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A comparative study of avian auditory brainstem responses: correlations with phylogeny and vocal complexity, and seasonal effects

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Abstract We conducted a comparative study of the peripheral auditory system in six avian species (downy woodpeckers, Carolina chickadees, tufted titmice, white-breasted nuthatches, house sparrows, and European starlings). These species differ in the complexity and frequency characteristics of their vocal repertoires. Physiological measures of hearing were collected on anesthetized birds using the auditory brainstem response to broadband click stimuli. If auditory brainstem response patterns are phylogenetically conserved, we predicted woodpeckers, sparrows, and starlings to be outliers relative to the other species, because woodpeckers are in a different Order (Piciformes) and, within the Order Passeriformes, sparrows and starlings are in different Superfamilies than the nuthatches, chickadees, and titmice. However, nuthatches and woodpeckers have the simplest vocal repertoires at the lowest frequencies of these six species. If auditory brainstem responses correlate with vocal complexity, therefore, we would predict nuthatches and woodpeckers to be outliers relative to the other four species. Our results indicate that auditory brainstem responses measures in the spring broadly correlated with both vocal complexity and, in some cases, phylogeny. However, these auditory brainstem response patterns shift from spring to winter due to species-specific seasonal changes. These seasonal changes suggest plasticity at the auditory periphery in adult birds.

Keywords Auditory brainstem response · Auditory evoked responses · Birds · Hearing · Vocal complexity

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Introduction

Vocal communication in animals requires at minimum a signaler, a signal, and a receiver. In avian species, many of which rely considerably on vocal communication, decades of research have uncovered developmental, morphological, ecological, and phylogenetic constraints and influences on vocal production (reviews in Hauser and Konishi 1999; Kroodsma and Miller 1982, 1996). Work indicates that the morphology of structures involved in vocal production can constrain the vocalizations species produce (Larsen and Goller 1999; Podos 1997, 2001; Suthers 1999). Relatively little work, however, has been devoted to addressing the reception side of vocal communication systems in birds (but see Dooling 1992; Dooling et al. 2000a). In the present study, we assessed the properties of avian vocal systems from the standpoint of receivers, using a comparative analysis of auditory brainstem responses (ABRs) among six species.

ABRs are a class of auditory-evoked responses generated by the peripheral auditory system – the auditory nerve and the auditory brainstem neurons – in response to acoustic stimuli. (ABRs are sometimes referred to in the literature as brainstem auditory evoked responses or brainstem auditory evoked potentials). ABRs in birds, as well as in mammals, are electrical potentials that are generated within 5–10 ms after the onset of the acoustic stimulus. The earliest peaks of the ABR response are generated by peripheral neurons and are not affected by subject state of arousal or by anesthetic agents or sedative drugs (Corwin et al. 1982; Hall 1992; see Sheykholeslami et al. 2001, who found an effect of anesthetic used in quail, *Coturnix* species, primarily in the later ABR peaks). Because early ABR peaks are relatively unaffected by anesthetic drugs, ABRs allow for relatively rapid testing of peripheral hearing responses in non-human animals. Additionally, ABR threshold measures correlate well with behavioral hearing thresholds, especially in the range 2000–4000 Hz (Dooling and

Walsh 1976; Konishi 1985; Saunders et al. 1980), a fact that supports the utility of this technique for assessing auditory reception at the periphery. We tested ABRs to broadband clicks with two protocols. The first varied click intensity while controlling for click rate (a standard testing protocol in human audiology), and the second varied click rate at constant intensity.

We measured ABRs in six species of birds (see Sibley and Ahlquist 1990 for a description of the relationships of these birds). Downy woodpeckers, *Picoides pubescens*, are the smallest North American woodpecker species, in the order Piciformes, family Picidae. The rest of the species tested are in the songbird order Passeriformes. Three of the passerine species tested, Carolina chickadees *Poecile carolinensis*, tufted timice *Baeolophus bicolor* (both in the family Paridae), and white-breasted nuthatches *Sitta carolinensis* (family Sittidae), are in the superfamily Sylvioidea. House sparrows, *Passer domesticus*, are in the superfamily Passeroidea, family Passeridae. European starlings, *Sturnus vulgaris*, are in the superfamily Muscicapoidea, family Sturnidae.

The six species comprising the present study therefore differed in their phylogenetic relationships, but they also differed in the complexity of their vocal repertoires. Our measures of vocal complexity for these species have a basis in Konishi's (1970) study of ten bird species using single-neuron recordings in the avian cochlear nuclei, nucleus magnocellularis and nucleus angularis. He found that the distribution of thresholds of single units generally correlated with behavioral audibility curves and with the range of vocal frequencies in the vocal repertoires of the birds – upper ranges of single unit characteristic frequencies correlated positively with vocal frequencies in these species (Konishi 1970). Psychoacoustic measures of best hearing ranges in birds also correspond well with the primary frequency ranges of vocal repertoires (e.g., Dooling and Saunders 1975; Farabaugh et al. 1998; Okanoya and Dooling 1988). As an index of vocal complexity in our six study species, we measured three frequency parameters of the vocal systems of these species (Table 1). The three frequency parameters were (1) the average high frequency of fundamentals of notes, calls, and songs; (2) the average frequency range within notes, calls, or songs; and (3) the complexity of frequency changes within note types (whether there were multiple frequency shifts within a single note type rather than simple ascending, descending, or chevron-shaped note types). White-breasted nuthatches and, to a lesser extent, downy woodpeckers, have the lowest frequencies and simplest vocal repertoires (Table 1). By these measures, Carolina chickadees, house sparrows, and European starlings have the highest frequencies and most complex vocal repertoires, with tufted titmice having relatively intermediate complexity.

We had three main goals with this study. First, we wished to assess basic peripheral hearing responses in these birds as a foundation for future work. Avian species are increasingly used as model systems for studying processes of hearing and recovery from hearing loss – birds can regenerate hair cells after loss due to overexposure to noise or to ototoxic drugs (Cotanche 1999; Cotanche et al. 1994, 1998; Dooling et al. 1997). Second, we sought to determine whether species differences in ABRs existed for this set of birds and if so, whether these differences might broadly be predicted by phylogeny and/or vocal characteristics of the species. If phylogeny is a strong predictor of ABRs in these species, we should find downy woodpeckers, house sparrows, and European starlings to be outliers in relation to the Sylvioidea, with the woodpeckers being the most disparate group. Indeed, audiograms from a diversity of avian taxa indicate that non-Passeriformes species in general have sharper high-frequency cutoffs than Passeriformes species, except for some exceptional species such as barn owls, *Tyto alba*, with very good high-fre-

Table 1 Measures of complexity in vocal repertoires of downy woodpeckers (dw), Carolina chickadees (cc), tufted titmice (tt), white-breasted nuthatches (nh), house sparrows (hs), and European starlings (st). Frequency measures (to the nearest 0.5 kHz) taken from sonograms published in cited sources

Species (no. of vocalizations analyzed)	Average high frequency (kHz) of fundamental ($\pm$SD)[1]	Average frequency (kHz $\pm$ SD)[2]	% Complex[3]	References[4]
dw (7)	3.6 $\pm$ 0.2	2.2 $\pm$ 0.8	42.9	g, m
nh (12)	2.1 $\pm$ 0.4	0.7 $\pm$ 0.3	33.3	j
tt (6)	5.0 $\pm$ 3.3	2.0 $\pm$ 2.7	33.3	f, i, k
cc (15)	7.1 $\pm$ 2.4	4.0 $\pm$ 2.2	46.7	l
hs (12)	4.8 $\pm$ 1.3	2.7 $\pm$ 0.6	58.3	h
st (15)	6.7 $\pm$ 2.2	5.4 $\pm$ 2.3	80.0	a, b, c, d, e

[1]For each note, or the fundamental of each note containing harmonic-like structures, the highest frequency of the note, averaged across all the vocalizations analyzed for each species

[2]For each note or string of notes (calls or songs), the range from the highest frequency (same as 1) to the lowest frequency, averaged across all the vocalizations for each species

[3]Within a note or string of notes (calls or songs), if the vocal type exhibited multiple changes of frequency rather than simple ascending, descending, or chevron-shaped frequency changes, it was classified as "complex"

[a]Adret-Hausberger (1988); [b]Adret-Hausberger and Jenkins (1988); [c]Adret-Hausberger et al. (1990); [d]Eens et al. (1991, 1992); [e]Feare (1984); [f]Grubb and Pravosudov (1994); [g]Mahan (1996); [h]Nivison (1978); [i]Offutt (1965); [j]Ritchison (1983); [k]Schroeder and Wiley (1983a, 1983b); [l]Smith (1972); [m]Winkler and Short (1978)

quency hearing (Köppl 1997; reviewed in Dooling et al. 2000b). Alternatively, if vocal complexity is a strong predictor of ABRs in these species, we should find that white-breasted nuthatches and perhaps downy woodpeckers show longer latency and lower amplitude responses than the other species. This is because the ABRs to click stimuli are primarily driven by the high-frequency, basal portions of the cochlea (or the basilar papilla in birds). Species with fewer hair cells devoted to higher frequencies would be expected to show responses with longer latencies (as the response would be generated in the more apical regions of the basilar papilla, and the time required for the traveling wave to reach those regions would be longer) and lower amplitudes (see Chen et al. 1994; Gleich and Manley 2000; Köppl and Manley 1997; Rubel and Ryals 1982). Further, variables such as differences in skull thickness or size will not affect ABR latency measures. Volume conduction of auditory-evoked potentials occurs through an essentially resistive medium – in the absence of reactive components of impedance, changes in recording configurations (or skull thickness) will only affect the response amplitude and not the response latency (Ananthanarayan and Durrant 1991; Schlag 1973). Third and finally, by testing birds in different parts of the year, we sought to assess whether these peripheral hearing responses might change across seasons – if so, this would suggest intriguing neural plasticity in the peripheral auditory system.

Materials and methods

Subjects

We divided our testing of 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 most species (e.g., Kroodsma and Miller 1982, 1996). ABRs were tested in adult birds – birds were aged according to Pyle (1997). We tested ABRs in 10 downy woodpeckers (5 in spring, 5 in winter), 9 white-breasted nuthatches (5 in spring, 4 in winter), 11 Carolina chickadees (5 in spring, 6 in winter), 13 tufted titmice (7 in spring, 6 in winter), 12 house sparrows (7 in spring, 5 in winter), and 8 European starlings (0 in spring, 8 in winter). We ran roughly equal numbers of males and females of each of the sexually dimorphic species (i.e., woodpeckers, nuthatches, house sparrows and starlings). Carolina chickadees and tufted titmice are sexually monomorphic; sex was not determined in these species. Given our relatively low sample sizes and approximately equal sex ratio in the sample, we did not differentiate sex in our statistical analysis but instead simply treated the potential of sex differences in ABRs as a source of residual variation. Except for 4 starlings, birds were captured in residential areas and in the Ross Biological Station of Purdue University, West Lafayette, IN. The other 4 starlings were captured with mist nets at one of Purdue's Agricultural research farms. Temporary seed stands with Potter traps (treadle traps) were baited with sunflower seed, safflower seed, mixed bird seed, or suet for several days to attract the desired species.

Captured birds were brought into captivity immediately after capture and housed indoors in individual 1-m^3 wire mesh home cages. In captivity, birds were never housed in complete isolation, and could always hear other birds of these species housed in other cages. Birds were kept under a light/dark cycle appropriate for their north-central Indiana capture area and the time of year. Each individual was provided with mixed seed, crushed oyster shell and grit, and was given two or three mealworms and fresh vitamin-treated water daily. Starlings and woodpeckers were also provided with suet. After ABR testing (described below), birds were housed in their individual cages an additional 2–3 days and then were released at their individual sites of capture. For each species (except the less sedentary starlings), we were able to observe many of our color-banded subjects in the field at their capture sites months, and in some cases over a year, after we had run them in our ABR tests.

Average masses (mean ± SD) of each species, collected from individuals immediately before we had tested their ABRs, were 10.0 ± 0.7 g for Carolina chickadees, 26.0 ± 1.2 g for downy woodpeckers, 69.4 ± 6.2 g for European starlings, 26.0 ± 1.6 g for house sparrows, 20.4 ± 1.9 g for tufted titmice, and 20.6 ± 1.3 g for white-breasted nuthatches.

Preparation and ABR testing of subjects

ABRs of birds were measured between 1300 and 1730 hours. Subjects were placed into black plastic carrying boxes and transported to the audiology laboratory where they were 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^{-1}, 0.1 ml of xylazine at 100 mg ml^{-1}, in 9.4 ml sterile saline). Dosage varied from 0.10 ml to 0.17 ml per 10 g of bird, depending on the species. Starlings, having considerably larger masses than the other five species, were given smaller amounts of a more concentrated mixture of ketamine/xylazine (1.5 ml of ketamine at 100 mg ml^{-1}, 1.5 ml of xylazine at 20 mg ml^{-1}, in 12ml of sterile saline). Dosage for starlings with this less-dilute mixture was 0.10 ml per 25 g of bird.

After injection, each subject was placed on a small hand towel inside its carrying box for 5–6 min until its eyes closed and it appeared fully anesthetized. After this time period, it was taken into the testing room, a walk-in IAC acoustic isolation room. Evoked responses were recorded differentially between 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). Another electrode placed under the skin of the back (nape) of the neck served as the common ground. After the electrodes were inserted and held firmly 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 the temperature at 35–40 °C for at least 30 min (longer than needed to complete the ABR tests) which is well within the thermoneutral zone for these small birds (e.g., see Chaplin 1974; Cooper and Swanson 1994; O'Connor 1975). The entire set of protocols, including some tone burst protocols not discussed here, took approximately 45 min to run per bird. For a subset of the birds in the study, we measured the ABR to a 112-dB sound pressure level (SPL) click stimulus at the beginning of 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 over the course of testing, which might be indicative of anesthetic effects. For the five species for which we did this second testing using a click at 112 dB SPL, only starlings showed a significant change in latency. Peak I and peak II were significantly longer for starlings at the onset of testing relative to roughly 30 min into testing – why this would be the case only for starlings is unknown.

ABRs were elicited with broad-band alternating click stimuli, using Intelligent Hearing Systems Smart EP (version 2.2). For the click intensity protocol, clicks were presented at 31.1 s^{-1}. Intensities presented were 112, 92, 72, 62, 52, and 42 dB SPL (these correspond to standard testing levels of the IHS system for humans of 80, 60, 40, 30, 20, and 10 dB nHL). For the click rate protocol, clicks were presented at 112 dB SPL, at rates of 31.1, 41.9, 61.9, 81.9, 101.9, 121.9, and 141.9 s^{-1}. The click stimuli were presented through an ER-3A-MS (Custom) 300-Ω insert earphone, coupled

to the ear of the subject with putty. The click stimuli used to energize the insert earphone produced a broadband stimulus with uniform output to 4000 Hz, dropping subsequently at a rate of about 35 dB/octave. An IHS Opti-Amp 8000 was used to amplify the EEGs. Interelectrode impedances were maintained below 7 kΩfor all of the species except chickadees, where the best we were able to achieve was below 20 kΩ. 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 were collected at each intensity and rate. Each response waveform represented 512 stimulus presentations over a 12.8-ms analyses window with a sampling rate of 40 kHz.

Measurement of ABR waveforms

Fig. 1 shows representative ABR waveforms for the six species. We measured characteristics of the first two positive peaks (designated I and II) and the first large negative peak (designated –III). Peaks I, II, and –III were highly conserved across individuals and across species. The generator site of peak I is likely the auditory nerve (Brown-Borg et al. 1987; Hall 1992). We do not know the generator sites of peak II and peak –III for these species, but we know they are, with increased latency of response, progressively further along the auditory brainstem. Peak II might be the proximal portion of the auditory nerve (this would make peak I the distal portion of the auditory nerve; see Brown-Borg et al. 1987 for a study in the white leghorn chick), or it might be a response primarily generated in the nucleus magnocellularis, an avian cochlear nucleus receiving direct projections from the auditory nerve. Some lesion studies with small mammals suggest that peak II might be generated even further along the auditory brainstem (Achor and Starr 1980). Lesion studies in passerine birds would be needed to determine the generators for these peaks. Regardless of precise generator sites, the conserved nature of peaks I, II, and –III across the six species in the present study strongly suggests they are generated at the same sites for these birds.

At each intensity and rate, we determined for each peak the latency in milliseconds and the amplitude in microvolts (see Fig. 1). The amplitude measure for peak I was from the peak to the following trough. Peak II amplitude was measured from peak II to peak –III (the negative peak). Peak –III amplitude was measured from peak –III to the next major positive peak (labeled with an asterisk in Fig. 1). Latency measures indicate how quickly the generators of the auditory brainstem respond to the onset of the broadband click stimuli. The various amplitude measures indicate the strength of the response for that particular generator, relative to the next generator. Because ABRs to click stimuli are driven primarily by basal, high-frequency portions of the avian basilar papilla, we assumed that species exhibiting shorter latencies and higher amplitudes of peaks would be those species with stronger auditory periphery responses at higher frequencies.

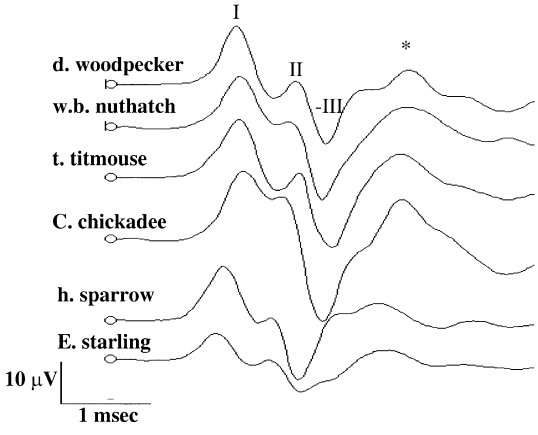

Fig. 1 Examples of auditory brainstem response (ABR) waveforms from each species used in the study. The three peaks reported in this study (I, II, and –III) are labeled on the waveform of the downy woodpecker. The *asterisk* indicates the peak used in the measurement of peak –III amplitude

Statistical analyses

A large amount of data was collected sequentially on each bird used in the study. We therefore used repeated measures analysis of covariance with first-order autoregressive structure (Proc MIXED; SAS Institute 1994) to test for the effect of our independent variables on our two dependent variables: peak latency and peak amplitude. We used three main effects in each regression model: (1) season (spring versus winter), (2) species, and (3) click rate or click intensity. We independently analyzed the data sets generated from varying click rate (at a fixed 112 dB SPL) and the sets generated by varying click intensity (at a fixed 31.1 clicks/s). Season and species effects were treated as discrete variables. Rate and intensity were treated as continuous variables. For the continuous variables, nonlinearity was tested by adding a squared term to the model and checking the residuals for the presence of higher-order effects. In all cases, the dependent variables were either linearly or quadratically (i.e., showing a significant squared term) correlated with the independent variables. Bird identity was used as the subject variable.

All two-way interactions between the main effects and the three-way interaction were added to the models. Non-significant interactions were dropped from the model in order of decreasing P value until all remaining interactions were significant ($\alpha = 0.05$). Note that the degrees of freedom may vary depending on the interaction terms left in the model. Normality of the model residuals was tested using Proc UNIVARIATE (SAS Institute 1990). In several cases, peak amplitude had to be log transformed to normalize the residuals. The residuals for peak latency were normally distributed for all tests. Where the season×species interaction (or the season×species×click-rate/click-intensity interaction) was significant, we tested for two sets of multiple comparisons using the DIFF option of the LSMEANS calculation in Proc MIXED: (1) seasonal changes in peak latency or peak amplitude within each species, and (2) species difference in peak latency or amplitude within each season.

Results

We analyzed six species in two seasons with seven levels of click rate and six levels of click intensity (note: no starlings were run in the spring). Species ABR profiles for click rates were largely similar to those for click intensity; we therefore report only the click intensity data. We focus on several components of the data set that we consider particularly relevant to this study: (1) peak latency versus click intensity and peak amplitude versus click intensity functions; (2) seasonal variation in mean latency and amplitude for each species; (3) within-season differences between species in mean latency and amplitude. The latter two components were estimated by taking least-squares means for each combination of species and season, controlling for click intensity. Most of the statistical models generated a significant three-way interaction between intensity, species and season. For example, the relationship between peak latency and click intensity varied between species, and this relationship changed from spring to winter. Nonetheless, there was relatively little variation in the general shape of the latency/amplitude versus intensity curves. Below, we

note where these three-way interactions are significant but only provide qualitative descriptions of the basis of the 3-way interactions.

Latency

Peak latency decreased non-linearly with an increase in click intensity (Figs. 2, 3: Table 2). For peaks I and II, this relationship between click intensity and peak latency was complicated by a significant species×season×intensity interaction (Table 2).

A significant species×season interaction was found for all three peaks (Table 2). Multiple comparisons indicate two trends in seasonal differences in peak latency within species: (1) the woodpeckers and nuthatches showed a reduction in latency from spring to winter for all three peaks (Fig. 4; Table 3); and (2) the chickadees, sparrows and titmice showed no significant change in peak latency from spring to winter (Table 3).

We used multiple comparisons of least-squares means to compare species differences within each season. In the spring, the sparrows exhibited the shortest latency of the species we studied for all three peaks (Fig. 4a, b, c; Table 4). The parids (chickadees and titmice) had intermediate latencies and the nuthatches and woodpeckers had the longest latencies, although for peak I none of the latter four species was significantly different from any other (Table 4).

The relationship between species was substantially different in the winter compared to the spring (Fig. 4d, e, f; Table 4). First, there was broader overlap in peak latency between species in the winter, resulting in non-significant differences for most species comparisons. Second, chickadees exhibited the longest latencies for all three peaks and starlings generally exhibited the shortest latencies.

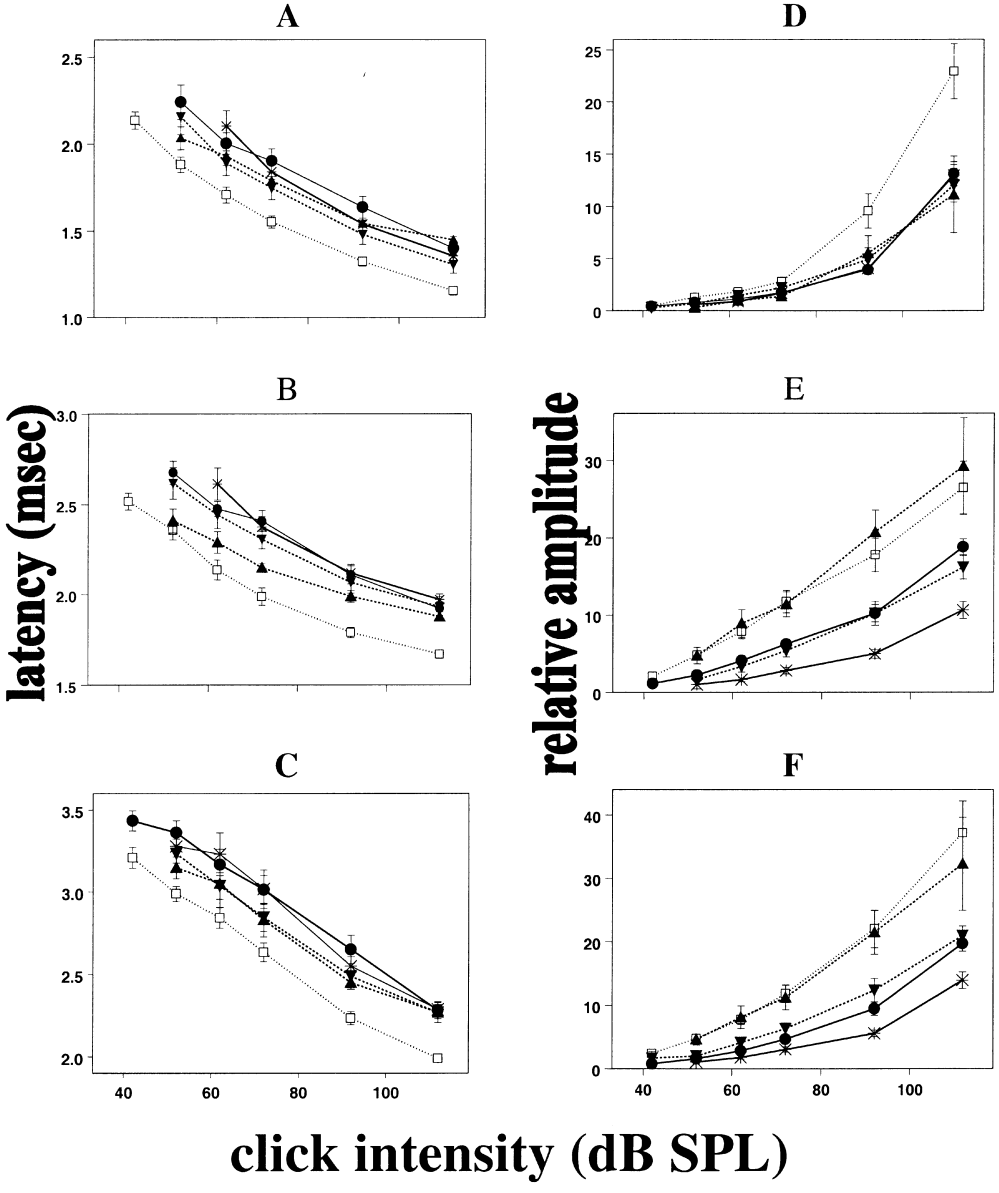

Fig. 2 Peak latency (**A**–**C**) and amplitude (**D**–**F**) as a function of click intensity for spring. **A**, **D** Peak I; **B**, **E** peak II; **C**, **F**: peak –III. Symbols represent mean and SE. *Crosses*: downy woodpecker; *filled circles*: white-breasted nuthatch; *filled triangles*: Carolina chickadee; *filled inverted triangles*: tufted titmouse; *open squares*: house sparrow

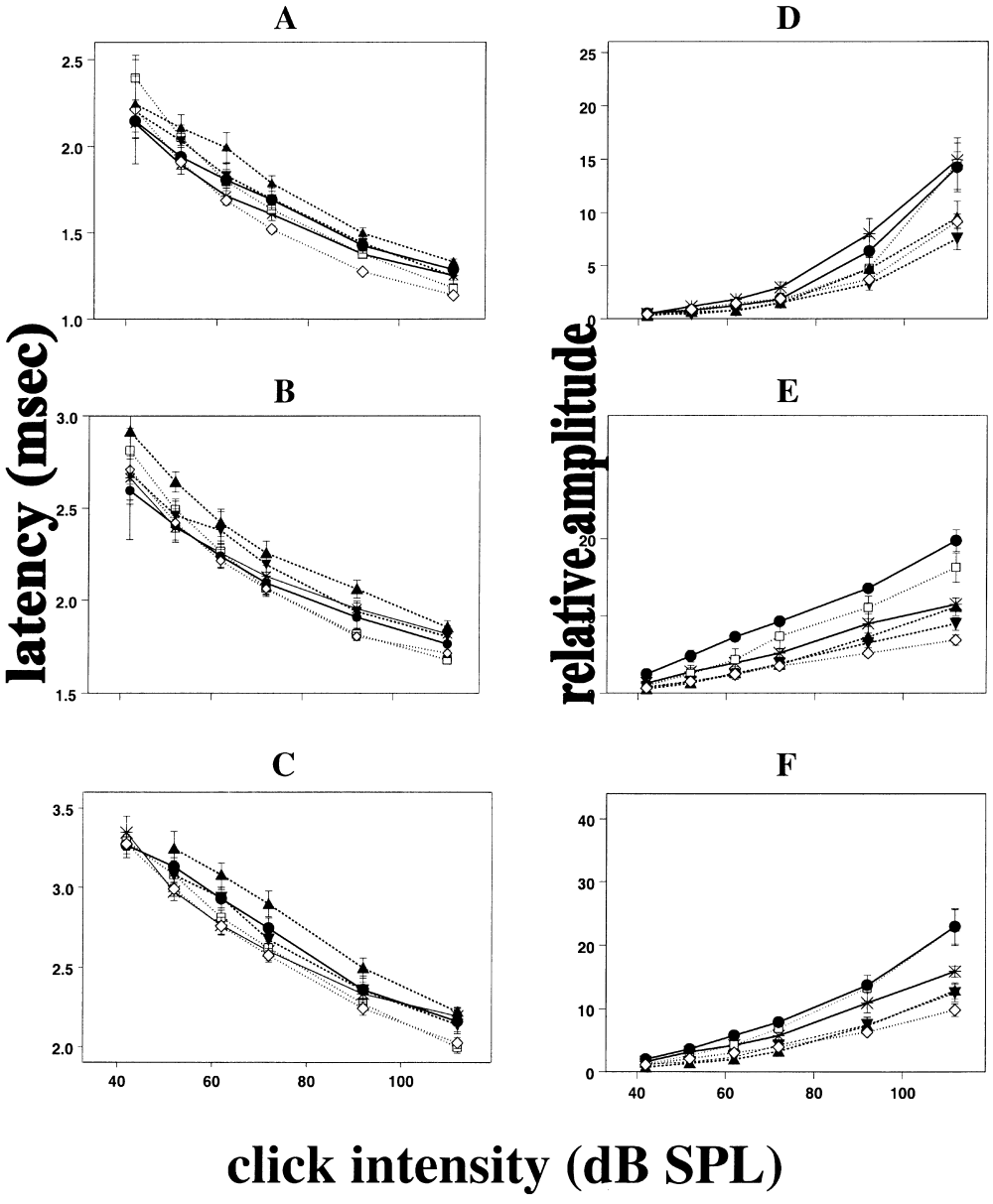

Fig. 3 Peak latency (**A**–**C**) and amplitude (**D**–**F**) as a function of click intensity for winter. **A**, **D** peak I; **B**, **E** peak II; **C**, **F** peak –III. *Open diamonds*: European starling. See Fig. 2 for a description of the other symbols

Table 2 Repeated measures ANCOVA tables for the effect of click intensity, species and season on peak latency and amplitude values of three different auditory brainstem response (ABR) peaks. The degrees of freedom (df) for the *F*-tests are given as subscripts after the *F* statistic; ns = interaction term not significant and therefore dropped from the model (see text)

Dependent variable	Independent variables	Peak I		Peak II		Peak –III	
		F_{df}	*P*	F_{df}	*P*	F_{df}	*P*
Latency	Intensity	$708.8_{1,52}$	< 0.0001	$530.3_{1,52}$	< 0.0001	$610.5_{1,52}$	< 0.0001
	Intensity^2	$200_{1,52}$	< 0.0001	$158.6_{1,52}$	< 0.0001	$97.3_{1,52}$	< 0.0001
	Species	$0.4_{5,52}$	0.83	$1.9_{5,52}$	0.11	$2.4_{5,52}$	0.053
	Season	$4.1_{1,52}$	0.048	$1.7_{1,52}$	0.19	$8.8_{1,52}$	0.005
	Species × Season	$7.3_{4,52}$	< 0.0001	$5.4_{4,52}$	0.001	$10.4_{4,52}$	< 0.0001
	Species×season×Intensity	$2.2_{10,52}$	0.029	$2.3_{10,52}$	0.025	ns	
Log(amplitude)	Intensity	$174.4_{1,57}$	< 0.0001	$59.4_{1,52}$	< 0.0001	$430.1_{1,52}$	< 0.0001
	Intensity^2	$11.4_{1,57}$	< 0.0001	$200.2_{1,52}$	< 0.0001	$110.0_{1,52}$	< 0.0001
	Species	$2.5_{5,52}$	0.042	$2.6_{5,52}$	0.035	$3.5_{5,52}$	0.009
	Season	$0.05_{1,52}$	0.82	$0.7_{1,52}$	0.40	$0.6_{1,52}$	0.43
	Intensity×species	$3.0_{5,57}$	0.019	ns		$2.5_{5,52}$	0.04
	Species×season	$3.8_{4,52}$	0.008	$13.2_{4,52}$	< 0.0001	$15.3_{4,52}$	< 0.0001
	Intensity×species×season	ns		$3.9_{10,52}$	0.004	$4.0_{5,52}$	0.004

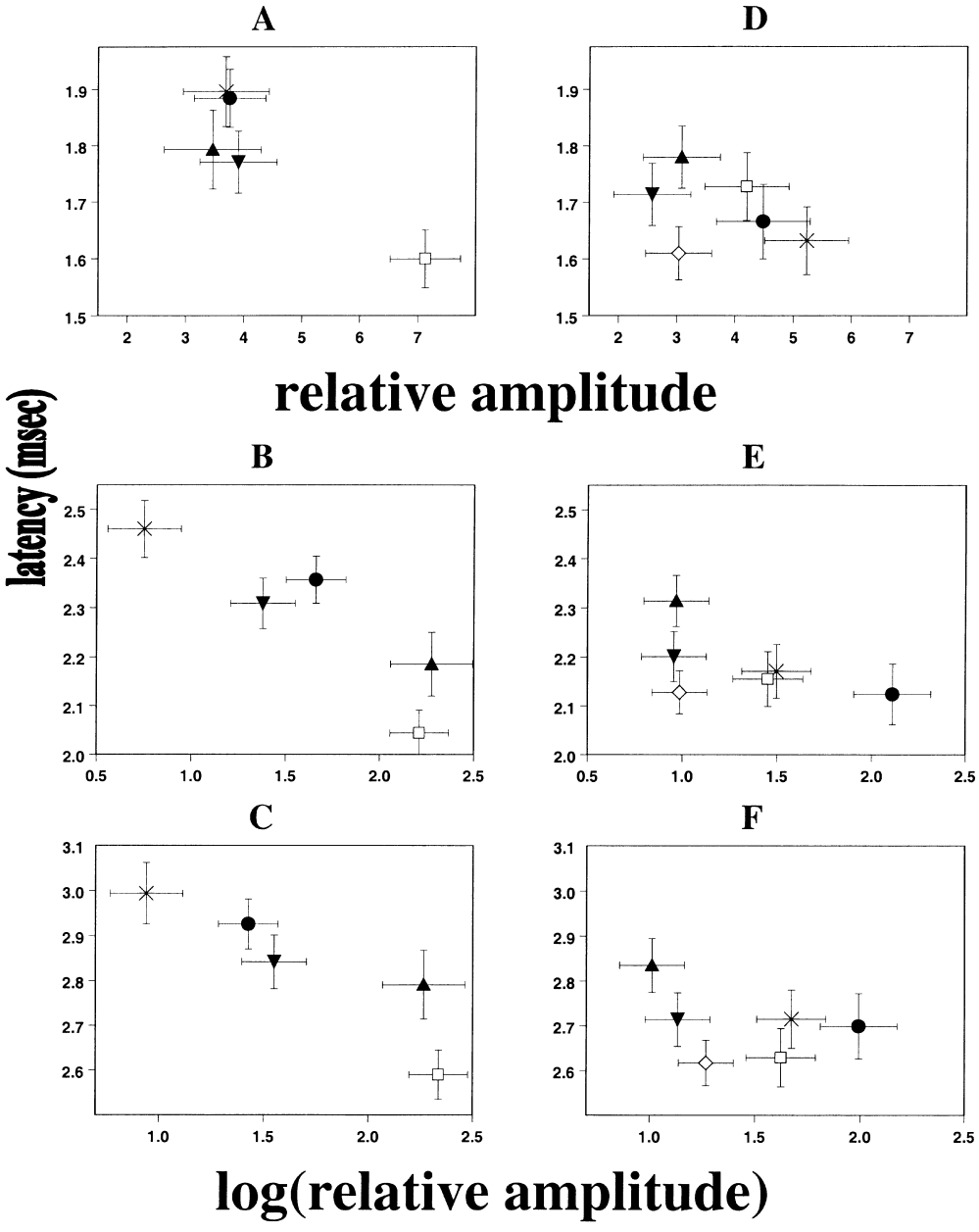

Fig. 4 Peak latency as a function of peak amplitude for spring (**A**–**C**) and winter (**D**–**F**). **A**, **D** peak I; **B**, **E** peak II; **C**, **F** peak –III. Symbols represent least-squares means and SEs. See Figs. 2 and 3 for description of symbols. Note that amplitudes for **B**, **C**, **E**, and **F** are log transformed

Amplitude

Peak amplitude increased non-linearly with an increase in click intensity for all three peaks (Figs. 2, 3; Table 2). However, the relationship between amplitude and click intensity varied significantly with season and species for all three peaks (as indicated by significant intensity× species and intensity×species×season interaction terms; Table 2). In general, the slope of the relationship for peak I was steeper for species with longer mean latencies (e.g., sparrows in spring, woodpeckers in winter, nuthatches in winter) compared to those with shorter latencies (e.g., titmice in winter, starlings and chickadees in winter). No pattern is discernable for peaks II or –III.

The species×season interaction was significant for all three peaks (Table 2). Multiple comparisons indicate three trends in the effect of season on peak amplitude within a species (Fig. 4): (1) in the nuthatches and woodpeckers, amplitude was significantly lower in the spring than in the winter for at least one peak (Table 3); (2) the titmice exhibited no significant change in peak amplitude from spring to winter (although there was a trend for amplitudes to be higher in spring than in winter, Table 3); and (3) amplitude was generally greater in the spring than in the winter for chickadees and sparrows (Table 3).

In the spring, the woodpeckers had significantly lower peak amplitudes than the other species (peaks II and –III), and the sparrows (peaks I, II, and –III) and chickadees (peaks II and –III) had significantly higher peak amplitudes than the other species (Table 4). These relationships are nearly reversed in the winter when the

Table 3 Effect of season on each species' ABR measures. $df = 52$ for all tests. Positive t values represent ABR measures in which spring values are larger than fall values; negative values represent spring values smaller than fall values

Dependent variable	Species	Peak I		Peak II		Peak –III	
		t	P	t	P	t	P
Latency	Downy woodpecker	3.1	0.003	3	0.004	3.6	0.0007
	White-breasted nuthatch	2.6	0.01	2.5	0.02	3.0	0.004
	Carolina chickadee	0.2	0.88	–0.5	0.65	–1.6	0.13
	House sparrow	–1.6	0.11	–0.5	0.65	–1.5	0.13
	Tufted titmouse	–0.7	0.47	1.5	0.14	1.5	0.14
Amplitude	Downy woodpecker	–2.2	0.03	–2.8	0.007	–3.1	0.003
	White-breasted nuthatch	–1.1	0.28	–1.8	0.09	–3.1	0.003
	Carolina chickadee	–0.8	0.40	4.7	< 0.0001	5.0	< 0.0001
	House sparrow	2.6	0.013	3.2	0.003	3.3	0.002
	Tufted titmouse	1.5	0.15	1.8	0.09	1.9	0.06

nuthatches, woodpeckers and sparrows had higher peak amplitudes for all peaks than the titmice, chickadees, and starlings (Table 4).

In summary, at the two extremes for these six species, downy woodpeckers tended to show longer latency and lower amplitude responses, and house sparrows tended to show shorter latency and greater amplitude responses. Overall species differences were difficult to determine, however, as there was such a pronounced effect of season. In general, woodpeckers and nuthatches showed shorter latency and greater amplitude responses in winter than in spring, whereas the other species tended to show longer latency and lower amplitude responses in winter than in spring.

Discussion

General ABR patterns

The changes in response amplitude and response latency with stimulus intensity and rate reported here are similar to those reported for humans and a wide range of non-human animals (e.g., Dooling and Walsh 1976; Hall 1992; Saunders et al. 1980). Specifically, as the intensity of the click stimulus increased, the latency of the response decreased and the amplitude of the response increased. As stimulus intensity increases, basal (higher frequency) portions of the basilar papilla are increasingly activated – this causes the modal value of the latency distribution across the avian cochlea to shift towards the base, thereby decreasing response latency. This basal spread of excitation with greater stimulus intensity also recruits more neurons that are synchronously activated, thereby increasing the amplitude of the response.

Species differences

This comparative study tested for species differences in evoked responses of the avian peripheral auditory system. We sought to determine if broad correlations exist between species differences in ABRs and phylogenetic relationships or vocal complexity. Taken together, the click intensity and click rate data suggest the general trend of downy woodpeckers, the one bird in the study not from the order Passeriformes, showing the longest latency and lowest amplitude responses to the click stimuli. These data suggest, therefore, that of these six species, woodpeckers would exhibit the weakest high-frequency hearing. To our knowledge, no tests of behavioral auditory thresholds exist for any woodpecker species, but various reviews of avian auditory thresholds would predict that, except for a few exceptional species, non-passerines would have sharper high-frequency cutoffs compared to passerines (e.g., Dooling et al. 2000b). Woodpeckers are known to have anatomical specializations in their middle ears, involving stiffening of the connections of the columella, thought to protect the inner ear from the concussive and repetitive shocks of

Table 4 Multiple comparisons of mean differences among species within each season in the click intensity experiment (see Fig. 4 for least-squares means). Species given the same letter are not significantly different ($P > 0.05$) (*cc* Carolina chickadee; *dw* downy woodpecker; *hs* house sparrow; *nh* white-breasted nuthatch; *st* European starling; *tt* tufted titmouse). Species are listed from lowest to highest mean value (LSM)

Variable	Season	ABR peak	Species comparisons					
Latency	Spring	I	hs	tt	cc	nh	dw	
			A	B	B	B	B	
		II	hs	cc	tt	nh	dw	
			A	AB	BC	CD	D	
		–III	hs	cc	tt	nh	dw	
			A	B	BC	BC	C	
	Winter	I	st	dw	nh	tt	hs	cc
			A	AB	AB	AB	AB	B
		II	nh	st	hs	dw	tt	cc
			A	A	A	AB	AB	B
		–III	st	hs	nh	tt	dw	cc
			A	A	AB	AB	AB	B
Amplitude	Spring	I	cc	dw	nh	tt	hs	
			A	A	A	A	B	
		II	dw	tt	nh	hs	cc	
			A	B	B	C	C	
		–III	dw	nh	tt	cc	hs	
			A	B	B	C	C	
	Winter	I	tt	st	cc	hs	nh	dw
			A	A	A	AB	AB	B
		II	tt	cc	st	hs	dw	nh
			A	A	A	B	B	C
		–III	cc	tt	st	hs	dw	nh
			A	A	AB	BC	C	C

pecking at hard surfaces (Kohllöffel 1984). This middle ear structural adaptation may account for the differences we see here in the ABRs of downy woodpeckers; measures of cochlear microphonic responses at 2 and 3 kHz for these six species, however, suggest comparable responding at the level of the basilar papilla (T.M. Freeberg et al., unpublished data). Regardless of the middle ear or inner ear mechanisms, our data indicate that generally longer latency responses are being generated by woodpeckers to the same acoustic stimuli with which the other species were provided. We also measured low ABR amplitudes for woodpeckers relative to the other species. Some of this variation between species could be caused by a relatively thicker skull in the woodpeckers.

Species differences were also exhibited within the species we tested in the order Passeriformes. In the spring, ABR characteristics broadly correlated with vocal complexity. In terms of latency, for example, nuthatches showed the longest responses, and chickadees and house sparrows showed the shortest responses. Thus, in the spring within these passerine species, ABR waveform characteristics seemed to correlate with vocal complexity and not so closely with phylogeny. However, these relationships did not hold for the winter data. All species tended to be more similar to one another in their ABRs in the winter compared to spring. In addition, the two parids, chickadees and titmice, tended to show longer latency and lower amplitude responses in the winter. Thus, the winter data failed to support either a phylogenetic or a vocal complexity hypothesis.

European starlings were tested only in the winter months, and therefore seasonal comparisons for this species cannot be made. In the winter months, starlings showed the shortest latency responses in the click rate protocol (often overlapping with house sparrows). Starling response amplitudes were generally small for all peaks, but this can be accounted for by their larger skull size compared to the other five species – as the extracranial electrodes were further from the generator sites, the amplitudes of the responses of those generators reaching the surface of the skull were expected to be smaller.

Seasonal changes in ABRs

Perhaps the most surprising finding to emerge from this comparative study was the effect season had on ABRs in these birds. All five species tested in both spring and winter exhibited seasonal variation in at least some of the early ABR peaks we measured. In addition, the birds with the simplest vocal repertoires, the nuthatches and woodpeckers, generally showed shorter latency and greater amplitude responses to clicks in the winter than in the spring. The pattern seen in nuthatches and woodpeckers is reversed in the birds with more complex vocal repertoires. This suggests the intriguing possibility that plasticity in the peripheral auditory system is correlated with vocal complexity.

How can we explain the differences across the seasons seen in the nuthatches and woodpeckers? At a proximate level, winter months are typically times of great energetic demands and physiological stress on non-migrant birds. Perhaps in woodpeckers and nuthatches, basic metabolic processing is significantly higher in the winter. Reduced anterior bloodflow in humans can produce longer latency ABRs (Mills and Ryals 1985); perhaps nuthatches and possibly woodpeckers have markedly increased cerebrovascular bloodflow in the winter months that the other species do not experience. At an ultimate level, white-breasted nuthatches also are socially the most stable species across the year. Females and males are paired year-round (also the case in downy woodpeckers, but with often less stable female-male pairs), typically in the same territory (Pravosudov and Grubb 1993). Chickadees and titmice form overwintering flocks of other conspecifics that may include floaters, resulting in more complex and often less stable social organization across the year (Smith 1991). House sparrows and European starlings form very large flocks that can change in composition over relatively short periods of time (Feare 1984; Lowther and Cink 1992). Thus, increased social stability may be related generally to seasonal changes in hearing, and more specifically to the seasonal patterns of auditory evoked responses documented here. Clearly, much more work is needed to determine the possible relationships between vocal and social complexity, energetic demands, and peripheral hearing processes and patterns across the year in these species.

Implications for vocal complexity and learning?

Seen in a broad perspective, the seasonal changes we describe here in the peripheral auditory system are consistent with a number of other seasonal changes in the avian brain. Nuthatches, chickadees, titmice, and, to a lesser extent, woodpeckers, cache food items in the fall and winter months that they can later retrieve to eat. This requires highly developed long-term memory (van der Wall 1990). Studies have shown that the hippocampus in caching species tends to be much larger than that of non-caching species (Clayton 1998; Krebs et al. 1996; Sherry et al. 1989), and that neural density in the hippocampus increases considerably in caching species as the days grow shorter as winter months approach (Barnea and Nottebohm 1994, 1996). The brood parasitic cowbirds, *Molothrus* species, do not cache, but require long-term memory of host nest site locations during the breeding season, and these species show seasonal changes in hippocampal volume as well (Clayton et al. 1997).

With regard to the vocal system, it is well known that brain regions devoted to vocal production and vocal learning change considerably in individuals – both young and adult – over the course of a year (Ball 1999; Nottebohm 1981, 1999). Starlings are open-ended

learners (Feare 1984) and chickadees are able to learn to modify their vocal signals as adults when their social context changes (Nowicki 1989). Thus, for these two species at least, we might expect important neural changes in the vocal learning and control systems across seasons (see also Brenowitz et al. 1998; Smith et al. 1997). Finally, unlike the case with mammals, hair cells are regenerated in birds following hair cell damage due to ototoxic drugs or acoustic overexposure (e.g., Cotanche et al. 1994) – perhaps our findings relate to normal processes of basilar papilla change in these species over seasons.

There is considerable evidence that hearing plays a major role in vocal learning in birds (e.g., Dooling 1982, 1992; Farabaugh and Dooling 1996). Changes in evoked responses at the auditory periphery in nuthatches (and woodpeckers) in the winter months may be an indication of increased auditory sensitivity at that time, possibly aiding in the perception of, and perhaps learning of neighboring conspecifics' vocal signals. This question could be answered by determining behavioral thresholds for acoustic stimuli across seasons in this species. To answer this question from the standpoint of auditory electrophysiology would require larger samples of individuals across the year, and ideally longitudinal samples of the same individuals across seasons. Furthermore, both behavioral and electrophysiological measures of hearing should be linked with detailed behavioral analyses of vocal and social interactions between neighboring pairs across those same time periods. A comparative study with other Sylvioidea species would be informative to this question. Unlike nuthatches, both chickadees and titmice show longer latencies and smaller amplitude responses (or no change) from spring to winter, have more complex social organization across the seasons, and have a vocal repertoire with higher frequency vocalizations.

This comparative study is our first step towards attempting to understand differences in auditory evoked responses and how those differences might map on to evolutionary, vocal, and perhaps social differences in these species. We have found species differences in early auditory evoked responses, and these differences in general seem to map onto phylogenetic differences and, taking into account seasonal changes, differences in vocal complexity. It would be beneficial to focus future comparative work on much larger numbers of more closely related species, that ideally vary in terms of the complexity and frequency ranges of their vocal repertoires, to determine the strengths of association between phylogeny, vocal complexity, and ABR profiles. Further, we need to take into account possible middle-ear differences in musculature and anatomy – since properties of the middle ear may be important for protection from self-vocalization and in acoustic feedback in vocal learning (Saunders et al. 2000). We also need to test for seasonal changes within species using larger numbers of individuals tested at several times over the year. Finally, the results from the present study suggest the possibility that processes at the auditory periphery may constrain the evolution of vocal communication systems. While these findings represent our initial step towards answering these many questions, we believe this study may have important implications for thinking about the evolution of vocal complexity and possibly vocal learning.

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