J Comp Physiol A (2007) 193:201–215
DOI 10.1007/s00359-006-0180-z

ORIGINAL PAPER

Seasonal variation in avian auditory evoked responses to tones: a comparative analysis of Carolina chickadees, tufted titmice, and white-breasted nuthatches

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Received: 20 June 2006 / Revised: 13 September 2006 / Accepted: 1 October 2006 / Published online: 26 October 2006

Abstract We tested for seasonal plasticity of the peripheral auditory system of three North American members of the Sylvioidea: Carolina chickadees (*Poecile carolinensis*), tufted titmice (*Baeolophus bicolor*), and white-breasted nuthatches (*Sitta carolinensis*). We measured three classes of auditory evoked responses (AER) to tone stimuli: sustained receptor/neural responses to pure-tone condensation waveforms, the frequency-following response (FFR), and the earliest peak of the AER to stimulus onset (tone onset response). Seasonal changes were detected in all classes of AERs in chickadees and nuthatches. Seasonal changes in titmice were restricted to the tone onset response. Interestingly, changes detected in chickadees (and to a lesser extent in titmice) were generally in an opposite direction to changes seen in nuthatches, with chickadees exhibiting greater amplitude AER responses in the spring than in winter, and nuthatches exhibiting greater amplitude AER responses in winter than in spring. In addition, the seasonal differences in the sustained responses tended to be broad-band in the chickadees but restricted to a narrower frequency range in nuthatches. In contrast, seasonal differences in the onset response were over a broader frequency range in titmice than in chickadees and nuthatches. We discuss some possible mechanistic and functional explanations for these seasonal changes.

Keywords Auditory evoked response (AER) · Frequency following response (FFR) · Seasonality · Bird hearing · Vocal complexity

List of abbreviations

AER	Auditory evoked response
CM	Cochlear microphonic
FFR	Frequency following response
FFR2	Second harmonic of the frequency following response
CM + FFR	Sustained response including both cochlear microphonic and frequency following response

Introduction

Neural regions devoted to vocal development and production in songbirds change substantially across seasons in both young and adult individuals (Bottjer and Johnson 1997; Ball 1999; Nottebohm 1999; Brenowitz 2004; Brenowitz and Beecher 2005). Hearing is known to play a fundamental role in vocal learning specifically and in vocal communication more generally (Dooling 1982, 1992; Dooling et al. 2000); however, we know relatively little about the role of the peripheral auditory

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system in vocal communication. In particular, few studies have addressed possible seasonal influences on the auditory periphery (Lucas et al. 2002; Sisneros and Bass 2003; Goense and Feng 2005).

In an earlier comparative study of six avian species (three of which are part of the current study), we tested for species differences in auditory evoked responses (AERs) generated by broadband click stimuli (Lucas et al. 2002). AERs are neural impulses generated by the peripheral auditory system—the auditory nerve and the auditory brainstem neurons—in response to acoustic stimuli. AERs in songbirds have been found to correlate with behavioral hearing thresholds, particularly in the region of best hearing from roughly 2 to 4 kHz, though AERs underestimate true hearing thresholds by about 30 dB over this range (Dooling and Walsh 1976; Saunders et al. 1980; Brittan-Powell et al. 2002). We found that species differences in AERs measured in the spring were correlated with both the complexity and the prevalence of high-frequency signals in the vocal repertoire of those species (Lucas et al. 2002). An unexpected finding of this earlier study was that click-evoked AER latency and amplitude were strongly affected by season (specifically whether birds were tested in winter vs. spring). Two more recent studies on midshipman fish, *Porichthys notatus* (Sisneros and Bass 2003; Sisneros et al. 2004) and leopard frogs, *Rana pipiens* (Goense and Feng 2005) suggest that seasonal variation in the acuity of the peripheral auditory system may be taxonomically widespread.

Our earlier study reported only click-evoked AERs (Lucas et al. 2002). As part of those same experiments, we measured AERs to tone burst stimuli, but these data were not included in the original manuscript. Nevertheless, these data are important because they fill in important details that are not available with click-evoked AERs. Specifically, clicks are broad-band stimuli. As such, we cannot distinguish whether seasonal changes detected with click-evoked AERs represent narrow-band or broad-band variation in the activity of the peripheral auditory system. AERs to tone burst stimuli provide this information.

Here we report tone-evoked AERs for three species. All three species are members of the Superfamily Sylvioidea of the Parvorder Passerida (Sibley and Ahlquist 1990). Carolina chickadees, *Poecile carolinensis*, and tufted titmice, *Baeolophus bicolor*, are members of the family Paridae, and white-breasted nuthatches, *Sitta carolinensis*, are members of the Sittidae. We chose these species because they are all relatively closely related, but vary substantially in their vocal repertoire (see Lucas et al. 2002). The nuthatches have simple calls and songs that are primarily produced with low frequencies (Ritchison 1983). Chickadees and titmice use a broader frequency range, with chickadees' vocal repertoire (Smith 1972; Bloomfield et al. 2005) more complex than that of titmice (Offutt 1965; Schroeder and Wiley 1983).

We assessed three classes of AERs to tone stimuli: sustained receptor/neural responses to tone burst stimuli with condensation onset polarity (here called CM + FFR), the frequency-following response (FFR), and the earliest peak of the AER to the onset of the stimuli. Sustained responses to tones potentially include both a receptor component (cochlear microphonic or CM) and a neural component (FFR). Both the receptor response (presumably an outer hair cell response) and the neural response (reflecting phase-locked neural activity in a population of brainstem neural elements) mimic the temporal waveform of the stimulus. The CM is elicited by periodic deflections of the basilar membrane; the amplitude of the CM measures the ability of the hair cells of the inner ear to respond to an AC electrical potential, and is strongly correlated with the intensity of the stimulus (Bekesy 1950). The amplitude of the FFR measures the ability of the peripheral auditory system to phase-lock to the stimulus frequency, and also varies with stimulus intensity (e.g. Krishnan 1999; Krishnan and Parkinson 2000). The earliest peak of an auditory brainstem response is likely generated by the auditory nerve (possibly the distal portion of the auditory nerve: Brown-Borg et al. 1987). Collectively, these three classes of AERs allow us to address seasonal effects on the auditory system.

Based on the complexity of the vocal repertoire of the species, we predicted that the spring increase in acoustic sensitivity seen in chickadees and titmice would be relatively broad-band, whereas the winter increase in sensitivity seen in nuthatches should be skewed to lower frequencies (~2 kHz).

Methods

Subjects and seasons

All birds were captured in the vicinity of West Lafayette, IN, USA. Only adult birds were used in our study—the birds were aged according to Pyle (1997). Sex was determined by color pattern for nuthatches (see Pyle 1997). For Carolina chickadees and tufted titmice, sex was based on size distributions of birds caught in the vicinity of our capture site (Thirakhupt 1985) and previously validated using visual inspection

of gonads (see Lucas et al. 1993, 2006). Immediately after capture in treadle traps baited with sunflower seed, birds were banded with unique colored leg rings and were brought into the laboratory and housed indoors in individual 1 m^3 wire mesh home cages in rooms containing other such cages housing individuals of these species (i.e. birds were never housed in complete isolation). All birds were tested within 3 days of capture and all birds were tested only once. While in the aviaries, birds were kept under a light/dark cycle appropriate for their west-central Indiana capture area and the time of year. Each individual was provided with mixed seed (shelled sunflower seed and safflower seed), crushed oyster shell and grit, and was given 1–3 mealworms and fresh vitamin-treated water daily. After AER testing (described below), birds were housed in their individual cages an additional 2–3 days to recover completely from the anesthetic and testing, and then were released at their individual sites of capture.

We divided our testing of the birds into two seasons, "winter" (October–January) and "spring" (February–April). This cutoff between January and February was chosen because around this time reproductive hormones begin to change and song rates begin to increase in this population (see Lucas et al. 2006; also see Ball 1999). This is also roughly the time of year in which, for chickadees and titmice, social flocks begin to break up into female-male pairs that establish breeding territories (Smith 1991). Four Carolina chickadees were tested in the spring (3 males, 1 female; 1 in February, 1 in March and 2 in April) and seven in the winter (3 males, 4 females; 1 in October and 6 in November). Seven tufted titmice were tested in the spring (4 males, 3 females; 2 in March and 5 in April) and six in the winter (3 males, 3 females; 2 in November and 4 in December). Four white-breasted nuthatches were tested in both spring (3 males, 1 female; 2 in March and 2 in April) and winter (2 males, 2 females; 1 in October and 3 in December). Average masses (mean ± SD) of each species, collected from individuals immediately before we had acquired their AERs, were 10.0 ± 0.7 g for Carolina chickadees, 20.4 ± 1.9 g for tufted titmice, and 20.6 ± 1.3 g for white-breasted nuthatches.

Procedure

We acquired AERs from all birds between 1300 and 1730 hours, from January 2000 to April 2001. Subjects were taken from their holding cages, weighed, placed into opaque carrying boxes, and transported to the Audiology Laboratory where they were briefly held until testing. Immediately prior to testing, each subject was removed from the carrying box and was injected in the breast muscle with a mixture of ketamine/xylazine (0.5 ml of ketamine at 100 mg/ml, 0.1 ml of xylazine at 100 mg/ml, in 9.4 ml sterile saline). Dosage varied from 0.10 ml to 0.12 ml per 10 g of bird.

After injecting a bird, we placed it on a small hand towel inside its carrying box for 5–6 min until its eyes closed and it appeared fully anesthetized. The bird was then taken into the testing room, a walk-in IAC acoustic isolation room kept at roughly 23°C. Evoked responses were recorded using subdermal needle electrodes (Nicolet Biomedical) placed under the skin of the crown directly above and midway between the eyes (positive electrode) and under the skin of the auricular region directly behind the ipsilateral external auditory meatus (negative electrode). The ground was an electrode placed under the skin of the back (nape) of the neck. After the electrodes were inserted and held in place by tape, the subject was placed inside a large plastic box, on top of pre-heated padding to help maintain body temperature. We did not measure body temperature in these experiments, but the pre-heated padding maintained a 35°C temperature for at least 45 min (longer than needed for us to complete the AER data acquisition), which is within the thermoneutral zone for these small birds (Chaplin 1974; Cooper and Swanson 1994). The first protocol we ran on each bird involved broad-band click stimuli—the results of these studies have been published previously (Lucas et al. 2002). Following the click stimuli protocols, we ran protocols using tone burst stimuli. For half of the birds in the study, we measured the onset response to a 95 dB SPL click stimulus just prior to the beginning of tone burst testing, and then again approximately 30 min later near the end of testing. We did this to determine whether there were systematic shifts in peak latencies and amplitudes over the course of testing, which might be indicative of anesthetic effects. None of the three species tested here showed a significant change in latency or amplitude of response to the click stimuli from the beginning to ending of testing, suggesting that changing anesthetic effects over our roughly 45 min of testing were negligible.

Responses were elicited with frequency specific tone bursts, using Intelligent Hearing Systems (IHS) Smart EP (Version 2.2). Tones were 20 ms in duration with 3.0 ms rise/fall time (extended cosine) presented at 31.13/s. The tone stimuli were presented through an ER-3A-MS (Custom) 300 Ω insert earphone, coupled to the ear of the subject with putty. The frequency response of the stimulus out of the insert earphone was flat to 4 kHz, and then dropped off in intensity by roughly 35 dB SPL/octave from 5 to 10 kHz**.** The tone

burst protocol included the presentation of five different frequencies (1, 2, 3, 4, and 5 kHz presented in that order) at each of each of four levels (95, 75, 55 and 35 dB SPL), calibrated with a Bruel and Kjoer sound level meter using a 2 CC coupler. True SPL levels were correct for 1–4 kHz tones but were 10 dB below these values for 5 kHz tones. Throughout the paper we will refer to the stimulus levels as 95, 75, 55 and 35 dB SPL.

An IHS Opti-Amp 8000 was used to amplify the EEGs. Interelectrode impedances were maintained below 7 kΩ for titmice and nuthatches and below 20 kΩ for chickadees. The basis for the species difference in impedance is unknown. Impedance is affected by skin and skull thickness and by the properties of the intercranial medium (the latter presumably is similar across species). We know of no evidence that impedance differences affect the results we are reporting here. The EEG inputs were amplified 200,000 times and were band-pass filtered from 100 to 5,000 Hz (6 dB/octave roll-off, RC response characteristics). Two response waveforms in condensation phase and two response waveforms in rarefaction phase were collected at each intensity and frequency. Each response waveform represented 512 stimulus presentations over a 25.6 ms sample window with a sampling rate of 55 kHz.

In all cases, the data from 75 to 55 dB were intermediate to the data from 95 to 35 dB. We therefore present analyses of the sustained responses for only the two extreme tone-burst intensities. However, we present data on latency and amplitude of the first peak in the tone burst onset response at 95 and 55 dB (not 35 dB), because the first peak was difficult to locate consistently across birds at 35 dB. Two measures of sustained responses to tones were estimated: CM + FFR and the FFR. 'CM + FFR' was the amplitude of a Fast Fourier Transform of the EEG at the frequency of tones presented into the ear as condensation-phase waveforms. This measure includes a sustained periodic signal from the cochlea (called the cochlear microphonic or CM) with a short latency, and phase-locking in the brainstem (called the frequency following response or FFR; Huis in't Veld et al. 1977) with a longer latency. Phase-locking in the brainstem is expected to begin only after the first positive AER peak. The CM contribution to the CM + FFR response can be minimized by adding the independent responses obtained using condensation and rarefaction onset polarity. The resulting residual should largely be the phase-locked neural component (FFR and FFR2). CM is minimized by adding waveforms of different polarity (rarefaction waveforms are 180° out of phase compared to condensation waveforms), because polarity is retained in the cochlear response waveform. Thus adding the waveforms nullifies the CM signal. Rarefaction and condensation waveforms are not perfectly out-of-phase in the brainstem because there is a non-linear transformation of signal from basilar papilla to auditory nerve. Thus, the FFR signal is retained by the addition of rarefaction and condensation waveforms.

Assuming that the transfer of signal from cochlea to auditory nerve includes a simple half-wave rectification of the signal, we expect to see a prominent second harmonic (at 2f; here called FFR2) in the rarefaction + condensation waveform. The second harmonic (FFR2) of the frequency following response may also result from the fact that the FFR is derived from several separate generators (see Gardi et al. 1979; Stillman et al. 1978). Thus, the amplitude of this harmonic corresponds to the magnitude of the 2f neural component (referred here as FFR2). The addition of the rarefaction and condensation waveforms also yields the onset component because 'noise' from the CM is damped, making the onset component easier to measure. Representative FFR waveform and a first peak of the onset response are illustrated in Fig. 1a.

There is some debate about whether the rarefaction + condensation waveform generates a true FFR. Although the CM can indeed be eliminated using this technique (e.g. Sohmer et al. 1977; Gardi et al. 1979), the technique may generate a distorted representation of the FFR if the transfer of signal from the cochlea to the auditory nerve is strongly non-linear (Chimento and Schreiner 1990). While we acknowledge this potential problem, the technique does provide at least an index of the FFR uncontaminated by CM. We view the results in this light. Nonetheless, the details of the representation of the FFR should be viewed with caution.

Statistical design

Multiple data were collected sequentially on each bird. Therefore, all data were tested using repeated measures analysis of variance with the individual bird as the subject using PROC MIXED in the SAS statistical program (SAS 1994). The data were sorted chronologically and analyzed using a first-order autoregressive variance/covariance matrix (ar(1) in PROC MIXED).

We used five different dependent variables in our analyses. Three were indices of a sustained response: CM + FFR, FFR and FFR2. The final two dependent variables were the latency and the amplitude of the

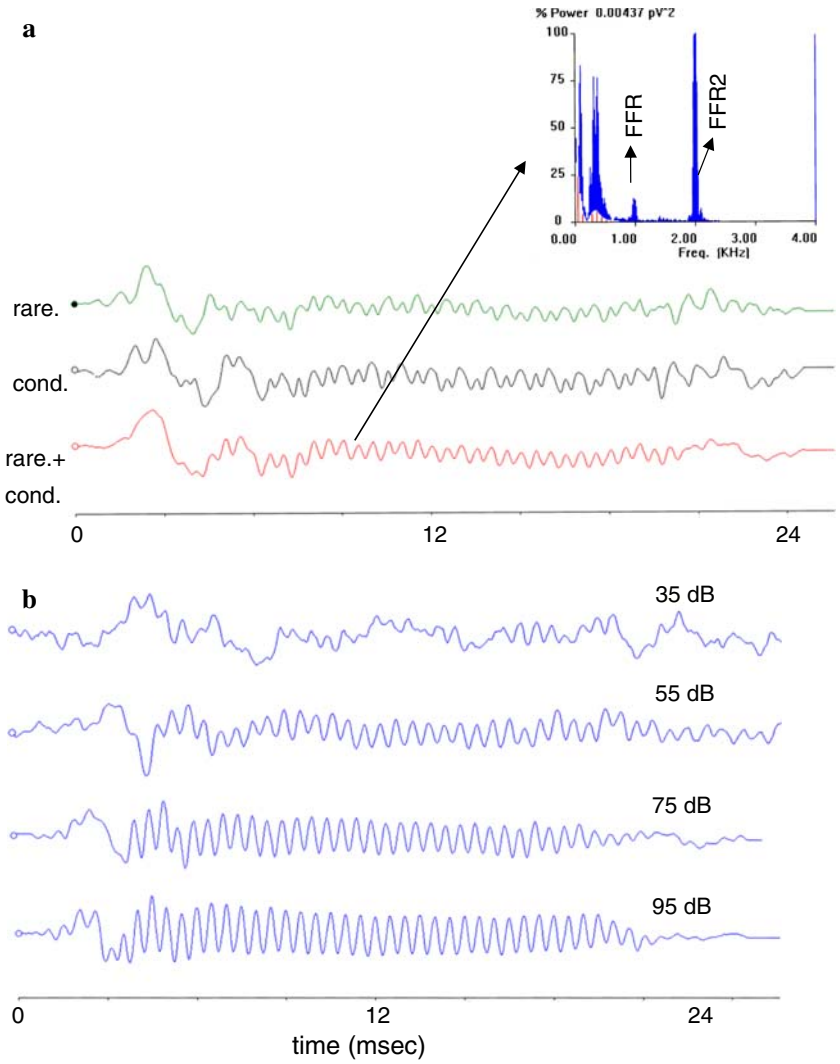

Fig. 1 **a** Examples of an FFR (where the rarefaction waveform is added to the condensation waveform) generated from the peripheral auditory system of a white-breasted nuthatch to a pure tone at 1,000 Hz and 95 dB. A power spectrum of the waveform indicates our metric of FFR and FFR2 strength. Note the stronger second harmonic (FFR2) compared to the fundamental (FFR). **b** Examples of CM + FFR waveforms (condensation phase only) of a white-breasted nuthatch at each of the four intensities used in this study

first positive AER peak, where latency was the time from the onset of the stimulus to the maximum amplitude of the first peak. Peak amplitude was measured as the relative amplitude difference from the first positive peak to the first negative peak (Fig. 1a).

We used four independent variables for each test: tone frequency, species of bird, sex of bird, and season. Separate analyses were run on the 35 and 95 dB data sets. All four independent variables were treated as class variables, and all possible interaction terms were added to the model. Non-significant interactions were dropped from the model in order of decreasing order of complexity (e.g. the 4-way interaction was dropped first if it was n.s.), and in order of increasing F value. Least squares means were generated using the LSMEANS option under PROC MIXED, and multiple comparisons were evaluated using the DIFF option of LSMEANS. CM + FFR, FFR, and FFR2 values were cube root transformed to normalize variance of the model residuals. No transformation was necessary for latency or amplitude of the first AER peak.

The five dependent variables we evaluated represent two sets of properties of the peripheral auditory response, the sustained response (characterized by CM + FFR, FFR and FFR2) and the auditory brainstem response (characterized by latency and amplitude of the first AER peak). An alternative method to the univariate approach described above, is to treat the vector of properties of each auditory response as a single dependent variable in a repeated measures MANOVA. We performed these analyses on the data to ensure that our primary conclusions (species-specific seasonal patterns in the response of the peripheral auditory system to sound) were robust. In the analyses, each dependent variable was converted to a Z-score, and repeated measures MANOVA analyses were performed on the Z-scores using Proc GLM (SAS 1994) using individual bird as the subject variable. Exact P values for Wilk's

Lamba were calculated using the 'mstat = exact' command within MANOVA. The full statistical model including main effects and interactions (described above) was tested, but to save space we report only the critical interaction terms. Tests of sphericity (an assumption of the MANOVA design) were performed using the 'printe' option in the 'repeated' statement.

Results

CM + FFR

The seasonal CM + FFR response was significantly different between species (Table 1). Averaging across frequencies, response amplitude for chickadees was significantly lower in winter compared to the spring (Fig. 2) at both 95 dB ($t_{24} = 2.86$, $P = 0.009$) and 35 dB ($t_{24} = 4.32$, $P = 0.0002$). In contrast, CM + FFR response amplitude for nuthatches was significantly greater in winter compared to the spring (Fig. 2) at 95 dB ($t_{24} = 2.47$, $P = 0.021$), but no significant difference was detected at 35 dB ($t_{24} = 0.48$, $P = 0.63$). Titmice showed no difference in CM + FFR between seasons for either intensity level (95 dB: $t_{24} = 0.15$, $P = 0.88$; 35 dB: $t_{24} = 1.23$, $P = 0.23$).

As expected, the decreased winter CM + FFR in the chickadees was distributed across a fairly broad range of frequencies (Fig. 3a), whereas the increased winter CM + FFR in the nuthatches was restricted to an enhancement at 2 kHz (Fig. 3c). The CM + FFR response to different tones was virtually identical across seasons for titmice (Fig. 3b). The difference between species in the seasonal response to tones is indicated in the significant frequency × species × season interaction (Table 1).

There was no overall effect of sex on the magnitude of the CM + FFR response (95 dB: $F_{1,23} = 0.57$, $P = 0.46$; 35 dB: $F_{1,23} = 0.01$, $P = 0.91$), nor were there any significant interactions between sex and the other independent variables (all $P > 0.10$). Given that sexes were equivalent in CM + FFR, we dropped this term from the repeated measures ANOVAs for the preceding analyses.

Note that there was one bird (a chickadee) tested in February. Our seasonal dichotomy between spring and winter is arguably stronger if we delete this bird during this transition month. Doing so does not change any of

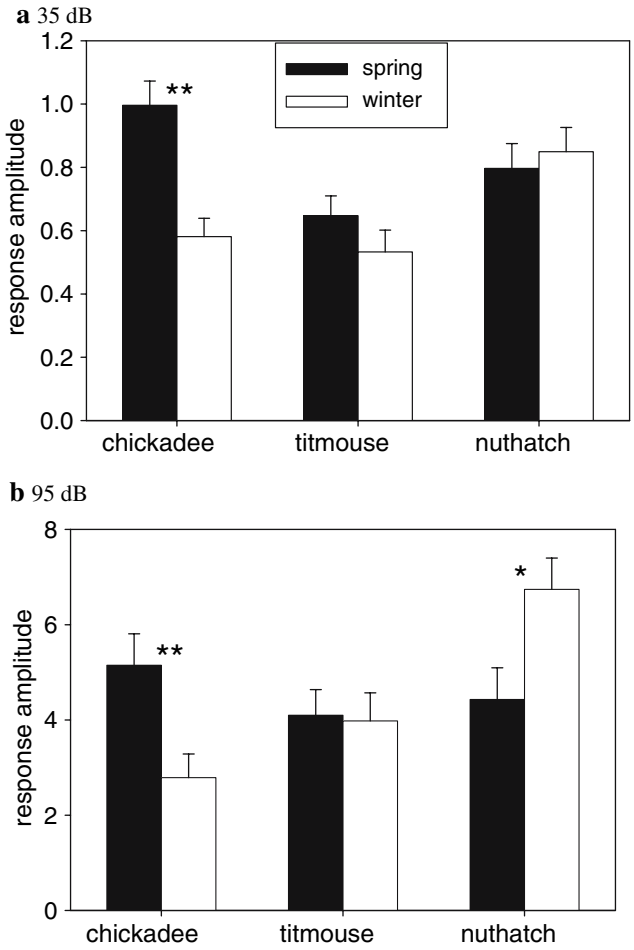

Fig. 2 Mean CM + FFR response as a function of season at **a** 35 dB and **b** 95 dB. Each value is the least squares mean (±SE) based on the statistical model from Table 1, averaged over all frequencies. Units for response amplitude are cube-root of ($pV^2 \times 10^4$). Significance of a seasonal change within species is denoted by *asterisks*: $^{**}P < 0.01$, $^{*}P < 0.05$. Note the different scales on the two figures

Table 1 Repeated measures ANOVA for cube-root transformed CM + FFR response (condensation wave-form) measured for 35 and 95 dB tone bursts

Independent variable	35 dB			95 dB		
	ndf, ddf	*F*	*P*	ndf, ddf	*F*	*P*
Frequency	4, 95	50.4	<0.0001	4, 95	27.3	<0.0001
Species	2, 24	6.7	0.005	2, 24	4.2	0.028
Season	1, 24	7.6	0.011	1, 24	0.01	0.91
Species × season	2, 24	5.5	0.011	2, 24	7.0	0.004
Species × frequency	8, 95	1.7	0.11	8, 95	2.8	0.009
Season × frequency	4, 95	1.8	0.14	4, 95	0.5	0.71
Species × freq × season	8, 95	2.4	0.020	8, 95	2.3	0.026

ndf Numerator degrees of freedom, *ddf* denominator degrees of freedom

Table 2 Repeated measures ANOVA for cube-root transformed frequency following response (condensation + rarefaction waveform)

Independent variable	35 dB			95 dB		
	ndf, ddf	*F*	*P*	ndf, ddf	*F*	*P*
(a) FRR						
Frequency	3, 86	34.2	<0.0001	3, 72	30.7	<0.0001
Species	2, 25	4.2	0.028	2, 21	5.1	0.016
Season	1, 25	5.9	0.023	1, 21	2.5	0.13
Sex	1, 25	0.5	0.49	1, 21	1.1	0.31
Species × sex	ns			2, 21	3.5	0.048
Species × season	ns			2, 21	3.2	0.063
Species × frequency	ns			6, 72	0.5	0.84
Season × frequency	ns			3, 72	1.5	0.24
Species × freq × season	ns			6, 72	4.6	0.001
(b) FFR2						
Frequency	3,86	52.4	<0.0001	3,72	49.1	<0.0001
Species	2,23	0.6	0.56	2,22	0.7	0.50
Season	1,23	5.4	0.030	1,22	0.1	0.72
Sex	1,23	4.1	0.056	1,22	0.6	0.46
Season × sex	ns			1,22	4.5	0.046
Species × season	2,24	6.3	0.006	2,22	6.1	0.008
Species × frequency	ns			6,72	1.4	0.24
Season × frequency	ns			3,72	0.8	0.52
Species × freq × season	ns			6,72	6.8	<0.0001

Data for the (a) fundamental (FFR) and (b) second harmonic (FFR2) were analyzed separately; Non-significant (ns) interactions were dropped from the model

our results. Nonetheless, the bird was left in the sample because the seasonal transition was determined a priori (as discussed in the Methods).

FFR: the avian brainstem

The data for the first and second harmonics of the frequency following response were similar but not identical to the results from the CM + FFR (Table 2). Chickadees had a significantly weaker FFR in the winter compared to the spring (FFR, 95 dB: $t_{22} = 3.4$, $P = 0.003$; 35 dB: $t_{22} = 3.2$, $P = 0.006$; FFR2, 95 dB: $t_{22} = 2.1$, $P = 0.044$; 35 dB: $t_{23} = 4.6$, $P = 0.0001$). Nuthatches tested at 95 dB had a stronger response amplitude in the winter only for FFR2 (Fig. 4) (FFR, 95 dB: $t_{22} = 1.3$, $P = 0.21$; 35 dB: $t_{22} = 0.5$, $P = 0.63$; FFR2, 95 dB: $t_{22} = 2.5$, $P = 0.020$; 35 dB: $t_{23} = 0.03$, $P = 0.98$). In titmice, there was no difference across seasons for either measure of FFR (Fig. 4) (FFR, 95 dB: $t_{22} = 0.5$, $P = 0.63$; 35 dB: $t_{22} = 0.99$, $P = 0.33$; FFR2, 95 dB: $t_{22} = 0.29$, $P = 0.79$; 35 dB: $t_{23} = 0.44$, $P = 0.67$). Note the amplitude of FFR2 is about the same as that of FFR (Fig. 4) at high intensities (95 dB) and about half the amplitude of FFR at low intensities (35 dB).

As with CM + FFR, the species differed in the degree to which their frequency-specific FFRs changed across seasons. In chickadees, seasonal FFR differences were more narrow-band than observed in CM + FFR: chickadees exhibited a stronger FFR in the spring (compared to winter) at 2 kHz and at both 1 and 2 kHz for FFR2. The seasonal effect in nuthatches is at a lower frequency than the CM + FFR response: both FFR and FFR2 were significantly weaker in the spring compared to the winter for only the 1 kHz tone (Fig. 5). No significant patterns were evident in titmice (Fig. 5). These species differences are significant as indicated by a significant frequency × species × season interaction (Table 2).

There was a weak effect of sex on the species differences in FFR at 95 dB (Table 2a). This is the result of a significant difference between nuthatch males and females (LSM ± SE: male, 0.011 ± 0.002; female, 0.020 ± 0.003; $t_{21} = 2.7$, $P = 0.015$). No sex differences were detected for chickadees ($t_{21} = 0.4$, $P = 0.70$) or titmice ($t_{21} = 0.7$, $P = 0.51$). There was also a weak effect of sex on seasonal differences in FFR2 at 95 dB (Table 2b). This resulted from females in winter showing a stronger FFR2 than males (LSM ± SE: female, 0.017 ± 0.003; male, 0.008 ± 0.003; $t_{22} = 2.4$, $P = 0.023$). Sex was not a significant factor in the model in spring ($t_{22} = 0.8$, $P = 0.41$). Note that if 'sex' is dropped from the analyses altogether, the other trends described above (which partial out these sex effects) are unchanged.

The fact that patterns detected in the FFR and CM + FFR differ suggests that the CM + FFR waveform includes a CM component. This can be verified by an analysis of the latency with which the sustained response is generated. Figure 1 illustrates that the CM + FFR response is generated in less than 1 ms, suggesting a CM component. When the CM is removed by adding condensation and rarefaction waveforms (Fig. 1a), the onset of the FFR occurs at about 3.5 ms. The magnitude of the

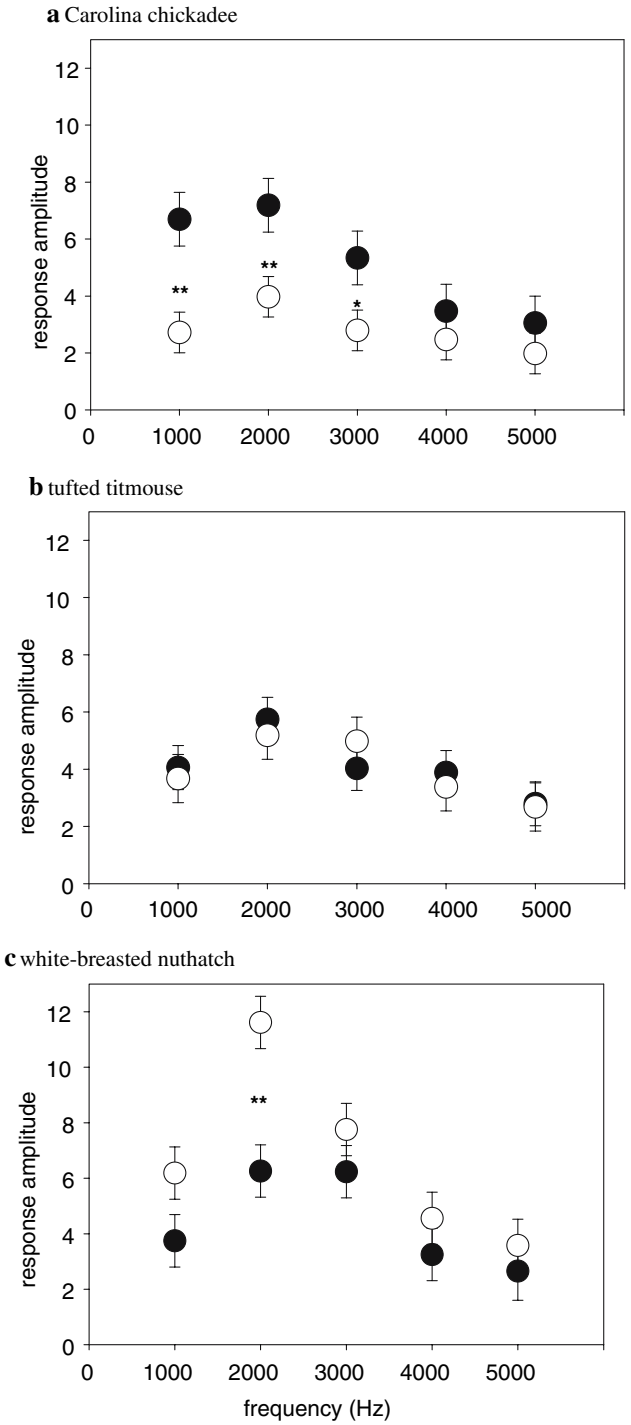

Fig. 3 CM + FFR response measured at 95 dB as a function of frequency for **a** Carolina chickadees, **b** tufted titmice, and **c** white-breasted nuthatches. Data represent least squares means (±SE) based on the statistical models in Table 1. *Closed symbols* spring; *open symbols* winter. Differences between spring and winter are indicated by *asterisk*: ** $P < 0.01$, * $P < 0.05$. Units for response amplitude are cube-root of ($pV^2 \times 10^4$)

CM component (as indicated by the onset latency of the sustained response) also appears to diminish with a reduction in signal intensity 1b).(Fig. 1b).

First peak of onset response (AER)—latency and amplitude

Averaged across frequencies, both chickadees (55 dB: $t_{24} = 2.0$, $P = 0.06$; 95 dB: $t_{24} = 3.9$, $P = 0.001$) and titmice (55 dB: $t_{24} = 1.8$, $P = 0.08$; 95 dB: $t_{24} = 4.5$, $P = 0.001$) showed a lower amplitude of the first peak of the onset response in the winter compared to the spring, whereas nuthatches showed the opposite pattern, at least at 95 dB (55 dB = $t_{24} = 1.4$, $P = 0.18$; 95 dB, $t_{24} = 2.3$, $P = 0.030$) (see Table 3, Fig. 6).

Seasonal patterns in onset amplitude were also frequency dependent (i.e. the season × freq × species interaction was significant, at least for 95 dB; Table 3, Fig. 7), although the patterns were somewhat different than those found with the sustained responses. Here, surprisingly, seasonal differences are evident over a broader range of frequencies in titmice (1–3 kHz) than in chickadees or nuthatches (1–2 kHz).

Averaged across frequencies, the species differed in mean latency (Fig. 6), but seasonal patterns in latency were not different between species (species × season interaction: 55 dB: $F_{2,\ 23} = 2.0$, $P = 0.16$; 95 dB: $F_{2,23} = 0.9$, $P = 0.42$), nor was there a significant season × freq × species interaction ($F_{6,72} = 1.6$, $P = 0.15$).

There was no overall effect of sex on the amplitude of the onset response (55 dB: $F_{1,23} = 0.69$, $P = 0.45$; 95 dB: $F_{1,23} = 0.41$, $P = 0.53$), nor were there any significant interactions with sex (all $P > 0.16$). There was also no overall effect of sex on the latency of the onset response (55 dB: $F_{1,23} = 3.12$, $P = 0.09$; 95 dB: $F_{1,23} = 0.80$, $P = 0.38$), nor were there any significant interactions with sex (all $P > 0.15$). Given that sexes were equivalent in the onset responses, we dropped this term from the repeated measures ANOVAs for the preceding analyses.

MANOVA analyses

We performed a repeated measures MANOVA analysis treating the three sustained-response dependent variables (CM + FFR, FFR, and FFR2) as a single vector. The species × season × Hz interaction was highly significant for the data collected both at 95 dB (Wilk's Lambda = 0.56, $P < 0.0001$; sphericity was not significant: $x^2_2 = 2.6$, $P = 0.27$), and at 35 dB (Wilk's Lambda = 0.74, $P < 0.0001$; however, sphericity was significant: $x^2_2 = 11.2$, $P = 0.004$). These results are consistent with the univariate statistics described above.

There was a weak but significant species × season × Hz interaction for the properties (amplitude and latency) of the first AER peak at 95 dB (Wilk's Lambda = 0.80, $P = 0.042$; sphericity is not calculated

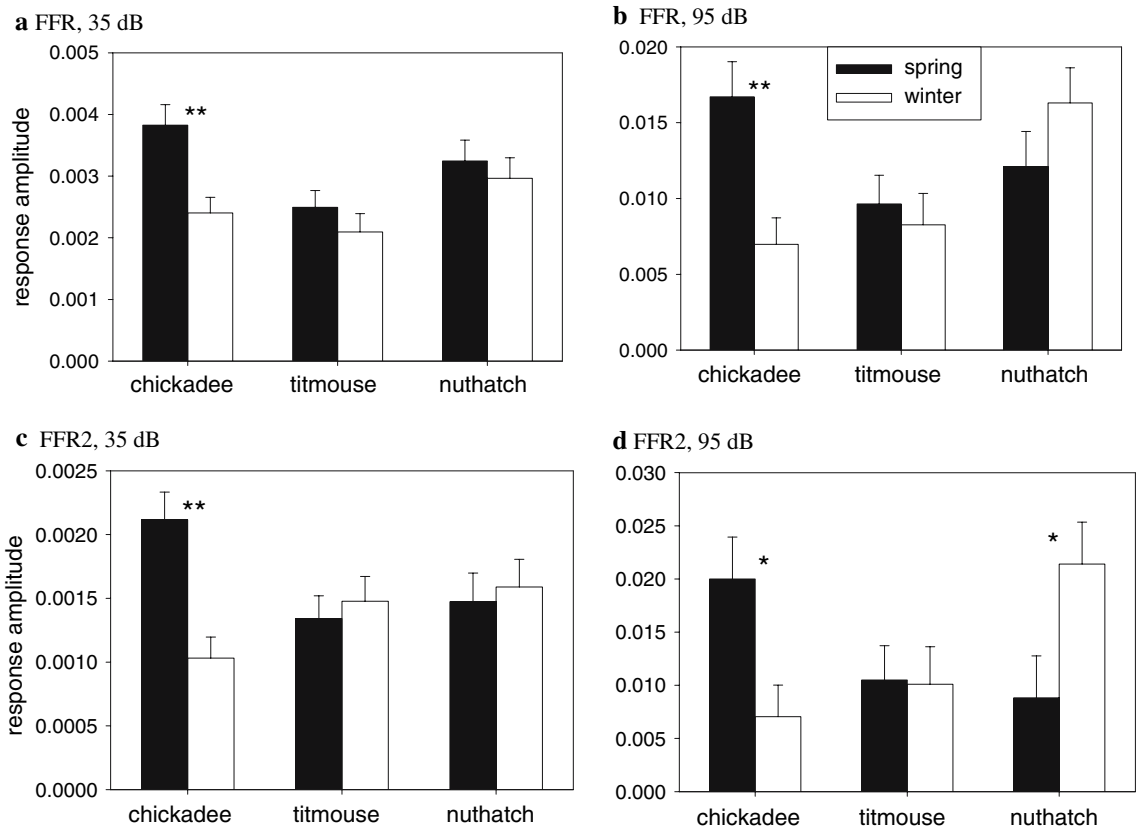

Fig. 4 **a, b** Fundamental (FFR) and **c, d** second harmonic (FFR2) of the condensation + rarefaction waveform as a function of season. Each value is the least squares mean (±SE) based on the statistical model from Table 1, averaged over all frequencies. Data are from 35 (**a, c**) and 95 (**b, d**) dB tone bursts. Units for response amplitude are cube-root of ($pV^2 \times 10^4$). Significance of a seasonal change within species is denoted by *asterisks*: $^{**}P < 0.01$, $^{*}P < 0.05$. Note that total range of each figure is different

with only 2 dependent variables), but the interaction was not significant at 35 dB (Wilk's Lambda = 0.80, $P = 0.070$). However, the species × season interaction was highly significant at both intensities (95 dB: Wilk's Lambda = 0.64, $P = 0.0001$; 55 dB: Wilk's Lambda = 0.64, $P = 0.0007$). These results are consistent with the univariate statistics described above.

Discussion

Our results support those of Lucas et al. (2002): there is seasonal variation in peripheral and brainstem auditory activity in several species of songbirds, with a spring increase in chickadees (and, to a lesser extent, in titmice) and a winter increase in nuthatches. However, our results add important details to this observation. In particular, the spring increase in chickadees is relatively broad-band, whereas the winter increase in nuthatches is narrowly tuned to 2 kHz (for the CM + FFR) or 1 kHz (for the FFR). Moreover, the spring increase shown in titmice is restricted to a relatively broad-band enhancement in the onset response to tones, and does not extend to seasonal differences in a sustained response. Titmice also show seasonal variation in AER responses to click stimuli (Lucas et al. 2002), suggesting that the differential response to tone-induced AERs versus FFRs is real. Finally, our results suggest that the seasonal patterns in sustained responses to tones result from both cochlear (CM) and neural (FFR) components.

Onset AER and FFR data represent different properties of signal processing

While the onset AER and FFR may have anatomically overlapping generators, they are fundamentally different in terms of the underlying neural activity producing these components (Hall 1992; Hoorman et al. 1992; Johnson et al. 2005). The onset AER is a transient response and reflects neural activity synchronized to the onset of the stimulus. Onset AER's have been used to indicate general sensitivity to sound (e.g., Woolley et al. 2001; Britten-Powell et al. 2005) and obviously are a component of onset responses to certain speech patterns (Johnson et al. 2005). The FFR, on the other

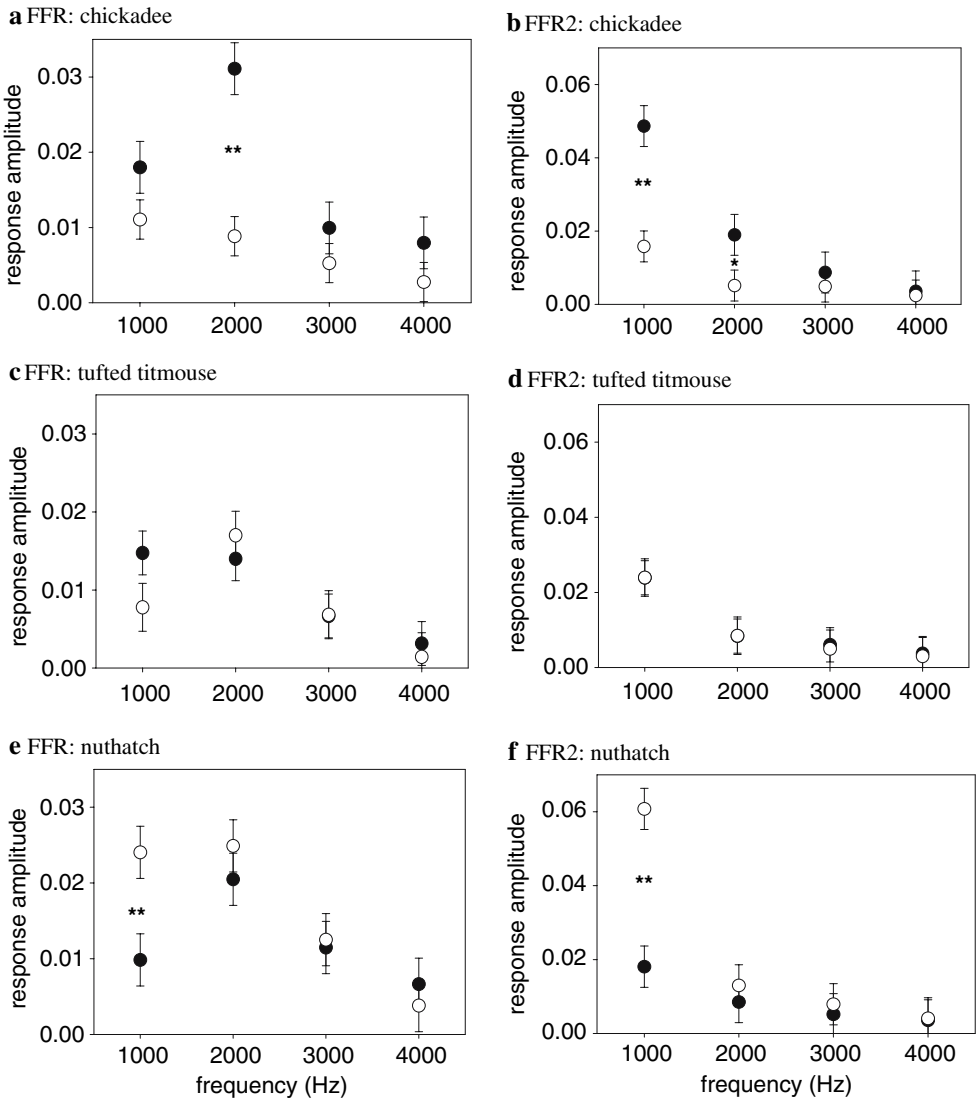

Fig. 5 **a, c, e** Fundamental (FFR) and **b, d, f** second harmonic (FFR2) of the frequency following response measured at 95 dB as a function of frequency for **a, b** Carolina chickadees, **c, d** tufted titmice, and **e, f** white-breasted nuthatches. Data represent least squares means (±SE) based on the statistical models from Table 1. *Closed symbols* spring; *open symbols* winter. Differences between spring and winter are indicated by *asterisk*: $^{**}P < 0.01$, $^{*}P < 0.05$. Units for response amplitude are cube-root of ($pV^2 \times 10^4$)

hand, is a sustained response that reflects neural phase-locking to the individual cycles of the stimulus waveform. Given this, the onset AER measure provides information about the functional integrity of peripheral and brainstem auditory structures. The FFR preserves more information about the acoustic features of complex stimuli and provides for a robust analytic window to evaluate neural encoding of complex sounds at the level of the auditory brainstem (e.g., in human voice encoding: Krishnan and Parkinson 2000, Young and Sachs 1979; Hoormann et al. 1992). Indeed, measurement of onset AER's and FFR's in the same (human) individual shows only a weak correlation between them (Hoorman et al. 1992), underscoring the difference between the indices.

These suggested differences between the function of onset AER's and FFR's are intriguing because seasonal variation in these properties in titmice were so different from those of chickadees. Chickadees showed strong seasonality in FFR's, whereas titmice showed strong seasonality only in onset AER's. One hypothesis for these species differences is that sexual selection on the complex vocal repertoire of chickadees (Ficken and Ficken 1978; Lucas and Freeberg 2007) has selected for enhanced processing of complex sound in the spring, whereas sexual selection on a simpler vocal repertoire in titmice has selected for enhanced sensitivity in the spring (as indicated by stronger onset AER's in spring vs. winter) but no enhancement in the FFR. This idea is easily testable. If the hypothesis is true, auditory responses to complex sounds (e.g., strong amplitude or frequency modulated sounds) should be more robust in chickadees than in titmice, but seasonal variation in behavioral thresholds to simple tones should be greater in titmice (as we demonstrated in this paper).

There is some precedence in the literature for seasonality in both the inner ear and in the auditory

Table 3 Repeated measures ANOVA for (a) AER peak 1 amplitude and (b) latency measured for 55 and 95 dB tone bursts

Independent variable	55 dB			95 dB		
	ndf, ddf	*F*	*P*	ndf, ddf	*F*	*P*
(a) Amplitude						
Frequency	3, 76	80.8	<0.0001	3, 72	108.8	<0.0001
Species	2, 24	5.5	0.011	2, 24	4.0	0.033
Season	1, 24	1.4	0.25	1, 24	10.8	0.004
Species × season	1, 24	3.3	0.053	2, 24	13.6	0.0001
Species × frequency	6, 76	6.0	<0.0001	6, 72	8.3	<0.0001
Season × frequency	ns			3, 72	1.0	0.39
Species × freq × season	ns			6, 72	4.2	0.0012
(b) Latency						
Frequency	3, 77	28.9	<0.0001	3, 81	28.8	<0.0001
Species	2, 26	13.2	0.0001	2, 26	4.6	0.019
Season	1, 26	0.3	0.58	1, 26	1.0	0.33
Species × frequency	6, 77	6.9	<0.0001	6, 81	2.9	0.012

Non-significant interaction terms (ns) were dropped from the model

brainstem. Seasonal changes in auditory acuity in the midshipman fish are related to changes in the inner ear (or sacculus; Sisneros et al. 2004). Seasonal changes in frequency tuning and temporal processing in the leopard frog were identified by single-cell recording in the midbrain (torus semicircularis; Goense and Feng 2005). Assuming that the results of these studies indicate general properties of vertebrate auditory systems, we should expect to see seasonal variation in the physiology of the auditory system at several locations.

Generalities

A number of aspects of our data set are representative of a diversity of taxa. For example, for a particular tone frequency, as the intensity of the stimulus increased, the amplitude of the response increased (and the latency of the first peak decreased; e.g., Dooling and Walsh 1976; Hall 1992). Also, the region of best hearing in many songbird species is between 1,000 and 4,000 Hz (Dooling 1982), and in general we found the strongest amplitude responses at 1,000–2,000 Hz, dropping off substantially by 4,000 Hz.

Our results raise two important questions. The first is the mechanistic basis of this seasonal variation. The second is the functional or evolutionary basis of these seasonal trends. We obviously cannot address either question definitively; however, we can offer our speculation on both points.

Mechanistic basis of seasonal patterns

It is important to note that the cochlea of adult songbirds is inherently plastic in that functional hearing can be restored after deafening due to hair cell damage caused by noise overexposure or ototoxic drugs (Cotanche et al. 1994; Dooling et al. 1997; Cotanche 1999). Perhaps it should not be too surprising that such a plastic system shows evidence of seasonal variability when so much of the species' behavior and physiology is tied to seasonal change. Two major circuits in the songbird brain are devoted to vocal production and to vocal learning (Nottebohm 1981; Ball 1999; Brenowitz 2004). One of these, the anterior forebrain pathway, is fundamental to song learning and song perception, and the starting point of that circuit involves neural input from auditory pathways to the HVC nucleus in the nidopallium (Margoliash 1997; Nottebohm 1999). Experimental manipulation of daylength causes changes in nuclei of the anterior forebrain pathway (Tramontin and Brenowitz 2000). Changes in daylength impact responses of HVC to playbacks of males' songs and of a bird's own songs in canaries, *Serinus canaria* (del Negro et al. 2000, 2005), and longer daylengths produce more rapid auditory discrimination of songs in zebra finches, *Taeniopygia guttata* (Cynx and Nottebohm 1992; however, zebra finches are not truly seasonal breeders, and changes in daylength in the seasonally-breeding song sparrow, *Melospiza melodia*, did not lead to such an effect on learned song discriminations—Reeves et al. 2003). Changes in daylength were also found to affect discrimination of conspecific and heterospecific song in starlings, *Sturnus vulgaris*, and in canaries, *Serinus canaria*, and these two species responded in different ways to those daylength changes (Calhoun et al. 1993). Furthermore, cues related to the breeding season, but unassociated with day length, have been shown to impact these brain regions in rufous-collared sparrows, *Zonotrichia capensis* (Moore et al. 2004).

In the midshipman fish, *Porichthys notatus*, females are attracted to a low frequency 'hum' of males during the breeding season, and female midshipman

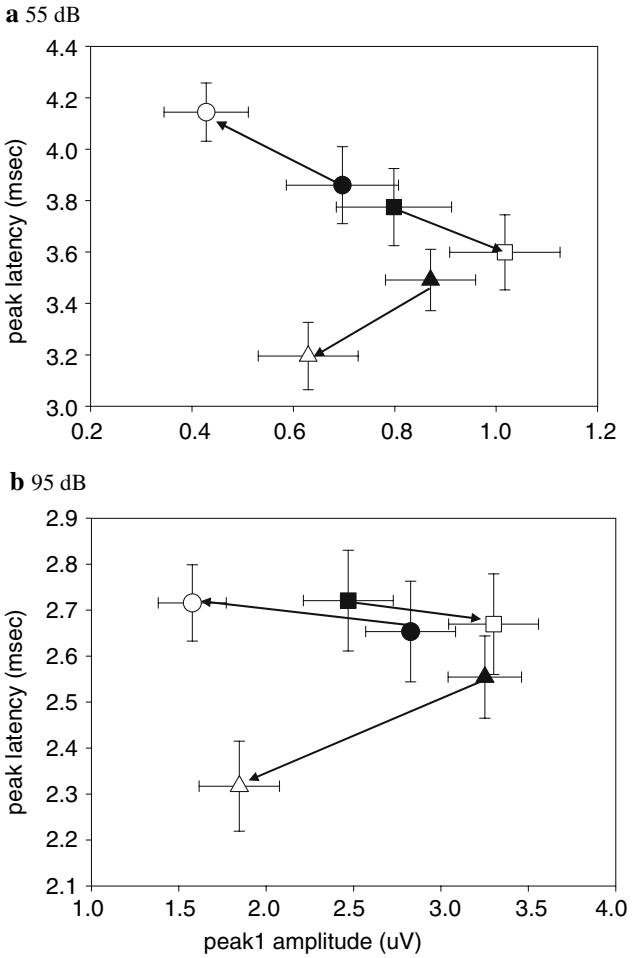

Fig. 6 Latency as a function of relative amplitude for the first positive AER peak measured at **a** 55 dB and **b** 95 dB for Carolina chickadees, tufted titmice, and white-breasted nuthatches. Each value is the least squares mean (±SE) based on the statistical models in Table 3, averaged over all frequencies. *Circles* chickadees; *triangles* tufted titmice; *squares* nuthatches. *Open symbols* winter; *closed symbols*: spring. *Arrows* indicate the change from spring to winter

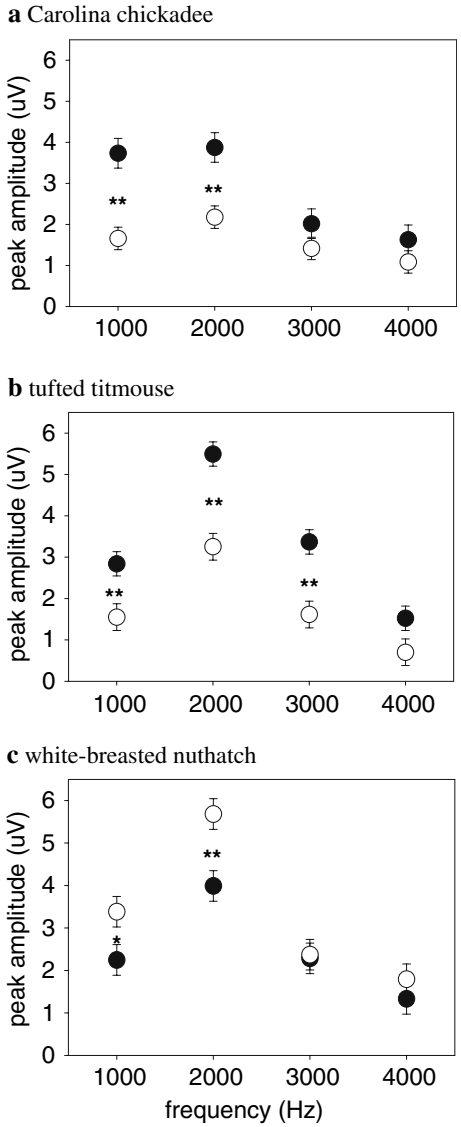

Fig. 7 Peak 1 amplitude measured at 95 dB as a function of tone frequency for **a** Carolina chickadees, **b** tufted titmice and **c** white-breasted nuthatches. Data represent least squares means (±SE) based on the statistical models from Table 3. *Closed symbols* spring; *open symbols* winter. Differences between spring and winter are indicated by *asterisk*: $^{**}P < 0.01$, $^{*}P < 0.05$

show increased sensitivity to the frequencies of the male hum when the breeding season approaches (Sisneros and Bass 2003). This behavioral change seems driven by changes in sensitivity of afferents from the sacculus (the fish's primary organ of hearing in the inner ear) to the auditory nerve. One possible mechanism of season-related neural plasticity in the peripheral auditory system of these fish stems from increased numbers of saccular afferents (Sisneros and Bass 2003). These changes may be facilitated by, or at least associated with, circulating levels of gonadal steroids that themselves change seasonally. Sisneros and Bass (2003) suggest that gonadal steroids influence changes in sensitivity of hair cells of the inner ear by regulating the enzymes that influence the action of ion channels of receptors, and hormone manipulation studies support this hypothesis (Sisneros et al. 2004; see Zakon 1987, for hormone effects in weakly electric fish, *Sternopygus macrurus*, and Yovanov and Feng 1983, for hormone effects in leopard frogs, *Rana pipiens*). If these proposed mechanisms generalize across taxa, parallel analyses—sampling multiple time points over the year—of reproductive behaviors, circulating androgens and estrogens, AERs, and cochlear and auditory system neuroanatomy should reveal strikingly different patterns for the three species of our study.

Functional basis of seasonal patterns

We suggest that the functional basis of our results involves two components of the life history of these birds, their vocal repertoire and the microhabitats that the birds occupy. From the perspective of the vocal repertoire, chickadees unequivocally have a more complex vocal repertoire than white-breasted nuthatches (chickadees: Smith 1972, Ficken et al. 1978; nuthatches: Ritchison 1983). Chickadees employ a wide range of frequencies (1–11 kHz) in their vocal repertoire, and they use a large number of elements from rapidly frequency-modulated tonal notes to an array of notes that are buzzes or stacked overtones (Nowicki and Nelson 1990). Nuthatches use a much narrower range of frequencies with very little frequency modulation and indeed with very little diversity between note types. Titmice are harder to place currently, as so little work has been done on their vocal system, but they appear to lie somewhat intermediate between chickadees and nuthatches in terms of vocal complexity (Lucas et al. 2002). We suggest that differences between chickadees and nuthatches in vocal repertoires select for broadband acuity in chickadees and narrower-band acuity in nuthatches (see Dooling 1982). Evidence published to date indicates that female black-capped chickadees, *Poecile atricapillus*, distinguish between males based on frequency and temporal properties of the song (Christie et al. 2004; male chickadees can also learn to discriminate individual songs Phillmore et al. 2002). Thus, sexual selection may be the driving force increasing chickadee acoustic acuity in the spring.

What aspect of the life history of white-breasted nuthatches is unusual with respect to the acuity of acoustic processing in the fall/winter? Nuthatches are bark foragers (Pravosudov and Grubb 1993). The use of tree trunks as a foraging site has been shown to impose high predation risk on woodpeckers by the inevitable reduction in the visual field caused by the tree trunk (Lima 1992). We suggest that this increased risk, particularly in the fall and winter after leaf fall, puts a premium on low-frequency acoustic acuity (e.g. for hearing the wing beats of avian predators). This conclusion is supported by auditory brainstem responses of downy woodpeckers, which also show increased acoustic acuity in the fall/winter compared to the spring (Lucas et al. 2002). Nuthatches may also use acoustic cues to find insect prey. If true, it may represent a second factor selecting for increased acuity in the winter, when food abundance is low. Finally, the shift in peak sensitivity to winter months may reflect an early mating season: courtship in nuthatches occurs months before actual reproduction (Pravosudov and Grubb 1993). At present we cannot distinguish these alternative hypotheses.

Summary

Our data support the general conclusion drawn by Lucas et al. (2002) that there is seasonal variation in the response to sound by the cochlear and brainstem auditory system of passerines. We found a significant effect of season on CM + FFR and FFR measures in chickadees (greater amplitude responses at a broad range of frequencies in spring) and in nuthatches (greater amplitude responses at lower frequencies in winter). There were also seasonal effects in all three species in the onset response to tones. The fact that this seasonality effect has also been demonstrated in both a fish species (Sisneros and Bass 2003) and a frog species (Goense and Feng 2005) implies that the phenomenon may be quite widespread. Along with Lucas et al. (2002), we believe that this is the first demonstration of the dynamic nature of the peripheral auditory system in birds from the standpoint of normally-occurring seasonal change. This result is important because it extends our view of a seasonally dynamic avian brain (Nottebohm 1981; Tramontin and Brenowitz 2000; Ball et al. 2002; Brenowitz 2004) into the brainstem and even into the inner ear.

Acknowledgments Thanks to Ben Fanson, Kerry Fanson, Ken Henry and Mark Nolen for feedback on the manuscript. Ken Henry helped with system calibration. This work was approved 18 June, 1999 by the Purdue University Animal Care Committee under IACUC no. 99–003. Todd M. Freeberg wishes to acknowledge postdoctoral support by an NIH training grant to the Department of Audiology and Speech Sciences at Purdue University that made this research possible.

References

Ball GF (1999) The neuroendocrine basis of seasonal changes in vocal behavior among songbirds. In: Hauser MD, Konishi M (eds) The design of animal communication. MIT Press, Cambridge, pp 213–253

Ball GF, Riters LV, Balthazart J (2002) Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. Front Neuroendocrinol 23:137–178

Bekesy G (1950) D-C potentials and energy balance of the cochlear partition. J Acoust Soc Am 22:576–582

Bloomfield LL, Phillmore LS, Weisman RG, Sturdy CB (2005) Note types and coding in parid vocalizations: III. The chick-a-dee call of the Carolina chickadee (*Poecile carolinensis*). Can J Zool 83:820–833

Bottjer SW, Johnson F (1997) Circuits, hormones, and learning: vocal behavior in songbirds. J Neurobiol 33:602–618

Brenowitz EA (2004) Plasticity of the adult avian song control system. Ann NY Acad Sci 1016:560–585

Brenowitz EA, Beecher MD (2005) Song learning in birds: diversity and plasticity, opportunities and challenges. Trends Neurosci 28:127–132

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

Britten-Powell EF, Lohr B, Hahn DC, Dooling RJ (2005) Auditory brainstem responses in the Eastern screech owl: an estimate of auditory thresholds. J Acoust Soc Am 118:314–321

Brown-Borg HM, Beck MM, Jones TA (1987) Origin of peripheral and brainstem auditory responses in the white leghorn chick. Comp Biochem Physiol 88A:391–396

Calhoun S, Hulse SH, Braaten RF, Page SC, Nelson RJ (1993) Responsiveness to conspecific and alien song by canaries (Serinus canaria) and European starlings (*Sturnus vulgaris*) as a function of photoperiod. J Comp Psychol 107:235–241

Chaplin SB (1974) Daily energetics of the black-capped chickadee, *Parus atricapillus*, in winter. J Comp Physiol 89:321–330

Chimento TC, Schreiner CE (1990) Selectively eliminating cochlear microphonic contamination from the frequency-following response. Electroencephalogr Clin Neurophysiol 75:88–96

Christie PJ, Mennill DJ, Ratcliffe LM (2004) Pitch shifts and song structure indicate male quality in the dawn chorus of black-capped chickadees. Behav Ecol Sociobiol 55:341–348

Cooper SJ, Swanson DL (1994) Seasonal acclimatization of thermoregulation in the black-capped chickadee. Condor 96:638–646

Cotanche DA (1999) Structural recovery from sound and aminoglycoside damage in the avian cochlea. Audiol Neurootol 4:271–285

Cotanche DA, Lee KH, Stone JS, Picard DA (1994) Hair cell regeneration in the bird cochlea following noise damage or ototoxic drug damage. Anat Embryol 189:1–18

Cynx J, Nottebohm F (1992) Role of gender, season, and familiarity in discrimination of conspecific song by zebra finches (*Taeniopygia guttata*). Proc Natl Acad Sci USA 89:1368–1371

Del Negro C, Kreutzer M, Gahr M (2000) Sexually stimulating signals of canary (*Serinus canaria*) songs: Evidence for a female-specific auditory representation in the HVc nucleus during the breeding season. Behav Neurosci 114:526–542

Del Negro C, Lehongre K, Edeline J (2005) Selectivity of canary HVC neurons for the bird's own song: modulation by photoperiodic conditions. J Neurosci 25:4952–4963

Dooling RJ (1982) Auditory perception in birds. In: Kroodsma DE, Miller EH (eds) Acoustic communication in birds. Academic, New York, pp 95–130

Dooling RJ (1992) Hearing in birds. In: Webster DB, Fay RR, Popper AN (eds) The evolutionary biology of hearing. Springer, New York, pp 545–559

Dooling RJ, Walsh JK (1976) Auditory evoked response correlates of hearing in the parakeet (*Melopsittacus undulates*). Physiol Psychol 4:224–232

Dooling RJ, Ryals BM, Manabe K (1997) Recovery of hearing and vocal behavior after hair-cell regeneration. Proc Natl Acad Sci USA 94:14206–14210

Dooling RJ, Lohr B, Dent ML (2000) Hearing in birds and reptiles. In: Dooling RJ, Fay RR, Popper AN (eds) Comparative hearing: birds and reptiles. Springer, Berlin Heidelberg, New York, pp 308–359

Ficken MS, Ficken RW, Witkin SR (1978) Vocal repertoire of the black-capped chickadee. Auk 95:34–48

Gardi HN, Merzenich MM, KcKean C (1979) Origins of the scalp-recorded frequency-following responses in the cat. Audiology 18:353–381

Goense JBM, Feng AS (2005) Seasonal changes in frequency tuning and temporal processing in single neurons in the frog auditory midbrain. J Neurobiol 65:22–36

Hall JW III (1992) Handbook of auditory-evoked responses. Allyn and Bacon, Boston

Hoorman J, Falkenstein M, Hohnsbein J, Blanke L (1992) The human frequency-following response (FFR): normal variability and relation to the click-evoked brainstem response. Hear Res 59:179–188

Huis in't Veld F, Osterhammel P, Terkildsen K (1977) Frequency following auditory brainstem responses in man. Scand Audiol 6:27–34

Johnson KL, Nicol TG, Kraus N (2005) Brain stem response to speech: a biological marker of auditory processing. Ear Hear 26:424–434

Krishnan A (1999) Human frequency-following responses to two-tone approximations of steady-state vowels. Audiol Neurootol 4:95–103

Krishnan A, Parkinson J (2000) Human frequency-following response: representation of tonal sweeps. Audiol Neurootol 5:312–321

Lima SL (1992) Vigilance and foraging substrate: antipredatory considerations in a nonstandard environment. Behav Ecol Sociobiol 30:283–289

Lucas JR, Freeberg TM (2007) "Information" and the chick-a-dee call: communicating with a complex vocal system. In: Otter KA (ed) Ecology and behaviour of chickadees and titmice: an integrated approach. Oxford University Press, New York, (in press)

Lucas JR, Peterson L, Boudinier R (1993) The effects of time constraints and changes in body mass and satiation on the simultaneous expression of caching and diet-choice decisions. Anim Behav 45:639–658

Lucas JR, Freeberg TM, Krishnan A, Long GR (2002) A comparative study of avian auditory brainstem responses: correlations with phylogeny and vocal complexity, and seasonal effects. J Comp Physiol A 188:981–992

Lucas JR, Freeberg TM, Egbert J, Schwabl H (2007) Corticosterone, body mass, and caching rates of Carolina chickadees from disturbed and undisturbed sites. Horm Behav 49:634–643

Margoliash D (1997) Functional organization of forebrain pathways for song production and perception. J Neurobiol 33:671–693

Moore IT, Wingfield JC, Brenowitz EA (2004) Plasticity of the avian song control system in response to localized environmental cues in an equatorial songbird. J Neurosci 24:10182–10185

Nottebohm F (1981) A brain for all seasons: Cyclical anatomical changes in song-control nuclei of the canary brain. Science 214:429–436

Nottebohm F (1999) The anatomy and timing of vocal learning in birds. In: Hauser MD, Konishi M (eds) The design of animal communication. MIT Press, Cambridge, pp. 63–110

Nowicki S, Nelson DA (1990) Defining natural categories in acoustic signals: comparison of 3 methods applied to chick-a-dee call notes. Ethol 86:89–101

Offutt GC (1965) Behavior of the tufted titmouse before and during the nesting season. Wilson Bull 77:382–387

Phillmore LS, Sturdy CB, Turyk MRM, Weisman RG (2002) Discrimination of individual vocalizations by black-capped chickadees (*Poecile atricapilla*). Anim Learn Behav 30:43–52

Pravosudov VV, Grubb TC Jr (1993) White-breasted nuthatch (*Sitta carolinensis*). In: Poole A, Gill F (eds) The birds of North America, No. 54. American Ornithologists' Union, Washington, pp 1–15

Pyle P (1997) Identification guide to North American birds. Slate Creek Press, Bolinas

Reeves BJ, Beecher MD, Brenowitz EA (2003) Seasonal changes in avian song control circuits do not cause seasonal changes in song discrimination in song sparrows. J Neurobiol 57:119–129

Ritchison G (1983) Vocalizations of the white-breasted nuthatch. Wilson Bull 95:440–451

Saunders JC, Pallone RL, Rosowski JJ (1980) Frequency selectivity in parakeet hearing: behavioral and physiological evidence. In: Nöring R (ed) Proceedings of the 27th International Congress of Ornithology. Deutsche Ornithologen-Gesellschaft, Berlin, pp 615–619

Schroeder DJ, Wiley RH (1983) Communication with shared song themes in tufted titmice. Auk 100:414–424

Sibley CG, Ahlquist JE (1990) Phylogeny and classification of birds: a study in molecular evolution. Yale University Press, New Haven

Sisneros JA, Bass AH (2003) Seasonal plasticity of peripheral auditory frequency sensitivity. J Neurosci 23:1049–1058

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

Smith SM (1991) The black-capped chickadee: behavioral ecology and natural history. Comstock, Ithaca

Smith ST (1972) Communication and other social behavior in *Parus carolinensis*. Publ Nuttall Ornithol Club 11:1–125

Sohmer H, Pratt H, Kinarti R (1977) Sources of frequency following responses (FFR) in man. Electroencephalogr Clin Neurophysiol 42:656–664

Stillman RD, Crow G, Moushegian G (1978) Components of the frequency-following potential in man. Electroencephalogr Clin Neurophysiol 44:438–446

Thirakhupt K (1985) Foraging ecology of sympatric parids: individual and populational responses to winter food scarcity. Ph.D. thesis, Purdue University, West Lafayette, Indiana

Tramontin AD, Brenowitz EA (2000) Seasonal plasticity in the adult brain. Trends Neurosci 23:251–258

Woolley SM, Wissman AM, Rubel EW (2001) Hair cell regeneration and recovery of auditory thresholds following aminoglycoside ototoxicity in Bengalese finches. Hear Res 153:181–195

Yovanov S, Feng AS (1983) Effects of estradiol on auditory evoked responses from the frog's auditory midbrain. Neurosci Lett 36:291–297

Young ED, Sachs MB (1979) Representation of steady-state vowels in the temporal aspects of the discharge patterns of populations of auditory-nerve fibers. J Acoust Soc Am 66:1381–1403

Zakon HH (1987) Hormone-mediated plasticity in the electrosensory system of weakly electric fish. Trends Neurosci 10:416–421