

# Auditory brainstem responses in adult budgerigars (*Melopsittacus undulatus*)

Elizabeth F. Brittan-Powell and Robert J. Dooling

Department of Psychology, University of Maryland, College Park, Maryland 20742

Otto Gleich

ENT Department, University of Regensburg, 93042 Regensburg, Germany

(Received 24 September 2001; revised 15 May 2002; accepted 23 May 2002)

The auditory brainstem response (ABR) was recorded in adult budgerigars (*Melopsittacus undulatus*) in response to clicks and tones. The typical budgerigar ABR waveform showed two prominent peaks occurring within 4 ms of the stimulus onset. As sound-pressure levels increased, ABR peak latency decreased, and peak amplitude increased for all waves while interwave interval remained relatively constant. While ABR thresholds were about 30 dB higher than behavioral thresholds, the shape of the budgerigar audiogram derived from the ABR closely paralleled that of the behavioral audiogram. Based on the ABR, budgerigars hear best between 1000 and 5700 Hz with best sensitivity at 2860 Hz—the frequency corresponding to the peak frequency in budgerigar vocalizations. The latency of ABR peaks increased and amplitude decreased with increasing repetition rate. This rate-dependent latency increase is greater for wave 2 as indicated by the latency increase in the interwave interval. Generally, changes in the ABR to stimulation intensity, frequency, and repetition rate are comparable to what has been found in other vertebrates. © 2002 Acoustical Society of America. [DOI: 10.1121/1.1494807]

PACS numbers: 43.64.Ri, 43.64.Tk [WWA]

## I. INTRODUCTION

Much is known about hearing in birds from behavioral studies (see the recent review in Dooling *et al.*, 2000). In general, birds hear best between 1000 and 5000 Hz, with absolute sensitivity approaching 0–10 dB SPL in the frequency of best hearing, which is typically around 2000–3000 Hz (see the review in Dooling *et al.*, 2000). However, behavioral testing is not always possible in all birds (e.g., young birds or birds in the field). Over the last few decades, auditory brainstem responses (ABR) have been used as a tool for studying the functionality of the auditory system in a wide variety of mammals (e.g., Burkard *et al.*, 1996a; Burkard and Voigt, 1989; Donaldson and Rubel, 1990; Jewett *et al.*, 1970; McFadden *et al.*, 1996; Mills *et al.*, 1990; Liu and Mark, 2001) and in other nonmammalian vertebrates (e.g., Corwin *et al.*, 1982; Kenyon *et al.*, 1998; Higgs *et al.*, 2002). Overall, these studies show that brain stem responses are similar across most vertebrate classes (e.g., Corwin *et al.*, 1982; Walsh *et al.*, 1992).

To date, ABR studies have only been conducted on a limited number of avian species. While complete reports on the effects of intensity and rate on latency and amplitude of the ABR waves or ABR-generated audiograms have been examined in depth in precocial birds (e.g., Dmitrieva and Gottlieb, 1992; Saunders *et al.*, 1973), much less is known about these measures in altricial birds (Aleksandrov and Dmitrieva, 1992; Moiseff *et al.*, 1996; Woolley and Rubel, 1999; Wooley *et al.*, 2001). To this end, we examined the ABR as a measure of auditory sensitivity in the adult budgerigar. The budgerigar is a small, altricial Australian parrot that is the most widely studied parrot species in the field of acoustic communication (see the review in Farabaugh and

Dooling, 1996; Heaton and Brauth, 1999; Hile and Striedter, 2000). This study consisted of two experiments that focused on ABR responses elicited by clicks and tone-burst stimuli in adult budgerigars. The response characteristics examined in experiment 1 were ABR threshold and the effects of intensity on latency and amplitude of the ABR waveform. Experiment 2 examined the effects of presentation rate on wave latency and amplitude in adult budgerigars. These data served as a baseline for assessing the development of hearing in nestling budgerigars.

## II. METHODS

Adult budgerigars (1–4 years of age) served as subjects in these experiments. The birds, bought through local pet stores or bred within our colony, were housed in an avian vivarium at the University of Maryland and kept on a photoperiod correlated with the season. They were given free access to food and water. All birds were sedated with an intramuscular injection of ketamine (50 mg/kg) and diazepam (2 mg/kg) prior to electrode placement. The animals remained relatively motionless for up to 75 min. Body temperature was maintained at  $41 \pm 0.5$  °C with a heating pad and monitored with a thermistor probe placed under the wing (Frederick Haer and Co., model 40-90, Bowdoinham, ME). After data collection was completed, the bird was placed in a heated therapy unit and allowed to recover from sedation. Once the animal was fully revived and eating, it was returned to the vivarium. The Animal Care and Use Committee at the University of Maryland approved all animal use.

The bird was positioned so that the speaker (KEF SP 3235, model 60S, frequency range 100 Hz to 20 kHz, KEF Electronics of America, Inc., Holliston, MA) was 30 cm

from the bird's right ear (90° azimuth relative to the bird's beak; 0° elevation relative to the bird's right ear). Standard platinum alloy, subdermal needle electrodes (Grass F-E2; West Warwick, RI) were placed just under the skin high at the vertex (active), directly behind the right ear canal (the ear ipsilateral to the speaker, reference), and behind the canal of the ear contralateral to stimulation (ground). Shielded electrode leads were twisted together to reduce electrical noise through common-mode rejection. The stimulus presentation, ABR acquisition, equipment control, and data management were coordinated using a Tucker-Davis Technologies (TDT, Gainesville, FL) modular rack-mount system controlled by an optical cable-linked 350-MHz Pentium PC containing a TDT AP2 digital signal process board and running TDT BIOSIG software. Sound stimuli were generated using TDT SIGGEN software and fed through a DA1 digital-analog converter, a PA4 programmable attenuator, and a power amplifier (HB6) which directly drove the speaker. The electrodes were connected to the TDT HS4 Headstage that amplifies and digitizes the signal before being relayed over fiberoptic cables to the TDT DB4 digital biological amplifier. This amplifier allows additional filtering and gain to be added. A TDT TG6 timing generator synchronized the A/D and D/A-conversion.

Stimulus intensities were calibrated in the free field by placing the  $\frac{1}{2}$ -in. microphone of a sound-level meter (System 824; Larson Davis, Inc., Provo, UT) at the approximate position of the bird's right ear. Continuous tones were generated using the TDT BIOSIG program and measured using the fast-weighting A scale on the sound-level meter (dB SPL). To determine the intensity of the click, we used the peak equivalent SPL of the click. This was determined using an oscilloscope and noting the peak-to-peak voltage of the click. A test tone, e.g., a 1000-Hz tone, was played and adjusted until the peak-to-peak voltage was the same as it was for the click. The SPL required to match the amplitude of the click, as indicated by the sound-level meter, was the peak equivalent SPL (dB pSPL) of the click stimulus.

For all experiments, only the first two wave components, designated by sequential Arabic numerals, were described by their amplitude and latency characteristics (Fig. 1). Positive evoked potential peaks were identified manually by cursor control, and associated latencies and amplitudes were automatically stored by the computer. Latencies to wave 1 and wave 2 were corrected for conduction delays between the sound source and the entrance of the ear canal of the animal (0.88 ms). The latency of the interwave interval (referred to as 1–2 interval) was calculated as the difference in latency from wave 1 to wave 2. ABR wave amplitudes were measured using baseline-to-peak for wave 1 and peak-to-peak (preceding trough) amplitude for wave 2.

### III. EXPERIMENT 1: EFFECTS OF INTENSITY AND FREQUENCY ON THE ABR OF BUDGERIGARS

#### A. Introduction

For all animals tested to date, increasing stimulus level resulted in decreases in ABR response latency and increases in response amplitude. Frequency-specific ABRs were gen-

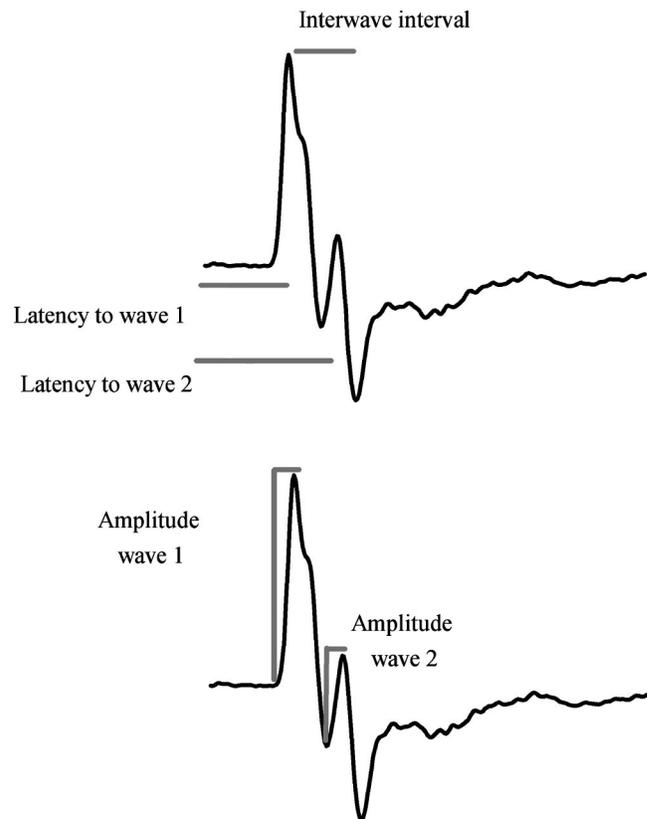


FIG. 1. Schematic showing how latency and amplitude measurements were taken for waves 1 and 2.

erated using brief tones. Experiment 1 evaluates the budgerigar ABR across stimulus intensities to determine whether the intensity dependence of evoked potentials in budgerigars is similar to those found in other animals.

#### B. Stimuli

Ten adult budgerigars were presented with multiple intensity stimulus trains (e.g., Mitchell *et al.*, 1996, 1999) that varied in frequency and intensity. Each train consisted of nine single clicks or frequency tone bursts that increased in intensity and were presented at a rate of 4/s. The rectangular-pulse broadband clicks were 0.1 ms (100  $\mu$ s) in duration with 25-ms interstimulus interval (ISI). Each individual tone burst was 5 ms in duration (1-ms rise/fall  $\text{COS}^2$ ) with a 20-ms ISI. The tone bursts used were 500, 1000, 1500, 2000, 2860, 4000, and 5700 Hz. The tone bursts were played through the speaker and sampled at 40 kHz into the A/D module of the TDT rack. Spectra were generated using 1024-pt fast Fourier transform (FFT). Spectral analysis shows all second and third harmonics were at least 30 dB down from the peak of the frequency of interest, except for the first harmonic of the 500-Hz stimulus, which was 18 dB down.

For frequencies below 2000 and above 2860 Hz, stimulus intensities began at 45–50 dB and increased in 5-dB steps to a maximum of 85–90 dB. Tone bursts of 2000 and 2860 Hz, as well as the click, were initially presented at 35–40 dB and increased in 5-dB steps to 65–75 dB; the remaining intensities were in 10-dB steps to a maximum of

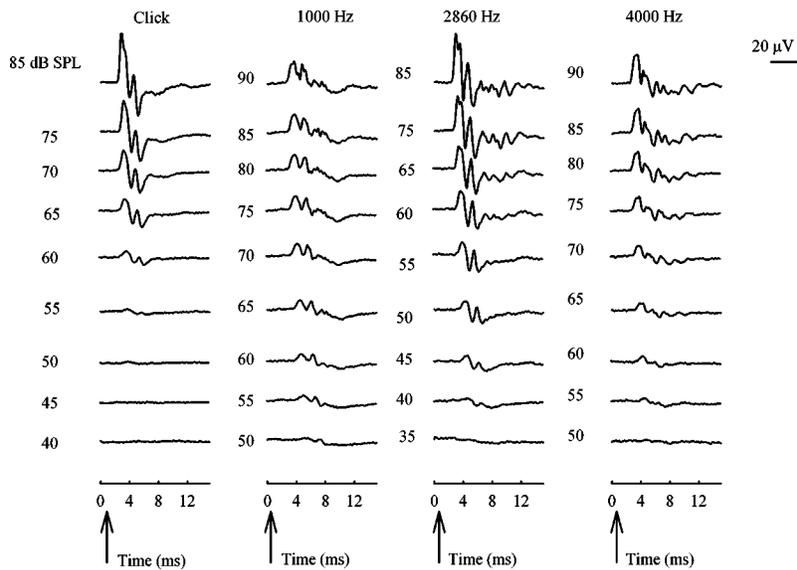


FIG. 2. Waveforms for a single bird for the click, 1000, 2860, and 4000 Hz as a function of SPL. Amplitude decreases and latency increases with decreasing SPL. Arrow denotes time at which stimulus reaches outer ear.

85 dB. Each ABR represents the average response of 1000 stimulus presentations (500 averages for each polarity/phase were added together to cancel the cochlear microphonic), sampled at 20 kHz for 235 ms following onset of the stimulus (allows for 25-ms recording time for each stimulus). The biological signal was amplified ( $\times 100$  K) and notch filtered at 60 Hz with the DB4 digital biological amplifier during collection. The signal was bandpass filtered below 30 Hz and above 3000 Hz after collection using the BIOSIG program.

### C. Analysis

ABR waveforms produced in response to high intensities were examined visually to determine which peaks would be used to measure latencies, amplitudes, and thresholds. A response was expected between 1 ms after the onset of the stimulus (travel time from speaker to the ear) and 10 ms because the response latency tends to increase at low SPLs. Using this time window, the wave components were described by their latency and amplitude characteristics.

ABR thresholds were estimated using several methods. First, thresholds were estimated using the visual detection method, i.e., the lowest intensity at which a response could be visually detected on the trace regardless of wave (Boettcher *et al.*, 1993) or 5 dB below the lowest intensity that elicited a measurable response (Walsh *et al.*, 1986). Second, a baseline-to-peak amplitude measure for wave 1 was taken for all intensities and stimuli (clicks and tone bursts). The amplitude-intensity functions generated from wave 1 amplitudes were used to estimate threshold ( $0\text{-}\mu\text{V}$  response) using linear regression. Last, thresholds were defined as the lowest stimulus intensity producing a response amplitude of  $0.5\ \mu\text{V}$ , which was at least 1 s.d. above the mean noise level (constant signal-to-noise ratio, S/N ratio of  $0.5\ \mu\text{V}$ ). The average level of noise in the ABR (the average amplitude difference between the largest peak and trough in the 2–4-ms range) was determined for 37 traces obtained to inaudible stimuli. The noise level of these recordings was  $0.28\ \mu\text{V}$  ( $\pm 0.16\ \mu\text{V}$

s.d.). Thresholds estimated from these methods were compared with each other (one-way analysis of variance, ANOVA) and to thresholds estimated using behavioral procedures.

### D. Results

Figure 2 shows typical ABR waveforms for an adult budgerigar click and three frequencies (1000, 2860, and 4000 Hz) as a function of intensity. Visual examination of the waveforms showed 2–3 prominent peaks that occurred within the first 5 ms after sound reaches the bird's external ear canal. Two major changes were evident for all waves. As the intensity of stimulation increased, latencies decreased and amplitudes increased.

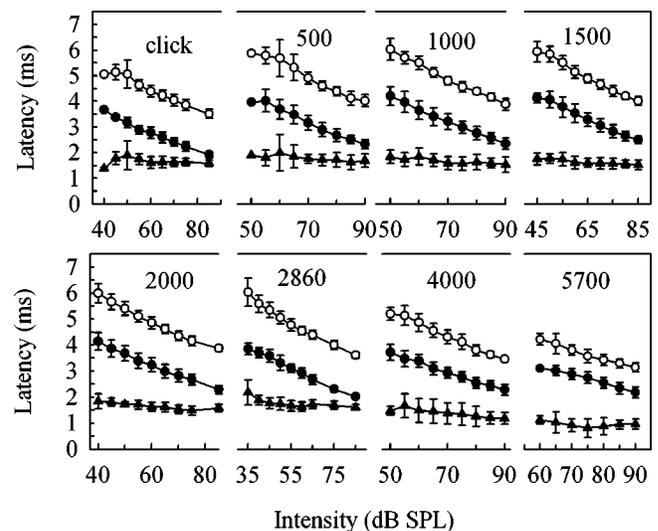


FIG. 3. Average latency as a function of intensity for the click and tone trains for ten adult budgerigars. The latency for wave 1 (closed circles) and wave 2 (open circles) increase as a function of decreasing level, while interwave interval (closed triangles) remains relatively constant. Error bars are standard deviations.

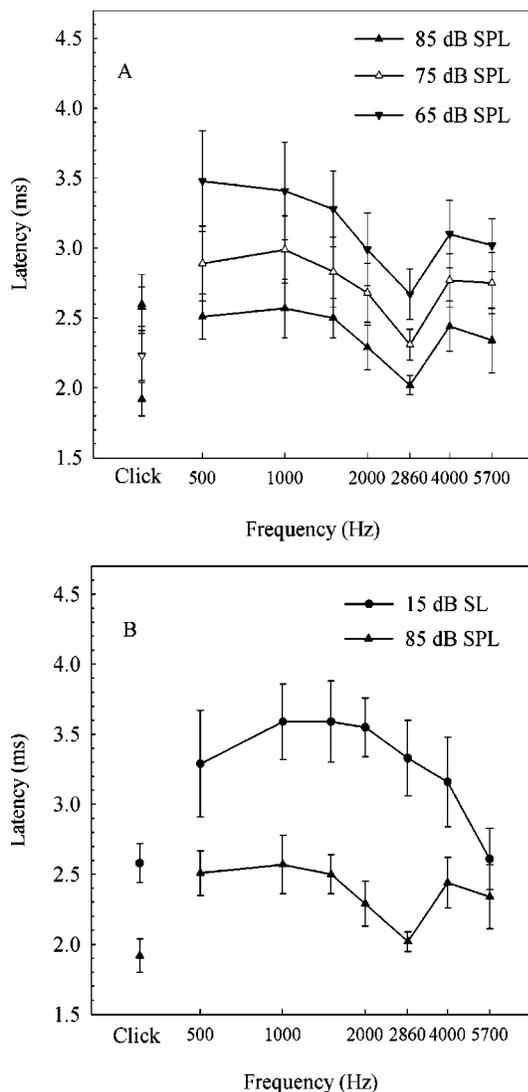


FIG. 4. (A) Average latency as a function of frequency for three constant SPL. There is not a consistent decrease in latency with increasing frequency. The lowest latencies are for 2860 Hz, which is the peak sensitivity of the behavioral audiogram as well as where most of the spectral energy of the contact call lies. (B) Average latency as a function of frequency using a constant SPL of 85 and 15 dB above threshold. Latency does decrease with increasing frequency if threshold is taken into account. Error bars are standard deviations.

Latency decreased monotonically as a function of increasing intensity for waves 1 and 2, while the interwave interval remained relatively constant across intensities (Fig. 3). There was not a consistent decrease in latency as frequency increased for constant SPL [Fig. 4(a)]. The shortest latencies are for 2860 Hz, the region of best sensitivity in both the ABR and behavioral audiograms. When a constant “sensation level” of 15 dB (i.e., 15 dB above threshold) was used, higher frequencies resulted in shorter latencies [Fig. 4(b)]. Interestingly, the latency to wave 1 at 500 Hz was more like that to higher-frequency tone bursts (2860 and 4000 Hz) than lower-frequency tone bursts (1000 and 1500 Hz). Given the spectral characteristics of the 500-Hz stimuli, only the second harmonic (1000 Hz) could influence the response, and it was 18 dB below the peak. All other harmonics were at least 30 dB down from the peak; therefore, har-

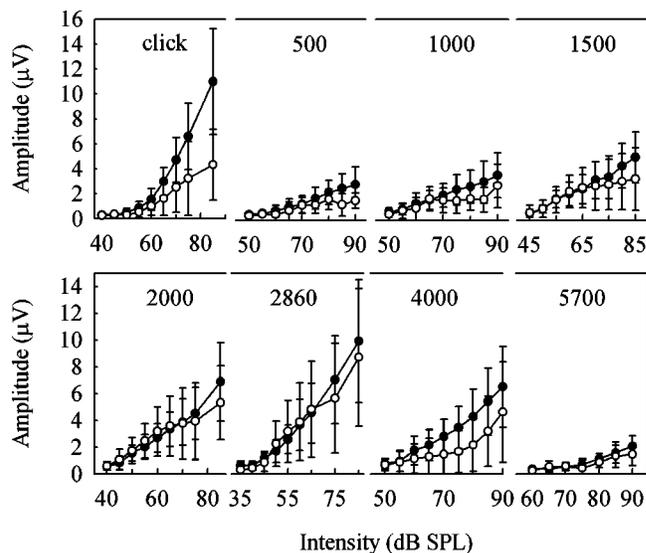


FIG. 5. Average amplitude as a function of intensity for the click and tone trains for ten adult budgerigars. Amplitudes for wave 1 (closed circles) and wave 2 (open circles) decrease rapidly with decreasing level. Error bars are standard deviations.

monics at higher frequencies cannot account for this latency discrepancy.

Figure 5 shows that the amplitude of waves 1 and 2 increased with increasing intensity. The slopes of the amplitude-intensity functions were frequency dependent, with clicks and midrange frequencies exhibiting steeper amplitude intensity functions than did low- or high-frequency tones. The highest average amplitudes were observed at frequencies in the budgerigar’s range of best hearing (i.e., 2000–4000 Hz) and in response to the click stimulus. Amplitude increased monotonically for wave 1 across stimuli and did not show saturation at any SPL tested. Wave 2 amplitudes did not show a similar monotonic increase, but approached saturation at 500, 1000, 1500, and 5700 Hz.

The coefficient of variation (CV) was used to determine the relative amount of dispersion in ABR latency and amplitude measures across the adult budgerigars used in this study. Latency measures across subjects varied less than 10%, while amplitude measures varied between 40%–80% across subjects. Wave 2 amplitudes were more variable than wave 1 amplitudes (Fig. 6).

Figure 7 shows a comparison of the average ABR-derived audiogram based on the three ABR threshold methods and the behavioral audiogram for budgerigars. As shown in this figure, threshold estimates by various methods were relatively consistent for the click stimuli. But, threshold estimates for the tone pips varied by 10–13 dB across methods [ $F(2,258) = 13.18$ ;  $p < 0.001$ ]. The response criterion of  $0.5 \mu\text{V}$  yielded significantly higher thresholds for the tone bursts than either the linear regression ( $p < 0.05$ ) or visual detection method ( $p < 0.05$ ). However, the response criterion and the visual detection methods more accurately paralleled the shape of the behavioral audiogram. The results from the regression method differed most from the U-shaped audiogram at the low-frequency end (500–2000 Hz). In general, all methods yielded threshold estimates that were 30–35 dB above behavioral estimates of hearing sensitivity.

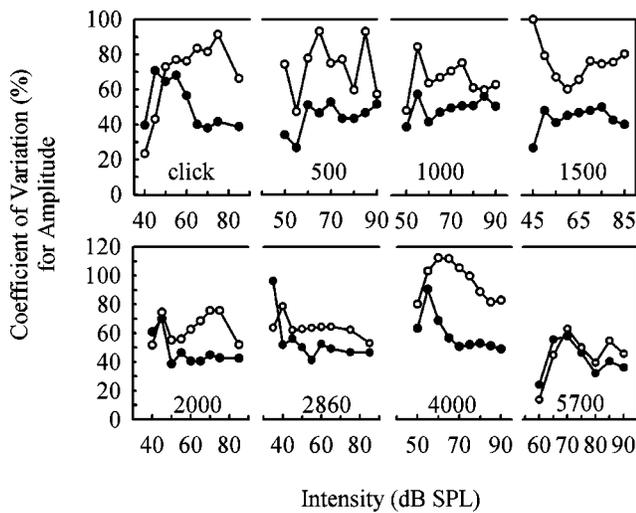


FIG. 6. Relative measure of amplitude dispersion across the ten adult budgerigars using the coefficient of variation (CV). Closed circles are for wave 1 measures. Open circles are for wave 2 measures. Overall, wave 2 amplitudes are more variable than wave 1 amplitudes.

#### IV. EXPERIMENT 2: THE RELATIONSHIP BETWEEN THE CM, CAP, AND ABR

##### A. Introduction

To date, there are no studies comparing the relationship between the cochlear microphonic (CM), compound action potential (CAP), and the ABR in the budgerigar. In this experiment, all three evoked potentials were recorded simultaneously and compared.

##### B. Stimuli

A single adult bird was presented with a multiple intensity stimulus train at 2860 Hz.

##### C. Analysis

The bird was anesthetized with IM injections of 20-mg/kg xylazine and 40-mg/kg ketamine. Additional doses of anesthetic (50% of initial dose) were supplemented as needed. The surgical procedures to gain access to the cochlea were described in detail for birds (Manley *et al.*, 1985). Briefly, feathers were removed from the head and around the external ear opening. An incision in the skin along the midline of the skull exposed the bone and it was cleared of connective tissue and dried. A small screw was cemented on the surface of the skull with dental cement to allow precise and stable placement of the head in a holding device and reproducible positioning of the ear canal opening in relation to the speaker.

For the CM and CAP potentials, the base of the cochlea was exposed by a dorsolateral approach. The core of a thin, Teflon-coated silver wire was exposed at the end and inserted through a tiny hole in the scala tympani to give direct electrical access to the perilymph. The Teflon insulation sealed the hole and prevented leaking of the perilymph. A subdermal electrode inserted into a neighboring neck muscle served as a reference. The electrodes for the ABR were placed as described in Sec. II, except the bird was 40 cm from the

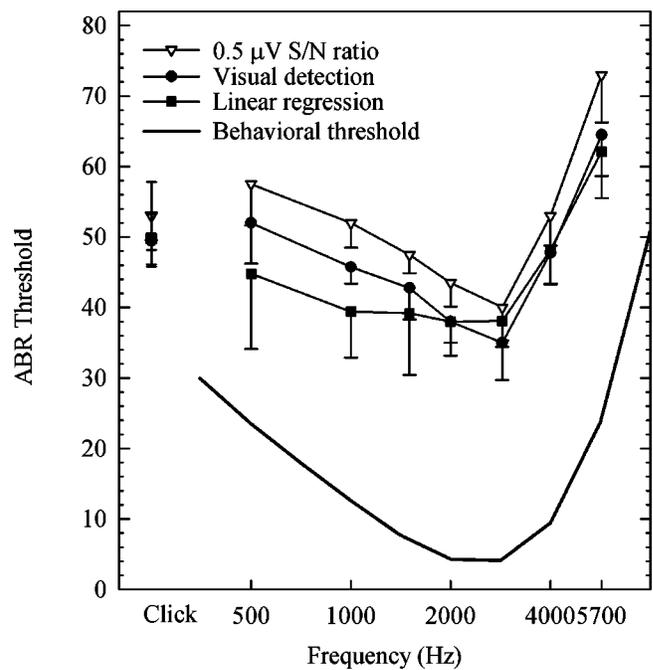


FIG. 7. Average ABR audiogram derived from the three methods: 0.5  $\mu$ V S/N ratio (open triangle), visual detection method (closed circle), and the linear regression (closed square) compared to the behavioral audiogram (dark solid line). The curve estimated by all methods is 30 dB higher than the behavioral curve, but the visual detection and criterion methods more closely approximate the shape of the behavioral audiogram. Error bars are s.d.

speaker. The stimulus presentation, data acquisition, equipment control, and data management were coordinated using the TDT setup described above, except where noted. Each ABR represents the average response of 600 stimulus presentations (300 averages for each), sampled at 20 kHz for 235 ms following onset of the stimulus (allows for 25-ms recording time for each stimulus). The biological signal was amplified ( $\times 20$  K) and notch filtered at 60 Hz with the DB4 digital biological amplifier during collection. The signal was bandpass filtered below 30 Hz and above 3000 Hz after collection using the BIOSIG program.

The CAP response component was derived by adding the response traces obtained for the 90° and 270° stimuli (canceling the CM component) and scaling the resulting response amplitude by half. This derived neural response component was then subtracted from the response trace to the normal stimulus to derive the CM response. The ABR response component was derived by adding the two phases of the stimulus together (as in experiment 1).

##### D. Results

Simultaneous recording of CM, CAP, and ABR in response to a 2860-Hz tone played at 60 dB SPL for a single budgerigar are shown in Fig. 8. The onset of the CM occurs first (2.35 ms) and lasts the length of the stimulus. The first negative wave of the CAP occurs 1.55 ms after the onset of the CM and is followed closely (0.3 ms later) by the first positive deflection of the ABR. This comparison strongly suggests that wave 1 of the ABR is the auditory nerve.

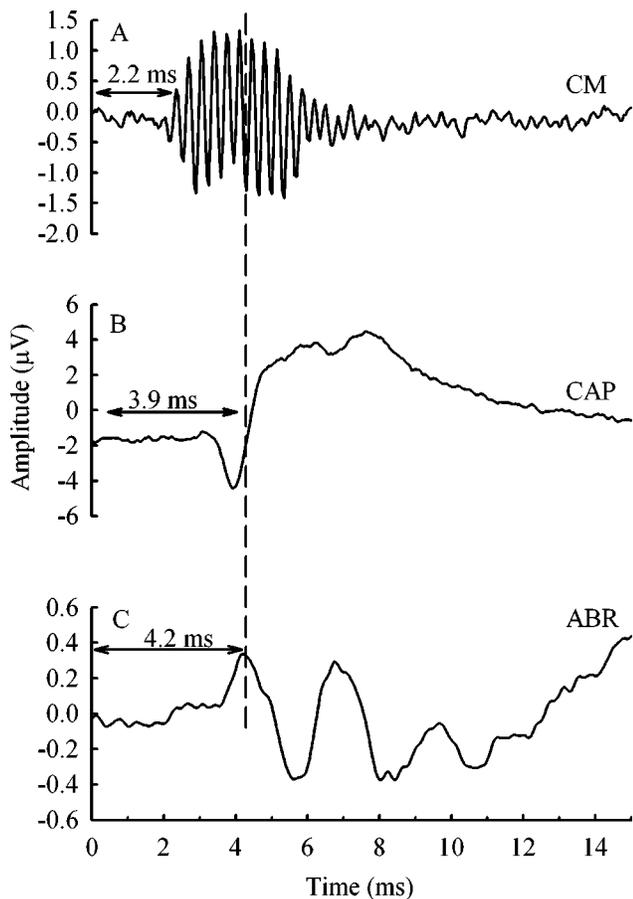


FIG. 8. Cochlear microphonic (A), compound action potential (B), and auditory brainstem response (C) recorded from a single budgerigar in response to a 2860-Hz tone played at 60 dB SPL. The negative deflection of the CAP corresponds well to the first positive deflection in the ABR.

## V. EXPERIMENT 3: EFFECTS OF CLICK REPETITION RATE ON THE ABR OF BUDGERIGARS

### A. Introduction

Studies of the ABR in humans and other mammals showed that higher click repetition rates caused increases in peak latency, decreases in peak amplitude, and alterations of wave morphology (e.g., Burkard and Voigt, 1989; Donaldson and Rubel, 1990; Jewett and Romano, 1972; and as reviewed in Hall, 1992). These changes may be a function of neural fatigue and adaptation (Hall, 1992). Knowing the baseline response of ABR latency and amplitudes to increasing presentation rate may allow investigators to use increased stimulation rate as a tool for detecting auditory neuropathologies.

The changes associated with increased presentation rate have been well studied in mammals. With the exception of the chick (Burkard *et al.*, 1994), few data exist for birds. However, like mammals, the chick shows similar rate-dependent changes. Experiment 2 extends the effects of click repetition rate on ABR latency and amplitude to the budgerigar.

The same ten birds were used in this experiment. All equipment and procedures were the same as in experiment 1, except where noted below.

### B. Stimuli

Click level was held constant at 100 dB pSPL. Short duration, broadband clicks (0.1 ms) were presented at five rates: 5, 10, 30, 60, and 90 per second (Hz). Each ABR represents the average response of 1000 stimulus presentations (500 averages for each polarity were added together to cancel the cochlear microphonic), sampled at 20 kHz for 10 ms following onset of the stimulus. As with experiment 1, the biological signal was amplified ( $\times 100$  K) and notch filtered at 60 Hz with the DB4 digital biological amplifier during collection. The signal was bandpass filtered below 30 Hz and above 3000 Hz after collection using the BIOSIG program.

### C. Analysis

Latency and amplitude measures were calculated for waves 1 and 2 for all repetition rates as described earlier. As a further comparison of the effects of increasing rate on the amplitude of the waves, the ratio of wave 1 to wave 2 was calculated.

### D. Results

Figure 9 shows a budgerigar's ABR waveforms to all click rates used in this study. As click repetition rate increased, latency increased and amplitude decreased to all waves. The waves also broadened, especially at rates higher than 10 Hz. Figures 10(a)–(c) show that with increasing repetition rate, the average latency to individual ABR waves increased. The latency of wave 1, wave 2, and the 1–2 interval were evaluated with a two-way ANOVA. Table I summarizes the results. Overall, as the repetition rate increased from 5 to 90 Hz, latency increased for wave 1 and wave 2 with a greater increase for wave 2, as shown by the increase in the wave 1–2 interval [Fig. 10(c)]. Pairwise comparisons using Student's *t* tests showed that latencies to waves 1 and 2 for repetition rates 30 Hz and above were significantly longer than for lower rates (10 Hz and below). The 1–2 interval increased significantly for repetition rates above 30 Hz.

The effects of increasing repetition rate from 5 to 90 Hz on amplitude are summarized in Figs. 10(d)–(f). Absolute amplitude decreased dramatically for both waves. The mean wave 1 to 2 amplitude ratio [Fig. 10(f)] shows that the amplitude of wave 1 was larger than the amplitude of wave 2, resulting in mean amplitude ratios greater than 1, but these ratios remained relatively constant across repetition rate. The amplitude of wave 1, wave 2, and the amplitude ratio of wave 1 to 2 were evaluated with a two-way ANOVA, and the results are summarized in Table I. Pairwise comparisons using Student's *t* tests showed that amplitudes of waves 1 and 2 were significantly larger for lower repetition rates (10 Hz and below) than for higher rates (30 Hz and above). The ratio of wave 1 to wave 2 amplitudes increased slightly, but this increase was not significant ( $p > 0.05$ ), suggesting that both waves were equally affected by increasing presentation rate.

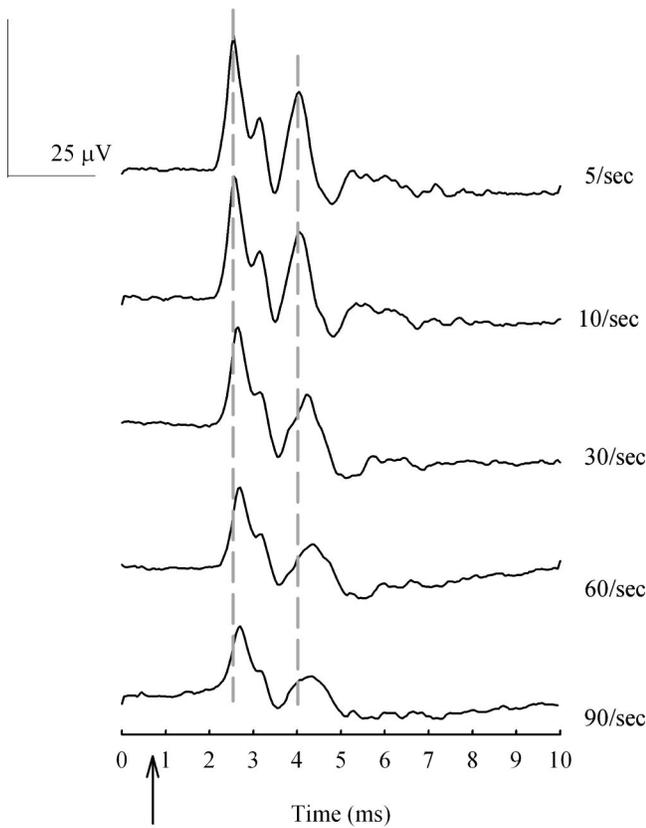


FIG. 9. Typical ABR trace for each repetition rate for an adult budgerigar. Increasing rate causes increased latencies and decreased amplitudes, with the biggest changes occurring above 10 Hz. Arrow denotes time at which stimulus reaches outer ear.

## VI. DISCUSSION

### A. Surface ABRs as an approach to the measurement of hearing in birds

The main purpose of this experiment was to measure hearing sensitivity in an adult bird using the ABR. Previous ABR studies have shown that while frequency-dependent ABRs are good predictors of audiogram shape, they are not necessarily predictive of absolute auditory sensitivity (e.g., Borg and Engström, 1983; Stapells and Oates, 1997; Wenstrup, 1984). ABR audiograms generated for the budgerigar were similar in shape to behavioral audiograms, showing a similar range of best hearing and a peak sensitivity at 2860 Hz (see the review in Dooling *et al.*, 2000). While the ABR audiogram was 30 dB less sensitive than the behavioral audiogram, much of this difference can be attributed to auditory temporal integration (Dooling *et al.*, 2000).

Figure 11 shows ABR audiograms for several species of vertebrates. Each audiogram reflects the bandwidth and shape of behavioral audiograms in the tested species, and all show a range of best sensitivity in the 2000–4000-Hz region. ABR thresholds from the current study are similar to those reported from Woolley and Rubel (1999) and Woolley *et al.* (2001) on Bengalese finches. Interestingly, thresholds for birds are higher than those of the mammalian species shown. Studies of tuning curve thresholds in birds show a larger spread of neural threshold at a given CF (review in Gleich and Manley, 2000; Manley *et al.*, 1985). Since the ABR is a

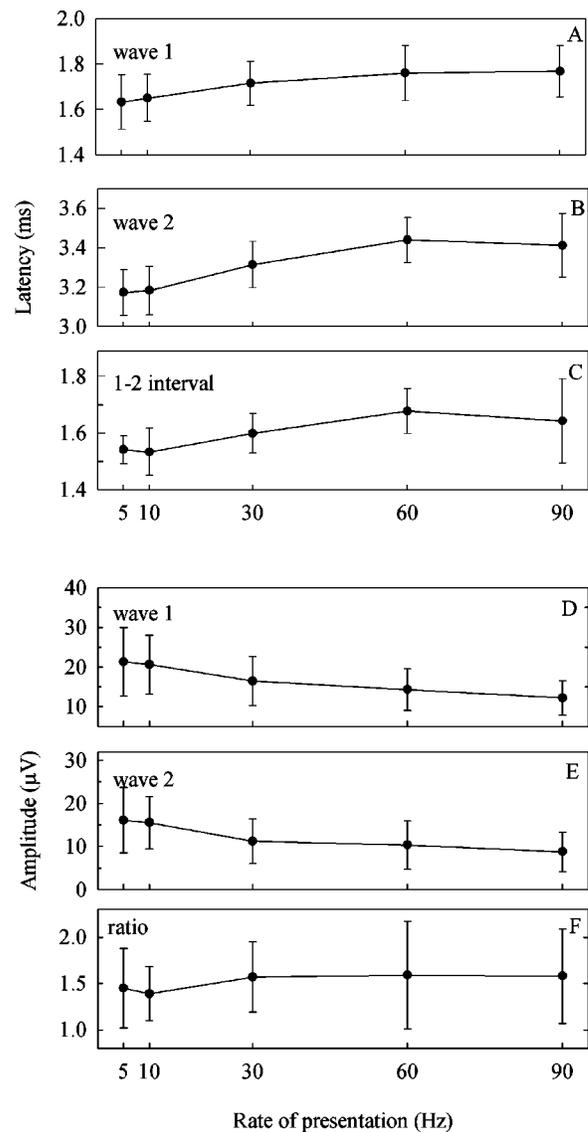


FIG. 10. Average latency of wave 1 (A), wave 2 (B), and the inter wave interval (C) plotted as a function of repetition rate for ten adult budgerigars. Latencies for all waves increased as rate increased, with greater increases seen in later waves (increase in interval). Average amplitude of wave 1 (D), wave 2 (E), and the ratio of wave 1 to 2 (F) plotted as a function of repetition rate for ten adult budgerigars. Amplitudes of all waves decreased as a function of increased rate. This decrease was similar for both waves 1 and 2. Error bars are standard deviations.

synchronized response, detecting a response near threshold requires synchronous activation of a sufficient number of fibers—on average this may be easier in mammals, where the proportion of fibers activated at threshold is likely greater

TABLE I. Results of ANOVA for latency and amplitude as a function of repetition rate.

Dependent variable	<i>N</i>	<i>F</i> ratio	Probability
Wave 1 latency	10	19.07	$p < 0.0001$
Wave 2 latency	10	24.08	0.001
Wave 1-2 Interval	10	7.40	0.0002
Wave 1 amplitude	10	24.73	0.0001
Wave 2 amplitude	10	13.36	0.0001
Wave 1/2 Ratio	10	1.13	NS

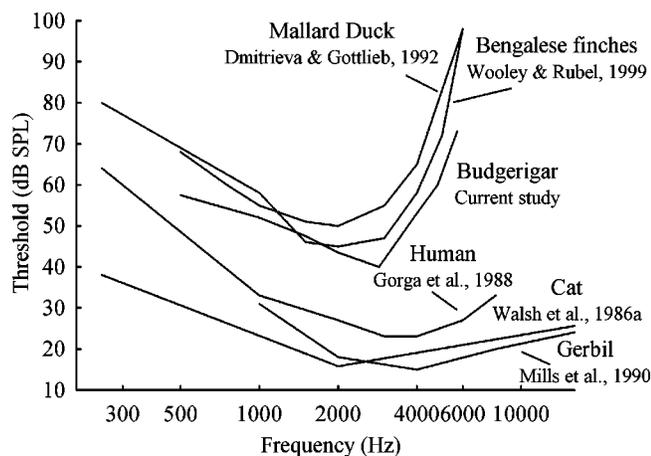


FIG. 11. ABR audiograms for several species of birds and mammals. All of the bird audiograms have similar shapes and thresholds. The bird audiograms are up to 30 dB higher than those of the mammals.

than it is in birds. Also, in absolute terms, the signal may be improved because the absolute number of fibers is higher in mammals (e.g., cats 50 000, Gacek and Rasmussen, 1961; see the review in Ryugo, 1992) than in birds (e.g., budgerigar 9800, Manley *et al.*, 1993).

### B. Effects of intensity on latency and amplitude for clicks and tone bursts

Typically, our budgerigar ABR waveform showed two measurable peaks that occurred within the first 4 ms after stimulation. This is similar to what was seen by Umemoto *et al.* (1993). A third peak was evident in some but not all traces, and was not examined further. In general, latency increased with decreasing SPL, and these increases occurred at the same rate for waves 1 and 2, such that the interwave interval remained constant across SPL. Similar results have been found in mammals (e.g., Burkard and Voigt, 1989; Burkard *et al.*, 1996a; Huang and Buchwald, 1978). There was not a consistent dependency of latency on frequency at constant SPL levels for budgerigars as has been shown in other studies (e.g., Gorga *et al.*, 1988). In fact, latencies were shortest for 2860 Hz, which is where the behavioral audiogram shows its most sensitive point and where most of the spectral energy of the budgerigar contact call is located. The amplitude of all peaks also decreased with decreasing SPL, but not at the same rate for wave 1 and 2. Overall, amplitude measures are more variable across subjects (relative dispersion between 40%–80%), while latency measures show little variation (less than 10%) across subjects (see Fig. 6). This is a typical finding in ABR studies.

There are no anatomical data to indicate why the largest amplitudes and shortest latencies were reported for the click and frequencies between 2000 and 4000 Hz, the region of greatest sensitivity in the budgerigar's audiogram. The basilar papilla contains about 5400 hair cells and is innervated by an estimated 9800 fibers (Manley *et al.*, 1993). Morphological features of the budgerigar papilla, such as hair-cell dimension, change gradually along the length and width, which does not provide an obvious basis for a dramatic change in function along the papilla. Likewise, the bases for

other known psychophysical specializations (such as increased sensitivity at 2860 Hz in the critical ratio; Dooling *et al.*, 2000) are also not evident at the level of the hair cells. As of yet, there are no detailed reports of the innervation pattern or innervation density of hair cells of different regions in the budgerigar's basilar papilla as compared to other species. Köppl *et al.* (2000) note that the budgerigar's fiber number/hair-cell number ratio is similar to that of the barn owl and suggest looking for regional increases in cochlear innervation densities in this species.

### C. Effects of click presentation rate on latency and amplitudes

With increasing click repetition rate, human, mammalian, and chick ABRs show increases in peak latencies and decreases in peak amplitudes (Burkard *et al.*, 1994; Burkard and Voigt, 1989; Burkard *et al.*, 1996a, b; Donaldson and Rubel, 1990; Lasky, 1997). The same effect was observed in budgerigars (Fig. 9). The decrease in amplitude occurred at the same rate for both waves [Fig. 9(f)].

As rate increased, there was a greater latency change for wave 2, which resulted in increasing interwave intervals with increasing repetition rate [Fig. 9(c)]. This has also been reported in humans (e.g., Burkard and Hecox, 1987), gerbils (Burkard and Voigt, 1989), cats (Burkard *et al.*, 1996a), and chicks (Burkard *et al.*, 1994). For humans, as rate increased from 10 to 90 Hz there was a mean I–V interval increase of 0.31 ms, from 3.71 to 4.02 ms (Burkard and Hecox, 1987). For gerbils, there was a 0.244-ms increase from 15 to 90 Hz for the i–v interval (Burkard and Voigt, 1989) and a 0.3-ms increase in the i–iv interval as rate increased from 10 to 100 Hz in cats (Burkard *et al.*, 1996a). As presentation rate increased from 10–90 Hz, the interval between wave 2 and 3 increased by approximately 0.10 ms in chicks and by 0.11 ms in budgerigars (from wave 1 to 2). As in other species tested, there was a greater latency change for later ABR peaks in the adult budgerigar. The constant ratio of amplitudes suggests that wave 1 and 2 were affected in the same way across the range of repetition rates used in the current experiment. Overall, these data suggest that the neural generators responsible for wave 1 and wave 2 in the ABR of budgerigars show similar rate-dependent ABR changes as mammals and other birds that have been tested (i.e., adaptation increases with increasing presentation rate, especially above 10 Hz).

### D. Neural correlates of ABR waves in budgerigars

While, to our knowledge, there have been no studies of ABR generators in budgerigars, it is possible to suggest possible neural generators for waves 1 and 2 based on latency data from other animals (e.g., chickens and cats). Wave 1 in animals is consistently attributed to auditory nerve. Wave 1 latencies were 1.3–1.4 ms for 103-dB SPL clicks (presented at 3.3 Hz) in chickens (Katayama, 1985) and 1.65 ms for 90-dB SPL clicks (presented at 5 Hz) in cats (Burkard *et al.*, 1996a). For the adult budgerigar, 100-dB SPL clicks presented at 5 Hz resulted in wave 1 latencies that are 1.6–1.7 ms. These latencies for budgerigars are similar to what has

been found for chickens and cats. Experiment 2 in the current study showed that the first negative deflection of the CAP corresponded well to the first positive deflection of the ABR waveform suggesting that the generators are the same. Therefore, we suggest that wave 1 of the budgerigar ABR is generated by the auditory nerve.

The source of wave 2 is somewhat more difficult to identify. Visual inspection of the chicken waveform suggests that Katayama's N4 might correspond to budgerigar wave 2. Nucleus laminaris is the suggested generator of N4 (latency 2.2–2.4 ms), and N4 in chickens has been suggested as equivalent to the third peak in the ABR of most mammals (Katayama, 1985; Burkard *et al.*, 1996a). In budgerigars, wave 2 latencies are 3.2–3.3 ms for a 100-dB click. Based on similarities in latency across these studies, wave 2 in the budgerigar may be generated by nucleus laminaris.

## VII. CONCLUSIONS

Audiograms based on scalp-recorded ABRs of adult budgerigars were similar in shape to behavioral audiograms (Fig. 7). As predicted from the lack of temporal integration in ABRs and the number of fibers needed for an ABR response, the ABR audiogram was less sensitive and underestimated behavioral thresholds by about 30 dB. However, the region of greatest sensitivity in the ABR audiogram was in the 2000–4000-Hz region, which matches the behavioral audiogram. This is also the region of peak energy in the adult contact calls (Dooling, 1986).

The effects of intensity on latency and amplitude of the ABR waves were similar to other animals studied to date. As intensity increased, latency to all waves decreased and amplitude increased. Analysis of the coefficient of variation showed that latency measures in this sample were more consistent than amplitude measures across subjects (Fig. 6). Last, increasing repetition rate in adult budgerigars resulted in longer latencies and decreased amplitudes. The latency changes were greater for wave 2 than for wave 1 (Fig. 10), indicating that generators of later waves are more affected by neural adaptation than wave 1. Wave amplitudes were equally affected by increasing presentation rate.

Overall, this study shows that the ABR is useful for assessing peripheral auditory system function and for estimating hearing thresholds in adult budgerigars. These procedures open the door to other investigations such as the recovery of hearing following hair-cell regeneration (Woolley and Rubel, 1999; Woolley *et al.*, 2001), the phase response of the basilar papilla (see for example, Dau *et al.*, 2000), and the development of hearing (Brittan-Powell, 2002). In fact, the noninvasive aspect of this procedure allowing repeated testing of the same individual makes it an ideal tool for assessing hearing during development. Hearing development using the ABR has been done in several mammals that are considered altricial, but not yet in altricial birds. Because budgerigars use auditory feedback to learn and modify their calls throughout life, knowing how the auditory system develops and what and when the animal hears provides insights into the role that hearing plays in the development of different types of vocalizations.

## ACKNOWLEDGMENTS

The authors would like to thank C. Carr, C. Moss, D. Yager, E. Walsh, K. Köppl, M. Leek, F. Kubke, M. Dent, D. Higgs, and J. Triplehorn for comments on earlier drafts and G. Manley and an anonymous reviewer for improvements made to this work. This work was supported in part by training grant No. DC-00046 from the National Institute of Deafness and Communicative Disorders of the National Institutes of Health to E.F.B.P. and National Institutes of Health Grant No. DC00198 to R.J.D. The work described here was in partial fulfillment of the requirement of a doctor of philosophy to E.F.B.P. (University of Maryland, College Park, 2002).

- Aleksandrov, L. I., and Dmitrieva, L. P. (1992). "Development of auditory sensitivity of altricial birds: absolute thresholds of the generation of evoked potentials," *Neurosci. Behav. Physiol.* **22**, 132–137.
- Boettcher, F. A., Mills, J. H., Norton, B. L., and Schmiedt, R. A. (1993). "Age-related changes in auditory evoked potentials of gerbils. II. Response latencies," *Hear. Res.* **71**, 145–156.
- Borg, E., and Engström, B. (1983). "Hearing thresholds in the rabbit," *Acta Oto-Laryngol.* **95**, 19–26.
- Brittan-Powell, E. F. (2002). "Development of auditory sensitivity in budgerigars," Department of Psychology, College Park, University of Maryland.
- Burkard, R., and Hecox, K. E. (1987). "The effect of broadband noise on the human brain-stem auditory evoked response. III. Anatomic locus," *J. Acoust. Soc. Am.* **81**, 1050–1063.
- Burkard, R., Jones, S., and Jones, T. (1994). "Conventional and cross-correlation brain-stem auditory evoked responses in the white leghorn chick: Rate manipulations," *J. Acoust. Soc. Am.* **95**, 2136–2144.
- Burkard, R., McGee, J., and Walsh, E. J. (1996a). "Effects of stimulus rate on feline brain-stem auditory evoked response during development. I. Peak latencies," *J. Acoust. Soc. Am.* **100**, 978–990.
- Burkard, R., McGee, J. and Walsh, E. J. (1996b). "Effects of stimulus rate on feline brain-stem auditory evoked response during development. II. Peak amplitudes," *J. Acoust. Soc. Am.* **100**, 991–1002.
- Burkard, R., and Voigt, H. F. (1989). "Stimulus dependencies of the gerbil brain-stem auditory-evoked response (BAER). I. Effects of click level, rate, and polarity," *J. Acoust. Soc. Am.* **85**, 2514–2525.
- Corwin, J. T., Bullock, T. H., and Schweitzer, J. (1982). "The auditory brain stem response in five vertebrate classes," *Electroencephalogr. Clin. Neurophysiol.* **54**, 629–641.
- Dau, T., Wegner, O., Mellert, V., and Kollmeier, B. (2000). "Auditory brain-stem responses with optimized chirp signals compensating basilar-membrane dispersion," *J. Acoust. Soc. Am.* **107**, 1530–1540.
- Dmitrieva, L. P., and Gottlieb, G. (1992). "Development of brainstem auditory pathway in mallard duck embryos and hatchlings," *J. Comp. Physiol., A* **171**, 665–671.
- Donaldson, G. S., and Rubel, E. W. (1990). "Effects of stimulus repetition rate on ABR threshold, amplitude, and latency in neonatal and adult Mongolian gerbils," *Electroencephalogr. Clin. Neurophysiol.* **77**, 458–470.
- Dooling, R. J. (1986). "Perception of vocal signals by Budgerigars (*Melopsittacus undulatus*)," *Exp. Biol.* **45**, 195–218.
- Dooling, R. J., Lohr, B., and Dent, M. L. (2000). "Hearing in birds and reptiles," in *Comparative Hearing: Birds and Reptiles*, edited by R. J. Dooling, A. N. Popper, and R. R. Fay (Springer-Verlag, NY), pp. 308–359.
- Farabaugh, S. M., and Dooling, R. J. (1996). "Ecology and evolution of acoustic communication in parrots: laboratory and field studies of budgerigars, *Melopsittacus undulatus*," in *Acoustic Communication in Birds*, edited by D. E. Kroodsma and E. H. Miller (Cornell University Press, Ithaca, NY), pp. 97–118.
- Gacek, R. R., and Rasmussen, G. L. (1961). "Fiber analysis of the statoacoustic nerve of guinea pig, cat and monkey," *Anat. Rec.* **139**, 455–463.
- Gleich, O., and Manley, G. A. (2000). "The hearing organ of birds and crocodilia," in *Comparative Hearing: Birds and Reptiles*, edited by R. J. Dooling, A. N. Popper, and R. R. Fay (Springer-Verlag, NY), pp. 70–138.

- Gorga, M., Kaminski, J., Beauchaine, K., and Jesteadt, W. (1988). "Auditory brain response to tone burst in normal hearing subjects," *J. Speech Hear. Res.* **31**, 89–97.
- Hall, J. (1992). *Handbook of Auditory Evoked Responses* (Allyn and Bacon, Boston, MA).
- Heaton, J. T., and Brauth, S. E. (1999). "Effects of deafening on the development of nestling and juvenile vocalizations in budgerigars (*Melopsittacus undulatus*)," *J. Comp. Psychol.* **113**, 314–320.
- Higgs, D. M., Soares, D., Brittan-Powell, E. F., Souza, M. J., Carr, C. E., Dooling, R. J., and Popper, A. N. (2002). "Amphibious auditory responses of the American Alligator (*Alligator mississippiensis*)," *J. Comp. Physiol., A* **188**, 217–223.
- Hile, A. G., and Striedter, G. F. (2000). "Call convergence within groups of female budgerigars (*Melopsittacus undulatus*)," *Ethology* **106**, 1105–1114.
- Huang, C., and Buchwald, J. (1978). "Factors that affect the amplitudes and latencies of vertex short latency acoustic responses in the cat," *Electroencephalogr. Clin. Neurophysiol.* **44**, 179–186.
- Jewett, D., and Romano, M. (1972). "Neonatal development of the auditory system: Potentials averaged from the scalp of rat and cat," *Brain Res.* **36**, 101–115.
- Jewett, D., Romano, M., and Williston, J. (1970). "Human auditory evoked potentials: Possible brainstem components detected on the scalp," *Science* **167**, 1517–1518.
- Katayama, A. (1985). "Postnatal development of auditory function in the chicken revealed by auditory brain-stem responses (ABRs)," *Electroencephalogr. Clin. Neurophysiol.* **62**, 388–398.
- Kenyon, T. N., Ladich, F., and Yan, H. Y. (1998). "A comparative study of hearing ability in fishes: the auditory brainstem response approach," *J. Comp. Physiol., A* **182**, 307–318.
- Köppl, C., Wegscheider, A., Gleich, O., and Manley, G. (2000). "A qualitative study of cochlear afferent axons in birds," *Hear. Res.* **139**, 123–143.
- Lasky, R. E. (1997). "Rate and adaptation effects on the auditory evoked brainstem response in human newborns and adults," *Hear. Res.* **111**, 165–176.
- Liu, G. B., and Mark, R. F. (2001). "Functional development of the inferior colliculus (IC) and its relationship with the auditory brainstem response (ABR) in the tammar wallaby (*Macropus eugenii*)," *Hear. Res.* **157**, 112–123.
- Manley, G. A., Gleich, O., Leppelsack, H. J., and Oeckinghaus, H. (1985). "Activity patterns of cochlear ganglion neurons in the starling," *J. Comp. Physiol., A* **157**, 161–181.
- Manley, G. A., Schabedissen, G., and Gleich, O. (1993). "Morphology of the basilar papilla of the budgerigar, *Melopsittacus undulatus*," *J. Morphol.* **218**, 153–165.
- McFadden, S. L., Walsh, E. J., and McGee, J. (1996). "Onset and development of auditory brainstem responses in the Mongolian gerbil (*Meriones unguiculatus*)," *Hear. Res.* **100**, 68–79.
- Mills, J. J., Schmiedt, R. A., and Kulish, L. F. (1990). "Age-related changes in auditory potentials of Mongolian gerbil," *Hear. Res.* **46**, 210–210.
- Mitchell, C., Kempton, J., Creedon, T., and Trune, D. (1996). "Rapid acquisition of auditory brain stem responses with multiple frequency and intensity tone bursts," *Hear. Res.* **99**, 38–46.
- Mitchell, C., Kempton, J., Creedon, T., and Trune, D. (1999). "The Use of a 56-stimulus train for the rapid acquisition of auditory brainstem responses," *Audiol. Neuro-Otol.* **4**, 80–87.
- Moiseff, A., Haresign, T., and Wang, J. (1996). "Sound localization from binaural cues by the barn owl auditory system," in *Neuroethological Studies of Cognitive and Perceptual Processes*, edited by C. F. Moss and S. J. Shettleworth (Westview Press, Boulder, CO), pp. 305–323.
- Ryugo, D. K. (1992). "The auditory nerve: Peripheral innervation, cell body morphology, and central projections," in *The Mammalian Auditory Pathway: Neuroanatomy*, edited by D. B. Webster, A. N. Popper, and R. R. Fay (Springer-Verlag, New York), pp. 23–65.
- Saunders, J. C., Coles, R. B., and Gates, G. R. (1973). "The development of auditory evoked responses in the cochlea and cochlear nuclei of the chick," *Brain Res.* **63**, 59–74.
- Stapells, D. R., and Oates, P. (1997). "Estimation of pure-tone audiogram by the auditory brainstem response: A review," *Audiol. Neuro-Otol.* **2**, 257–280.
- Umemoto, M., Sakagami, M., Ashida, K., Fukazawa, K., Matsunaga, T., Senda, T., and Fujita, H. (1993). "The ultrastructure of the basilar papilla of the budgerigar's inner ear," *Acta Oto-Laryngol.* **501**, 66–71.
- Walsh, E. J., McGee, J., and Javel, E. (1986). "Development of auditory-evoked potentials in the cat. I. Onset of response and development of sensitivity," *J. Acoust. Soc. Am.* **79**, 712–724.
- Wenstrup, J. J. (1984). "Auditory sensitivity in the fish-catching bat, *Noctilio leporinus*," *J. Comp. Physiol., A* **155**, 91–101.
- Woolley, S. M. N., and Rubel, E. (1999). "High-frequency auditory feedback is not required for adult song maintenance in Bengalese Finches," *J. Neurosci.* **19**, 358–371.
- Woolley, S. M. N., Wissman, A. M., and Rubel, E. (2001). "Hair cell regeneration and recovery of auditory thresholds following aminoglycoside ototoxicity in Bengalese Finches," *Hear. Res.* **153**, 181–195.