110 Hz, each with a 50 msec duration. The 20 Hz FM rate is analogous to the modulation in the "peter-peter" titmouse song. The 110 Hz FM rate was designed to match the FM rate of the house sparrow contact call.

3) **Two- and three-tone chords** (30 msec duration), constructed from 3 tones (1.2, 1.8 and 2.4 kHz – with the full 3-tone chord and all 3 combinations of 2-tone chords), were used to test for the processing of harmonic stacks. All three species use stacked overtones in some of their

vocalizations. This particular set of overtones most closely matches nuthatch song and some house sparrow contact calls.

4) **Schroeder-phase complexes** (50 msec duration) were used to test for the processing of complex harmonics. They are composed of 49 different pure tones that are stacked overtones 100 Hz apart starting at 200 Hz. With the proper initial phase of each tone (described in Schroeder 1970; see Lauer et al., 2006; Recio and Rhode 2000; Dooling et al. 2002), the Schroeder-phase complexes produce either upward or downward instantaneous frequency sweeps. While the complex can be manipulated to alter the speed of the frequency sweeps, we used only a single velocity sweep ($c = \pm 1$ in Lentz and Leek 2001). Thus with 49 pure, fixed tones, there is both amplitude modulation and instantaneous frequency modulation. Schroeder-phase complexes are important because they give us some indication about how the auditory system works when a large part of the cochlea is stimulated simultaneously.

Statistical Design

Data analysis required a 3-step process. First, AEP waveforms were output from Biosig RP (ver. 4.4.1, Tucker Davis Technologies, Inc.) to a text file using a 40 kHz sampling frequency. This text file was read into Praat software (ver. 4.6; Boersma, 2001). ABR amplitude and latency were measured directly from the waveform (see Fig. 1). The frequency of phase-locking over time was extracted from AEP responses to linear and sinusoidal FM stimuli using the "Pitch (ac)" command in Praat. This algorithm uses autocorrelation to estimate acoustic periodicity (Boersma, 1993); with our tonal FM this simplifies to the fundamental frequency of phase-locking over time. We used a time step of 0.125 msec and Gaussian windows to generate the autocorrelation. The software generates the fundamental frequency and strength of

autocorrelation (ranging from 0 to 1) for the response waveform at each time step. Autocorrelation strength is analogous to a correlation coefficient. Phase-locking to stacked overtones in 2- and 3-tone chords was analyzed using a Fourier Transform ('Spectrum" in Praat) with a Nyquist frequency of 20 kHz and a resolution of 8 Hz.

Tests of hypotheses with a single dependent variable used repeated measures ANOVA using the Kenward-Roger method to calculate degrees-of-freedom and a compound symmetric variance-covariance matrix (Proc Mixed; SAS for Windows, ver. 9.1.3). Interaction terms between all independent variables were included in the repeated measures ANOVA's, and non-significant terms were deleted in order of decreasing F value. Where appropriate, posthoc tests for pairwise comparisons were estimated using the 'LSMEANS/diff" command within Proc Mixed. Least Squares Means (LSMeans) were also generated with this command.

For linear FM signals, we used slope of the relationship between phase-locking frequency and auditory input frequency as a dependent variable in our analysis. These slopes indicate the resolution with which the auditory system matches frequency modulation in input sounds; a slope of one indicates a perfect match. Slopes were generated for each replicate separately using linear regression (Proc GLM in SAS).

The auditory response to 2- and 3-tone chords was taken as the intensity of phase-locking to the different input tones. We used repeated-measures MANOVA (Proc GLM in SAS) to test for species, sex, and chord-type effects because the dependent variables (phase-locking intensity to each tone in the chord) were multivariate. If the repeated measures MANOVA was significant, we ran univariate repeated measures ANOVA's to identify specific patterns for each separate peak. In addition to phase-locking to the input tones, harmonics are often present in the phase-locking response even if they are not found in the original signal (e.g., Henry 1997; Galbraith

1994). We ran separate analyses for the first two harmonics (3.0 and 3.6 kHz) with a statistical design similar to our analysis of phase-locking to tones. Finally, the 0.6 kHz spacing between our input tones generates amplitude modulation at 0.6 kHz (Viemeister and Plack 1993) and the auditory system can potentially phase-lock to this AM signal (Simmons and Buxbaum 1996). We tested species and sex differences in the intensity of phase-locking to AM by extracting the intensity of the 0.6 kHz signal from the AEP using Fourier analysis, then subjecting these data to a repeated measures ANOVA (Proc Mixed in SAS).

Schroeder-phase complexes are composed of 49 different tones. We analyzed the auditory response to these sounds by cross-correlating the entire AEP waveform for all pairs of birds and included in the samples the original input waveform (scaled to the same sampling frequency as the AEP waveforms). Cross-correlations were calculated using Praat software. We then used multidimensional scaling (Proc MDS in SAS) to reduce the dimensionality of the cross-correlation matrix. Linear distances between birds' position and the input sound, using the first two dimensions of the MDS analysis, were used to describe the relative fit of a bird's AEP waveform to the input sound. Repeated measures MANOVA was used to document the overall difference between birds in MDS 2-dimensional space.

RESULTS

Auditory Brainstem Response (ABR) to FM Stimuli

Tests of FM sounds were conducted on 10 titmice (5 males and 5 females collected from June through January), 14 house sparrows (9 males and 5 females collected from June through November), and 8 nuthatches (7 males and 1 female collected from July through January).

All frequency-modulated auditory inputs (except those starting at 6 kHz) generated a robust ABR (e.g., Fig. 1). To give some indication of the species-specific ABR properties in our sample, we simply calculated the mean latency of the first positive ABR peak (P1) for each individual bird measured from the ABR of all FM sounds, and we estimated peak amplitude using the mean difference in voltage between P1 and the first negative peak (P-1). Note that taking the mean of several ABR peaks gives a more robust estimate of the ABR because it combines 10 ABR's (5 stimuli × 2 replicates), although we obviously cannot distinguish ABR properties for each individual stimulus type.

Neither species ($F_{2,25} = 1.95$, $P = 0.16$) nor sex ($F_{1,25} = 0.0$, $P = 0.96$) accounted for a significant amount of variation in peak amplitude. In contrast, there was a highly significant difference between species in P1 latency ($F_{2,25} = 12.3$, $P = 0.0002$; Fig. 3), with nuthatches having a significantly longer latency than both titmice ($t_{25} = 3.7$, $P = 0.001$) and house sparrows ($t_{25} = 4.7$, $P = 0.0001$). There was no significant difference between titmice and house sparrows ($t_{25} = 0.13$, $P = 0.89$), nor was there an effect of sex on peak latency ($F_{1,25} = 1.0$, $P = 0.34$).

Phase-locking to Sinusoidal FM Stimuli

We analyzed three properties of phase-locking to sinusoidal frequency modulation: the latency before the auditory system matched input frequency, the relative strength of phase-locking to the input stimulus, and phase-locking intensity to the FM rate itself (for 110 Hz FM signals). The first two variables were derived from a best-fit autocorrelation, and the third was derived from a Fourier transform of the AEP waveform (see Methods). Both the input stimulus and the resulting phase-locking intensity are functions of time. We estimated the time delay in the input function that would result in a minimal squared deviation between the input stimulus and phase-

locking functions (in effect shifting the input stimulus in Fig. 4 to the right until it best-fit the phase-locking response). The magnitude of the time shift is the latency of phase-locking to input.

Our results show a significant difference between species ($F_{2,26} = 5.8$, $P = 0.008$) with a significantly greater latency in titmice compared to house sparrows ($t_{26} = 3.3$, $P = 0.003$) and compared to nuthatches ($t_{26} = 2.5$, $P = 0.020$; Fig. 5). There was no difference between nuthatches and house sparrows ($t_{26} = 0.4$, $P = 0.69$). There was a weak effect of modulation rate on latency (LSmeans±SE in sec; 20 Hz: 0.0020±0.0001; 40 Hz: 0.0018±0.0001; 70 Hz: 0.0016±0.0001; 110 Hz: 0.0018±0.0001; $F_{3,80} = 2.7$, $P = 0.051$). However, there was no effect of sex on latency ($F_{1,26} = 0.6$, $P = 0.45$), and no interaction terms were significant.

The species also differed in the relative strength of phase-locking to the sinusoidally FM tones. We can estimate this using the strength of the autocorrelation used to estimate the fundamental frequency at each time point in Fig. 4. Several important patterns emerge from our analyses. First, house sparrows (strength = 0.84 ±0.002) and nuthatches (strength = 0.81 ±0.02) have overall significantly stronger phase-locking than titmice (strength = 0.75 ±0.02; $F_{2,27} = 4.6$, $P = 0.019$). In addition, the sinusoidal FM signal ranges from 1.7 to 2.3 kHz, and birds in all three species phase-locked better in the higher (i.e., >2 kHz) frequency range compared to lower frequencies (see Fig. 1a for an example). We therefore distinguished between upper (>2 kHz) and lower (<2 kHz) frequencies in our analyses. With the exception of a non-significant sex effect, all other main effects (species, FM rate, and frequency range) were significant, as were all 2-way and 3-way interaction terms (Table 1). Phase-locking strength at the highest frequencies was greater for nuthatches and house sparrows than for titmice, with little effect of FM rate on strength (Table 1; Fig. 6a). In contrast, phase-locking strength was overall lower at frequencies

below 2 kHz, with a decrease in strength with increasing FM rates, a pattern that was strongest in nuthatches (Table 1; Fig. 6b). This can be seen in the spectrograms of the AEP waveforms: the strength of low frequency phase-locking decreased more in nuthatches (Fig. 1a) than in house sparrows (Fig. 1b).

There were 5 full cycles in the 110 Hz FM signal. Interestingly, we measured phase-locking in the Fourier transform of the AEP's to this FM rate. This phase-locking to FM rate can be seen as the shifting AEP baseline voltage in Figs. 1a and 1b. The intensity of phase-locking to FM rate varied between species ($F_{2,26}$=3.4, P=0.050). Nuthatches showed relatively strong phase-locking to 110 Hz FM rates (Fig. 7; compared to h. sparrows: t_{26} = 2.6, P=0.016; compared to titmice: t_{26} = 1.8, P=0.093). No sex effects were evident ($F_{1,26}$=0.1, P=0.72).

In summary, nuthatches and house sparrows have phase-locking that matches sinusoidal FM input well at low FM rates. Titmice showed the longest latency and weakest phase-locking of the three species. Only the house sparrow performs relatively well at matching FM tones at high FM rates, however the nuthatches show relatively strong phase-locking to the FM rates themselves at these high FM rates.

Phase-locking to Linear FM Sweeps

The linear frequency-modulated sweep ranged from either 1 to 6 or 6 to 1 kHz in either 30 or 50 msec. Note that this is a broader range of frequencies than was used in the sinusoidal FM tests. We used an autocorrelation technique to estimate the fundamental frequency of the phase-locked response in time bins of 0.125 msec (see Methods), giving us phase-locking frequency as a function of time. We then calculated the regression line for phase-locking fundamental as a function of input frequency for each replicate of each bird separately. The slope of this function

was used as an indication of the degree to which changes in the frequency of phase-locking matched changes in input sound frequency. It was clear from visual inspection of the AEP waveforms that phase-locking was much weaker above 3.0 kHz than below this threshold, and that phase-locking was relatively strong below 2.8 kHz. We therefore ran separate regression analyses for each frequency range.

We first focus on the higher frequency range of the sweeps (3.0 to 6.0 kHz). Our results showed that neither species ($F_{2,21}$ = 1.8, P = 0.20) nor sex ($F_{1,21}$ = 1.1, P = 0.32) affected phase-locking slope. However, there was a significant effect of input sweep type on the slope ($F_{3,171}$ = 5.0, P = 0.002), with lower slopes for the fast sweeps compared to the slow sweeps (LSM's, upsweep: 50-msec slope = 0.33 ±0.15 vs. 30-msec slope = 0.19 ±0.16; downsweep: 50-msec slope = 0.29 ±0.15 vs. 30-msec slope = -0.12 ±0.15). Nonetheless, these results suggest very poor matching of phase-locking to input at frequencies above 3.0 kHz. Indeed, only the house sparrow had a LSMeans slope significantly greater than 0 (LSMeans for slope: h sparrow, 0.42±0.15; titmouse, 0.19±0.25; nuthatch, -0.09±0.24).

In contrast, the results from the lower frequency range (1.0 – 2.8 kHz) indicate a significant species effect ($F_{2,26}$ = 9.4, P = 0.001), sweep type effect ($F_{3,78}$ = 43.9, P <0.0001) and a significant species × sweep-type interaction ($F_{6,78}$ = 3.4, P = 0.005). Overall, house sparrows showed a significantly better fit than nuthatches (LSM's, sparrows: 0.99 ±0.01, nuthatches: 0.92 ±0.01; t_{26} = 4.2, P = 0.0002), with titmice intermediate and significantly greater than nuthatches (LSM: 0.97 ±0.01; t_{26} = 2.2, P = 0.038) but not different than house sparrows (t_{26} = 1.8, P = 0.08). Sex did not account for a significant amount of variation in the slope ($F_{1,26}$ = 0.1, P = 0.93).

The significant species × sweep-type interaction results from all three species performing relatively well with the slow upsweep but nuthatches performing more poorly (i.e., lower slope) than the other species with rapid upsweeps and downsweeps (Fig. 8a). In addition, house sparrows showed a slope slightly greater than 1.0 on both slow and fast downsweeps, and the titmouse had a slope greater than 1.0 for the rapid downsweep. In all three cases, these high slopes were associated with high intercepts in regressions of phase-locking frequency as a function of time (Fig. 8b; slope-type × species interaction: $F_{6,81} = 10.29$, $P<0.0001$).

We can use the strength of the autocorrelation as a function of input frequency to estimate the frequency where phase-locking is strongest during the sweep. We estimated this for the frequency upsweeps by best fitting phase-locking strength as a function of input frequency using Loess regression, then estimating the frequency where the regression line peaked. Our results suggest that the species do not differ overall in best frequency ($F_{2,21} = 0.9$, $P = 0.41$), though (perhaps surprisingly) best frequency was lower in response to the slow sweep compared to the fast sweep ($F_{1,22} = 27.7$, $P< 0.0001$) and there was a significant species × sweep-type interaction ($F_{2,22} = 7.3$, $P= 0.004$; Fig. 9). The significant interaction resulted from both titmice ($t_{22}=2.1$, $P=0.049$) and nuthatches ($t_{22}=5.4$, $P<0.0001$) having higher best frequencies for the more rapid frequency sweep than for the slower sweep (Fig. 9). House sparrows showed no difference in best frequencies for the two sweep types ($t_{22}=1.3$, $P=0.21$).

In summary, none of the species was particularly good at processing the higher frequency range of these 1-6 kHz sweeps, and house sparrows were the only species to show any significant match of phase-locking frequency to the 3-6 kHz range of the input frequencies. Nuthatches performed more poorly than the other species to the lower frequencies in the sweeps, particularly when the sweeps were rapid (30 msec).

Two-and Three-Tone Chords

We used four different pure-tone chords as one test of our birds' auditory response to harmonic complexes. There were three notable components to these responses: phase-locking to the input tones, higher harmonics represented in the Fourier transform of the AEP (which were not in the input sound), and phase-locking to the 600 Hz AM envelope. We tested each of these components separately.

Phase-locking to the tones in each of the four chords was calculated using a Fourier transform of the AEP waveform. The data were collected from birds captured from July through December: 6 titmice (with 3 of each sex), 12 house sparrows (with 5 females and 7 males), and 7 nuthatches (with 1 female and 6 males). A repeated measures MANOVA, with phase-locking intensity to each of the tones in the chord as the dependent variables, indicated a strong difference between species ($F_{18,22} = 5.1$, $P = 0.002$), but no sex effect ($F_{9,11} = 2.0$, $P = 0.13$). The species effect results from stronger phase-locking to 1.8 kHz tones in nuthatches compared to titmice or house sparrows but only when the 1.8 kHz tone was presented simultaneously with a 2.4 kHz tone (Table 2). This result is supported by univariate repeated-measures ANOVA's: the intensity of 1.8 phase-locking shows a significant species effect for the *1.8+2.4 kHz* chord ($F_{2.21} = 6.1$, $P = 0.008$) and for the *1.2+1.8+2.4 kHz* chord ($F_{2.21} = 7.3$, $P = 0.004$), but there is no species effect for any other peak, nor is there a significant sex effect for any peak in our input stimuli (all $P > 0.05$).

One possible mechanism that would generate an enhancement of phase-locking to 1.8 kHz tones when coupled with additional tones is lateral inhibition (i.e. the reduction in intensity of phase-locking at frequencies near a given tone – see Discussion). We tested for this using

repeated-measures ANOVA models testing for an effect of stimulus chord type on phase-locking intensity to either the 1.2 kHz or 2.4 kHz tones (each was run separately). We also tested each species separately. Contrary to the lateral inhibition hypothesis, phase-locking intensity to 2.4 kHz tones was not affected by stimulus type (and therefore to the presence of a 1.8 kHz tone) in any species (nuthatch: $F_{2,12}$=0.25, P=0.78; titmouse: $F_{2,10}$=0.68, P=0.53; h. sparrow: $F_{2,22}$=0.21, P=0.81). In contrast, phase-locking intensity to 1.2 kHz tones was significantly affected by stimulus type, but only in the nuthatch (nuthatch: $F_{2,12}$=4.61, P=0.033; titmouse: $F_{2,10}$=0.98, P=0.41; h. sparrow: $F_{2,22}$=0.26, P=0.26). Moreover, phase-locking intensity in nuthatches to the 1.2 kHz tone was significantly *increased* when this tone was coupled to a chord with a 1.8 kHz tone (LSM's±SE, *1.2+1.8kHz* chord: 25.8±2.2 dBnV; *1.2+1.8+2.4kHz* chord: 26.7±2.2dBnV) compared to chords without a 1.8 kHz tone (LSM's±SE, *1.2+2.4kHz* chord: 18.2±2.2 dBnV). The data suggest that, in nuthatches, tones are enhanced by a second tone 600 Hz higher than the first, and there is no indication of a reduction in phase-locking intensity resulting from the presence of any other tone. As such, there is no indication of lateral inhibition.

We ran a separate repeated-measures MANOVA on the dB voltage levels of the Fourier transform peaks of the first two harmonics (3.0 and 3.6 kHz) of the AEP. We deleted data from the *1.2+2.4 kHz* chord from this analysis because no 3.0 kHz harmonic is expected (nor was one found) for this 2-tone sound. The results suggest both a significant difference between species ($F_{12,28}$ = 2.5, P = 0.021) and a significant sex × species interaction ($F_{12,28}$ = 2.3, P = 0.035) but no main sex effect ($F_{6,14}$ = 2.0, P = 0.14). The species effect is clear: nuthatches had the strongest harmonics for both peaks of all stimuli except 3.0 kHz for the *1.2+1.8 kHz* chord (Table 2). This outlier results from the only female nuthatch in our sample. This pattern (higher intensity harmonics for nuthatches) is significant for two peaks when separate ANOVA's are run for each

combination of input stimulus and harmonic (*1.8+2.4 kHz*, 3.6 kHz peak: $F_{2,19} = 3.5$, $P = 0.050$; *1.2+1.8 kHz*, 3.6 kHz peak: $F_{2,19} = 3.8$, $P = 0.041$; species effect in all other ANOVA's: $P > 0.05$). The sex × species interaction results from stronger harmonics in male house sparrows compared to female house sparrows, but no consistent sex differences in titmice.

Finally, our chords with 0.6 kHz spacing between tones (i.e. all but the *1.2+2.4 kHz* chord) generate a 0.6 kHz amplitude modulation. We tested for species- and sex-related differences in the phase-locking to this AM signal. Overall, there was no main species effect on 0.6 kHz phase-locking intensity ($F_{2,21} = 0.8$, $P = 0.45$), but there was a significant stimulus effect ($F_{2,44} = 12.6$, $P < 0.0001$) and a significant stimulus × species effect ($F_{4,44} = 8.8$, $P < 0.0001$). The species were similar for both *1.2+1.8kHz* and *1.8+2.4kHz* chords, and most different for the *1.2+1.8+2.4kHz* chord where house sparrows had a significantly greater AM phase-locking than nuthatches ($t_{27} = 2.8$, $P = 0.010$; all other comparisons: $P > 0.05$; Fig. 10). There was no effect of sex on phase-locking intensity ($F_{1,21} = 0.3$, $P = 0.59$).

In summary, nuthatches strongly phase-lock to 1.8 kHz tones when these tones are presented with 2.4 kHz tones, and their AEP responses included strong harmonics not in the original input. There was no indication that phase-locking at 1.8 kHz inhibited phase-locking at the other two harmonics in the input chords. Indeed, the presence of a 1.8 kHz tone actually increased the intensity of phase-locking to 1.2 kHz. However, these patterns do not extend to the 0.6 kHz AM signal, where nuthatches are either not different than the other species or show weaker AM phase-locking (to the full *1.2+1.8+2.4kHz* chord) compared to house sparrows.

Schroeder-phase complexes

We have auditory responses for 3 titmice (1 male, 2 females), 8 house sparrows (5 males, 3 females), and 3 nuthatches (all males) for both positive and negative Schroeder-phase complexes. The AEP from each bird was cross-correlated with the AEP from each other bird and with the original Schroeder-phase sound file. The matrix of correlation coefficients was then condensed using multiple dimensional scaling. As an index of fit to the sound, we measured the linear distance (in 2-dimensional multidimensional-scaling space) between the birds and the sound.

A graphical depiction of our analyses shows fairly tight clustering of species and sex within species (Fig. 11a,b). While the patterns were slightly different between the positive and negative Schroeder-phase complexes, nuthatches exhibited the smallest distance to the sound for both stimuli (species effect: negative $F_{2,24} = 3.4$, $P = 0.05$; positive: $F_{2,24} = 8.0$, $P = 0.002$). A repeated measures MANOVA conducted on the data shows significant species effects (positive: $F_{7,44} = 18.0$, $P < 0.0001$; negative: $F_{7.94} = 16.9$, $P < 0.0001$), sex effect for one of the stimuli (positive: $F_{2,22} = 3.6$, $P = 0.04$; negative: $F_{2,22} = 2.6$, $P = 0.10$), and a sex × species interaction for both stimuli (positive: $F_{2,22} = 10.7$, $P = 0.0006$; negative: $F_{2,22} = 24.4$, $P < 0.0001$). Given the limited data from nuthatches and titmice, we cannot rule out the possibility that this interaction term may be the result of an outlier. Nonetheless, species clearly differ from one another in response to Schroeder-phase complexes, and male and female house sparrows differ from one another as well.

In summary, the AEP of nuthatches matches the input stimulus better than titmice or house sparrows.

Discussion

Overview of results

We suggested in two earlier papers (Lucas et al. 2002, 2007) that white-breasted nuthatches had relatively simple vocalizations, house sparrows had more complex vocalizations, and tufted titmice vocalizations were intermediate in complexity. While some evidence supports the idea that auditory physiology matches vocal signals (e.g. AEP results from clicks and tones, Lucas et al. 2002, 2007), the results we report here show that auditory performance of these species is much more subtle than our simple generalization suggests. White-breasted nuthatches have poor processing of rapid FM tones and a long ABR latency, but they excel at processing of 1.8 kHz tones embedded in chords with 2.4 kHz tones, they have strong harmonic peaks in response to these chords, and they also excel at processing Schroeder-phase complexes. In contrast, house sparrows perform better than the other two species in processing rapid FM signals for both linear and sinusoidal FM. Finally, tufted titmice have the shortest latency ABR peaks, but they are poor relative to nuthatches and house sparrows in the processing of FM signals (at all modulation rates) and multi-tone chords. Interestingly, this poor performance by titmice supports a conjecture we made earlier (Lucas et al. 2007), based solely on phase-locking to pure tones, that titmice are relatively poor at processing complex sounds but nonetheless relatively sensitive to a broad range of frequencies. Henry and Lucas (submitted) describe audiograms that support this latter statement.

These auditory properties match general properties of these birds' vocalizations. The vocal repertoire of nuthatches is rich in overtones but has very little FM or note diversity (Ritchison 1983). In contrast, the song of the house sparrow has rapid FM and rapid modulation of harmonic overtones (Lowther and Cink 1992). Tufted titmice have a simple, pure-tone song

(Offutt 1965), and a relatively simple chick-a-dee like vocal system (see below). Thus, the correlation between details of auditory physiology and vocal signals implies tight coevolution between signal production and receiver physiology. At the very least, our results underscore that the processing of a diversity of signal properties (Nelson and Marler 1990) may be multidimensional. As emphasized by Møller (2006), our experimental protocol needs to match this level of diversity.

Assumptions of the study

We are assuming in our study that information we derive from AEP's is sufficient to characterize auditory physiology. Put in a slightly different way, we are assuming that onset responses such as the Auditory Brainstem Response and phase-locking to tones – both fundamental properties of the AEP – are more salient to the fine-scale processing of sound than is information derived from the tonotopic mapping of sound in the basilar papilla (see Møller 2006 for a review). This assumption is critical because ABR's and phase-locking are easily measured with AEP's, whereas tonotopic mapping is not. Nonetheless, a number of studies support our assumption. Single-unit studies from the auditory nerve show that neural phase-locking is a primary basis for encoding steady-state speech-like sounds (Young and Sachs, 1979). Subsequent studies have shown that auditory components of a wide variety of properties of human speech are encoded in neural signals measurable in AEP's (Krishnan and Parkinson, 2000; Johnson et al. 2005). Indeed, differences in tonal perception between humans who speak Mandarin (a tonal language) and those who speak English (a non-tonal language) are detectable in the phase-locking properties of AEP's (Krishnan et al. 2005). Humans also perceive a clear musical pitch only at frequencies where phase-locking occurs (below 5 kHz); above this, tone frequency is too high to

elicit phase-locking (Semal and Demany 1990; also see Simmons and Buxbaum 1996). The point is that phase-locking in the auditory periphery carries fine-scale details about an acoustic signal to the cortex.

Conspecific vs. heterospecific call recognition

There is an interesting parallel between our system and the budgerigar/canary/zebra finch system studied by Dooling and colleagues, among others, using behavioral measures of auditory perception. Budgerigars have vocalizations characterized by rapid FM tones without harmonics (Dooling 1986); similar to the rapid FM in house sparrows and the pure tone sweeps in titmice. Like nuthatches, zebra finches have vocalizations rich in harmonics, but only male calls have any appreciable FM (Simpson and Vicario 1990). Okanoya and Dooling (1991) showed that each species in the budgerigar/canary/zebra finch study system discriminates their own versus heterospecific calls and that each species is better at distinguishing different calls of their own species compared to different heterospecific calls. Lohr et al. (2003) found a similar pattern in these three species, although enhanced within-species discrimination was only evident at high signal-to-noise ratios. Dooling et al. (2002) found that zebra finches are better than canaries and budgerigars in detecting positive from negative Schroeder-phase complexes, and that budgerigars are better than canaries. This trend matches the extent of use of harmonics in each species' vocal repertoire. Similar results were described for species-specific responses to human speech sounds: Budgerigars discriminate F3 tones from human vowels better than zebra finches, but zebra finches outperform budgerigars on full formant speech which is characterized by a rich assortment of overtones (Dooling et al. 1995).

Our results are complementary to these behavioral studies. By decomposing "signal space" into its component parts, our data show that auditory filtering in the brainstem can account for some aspects of enhanced conspecific song recognition. For example, the strong match in nuthatches of the AEP waveform in response to Schroeder-phase complexes parallels the enhanced behavioral response to these complex sounds in zebra finches. Importantly, both species feature harmonic overtones in their vocal repertoire. The lack of a strong response to rapid FM tones in both titmice and nuthatches also mirrors properties of their conspecific vocal repertoire. We should note, however, that enhanced conspecific song recognition is not universal. In a test of auditory scene analysis, Hulse et al. (1997) showed that starlings are no better at learning starling song than 4 other species' songs. In this respect, it would be interesting to compare auditory processing in vocal mimics (such as starlings) versus non-mimicking species (including all other species discussed here).

Suppression/facilitation in harmonic overtones

The results from our 3-tone chords raise a related issue. Nuthatches have enhanced phase-locking (compared to house sparrows and titmice) to 1.8 kHz tones when these tones are coupled with 2.4 kHz tones. The highest intensity tones in nuthatch calls are at about these frequencies (Henry and Lucas submitted). Krishnan (2002) found similar results in human phase-locking to vowel sounds which are composed of harmonic overtones. He showed that phase-locking to formants 1 and 2, the formants most critical for detecting vowels, is enhanced compared to other harmonics. Krishnan (2002) suggested that this may result from selective suppression of non-formant harmonics through lateral inhibition. However, our data, at least for the 2- and 3- tone chords, fail to show any sign of lateral inhibition. If anything, the addition of 2.4 kHz tones

increases phase-locking intensity at 1.8 kHz, and the addition of 1.8 kHz tones increases phase-locking intensity at 1.2 kHz – but this pattern was shown only for nuthatches.

These trends may reflect differences in auditory filter characteristics. The peripheral auditory system acts as if it contained a bank of overlapping bandpass filters (Moore 1993). [A bandpass filter is a filter that retains frequencies within a given range above and below some central frequency. Frequencies outside this band are filtered out.] Wider filters in nuthatches (compared to the other species) may result in a spread of excitation enhancing phase-locking of harmonics. We are currently investigating this possibility.

Sex effects

There were a number of patterns in the sex-specific aspects of AEP's. We found no sex differences in any of the FM tones. In contrast, there were sex differences in the intensity of phase-locking harmonics to the 2- and 3-tone chords (male house sparrows had stronger phase-locking than females) and the fit to the Schroeder-phase complexes was different as well. Sex differences in hearing have been described in the literature. For example, in both humans and mice, females tend to have more sensitive hearing than males (Hultzcrantz et al. 2006; Henry 2002). In contrast, zebra finch males are better than females at detecting the presence or absence of second harmonics (Nottebohm et al. 1990) and at heterospecific call discrimination (Okanoya and Dooling 1991). Moreover, males and females in some species appear to respond to different spectral properties in calls (e.g., chickadees: Weisman and Ratcliffe 2004). Our results suggest that some of this sex-specific auditory processing may be occurring in the periphery or brainstem.

Habitat effects

Of the three species used in our study, nuthatches and titmice occupy woodland and house sparrows occupy urban/suburban habitats. The use of relatively low frequencies and simplicity of the song of both white-breasted nuthatches and tufted titmice is consistent with the well-established constraints imposed by propagation of sound through woodland habitats (Wiley 1991; Naguib 2003; Brown and Handford 2000). The songs of both nuthatches and titmice also primarily fall in a frequency range that matches the frequencies of peak phase-locking (1.5 – 3 kHz; Offutt 1965; Ritchison 1983; Schroeder and Wiley 1983). While it may seem obvious to see such a match between song and auditory performance (e.g., Dooling 1982), the house sparrow "chirrup" song has peak energy ranging from 3.5 – 5 kHz (Lowther and Cink 1992). We show here that this is outside the range of frequencies where phase-locking to FM sweeps is maximal. Nonetheless, this high frequency range has been described in a population of urban dark-eyed juncos (*Junco hyemales*) in which frequencies below 3 kHz that are found in forest areas are dropped in urban song (Slabbekoorn et al. 2007). Slabbekoorn et al. (2007) speculated that the increase in minimal song frequencies is selected as a result of enhanced reflection of low-frequency sounds off buildings. House sparrow song would likely be subject to the same selection pressure. However, auditory physiology adds an important component to this scenario because processing of the fine-structure detail in song is facilitated by a robust phase-locking response, as discussed above. Yet we failed to find phase-locking in the frequencies characteristic of song.

There are three possible explanations for the disparity in house sparrows between song properties and auditory properties. One is that the fine structure in song is simply not processed. ABR audiograms of house sparrows (Henry and Lucas, submitted) show sensitivity to

frequencies at least as high as 5 kHz, so there is little doubt that the birds hear these tones. Nonetheless, our phase-locking results suggest that these birds are poor at processing fine details in the song. Of course, the definitive answer will come with psychophysical studies of auditory detection (e.g., Moore 1993; Lohr et al. 2003).

Theunissen and Doupe (1998) provide a second explanation. In zebra finches, frequency cues appear to be less relevant than amplitude envelope cues in the cortical processing of a bird's own song. Thus, the relevant information in the house sparrow song could be in the amplitude envelope and not frequency. We show elsewhere (Henry and Lucas, submitted) that house sparrows can process rapid AM signals and this processing is consistent with AM rates in their song. Of course, there are many birds (e.g. chickadees, titmice and canaries; see discussion above) whose song is primarily tonal, so AM processing cannot be the sole criterion for processing of song in birds. It is currently unknown whether AM components in the house sparrow song carry more information than the FM components.

A third explanation for this disparity is seasonality. The results we report here are for birds captured late summer through winter. We have shown that house sparrows process clicks more strongly in the spring than in winter (Lucas et al. 2002), and that chickadees (but not titmice) phase-lock more strongly in the spring compared to winter (Lucas et al. 2007). This raises the intriguing possibility that a seasonal change in auditory physiology may enhance the ability of house sparrows to process their own song. Sisneros et al. (2004) provide an exceptionally detailed picture of just such a shift in female plain midshipman fish. These females cannot detect the relatively high frequency male "song" in winter, but increased estrogen levels in spring essentially shift the audiogram up in frequency to a range where the song is perceived. Our own work demonstrates significant up regulation of auditory performance during the

breeding season in several avian species (Lucas et al. 2002, 2007). We plan to test the potential for seasonal regulation of auditory processing in the coming year.

Social effects

The poor performance of titmice in our auditory tests is intriguing because titmice are closely related to chickadees (both belong to the family Paridae, Slikas et al 1996). Chickadees have unusually complex vocalizations (e.g. the gargle call, Baker and Gammon 2007), and they have one of the very few examples of a syntactically complex non-song vocal system (the chick-a-dee call) known in any bird (or even any animal; Lucas and Freeberg 2007). Tufted titmice share a chick-a-dee-like vocal system, but it is substantially less complex than the chickadee call, and the spectral complexity of the note types in tufted titmice is far reduced compared to chickadee chick-a-dee note types (Lucas and Freeberg 2007; Owens and Freeberg 2007). The simplicity of the titmouse vocal system compared to the chickadee vocal system appears to be driven by differences in social system (Lucas et al. 2007; see Ord et al. 2002, McComb and Semple 2005, Freeberg 2006). Chickadees have a relatively fluid social system with pair associations in spring and summer followed by territory defense by flocks of 2 – 10 unrelated birds in fall and winter (Smith 1991). The complexity of the chick-a-dee vocalization in chickadees may facilitate rapid coordination of flocks between individuals who have not associated, except perhaps as neighbors, before flock formation (Lucas and Freeberg 2007). Tufted titmice do not show this fluid social system. Instead, kin tend to associate throughout the winter (Pravosudov and Grubb 1993), and these tight social interactions correlate with a simple vocal repertoire (Lucas et al. 2007). Our data, and those reported in Lucas et al. 2007, show that brainstem physiology reflects the relative simplicity of the vocal system.

Summary

In summary, we demonstrate here and in Henry and Lucas (submitted) that we can use sound-induced AEP's to get a broad characterization of auditory filtering of species-relevant auditory signals. The general characteristics of the vocal repertoire, and in some cases the song repertoire in particular, appear to match auditory performance to a variety of FM tones and multi-tone chords. The only exception to this pattern was the lack of phase-locking in house sparrows over the frequency range of the song. We should emphasize that our AEP results give us insight into auditory filtering through the level of the brainstem. Higher levels of processing are not reflected in our measurements. Nonetheless, the filtering properties illustrated by AEP's can give us a robust view of the auditory processing of sound and help us close the gap in our understanding of the receiver side of signaler/receiver systems.

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Table 1. Repeated measures ANOVA for relative phase-locking strength in response to sinusoidally FM tones. Three species and four FM rates were used (see text). The data included in the analyses are mean strengths for each combination of species, FM rate, and frequency range (a dichotomous variable: upper= 2.0-2.3 kHz and lower=1.7-2.0 kHz). ndf = numerator degrees of freedom. ddf = denominator degrees of freedom.

Independent variable	ndf,ddf	F	P
FM rate	3,187	52.3	<0.0001
Species	2,26	4.3	0.024
Frequency range	1,187	235.8	<0.0001
Sex	1,26	0.1	0.77
FM rate × species	6,187	4.5	0.0003
FM rate × freq. range	3,187	16.7	<0.0001
Species × freq. range	2,187	7.2	0.001
Species × FM rate × freq. range	6,187	2.5	0.026

Table 2. (A) LSMean (±SE) phase-locking intensity to each tone in the 2- or 3-tone chords. (B) LSMean (±SE) phase-locking intensity of the 3.0 and 3.6 kHz FFR harmonics generated by the auditory system. All peak intensities are given in dBnV. The left-most column gives phase-locking (FFR) frequency and the chord for which intensity at that frequency was measured (in italics). The data for each combination of species and sex are presented separately. ETTI=titmouse; HOSP=house sparrow; WBNU=nuthatch. Significant outliers in each row (when they exist) are highlighted (see Results). Note: there was only one WBNU female in the sample, hence the lack of SE estimates.

(A)

Variable: FFR freq. / tones in input chord	Peak intensity: ETTI female	Peak intensity: ETTI male	Peak intensity: HOSP female	Peak intensity: HOSP male	Peak intensity: WBNU female	Peak intensity: WBNU male
1.8/*1.8+2.4*	24.7±1.4	26.2±4.6	30.9±2.1	30.2±1.9	35.9	34.7±1.1
2.4/*1.8+2.4*	20.8±0.6	19.7±4.1	20.0±3.9	24.1±2.4	20.7	22.6±1.7
1.2/*1.2+2.4*	22.8±1.5	18.5±6.7	17.8±3.3	20.0±1.6	11.8	21.1±2.4
2.4/*1.2+1.8*	22.5±0.8	22.3±2.9	20.1±3.7	24.5±3.0	20.8	22.3±1.9
1.2/*1.2+1.8*	23.8±2.7	24.7±3.4	21.5±1.6	22.8±1.6	17.0	26.6±1.2
1.8/*1.2+1.8*	25.3±2.3	19.2±5.7	30.0±2.7	31.7±1.8	26.5	27.4±1.6
1.2/*1.2+1.8+2.4*	21.9±5.2	24.6±3.3	17.8±2.0	24.6±1.5	15.6	28.3±0.9
1.8/*1.2+1.8+2.4*	23.8±0.9	24.0±3.6	29.1±2.9	28.7±2.2	34.3	34.2±1.1
2.4/*1.2+1.8+2.4*	19.1±0.6	15.6±4.7	20.3±2.8	21.3±2.4	22.4	19.6±1.2

(B)

Variable: Harmonic freq. / tones in input chord	Peak intensity: ETTI female	Peak intensity: ETTI male	Peak intensity: HOSP female	Peak intensity: HOSP male	Peak intensity: WBNU female	Peak intensity: WBNU male
3.0/*1.8+2.4*	11.6±4.4	11.1±6.6	7.2±4.5	13.4±3.3	22.2	19.0±3.5
3.6/*1.8+2.4*	-3.7±3.5	-5.7±3.6	0.1±2.8	1.9±2.3	9.2	3.2±2.7
3.0/*1.2+1.8*	1.1±5.9	-9.8±8.9	4.7±1.6	6.5±1.3	-6.9	6.3±2.7
3.6/*1.2+1.8*	-13.0±3.8	-2.6±3.4	-6.1±1.0	-2.4±1.3	0.5	-0.9±1.1
3.0/*1.2+1.8+2.4*	12.0±2.5	8.8±6.4	8.5±4.2	13.8±2.4	19.1	18.7±3.2
3.6/*1.2+1.8+2.4*	-3.5±4.7	-4.2±1.5	-1.0±3.9	1.1±1.9	3.3	1.1±2.4

Figure Legends

Figure 1. Representative AEP waveform (top) and spectrogram (bottom) derived from the waveform view. These are from (a) a nuthatch and (b) a house sparrow. Both were played a 110 Hz sinusoidal FM tone.

Figure 2. Examples of spectrograms of calls from (A) nuthatch, (B, C) titmice and (D) house sparrows. The frequency range (Y axis) is 0-11 kHz. Time (X axis) varied between calls.

Figure 3. LS means (± SE) ABR peak P1 latency as a function of LS means (± SE) peak P1 amplitude for titmice (closed squares), nuthatches (closed circles), and house sparrows (open triangles).

Figure 4. Representative phase-locking frequency as a function of time from a 20 Hz sinusoidal FM signal played to a house sparrow. The input stimulus was adjusted so that time=0 is when the sound reaches the tympanum.

Figure 5. LS means (± SE) phase-locking latency to match sinusoidal FM signals. Latency was estimated from cross correlation between AEP phase-locking frequency and the input frequency. Symbols with the same letters are not significantly different (α =0.05) based on post hoc tests (see Methods).

Figure 6. LS mean (± SE) phase-locking strength in response to sinusoidal FM tones plotted as a function of frequency modulation rate. Data are given separately for (A) input frequencies from 2.0 to 2.3 kHz, and (B) input frequencies from 1.7 to 2.0 kHz. See Fig. 3 for symbol definitions.

Figure 7. LS mean (± SE) phase-locking intensity at 110 Hz in response to a 110 Hz sinusoidal FM tone (i.e., phase-locking to the FM signal itself).

Figure 8.(A) LS mean (± SE) slope of the relationship between phase-locking frequency and

input frequency for linear FM sweeps, and (B) LS mean (± SE) intercept of the relationship between phase-locking frequency and time. Four sweep types (slow/up, fast/up, slow/down, fast/down) are indicated. In (A), a slope of 1.0 indicates a perfect match between phase-locking frequency and the frequency of the input stimulus. In (B), the Y-axis (left axis) represents FM upsweeps; the Z-axis (right axis) represents downsweeps. See Fig. 3 for symbol definitions.

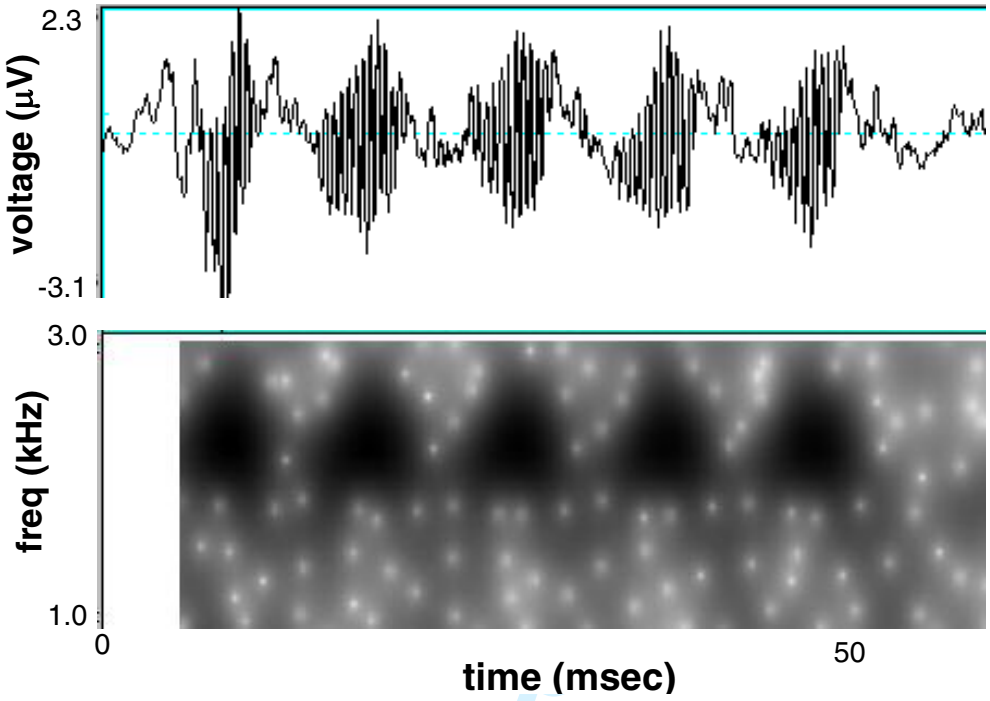
Figure 9. Estimated frequency (LS means± SE) for the linear FM sweep where the strength of phase-locking is maximal. Data for slow (50 msec) and fast (30 msec) sweeps are given separately. See Fig. 3 for symbol definitions.

Figure 10. Phase-locking intensity (LS means± SE) to the 600 Hz AM signal for the 2- and 3-tone chords. Note: lines are added to facilitate comparison of species.

Figure 11. First two dimensions from a multidimensional scaling of the cross-correlations between AEP waveforms of birds and the input Schroeder phase complex sound. Two types of Schroeder phase complexes were used: (A) negative Schroeder phase complex and (B) positive Schroeder phase complex. Each symbol represents a separate bird. Males (M) and females (F) are indicated next to the symbol: titmice (closed squares), nuthatches (closed circles), house sparrow (open triangle).

Figure 1

(A) white-breasted nuthatch



(B) house sparrow

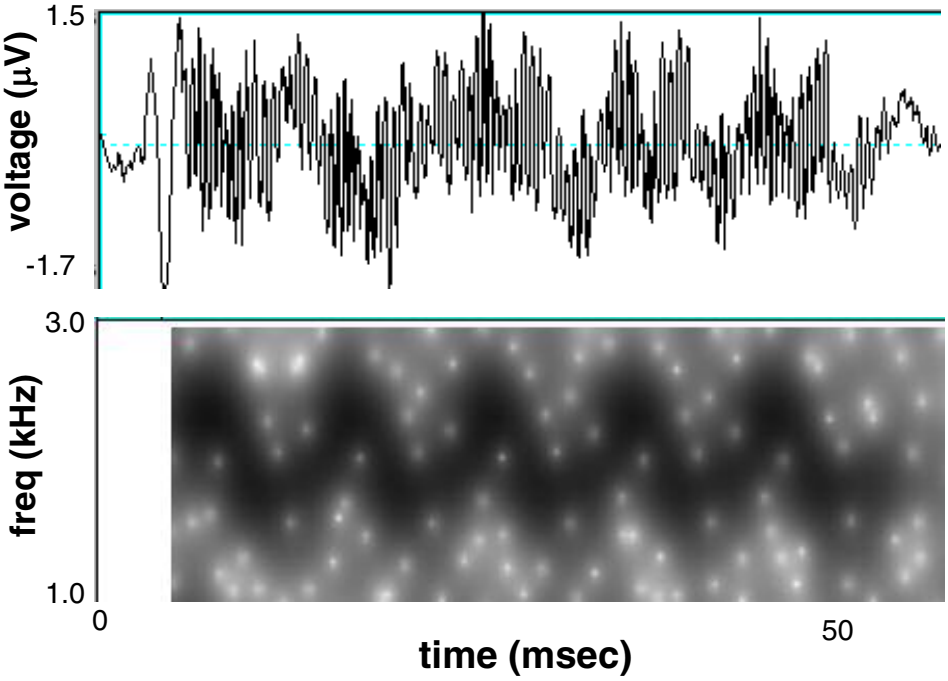
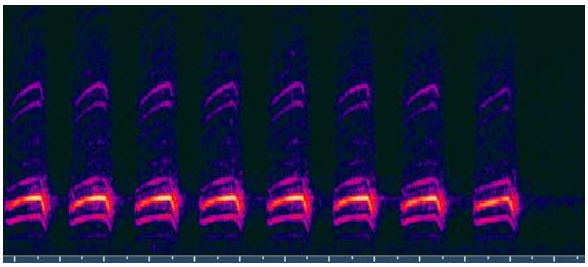
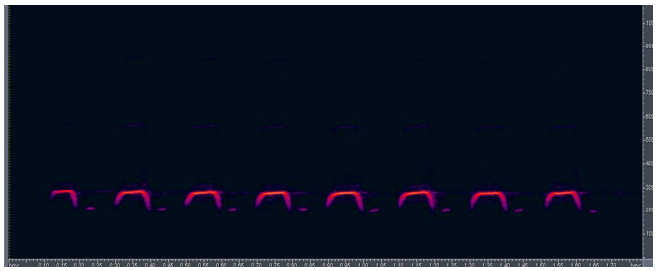


Figure 2

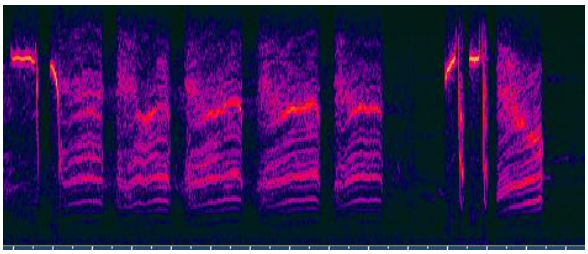
(A) white-breasted nuthatch song



(C) tufted titmouse ‘peter-peter’ song



(B) tufted titmouse ‘chick-a-dee’ call



(D) house sparrow ‘chirrup’ song

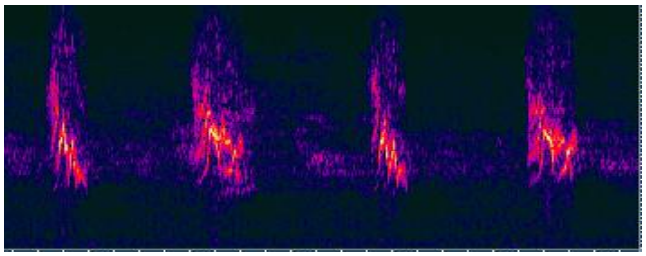


Figure 3

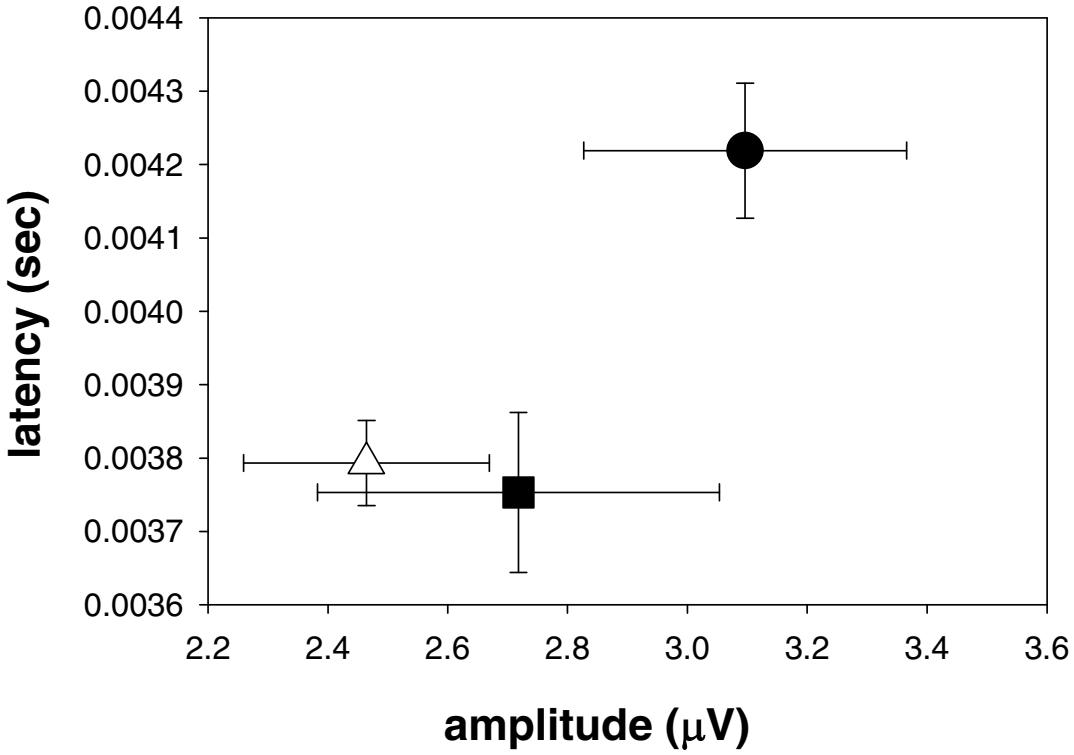


Figure 4

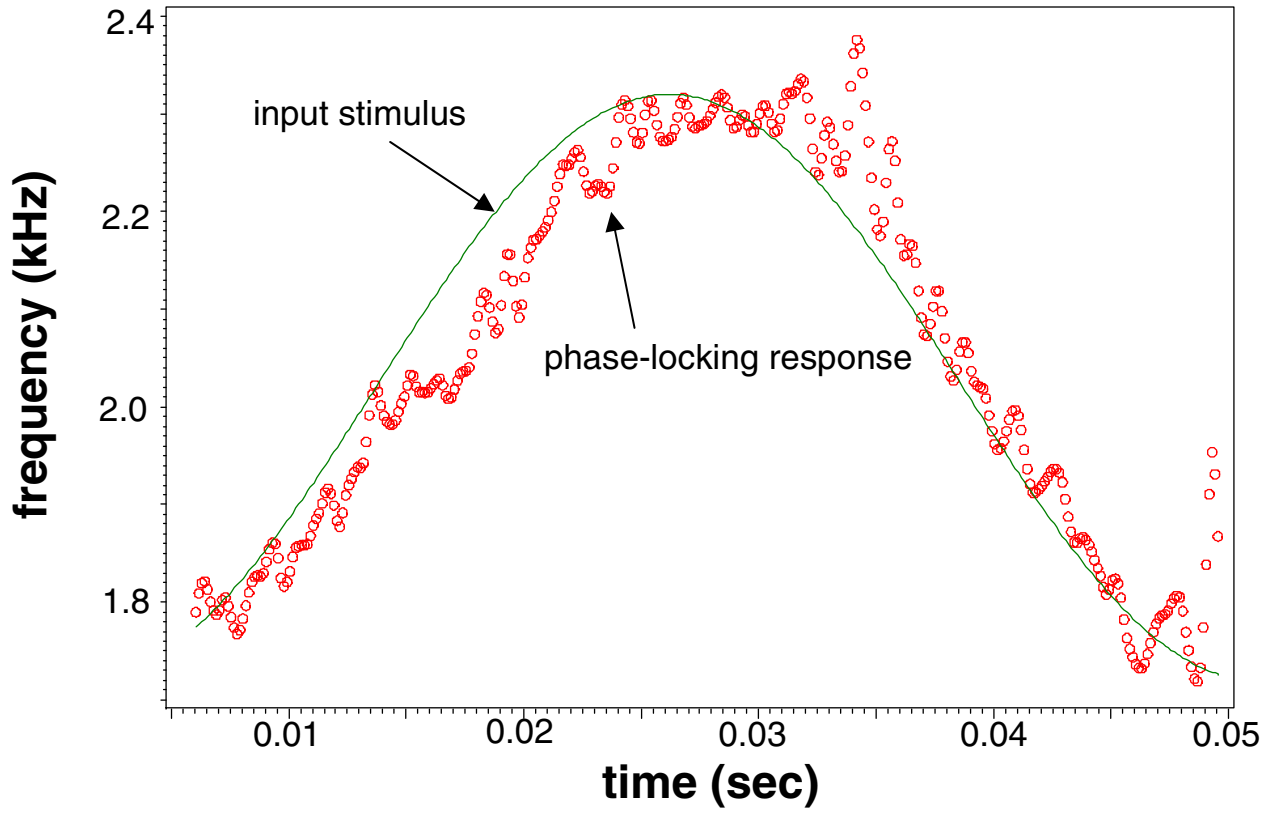


Figure 5

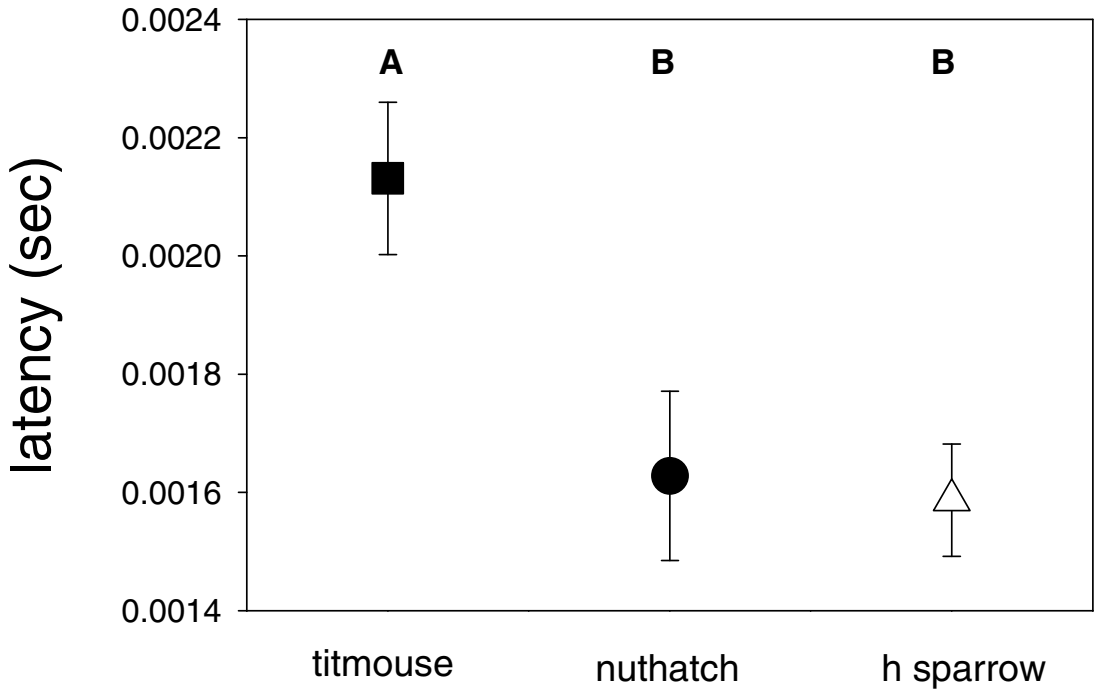
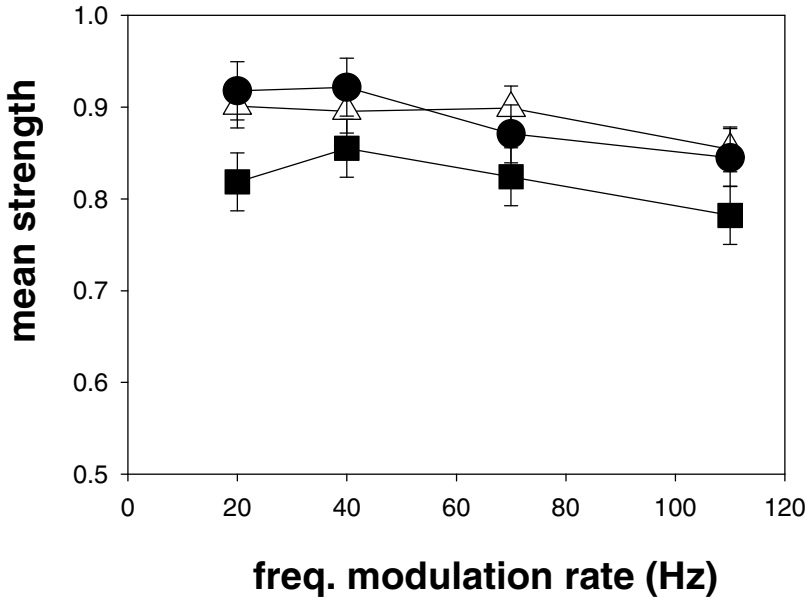


Figure 6

(A) 2.0 – 2.3 kHz



(B) 1.7 – 2.0 kHz

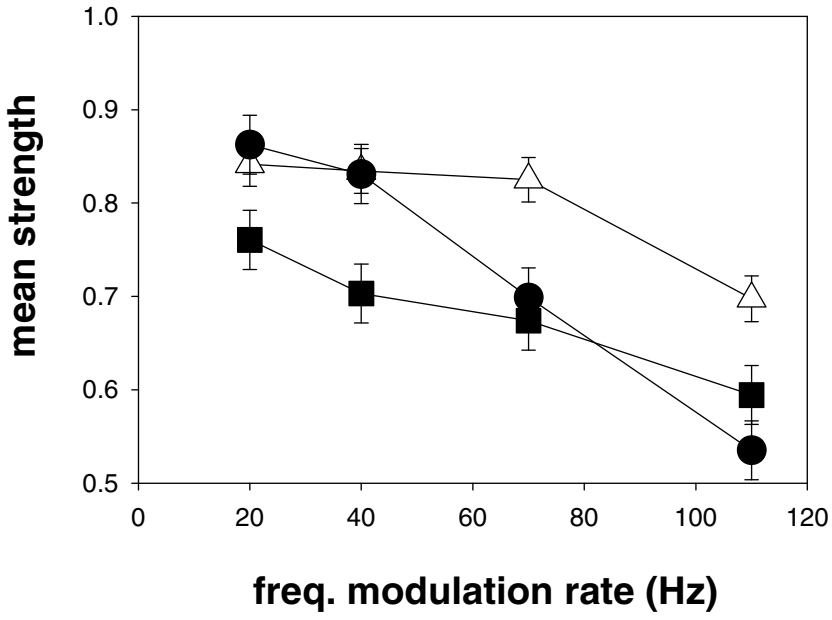


Figure 7

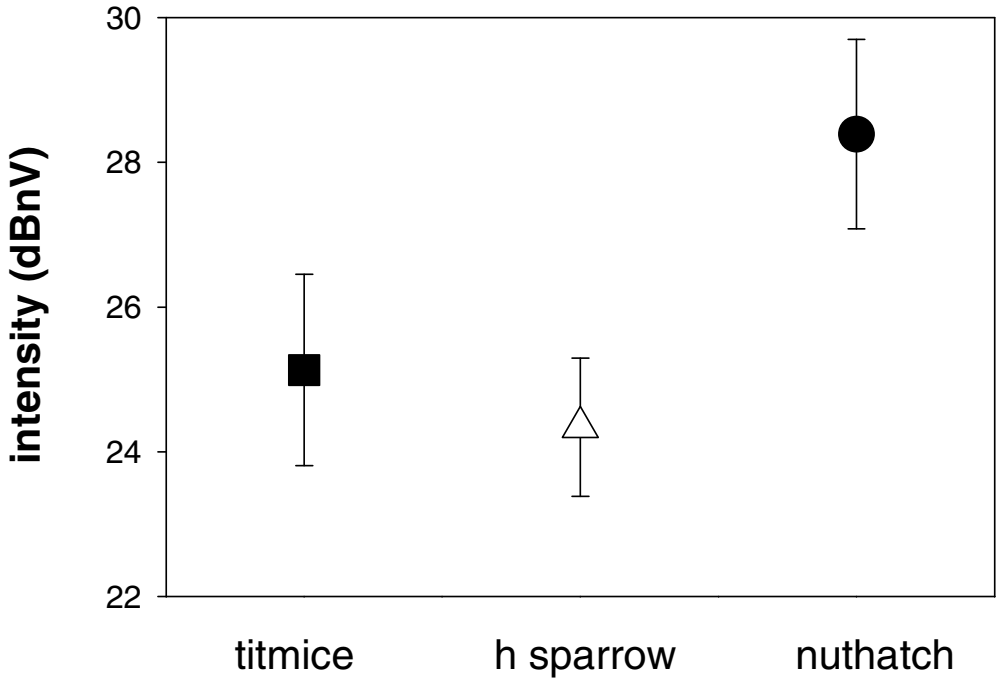
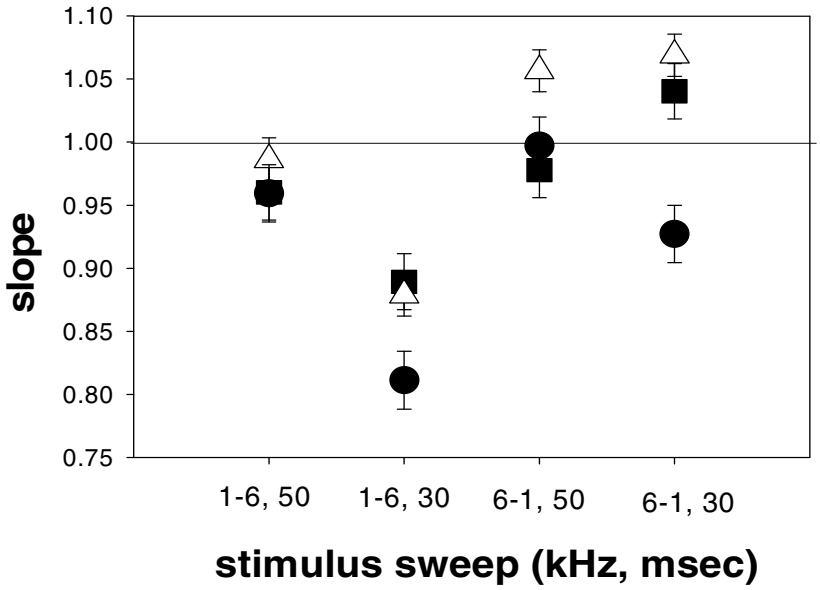


Figure 8

(A)


1.10
1.05
1.00
0.95
0.90
0.85
0.80
0.75
slope
1-6, 50
1-6, 30
6-1, 50
6-1, 30
stimulus sweep (kHz, msec)

(B)

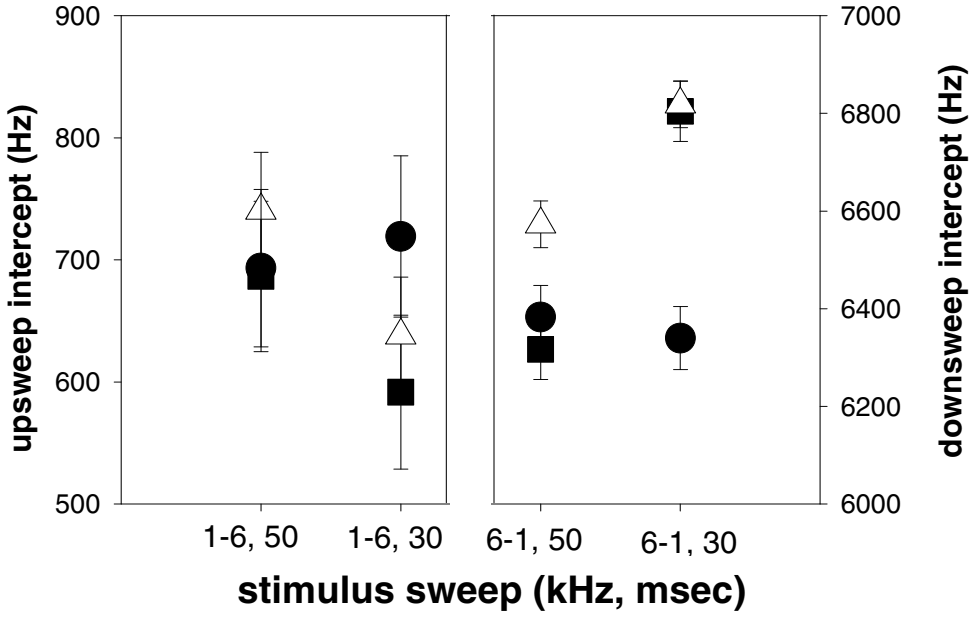

900
800
700
600
500
upsweep intercept (Hz)
7000
6800
6600
6400
6200
6000
downsweep intercept (Hz)
1-6, 50
1-6, 30
6-1, 50
6-1, 30
stimulus sweep (kHz, msec)

Figure 9

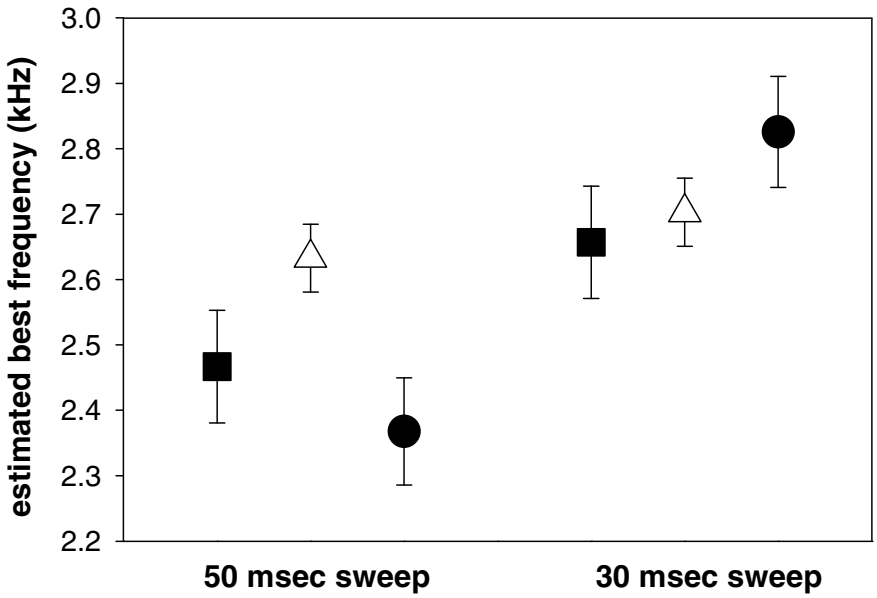


Figure 10

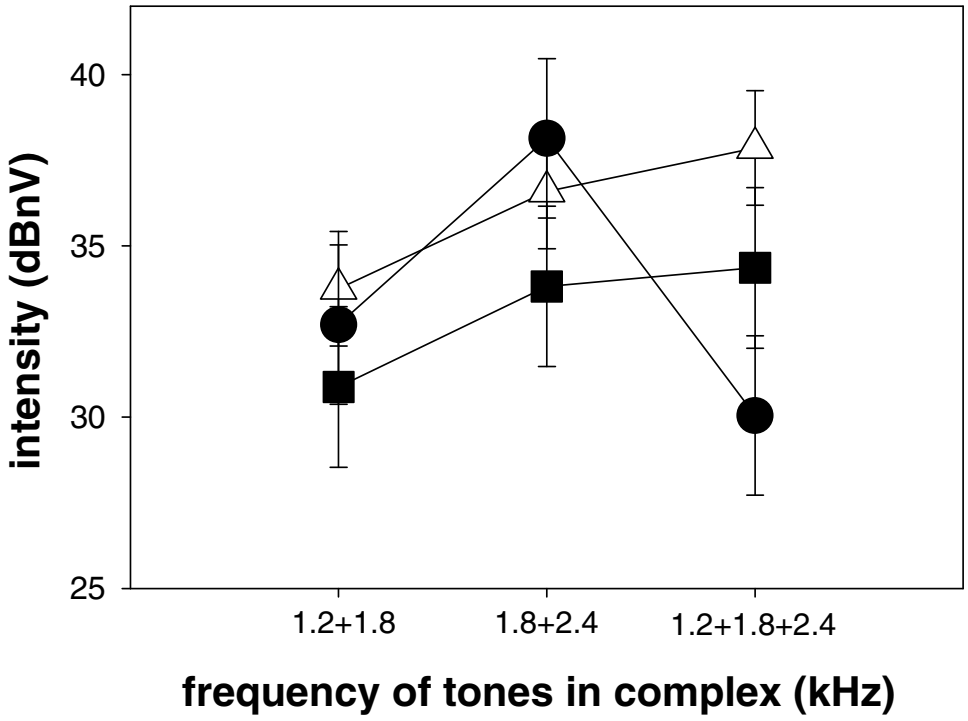
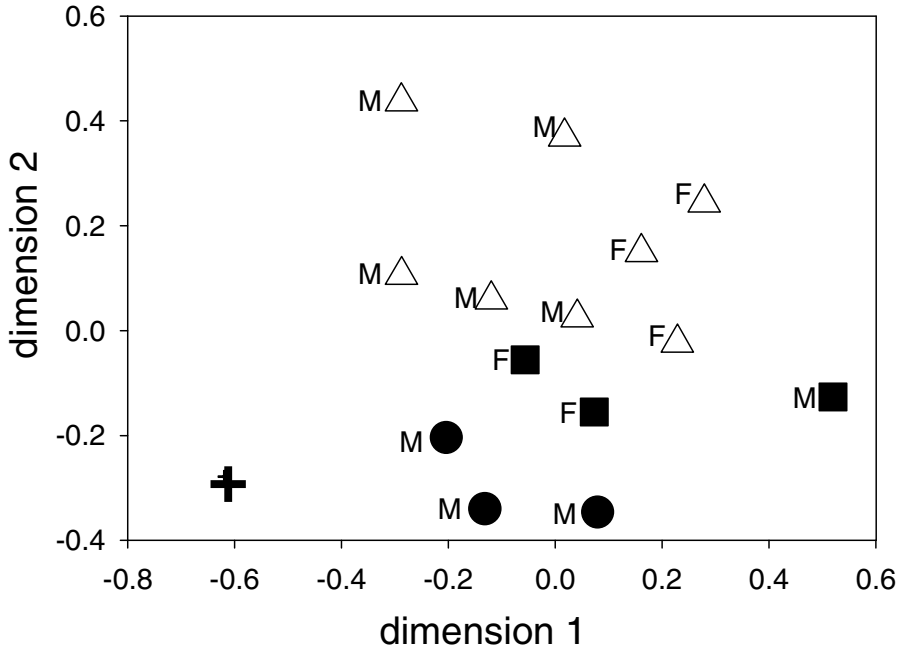


Figure 11

(A) negative Schroeder-phase waveform



(B) positive Schroeder-phase waveform