TITLE: Auditory Evoked Potentials to Complex Stimuli in Three Songbirds

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ABSTRACT

We characterized the processing of acoustic signals using auditory evoked potentials (AEPs) in response to frequency modulated (FM) tones, and 2- and 3- tone chords. Auditory brainstem responses (ABRs) were measured for FM tones, and frequency-following response (FFR) amplitude and FFR frequency were measured for all input stimuli. Comparisons of AEPs from tufted titmice, white-breasted nuthatches, and house sparrows indicate that auditory processing enhances most species-specific properties of vocal signals. Nuthatches had weak FFR amplitudes in response to rapid FM tones and a long ABR latency. However, they were the only species where higher frequency tones in chords increased FFR amplitude in response to lower frequency tones and they had strong FFR harmonic peaks in response to these chords. Sparrows had the strongest FFR in response to rapid FM signals, but surprisingly showed a weak FFR amplitude in the frequency range of their song (3.5-5 kHz). Titmice had the shortest latency ABR peaks, but had weak FFR amplitudes in response to 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. We discuss these results in light of species-specific vocal patterns, habitat use, and social behaviour.

Keywords: Auditory evoked potential (AEP), frequency following response (FFR), Auditory Brainstem Response (ABR), bird hearing, vocal complexity, frequency modulation, tufted titmouse (*Baeolophus bicolor*), white-breasted nuthatch (*Sitta carolinensis*), house sparrow (*Passer domesticus*)

The reigning paradigm for understanding communication systems is a focus on the sender and receiver of a signal (Bradbury & 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 & Slabbekoorn 2004). This is particularly true of vertebrates with complex acoustic signals. 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, 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 are composed of a myriad of properties (Nelson & 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 temporal properties of a complex signal the receiver uses 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); 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 (AEPs) measured from a variety of input stimuli can help bridge the gap between our depth of understanding of sender and receiver coding. AEPs are voltage changes, measured with surface electrodes on the scalp (Hall 1992), resulting from hair cell (i.e., cochlear) or neural (i.e., auditory nerve, brainstem, and possibly midbrain) activity caused by acoustic input. The two most basic characteristics of the AEP are an onset response (called the Auditory Brainstem Response, or ABR) and a sustained (or phase-locking) response to tonal inputs where populations 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 (e.g. *P1* and *P-1* in Fig. 1c) 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). 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. Therefore, we will refer to the sustained response as the FFR.

The value of AEPs for studies of communication was nicely illustrated by Kraus & Nicol (2005), who showed that consonants in human speech are encoded with the onset response whereas vowels are encoded with the FFR. 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 & Nicol 2005, as first described by Ananthanarayan 1999). This fact makes

AEPs extraordinarily powerful indices of the processing of auditory signals. We will focus primarily on the FFR here.

In addition to human speech (Cunningham et al. 2002; Krishnan 2002), AEPs have been used to characterize audiograms in birds (Brittan-Powell et al. 2002, 2005; Henry & Lucas 2008), 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), gerbils (Dolphin & Mountain 1993), and birds (Henry & Lucas 2008), auditory filter shape in porpoises (Popov et al. 2006), detection of amplitude envelopes in mice (Henry 2002), processing of clicks on tonal backgrounds in humans (Junius & 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 AEPs from three species: tufted titmice (*Baeolophus bicolor*), white-breasted nuthatches (*Sitta carolinensis*), and house sparrows (*Passer domesticus*). Three kinds of input stimuli (sinusoidal frequency modulated tones, linear frequency sweeps, and 2- and 3-tone chords) 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 properties of species-specific vocalizations should correlate with species-specific auditory responses to our input stimuli. In particular, the nuthatch auditory system should be most responsive to chords; the house sparrow auditory system should be most responsive to rapid frequency modulation; and titmice should be relatively 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

The three species we used in our study are not closely related taxonomically. Tufted titmice are members of the family Paridae, superfamily Sylviodea (Sibly & Ahlquist 1990). Nuthatches are in the family Sittidae, superfamily Muscicapoidea (Jønsson & Fjeldså 2006). House sparrows belong to the family Passeridae in the superfamily Passeroidea (Sibly & Ahlquist 1990).

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 & Wiley 1983), with a chick-a-dee-like vocalization that lacks the note complexity characteristic of chickadees (Owens & 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 & Cink 2006; Henry & Lucas 2008). They also use several different contact calls, one of which incorporates fairly rapid trills.

AEP responses to broad-band 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., the envelope-following response to AM signals at different AM rates) in all three species are described in Henry & Lucas (2008).

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 limited our sampling to the non-breeding season, July through January (for FM signals) or July through December (for chords), to avoid the confounding effect of seasonal changes in AEPs (Lucas et al. 2002, 2007). Bird weights (means±SD) on day of capture were as follows: titmice females = 21.0 ± 1.1g (n=5), titmice males = 21.6 ± 0.7g (n=5), house sparrow females = 25.3 ± 2.6g (n=5), house sparrow males = 27.6 ± 2.6g (n=9), nuthatch females = 19.6g (n=1), and nuthatch males = 20.9 ± 1.5g (n=7).

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; juvenile status in summer through October was determined using outer retrix 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 10 min (e.g., eyes open or wings flapping), the bird was not tested. The data from a bird was also not used if the bird woke up before testing finished. A total of three house sparrows and three nuthatches were 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 at 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 or removing layers of towel.

The test chamber consisted of a 1.2 ×1.4 ×1.2 m box lined with acoustic tile and 7.2 cm-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

174 7 K for the test to proceed. If the impedance was too high, the electrodes were repositioned to
175 ensure impedances below threshold.

176 Stimuli were calibrated to within ± 2 dB SPL at all relevant frequencies using a Bruel and
177 Kjaer model 1613 Precision Sound Level Meter and model 4131 2.6-cm condenser microphone
178 placed at the approximate location of the bird's ear. We tested the frequency output of the system
179 using a Sennheiser ME62 microphone run through a Marantz PMD690 digital recorder.

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

186 The analyses of input stimuli are broken into two sets of experiments based on input
187 stimuli: frequency modulated tones, and 2- and 3- tone chords. Sound files were constructed in
188 Praat (ver 4.6; Boersma 2001) using the "create sound from formula" option. Sounds were
189 filtered with Cool Edit Pro (ver 2.0) graphic equalizer to ensure that the signal was 80 dB SPL at
190 all frequencies. All input stimuli had 3 ms $\cos^2$ rise/fall times. The FM signals were presented at
191 11.13 stimuli/sec; the 2- and 3-tone chords were presented at 13.13 stimuli/sec. AEPs were
192 sampled at 40 kHz with a response amplification of 200k, high-pass filtered at 100Hz, and low-
193 pass filtered at 10kHz with a notch filter at 60 Hz. AEP waveforms used in our analyses were
194 based on averages of 500 stimulus presentations, and two waveforms (replicates) were collected
195 for each stimulus. Each experimental set will be addressed in a separate section where we
196 describe the test stimuli, stimulus-specific statistical methodology and results.

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 ANOVAs, and non-significant terms were deleted in order of decreasing F value. Normality of residuals and homogeneity of variances were tested using Proc Univariate (SAS for Windows, ver 9.1.3). In all cases, our analyses conformed to the assumptions of the repeated measures ANOVA so no transformations were used. 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. Note that least squares means are useful in describing patterns associated with a specific independent variable (e.g. effect of frequency on FFR amplitude) holding other factors (e.g. sex, individual effects, etc.) constant.

Ethical note: This work was approved 19 July, 2006 by the Purdue University Animal Care Committee under IACUC no. 05-058. Birds were kept in aviaries for at most 3 days before being released at the site of capture.

EXPERIMENT 1: FREQUENCY MODULATED TONES

FM stimuli

We used two classes of frequency-modulated (FM) tones. (1) **Sinusoidal FM tones:** four types were used that varied in modulation rate. Each type ranged from 1.7 to 2.3 kHz with an onset frequency of 1.7 kHz. Four FM rates were used: 20, 40, 70, and 110 Hz, each with a 50 msec duration. Pure tones in this frequency range elicit a strong FFR in these species (Lucas et

al. 2007). 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. (2) **Linear FM sweeps:** four types were used that varied in sweep direction and sweep rate. Two 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 & 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.

FM 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. 1c). 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 sinusoidal FM sounds, and we estimated peak amplitude using the mean difference in voltage between P1 and the first negative peak (P-1). We limited ABRs to only the sinusoidal FM sounds because these sounds generated a robust ABR and all of these sounds began at the same frequency. Note that taking the mean of several ABR peaks gives a more robust estimate of the ABR because it combines 8 ABRs (4 stimuli × 2 replicates), although we obviously cannot distinguish ABR properties for each individual stimulus type.

Second, the frequency of the FFR over time was extracted from AEP responses 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 the FFR 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 (see Fig 4b for an example). The autocorrelation strength reflects the degree of periodicity for a specific frequency, and ranges from 0 (aperiodic) to 1 (perfect sinusoidal periodicity).

Both the input stimulus and the resulting AEP waveform are functions of time. We estimated the time delay in the input function that would result in a minimal squared deviation between the frequency of the input stimulus and FFR functions (in effect shifting the input stimulus in Fig. 4a to the right until it best-fit the FFR response). The magnitude of the time shift is the latency of FFR to input.

Third, FFR amplitude to the FM rate itself was derived from a power spectrum generated by Fast Fourier transform of the entire AEP waveform. We measured this for 110 Hz FM signals only because there were 5 full cycles at this rate and fewer at all other rates (note: the duration of all stimuli was 50 msec).

For responses to linear sweeps, we estimated the average strength of the FFR for each combination of sweep direction and duration. On visual inspection of the data, strength was much lower at frequencies above 3 kHz than below this, so we used frequency range (1 – 3 kHz vs. 3 – 6 kHz) as an independent factor in the analysis.

The frequency of the FFR approximately matches input frequency after some time delay. We estimated the frequency-dependent properties of the delay by regressing FFR frequency as a

function of input frequency (see Fig. 7c for an example). A slope of less than 1 for the upsweep stimulus results from an increasing delay with an increase in frequency (as in Fig. 7c). A slope of greater than 1 for the downsweep indicates the same: longer delays at higher frequencies. These regressions were only calculated for the lower frequency range (1 – 3 kHz), and data were included in the model only if the FFR strength was > 0.70. This latter criterion assures that the FFR frequencies are accurate. Regression analyses were conducted separately for each replicate of each bird.

Finally, we used the strength of the autocorrelation as a function of input frequency to estimate the frequency where FFR is strongest during the linear sweeps. We estimated this for the frequency upsweeps by best fitting FFR strength as a function of input frequency using Loess regression, then estimating the frequency where the regression line peaked (see Fig. 8a for an example).

FM 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 stimulus inputs (except those starting at 6 kHz) generated a robust ABR (e.g., Fig. 1). 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).

FM results: FFR for sinusoidal FM stimuli

Our results show a significant difference between species in the latency between input frequency and FFR frequency ($F_{2,26}$ = 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. 4c). 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 (all P>0.05).

The latency effects in Fig 4c were calculated based on the fit between the entire input stimulus and the resulting AEP. However, latency in Fig. 4a appears shorter for the frequency downsweeps than for the upsweeps. We tested for asymmetries in latency with sweep direction for 20 and 40 Hz FM rates (where the FFR was strongest). Our results show a strong effect of sweep direction on latency ($F_{1,22}$ = 104.0, P<0.0001) with upsweep latency almost twice the duration of downsweep latency (Fig. 4d). There was also a significant species × sweep-direction interaction ($F_{2,22}$ = 4.5, P=0.023): species differences in latency were greater for downsweeps than they were for upsweeps (Fig. 4d). Sex effects on latency were not significant ($F_{1,21}$ = 0.3, P=0.6) and no interaction terms including sex were significant (all P>0.05).

The species also differed in the relative strength of the FFR to the sinusoidal FM tones. 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 FFR strength than titmice (strength = 0.75 ±0.02; $F_{2,27} = 4.6$, P = 0.019). In addition, the FFR was stronger above 2 kHz than below this frequency in all three species (see Fig. 1a for an example). We therefore added a dummy variable to our analysis distinguishing between upper (>2 kHz) and lower (<2 kHz) frequencies. 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). FFR strength above 2 kHz was greater for nuthatches and house sparrows than for titmice, with little effect of FM rate on strength (Table 1; Fig. 5a). In contrast, FFR strength below 2 kHz decreased in strength with increasing FM rates (Table 1; Fig. 5b). This pattern was strongest in nuthatches, as can be seen in the spectrograms of the AEP waveforms: the strength of low frequency FFR decreased more in nuthatches (Fig. 1a) than in house sparrows (Fig. 1c).

The birds generated an FFR to the 110 Hz FM rate itself; this can be seen as the shifting AEP baseline voltage in Figs. 1a and 1c. The amplitude of the FFR to FM rate varied between species ($F_{2,26} = 3.4$, P=0.050). Nuthatches showed relatively strong FFR to 110 Hz FM rates (Fig. 6; 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 had a strong FFR at low FM rates. Titmice showed the longest latency and weakest FFR of the three species. For the high frequency range of the FM tones (> 2kHz), FFR strength was independent of the stimulus modulation rate. For the lower range of frequencies in the FM tones (<2 kHz), only the house sparrow had a strong FFR at high FM rates. Nonetheless, nuthatches show relatively strong FFR to the FM rates themselves at these high FM rates.

334

335 *FM results: FFR to linear FM sweeps*

336 We first focus on the strength of the FFR in response to linear sweeps. This tells us how
337 strongly the auditory system follows each increment in frequency change throughout the sweep.
338 Not surprisingly, there was a strong frequency-range effect ($F_{1,25} = 920$, $P < 0.0001$), although
339 this was complicated by a significant sweep-type × frequency-range interaction ($F_{3,73} = 156$, $P <$
340 0.0001). Upsweeps had stronger FFR's than downsweeps at low frequencies (Fig. 7a), but
341 upsweeps had weaker FFR's than downsweeps at high frequencies (Fig. 7b).

342 While there was no significant main species effect ($F_{2,24} = 2.3$, $P = 0.12$), there was a
343 significant interaction between species, sweep-type, and frequency-range ($F_{6,73} = 3.1$, $P = 0.010$).
344 Where differences were significant, house sparrows showed a slightly stronger FFR within
345 sweep-type/frequency-range treatments (Fig. 7a,b). No sex differences were found ($F_{1,24} = 0.6$, P
346 = 0.43; no significant interactions with sex: all $P > 0.10$).

347 The slope of the regression of FFR frequency as a function of input frequency was used to
348 estimate the frequency-dependent latency of FFR rate (see Fig. 7c). Repeated measures
349 ANOVA's indicate a significant species effect ($F_{2,28} = 11.7$, $P = 0.0002$), sweep-type effect ($F_{3,85}$
350 = 51.0, $P < 0.0001$) and species × sweep-type interaction ($F_{6,85} = 5.9$, $P < 0.0001$). For
351 upsweeps, the slope of the regression was less than 1.0 for all species and sweep duration
352 combinations (Fig. 7d). These results indicate a longer latency at higher frequencies (as
353 illustrated in Fig. 7c). There were no significant species differences for the 50 msec upsweep
354 (Fig. 7d). In contrast, nuthatches showed a significantly lower slope than both house sparrows
355 and titmice (fig. 7d) for the 30 msec upsweep.

The results are mixed for downsweeps. House sparrows showed a slope greater than 1.0 for both sweep durations (Fig. 7d), indicating longer delays at higher frequencies. For titmice and nuthatches, the slopes were approximately 1.0 for the 50 msec downsweep and less than 1.0 for the 30 msec downsweep. Thus in titmice and nuthatches, the delays increased as frequency dropped during the sweep.

Our results suggest that the species do not differ overall in frequency where the FFR strength is maximal ($F_{2,21} = 0.9$, $P = 0.41$; see Fig. 8a for an example). However, the 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. 8b). 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. 8b). 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 as reflected in a weak FFR strength. The relationship between FFR rate and input frequency indicated an asymmetry in the latency of the FFR in titmice and nuthatches: latency was longer as frequency increased on the upsweeps, but latency was shorter as frequency increased on the downsweeps. No such asymmetry was observed in titmice.

EXPERIMENT 2: TWO- AND THREE-TONE CHORDS

Chord stimuli

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 shares properties with nuthatch song and some house sparrow contact calls.

Chord statistical design

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 and read into Praat software (ver. 4.6; Boersma, 2001). The strength of the FFR in response to stacked overtones was analyzed using a power spectrum generated from a Fast Fourier Transform ('Spectrum" in Praat) with a Nyquist frequency of 20 kHz and a resolution of 8 Hz. The power spectrum yields FFR amplitude at each frequency in dBnV.

We measured the amplitude of FFR to each frequency in the stimulus. A repeated measures MANOVA (Proc GLM in SAS) was used to test for species, sex, and chord-type effects because the dependent variables (FFR amplitude to each tone in the chord) were multivariate. If the repeated measures MANOVA was significant, we ran univariate repeated measures ANOVAs to identify specific patterns for each separate tone in the chord. In addition to FFR to the input tones, harmonics are often present in the FFR response even if they are not found in the original signal (e.g., Galbraith 1994; Henry 1997). We ran separate analyses for the first two harmonics (3.0 and 3.6 kHz) with a statistical design similar to our analysis of FFR 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 amplitude of the FFR in response to AM using a repeated measures ANOVA (Proc Mixed in SAS).

Chord results

The data on FFR to chords 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 FFR amplitude 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 FFR 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 ANOVAs: the amplitude of the FFR to the 1.8 kHz tone 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. There was no significant sex effect for any peak in our input stimuli (all P >0.05).

We tested for possible interaction effects between tones in each chord using repeated measures ANOVA models. Specifically, we tested for an effect of stimulus chord type on FFR amplitude to either the 1.2 kHz or 2.4 kHz tones (each was run separately). We also tested each species separately. FFR amplitude 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, FFR amplitude 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,

FFR amplitude 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±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±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; there is no indication of a reduction in FFR amplitude resulting from the presence of any other tone.

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 for an outlier at 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 amplitude harmonics for nuthatches) is significant for two peaks when separate ANOVAs are run for each combination of input stimulus and harmonic (*1.8+2.4 kHz* chord, 3.6 kHz peak: $F_{2,19}$ = 3.5, P = 0.050; *1.2+1.8 kHz* chord, 3.6 kHz peak: $F_{2,19}$ = 3.8, P = 0.041; species effect in all other ANOVAs: 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, we tested for species- and sex-related differences in the FFR to the 600 Hz AM signal generated by our 2- and 3- tone chords. Overall, there was no main species effect on FFR amplitude to 600 Hz AM ($F_{2,21}$ = 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 FFR strength than nuthatches (t_{27} = 2.8, P = 0.010; all other comparisons: P >0.05; Fig. 9). There was no effect of sex on FFR amplitude ($F_{1,21}$ = 0.3, P = 0.59).

In summary, nuthatches showed a strong FFR in response 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. The presence of a 1.8 kHz tone also increased the amplitude of the FRR to 1.2 kHz, but only in nuthatches. 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 FFR strength (to the full *1.2+1.8+2.4kHz* chord) compared to 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 species differences in auditory performance are much more subtle than our simple generalization suggests. White-breasted nuthatches have weak FFRs in response to rapid FM tones and a long ABR latency, but they exhibit stronger FFRs to tones when higher frequencies are added to the stimulus. Nuthatches also have strong harmonic peaks in response to these chords. In contrast, house sparrows perform better than the

other two species in processing rapid FM signals for both linear sweeps and sinusoidal FM. Finally, tufted titmice have the shortest latency ABR peaks, but they have generally weaker FFRs relative to nuthatches and house sparrows in response to 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 the FFR to pure tones, that titmice are relatively poor at processing complex sounds but nonetheless relatively sensitive to a broad range of frequencies. Henry & Lucas (2008) describe audiograms that support this latter statement.

These auditory properties match general properties of species-specific 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 & Cink 2006). Tufted titmice have a simple, pure-tone song (Offutt 1965), and a relatively simple chick-a-dee like vocal system with broad-band elements that are less structured than typically seen in chickadees (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 the contention that the processing of a diversity of signal properties (Nelson & Marler 1990) may be multidimensional. As emphasized by Møller (2006), experimental protocol needs to match this level of diversity.

The three species we tested were similar in that all showed maximal FFR amplitude at 2 - 3 kHz in the linear FM sweeps. These results are consistent with data reported in Lucas et al. (2007) on FFR to pure tones and on ABR-derived audiograms reported in Henry & Lucas (2008). However, the mechanisms underlying these two observations may not be identical.

Indeed, there is evidence that the processing of pure tones may be different from the processing of tone sweeps (Sek & Moore, 1999). In particular, detection of tone sweeps (or glides) is dependent on a number of factors, including frequency range relative to auditory filter widths (ERB's), FM rates, and filter asymmetry, phaselocking vs. excitation pattern in generating the auditory signal, and center frequency of the sweep (Sek & Moore, 2000; Thyer & Mahar, 2006). For example, FM sweeps whose range exceeds the width of auditory filters are harder to detect than those that vary over a range that falls within the filters. Thus, auditory filter properties should determine the auditory performance in response to frequency modulation, in addition to a simple response to specific tones.

The species differences we found in frequency-dependent latencies in the linear sweeps suggests, in part, that the species vary in filter width. We have independent data based on filter widths derived from notched noise that house sparrows have wider filters than the other two species (Henry & Lucas, in preparation). Filter width (ERB) at 2 kHz is about 500 Hz in house sparrows, 350 Hz in titmice and 300 Hz in nuthatches. These widths fit the data on sinusoidal FM well: the 1700-2300 Hz FM signal was within about 1 ERB for the sparrow but greater than 1 ERB for the other two species. Significantly, only the house sparrow showed a strong FFR for the 110 Hz FM rate throughout the entire sinusoidal FM signal.

The birds showed asymmetries in their response to up-sweeps compared to down-sweeps for both the linear sweeps and the sinusoidal FM. Thyer & Mahar (2006) suggested that asymmetries in discrimination of up- vs. down-sweeps (humans detect down-sweeps at lower thresholds than up-sweeps) could result from the traveling wave on the basilar membrane masking decreases in excitation level when the wave travels from high to low frequency; no masking would be expected for up-sweeps. Our results for the sinusoidal FM and for the higher

516 frequency range of the linear FM-sweeps support this hypothesis. However, the hypothesis is
517 not supported from our results from the lower frequency range of the linear FM-sweeps (also see
518 Krishnan & Parkinson 2000 who showed stronger FFR in humans to down-sweeps). One
519 possible explanation of these differences is the relative contribution of patterns of excitation on
520 the cochlea versus phase-locking cues in the auditory nerve (e.g. Sek & Moore 2000).
521 Our results from the linear up-sweeps suggest that the FFR latency increases with
522 increasing frequency. Of course, a priori, one would expect latency to be shortest at high
523 frequencies, given the excitation pattern of the cochlea (see Møller 2006). However, the length
524 of the avian cochlea is much shorter than the mammalian cochlea (Gleich et al. 2005), so factors
525 other than excitation pattern should be more important in birds. Indeed, the latency will be
526 affected by factors such as local masking on the basilar membrane (Thyer & Mahan, 2006),
527 variation in filter width across the cochlea, and variation in the number of auditory neurons that
528 are stimulated at each frequency. We have too little information on these properties at present to
529 describe the mechanism behind our results.
530 We presented stimuli at a fixed level (80 dB SPL) in all of our tests. We chose this level
531 because previous results on clicks and pure tones indicated that AEPs to 80 dB stimuli are
532 robust, are not overly contaminated by a cochlear microphonic signal, and species relationships
533 are generally unaffected when lower level stimuli are used (Lucas et al. 2002, 2007). Moreover,
534 ABR audiograms generated with tone pips suggest that auditory sensitivity is broadly similar
535 between the three species in our study over the range of 1-3 kHz, although sensitivity in titmice
536 is greater than that of the other two species at 4-6.4 kHz (Henry & Lucas 2008). Nonetheless,
537 we acknowledge that species-specific differences in sensation level may exist with the stimuli
538 used in our study, so the results should be viewed within the limited scope of a fixed sound level.

539

540 *Conspecific vs. heterospecific call recognition*

541 There is an interesting parallel between our system and the budgerigar/canary/zebra finch
542 system studied by Dooling and colleagues, among others, using behavioural measures of
543 auditory perception. Budgerigars have vocalizations characterized by rapid FM tones with and
544 without harmonics (Dooling 1986; Lavenex 1999); similar to the rapid FM in house sparrows
545 and the pure tone sweeps in titmice. Like nuthatches, zebra finches have vocalizations rich in
546 harmonics, but only male calls have any appreciable FM (Simpson & Vicario 1990). Okanoya &
547 Dooling (1991) showed that each species in the budgerigar/canary/zebra finch study system
548 discriminates their own versus heterospecific calls and that each species is better at
549 distinguishing different calls of their own species compared to different heterospecific calls.
550 Lohr et al. (2003) found a similar pattern in these three species, although enhanced within-
551 species discrimination was only evident at high signal-to-noise ratios. Dooling et al. (2002)
552 found that zebra finches are better than canaries and budgerigars in detecting positive from
553 negative Schroeder-phase complexes, and that budgerigars are better than canaries. This trend
554 matches the extent of use of harmonics in each species' vocal repertoire.

555 Our results are complementary to these behavioural studies. By decomposing "signal space"
556 into its component parts, our data show that auditory filtering in the brainstem can account for
557 some aspects of enhanced conspecific song recognition. For example, there are several
558 components of the AEP waveform in nuthatch response to multi-tone complexes that exceed the
559 responses of titmice and house sparrows. Importantly, all nuthatch vocalizations have harmonic
560 overtones. The lack of a strong response to rapid FM tones in both titmice and nuthatches also
561 mirrors properties of their conspecific vocal repertoire. We show elsewhere that modulation rate

562 transfer functions also correlate with species-specific call properties: AEP-derived FFR to
563 amplitude modulation is stronger at high AM rates (>1 kHz) in house sparrows and titmice
564 compared to nuthatches, and this matches AM signals in the vocal repertoire of these birds
565 (Henry & Lucas 2008). We should note, however, that enhanced conspecific song recognition is
566 not universal. In a test of auditory scene analysis, Hulse et al. (1997) showed that starlings are
567 no better at learning starling song than 4 other species' songs.
568
569 *Suppression/facilitation in harmonic overtones*
570 The results from our 3-tone chords raise a related issue. Nuthatches have enhanced FFR
571 amplitude (compared to house sparrows and titmice) to 1.8 kHz tones when these tones are
572 coupled with 2.4 kHz tones. The highest amplitude tones in nuthatch calls are at about these
573 frequencies (Henry & Lucas 2008). Krishnan (2002) found similar results in human FFR to
574 vowel sounds which are composed of harmonic overtones. He showed that FFR strength in
575 response to formants 1 and 2, the formants most critical for detecting vowels, is enhanced
576 compared to other harmonics. Krishnan (2002) suggested that this may result from selective
577 suppression of non-formant harmonics through lateral inhibition. However, our results do not
578 seem to be caused by lateral inhibition. Indeed, the addition of 2.4 kHz tones increases FFR
579 amplitude at 1.8 kHz, and the addition of 1.8 kHz tones increases FFR amplitude at 1.2 kHz –
580 but this pattern was shown only for nuthatches. The simplest explanation of our results is that
581 nuthatches, compared to the other species, show stronger distortion products (which in our
582 experiments are all multiples of 600 Hz) that result from our use of multiple-tone inputs.
583
584 *Sex effects*

There were only limited sex-specific aspects of AEPs. We found no sex differences in any of the FM tones. In contrast, there were sex differences in the amplitude of FFR harmonics to the 2- and 3-tone chords (male house sparrows had a stronger FFR than females). 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 (Henry 2002; Hultzcrantz et al. 2006). 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 & Dooling 1991). Moreover, males and females in some species appear to respond to different spectral properties in songs (e.g., chickadees: Weisman & Ratcliffe 2004). Despite these examples, we will need to study a much broader range of input stimuli and species before we have a comprehensive understanding of sex-specific effects.

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; Brown & Handford 2000; Naguib 2003). The songs of both nuthatches and titmice (Offutt 1965; Ritchison 1983; Schroeder & Wiley 1983) also primarily fall in a frequency range (1.5 – 3 kHz) that matches the frequencies of maximal FFR amplitude (this study) and auditory sensitivity (Henry & Lucas 2008). 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 maximal

energy ranging from 3.5 – 5 kHz (Lowther & Cink 2006). We show here that this is outside the range of frequencies where the strength of the FFR to FM sweeps is maximal.

A high frequency range used in song 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 additional dimension to this scenario because processing of the fine-structure detail in song is facilitated by a robust FFR. Yet we failed to find a strong FFR 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 & Lucas 2008) show sensitivity to frequencies at least as high as 5 kHz, so there is little doubt that the birds hear these tones. Nonetheless, our FFR results, and the ABR data from Henry & Lucas (2008) suggest that these birds are relatively poor at processing high frequency sounds. Of course, the definitive answer will come with psychophysical studies of auditory detection (e.g., Moore 1993; Lohr et al. 2003).

Theunissen & 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 (see Lavenex 1999 for a discussion of AM signals in budgerigar calls). Thus, the relevant information in the house sparrow song could be in the amplitude envelope and not in the frequency properties per se. We show elsewhere (Henry & Lucas 2008) that house sparrows can indeed 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) have stronger FFR amplitudes in response to tones 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 plainfin midshipman fish (*Porichthys notatus*). These females do not phase lock to the relatively high frequency male "song" in winter, but increased estrogen levels in spring dramatically increase phase-locking in the frequency range of the song.

Social effects

The relatively weak auditory responses of titmice in our study are 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 & 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 & 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 & Freeberg 2007; Owens & 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 & 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 & Freeberg 2007). Tufted titmice do not show this fluid social system. Instead, kin tend to associate throughout the winter (Pravosudov & 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, suggest that auditory physiological responses may match the simplicity of the vocal system.

SUMMARY

In summary, we demonstrate here and in Henry & Lucas (2008) that AEPs provide a broad characterization of auditory processing of species-relevant acoustic signals. The general characteristics of the vocal repertoire, and in particular the song repertoire, appear to match auditory responses to a variety of FM tones and multi-tone chords. The only exception to this pattern was the lack of a strong FFR in house sparrows over the frequency range of the song.

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

867

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

868

Table 2. (a) LSMean (±SE) FFR amplitude to each tone in the 2- or 3-tone chords. (b) LSMean (±SE) FFR amplitude of the 3.0 and 3.6 kHz FFR harmonics generated by the auditory system. All FFR amplitudes are given in dBnV. The left-most column gives FFR frequency and the chord for which amplitude 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	FFR amplitude: ETTI female	FFR amplitude: ETTI male	FFR amplitude: HOSP female	FFR amplitude: HOSP male	FFR amplitude: WBNU female	FFR amplitude: 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	FFR amplitude: ETTI female	FFR amplitude: ETTI male	FFR amplitude: HOSP female	FFR amplitude: HOSP male	FFR amplitude: WBNU female	FFR amplitude: 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. (a) and (c) are representative AEP waveforms (top) and spectrograms (bottom) derived from the waveform view. These are from (a) a nuthatch and (c) a house sparrow. Both were played a 110 Hz sinusoidal FM tone. Figure (b) is a spectrogram of the input stimulus. Note that the input stimulus was aligned to match the frequency response of the AEPs in (a) and (c). The first major ABR positive peak (P1) and negative peak (P-1) are labeled for the house sparrow. The spectrograms have a dynamic range setting (in Praat software) of 20 dB.

Figure 2. Examples of spectrograms of calls from (a) nuthatch, (b, c) titmice and (d) house sparrows.

Figure 3. LS mean (± SE) ABR peak P1 latency as a function of LS mean (± SE) peak P1 amplitude for titmice, nuthatches, and house sparrows.

Figure 4. (a) Representative FFR 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. (b) FFR strength as a function of time for the AEP represented in (a). (c) LS mean (± SE) FFR latency to match sinusoidal FM signals averaged across all FM rates tested. (d) LS mean (± SE) FFR latency to match sinusoidal FM signals averaged across 20 and 40 Hz signals. In (d), latency was calculated separately for upsweep and downsweep parts of the sinusoidal FM signal. Latency was estimated from cross correlation between FFR frequency and the input frequency. In (c), symbols with the same letters are not significantly different (α =0.05) based on post hoc tests (see Methods). In (d), lines above symbols indicate significant differences between species within each sweep direction: *=P<0.05; **=P<0.01.

Figure 5. LS mean (± SE) FFR 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.

Figure 6. LS mean (± SE) FFR amplitude at 110 Hz in response to a 110 Hz sinusoidal FM tone (i.e., FFR to the FM signal itself). Symbols with the same letters are not significantly different (α =0.05) based on post hoc tests (see Methods).

Figure 7. LS mean (± SE) FFR strength for (a) the lower frequency range (1-3 kHz) and (b) upper frequency range (3-6 kHz) for linear FM sweeps. (c) Example of the regression of FFR frequency as a function of input frequency measured on a nuthatch tested with a 50 msec linear FM upsweep. The symbols represent raw data; the solid line is the linear regression fit through the data; the dotted line represents the input stimulus with a slope=1.0 (Note: time zero is when the input stimulus reaches the tympanum. The elevation of the input stimulus relative to the FFR response is caused by a temporal delay in neural and cochlear response to the input stimulus). (d) LS mean (± SE) slope of the linear regression of FFR frequency as a function of input frequency for linear FM sweeps. The regression analyses were for the lower frequency range of sweeps (<2.8 kHz) only. A slope of 1.0 indicates a perfect match between FFR frequency and the frequency of the input stimulus. In (a), (b) and (d): four sweep types (slow/up, fast/up, slow/down, fast/down) are indicated. Lines above symbols indicate significant differences between species within each sweep type: *=P<0.05; **=P<0.01.

Figure 8. (a) Example of loess regression calculated from strength of FFR as a function of frequency for a 50 msec linear upsweep measured from a nuthatch. The symbols represent raw data and the line represents the loess regression fit through the data points. (b)

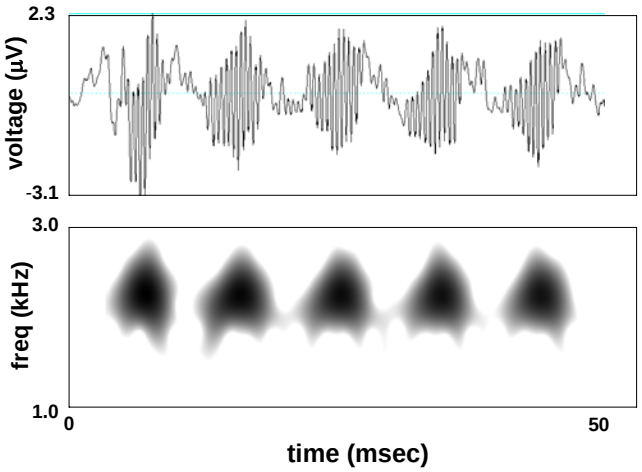
929 Estimated frequency (LS means± SE) for the linear FM upsweep where the strength of FFR
930 is maximal. Data for slow (50 msec) and fast (30 msec) sweeps are given separately. Lines
931 above symbols indicate significant differences between species within each sweep type:
932 *=P<0.05; **=P<0.01.

933 Figure 9. FFR amplitude (LS means± SE) to the 600 Hz AM signal for 2- and 3-tone chords.
934 Lines above symbols indicate significant differences between species within each sweep
935 type: *=P<0.05; **=P<0.01.

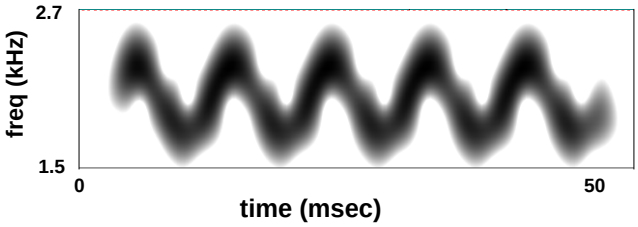
936

937 Figure 1
938

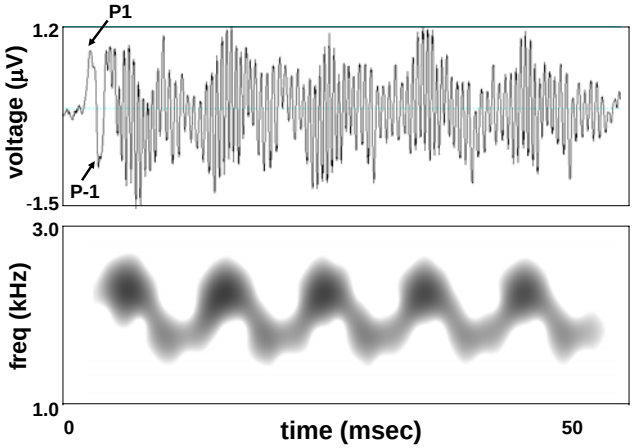
(a) white-breasted nuthatch



(b) input stimulus



(c) house sparrow



939
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941 Figure 2
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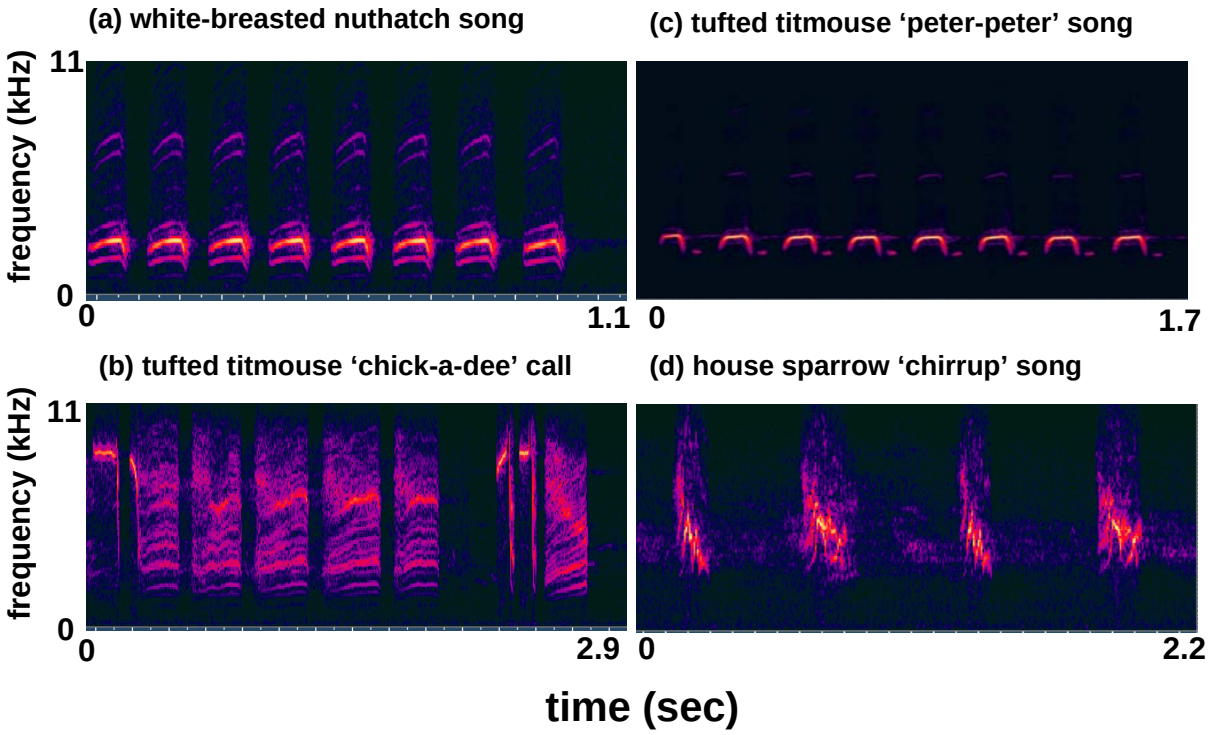

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944 Figure 3
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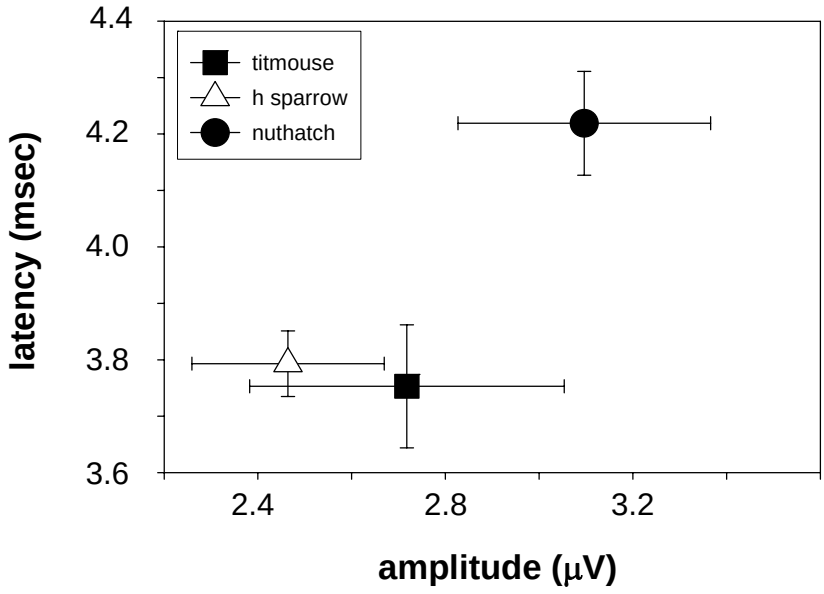

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948 Figure 4
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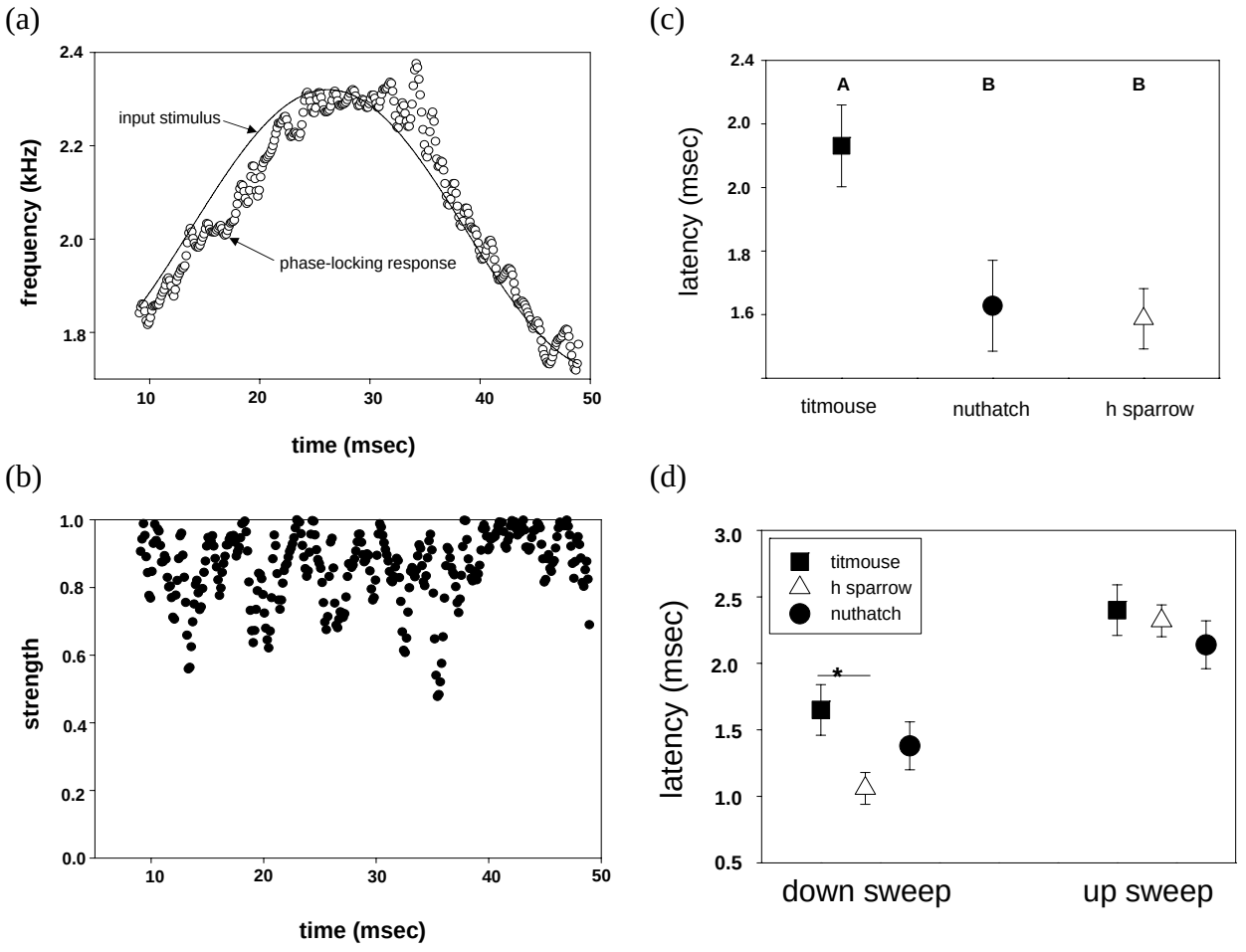
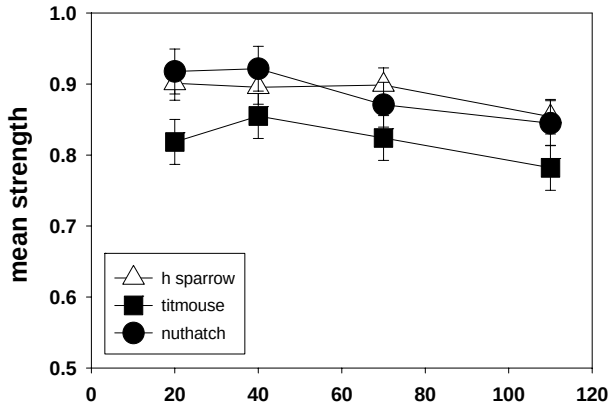


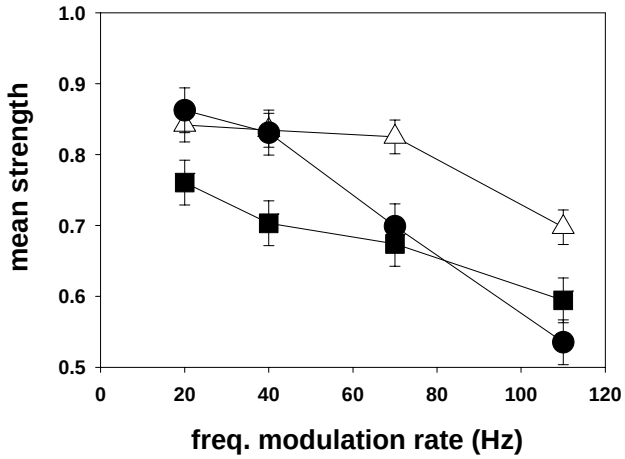
950

951 Figure 5
952

(a) Frequency range: 2.0 – 2.3 kHz



(b) Frequency range: 1.7 – 2.0 kHz



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954 Figure 6
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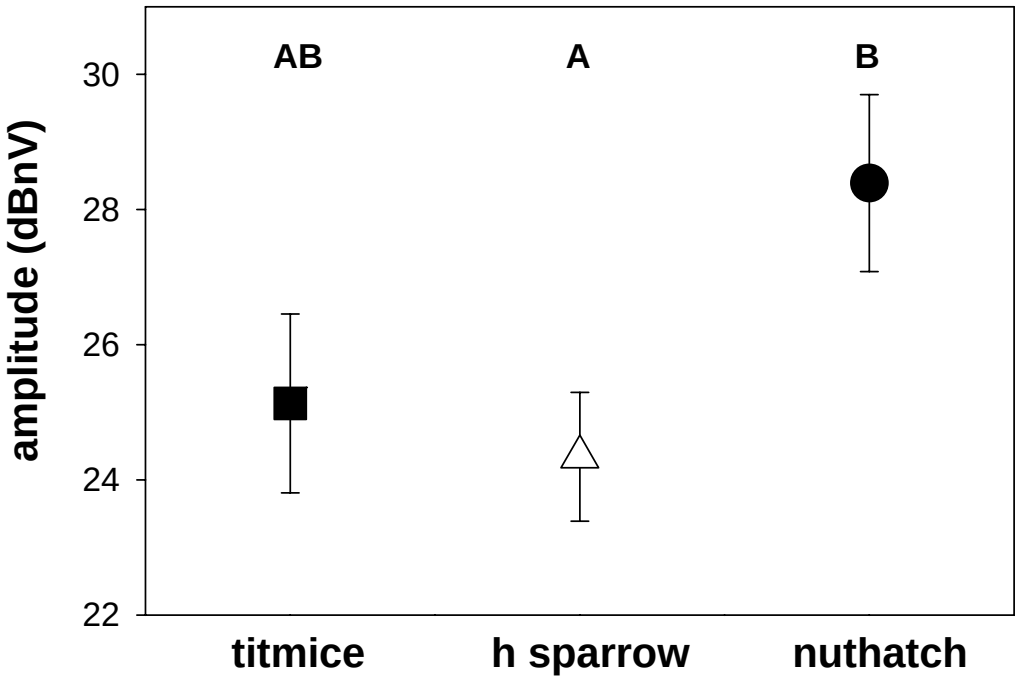

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957 Figure 7
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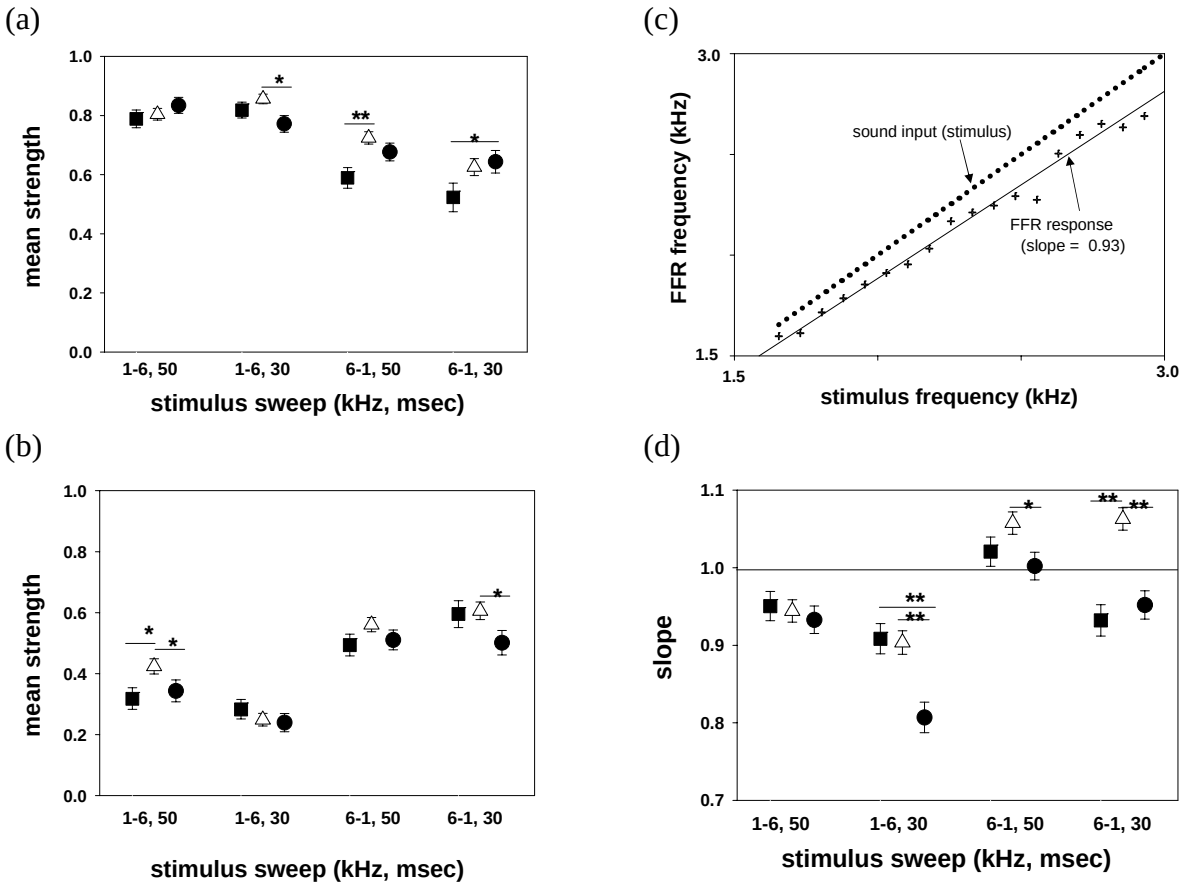

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961 Figure 8
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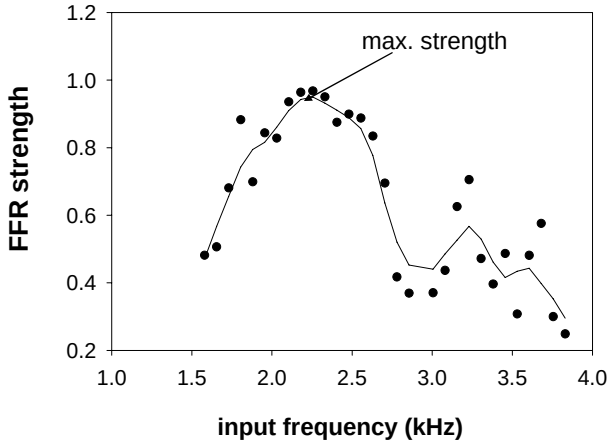
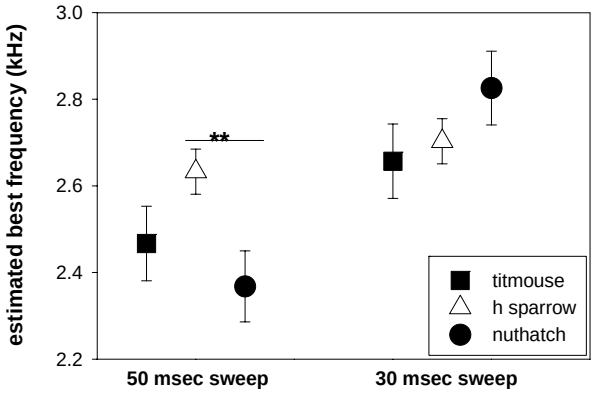
(a)



(b)



963

964 Figure 9
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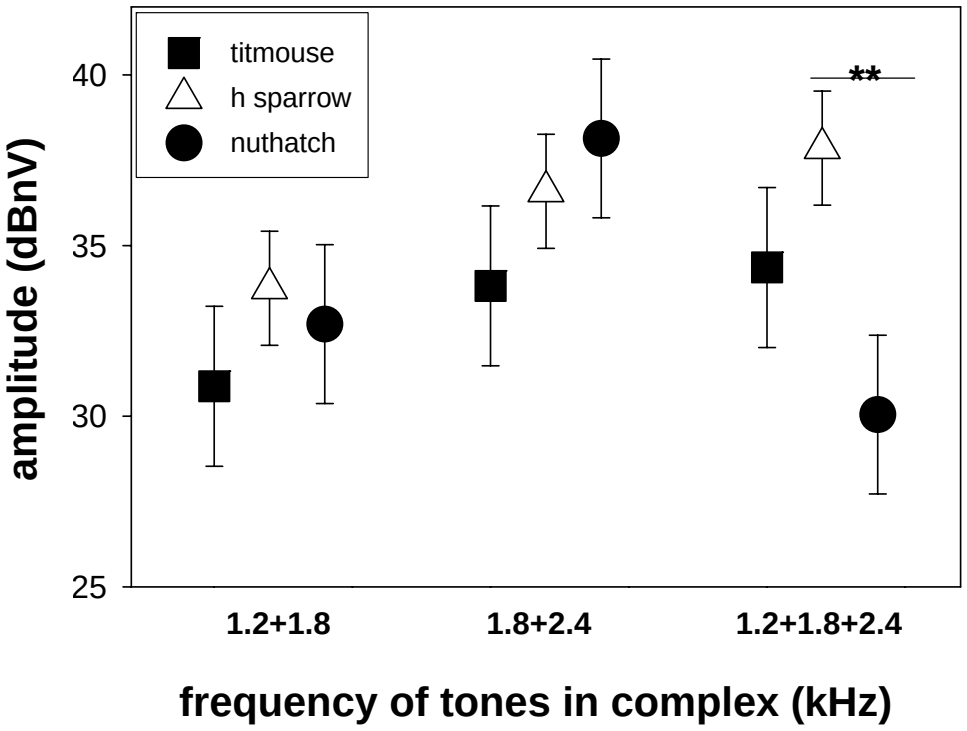

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