Normal Multiple Auditory Steady-State Response Thresholds to Air-Conducted Stimuli in Infants

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Abstract

Background and Purpose: Multiple auditory steady-state responses (ASSRs) to stimuli modulated at ~80 Hz are a promising technique for threshold estimation in infants, but additional data are required.

Research Design: We obtained multiple ASSRs to air-conducted (AC) stimuli.

Study Sample: There were 54 children in two age groups: > six months (N = 32) and ≤ six months (N = 22). All infants had normal hearing by tone-evoked auditory brain stem response.

Results: ASSR thresholds, estimated from 50 percent using cumulative percent present distributions, were 36, 30, 24, and 15 dB HL at 500, 1000, 2000, and 4000 Hz, respectively. Most (≥ 90%) of the infants showed present ASSRs at 49, 45, 36, and 32 dB HL at 500, 1000, 2000, and 4000 Hz, respectively, with no differences in the results of younger versus older infants. When responses were present for all stimuli for both ears, most infants showed all eight responses within five minutes. Compared to ipsilateral responses, ASSRs in the contralateral EEG (electroencephalogram) channel were smaller and often absent.

Conclusions: Based upon these data and the literature, normal AC ASSR “screening” levels would be 50, 45, 40, and 40 dB HL at 500, 1000, 2000, and 4000 Hz, respectively. Using the multiple-stimulus ASSR, infants with normal hearing referred for diagnostic electrophysiological threshold assessment can now be quickly confirmed as having normal thresholds for four frequencies in both ears.

Key Words: Auditory steady-state response, infants, normal hearing, tone-evoked auditory brain stem response

Abbreviations: ABR = auditory brain stem response; AC = air conducted; AEP = auditory evoked potential; AM = amplitude modulated; ASSR = auditory steady-state response; BCEHP = British Columbia Early Hearing Program; FFT = fast Fourier transform; FM = frequency modulated; IHS = Intelligent Hearing Systems; SNR = signal-to-noise ratio

Means of objectively estimating hearing thresholds are required for infants who are too young to provide reliable behavioral responses. Early identification and habilitation of hearing loss for improved access to auditory stimuli and for positive prognosis of speech and language are well established in the literature (Yoshinaga-Itano et al, 1998; Yoshinaga-Itano and Gravel, 2001; American Speech-Language-Hearing Association [ASHA], 2004; Hyde, 2005; Kennedy et al, 2006; Joint Committee on Infant Hearing, 2007). As a result of the importance of early identification of hearing loss, many countries have established newborn hearing screening programs. Diagnostic audiologic assessment is required for follow-up for infants who do not pass newborn hearing screening. The goal of many newborn hearing screening programs, including the British Columbia Early Hearing Program (BCEHP), is identification of permanent hearing loss (of a mild degree or worse) by age three months and amplification by the age of six months.

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months. Due to the high inter- and intrasubject variability of behavioral responses, objective measures are recommended for the estimation of hearing thresholds in young infants (ASHA, 2004).

An auditory evoked potential (AEP) with high correlation to behavioral threshold is essential for the young infant population and for those older infants and children for whom accurate behavioral thresholds cannot be obtained. Although the goals of universal newborn screening are identification by three months and habilitation by six months, most of the world (especially the developing world but also much of the developed world) does not currently have universal newborn hearing screening and follow-up. Thus, there remains a great need for objective testing that goes beyond the age of six months. At this time, there are two frequency-specific AEPs being considered for infant threshold estimation: the tone-evoked auditory brain stem response (ABR [Stapells, 2000a, 2000b]) and the auditory steady-state response (ASSR) to stimuli modulated from 80 to 110 Hz (Lins et al, 1996, Havana data; Savio et al, 2001; Cone-Wesson et al, 2002; John et al, 2002; Rance and Rickards, 2002; Stapells et al, 2005; Rance et al, 2006). Auditory brain stem responses are transient evoked potentials where a brain stem response to a tone or click occurs from 1 to 20 msec following a stimulus. Auditory steady-state responses are typically elicited by amplitude-modulated (AM) and/or frequency-modulated (FM) stimuli. The stimuli are presented at a high enough rate to cause overlapping of the transient responses to successive stimuli. The ASSR is detected using statistical measures of the response signal-to-noise ratio (SNR), typically either the amplitude of the response compared to background EEG (electroencephalogram) noise or its phase variability. A sample of responses or an averaged response can be subjected to a fast Fourier transform (FFT) allowing for the measurement of amplitude and phase. If the amplitude of the ASSR yields a significant SNR, a response is deemed to be present. No visual waveform identification is required. Transient evoked potentials (i.e., the ABR), on the other hand, typically require that a waveform be analyzed in the time domain with response presence (or absence) visually identified by a clinician. The ABR is widely used and is effective for threshold estimation in infants and children (Stapells, 2000a). Recording the ABR to brief tones rather than clicks greatly enhances the frequency specificity, and thus the accuracy, of threshold estimation (Stapells and Oates, 1997; Stapells, 2000a, 2000b).

The ASSR and its use for threshold estimation have been investigated for many years, since the work of Galambos et al (1981). Particular focus during the last decade has been on the ASSRs to multiple simultaneous stimuli modulated in the 70–110 Hz range (the “80 Hz multiple ASSR”). The “multiple ASSR” shows considerable promise as an accurate means of estimating hearing thresholds for infants and adults (for reviews, see Picton et al, 2003; Stapells et al, 2005; Tlumak et al, 2007b). In the multiple ASSR technique, responses to stimuli with multiple carrier frequencies are evaluated simultaneously; typically, they are evoked with a stimulus composed of multiple AM tones at least one octave apart in frequency and modulated at different rates, combined to form one stimulus for each ear and then presented to one or both ears. The multiple ASSR appears to be advantageous over the single-stimulus ASSR in that it enables evaluation of at least four frequencies for both ears simultaneously, resulting in faster threshold estimation compared to single-stimulus ASSR (Lins and Picton, 1995; John et al, 1998; Herdman and Stapells, 2001, 2003; Perez-Abalo et al, 2001; Dimitrijevic et al, 2002; John et al, 2002).

ASSRs to stimuli modulated at 70–110 Hz have been investigated for threshold estimation in infants in numerous research labs and clinics worldwide (Lins et al, 1996; Savio et al, 2001; Cone-Wesson et al, 2002; John et al, 2002; Rance and Rickards, 2002; Vander Werff et al, 2002; John et al, 2004; Rance et al, 2005; Swanepeol and Steyn, 2005; van der Reijden et al, 2005; Van Maanen and Stapells, 2005; Han et al, 2006; Luts et al, 2006; Rance et al, 2006; Small and Stapells, 2006), and several equipment options are commercially available for recording the ASSR. However, review of the literature indicates that there are few normative 80 Hz ASSR data sets for infants, especially for the multiple ASSR. Additionally, there are few studies of the 80 Hz ASSR in hearing-impaired infants, leading to the conclusion that, at present, the ASSR must be used in conjunction with the tone ABR for diagnosis of hearing loss in infants too young for behavioral testing (Stapells et al, 2005).

Although research has indicated that ASSR is a promising technique for threshold estimation in normal infants, variability among the studies makes direct comparisons difficult. Differences between studies include single versus multiple stimuli, ages of the subjects, equipment, monaural versus binaural testing, test environment, response and noise criteria, and method (or “gold standard”) for confirming normal hearing.

Several existing studies have a wide variety of age of subjects, with few in the age range of primary interest clinically (i.e., under two years of age). Those studies that tested relatively large numbers of infants used single stimuli (Cone-Wesson et al, 2002; Rance et al, 2005; Rance et al, 2006), whereas those using multiple stimuli tested small groups of infants (Swanepeol and Steyn, 2005; Luts et al, 2006) or tested infants in noisy environments (Lins et al, 1996, Havana data; Savio et
al, 2001; John et al, 2004). Finally, many studies confirmed normal hearing using click-evoked ABR (John et al, 2004; van der Reijden et al, 2005; Han et al, 2006; Savio et al, 2006), rather than tone ABR. Rance et al (2006) used tone ABR at 500 and 4000 Hz; however, whether their infants had normal hearing by tone ABR results is not explicitly indicated.

Previous infant ASSR research has employed different EEG electrode montages. Most single-stimulus studies have used a forehead–ipsilateral mastoid single channel (e.g., Cone-Wesson et al, 2002; Rance et al, 2005; Rance et al, 2006). Most multiple-stimulus studies have used a forehead–nape (midline) single channel (e.g., John et al, 2004; Luts et al, 2006), although recently clinicians and researchers have begun to use two EEG channels: forehead–ipsilateral and forehead–contralateral mastoid channels (e.g., Han et al, 2006; Mo and Stapells, 2008). Normative two-channel data for infants are required for the multiple-stimulus (monotic or dichotic) ASSR. Only two previous studies have presented results for ipsilateral versus contralateral EEG channels for the infant ASSR to air-conducted (AC) stimuli (van der Reijden et al, 2005; Small and Stapells, 2008). Neither study provides these results for dichotic stimuli or for several intensities.

Despite recent interest, there is still a lack of information pertaining to the multiple ASSR in normal infants (Rance, 2008; Vander Werff et al, 2008). The purpose of the present study was threefold. First, the study aimed to establish normative data (thresholds and “normal” levels) for multiple ASSR presence in a relatively large group (N = 54) of young infants showing normal tone-evoked auditory brain stem response results. Presently, there are few data for the multiple ASSR with the tone ABR as the “gold standard” for normal hearing in infants. Second, the study estimated the recording times at each intensity and, especially, the time needed to obtain significant (i.e., present) responses in normal infants, as this information is needed in order to evaluate the viability of multiple ASSR as a clinical procedure. Third, this study compared responses obtained simultaneously in the EEG channels ipsilateral and contralateral to the stimulated ear.

METHOD

Subjects

Tone ABRs and multiple ASSRs were obtained for 54 infants (99 ears) with an average age of 18.3 months. The median age was 12 months, with a range of 0.7–66.2 months. The infants were divided into two age groups: >six months (N = 32, mean age = 28.8 months) and ≤six months (N = 22, mean age = 3.1 months). The subjects who were ≤six months were tested under natural sleep, and all but one of those who were >six months were tested under conscious sedation (using chloral hydrate). All subjects were tested at British Columbia Children’s Hospital as part of clinical testing to confirm hearing status. Subjects were considered normal if ABRs were obtained to AC brief tones at 35 dB nHL at 500 Hz, 30 dB nHL at 2000 Hz, and 25 dB nHL at 4000 Hz for both ears. Detailed descriptions of our tone ABR protocols and parameters are available on the Internet (BCEHP, 2008; Stapells, 2008) and in the literature (Stapells and Oates, 1997; Stapells, 2000a). There were five infants with unilateral hearing loss; the ASSR data are only included for the ear with the normal tone ABR.

ASSR Stimulus Parameters and Calibration

Stimuli were generated using the Intelligent Hearing Systems SmartEP-ASSR. Individual AC stimuli for ASSR were calibrated in reference equivalent threshold sound pressure levels in dB HL (American National Standards Institute, 1996). Air-conducted stimuli were presented to both ears through ER-3A insert earphones. Rather than the SmartEP-ASSR default brief-tone stimuli, we created stimuli that more closely resembled continuous AM stimuli (i.e., approximately one modulation cycle in duration), based on our recent research suggesting that brief-tone stimuli were not optimal for the 80 Hz ASSR (Mo and Stapells, 2008). ASSR stimuli in the current study were cosine3-windowed sinusoids that were 12 msec in total duration and calibrated in dB HL. Carrier frequencies were 500, 1000, 2000, and 4000 Hz, modulated at 81.05491, 88.86719, 96.67969, and 105.4688 Hz for the right ear and 77.14844, 84.96094, 92.77344, and 100.5859 Hz for the left ear. The digital-to-analog conversion rate was 44 kHz. The intensity of the tones varied from 0 to 70 dB HL.

ASSR Recording Parameters and Analysis

Four disk electrodes were placed on the subject’s scalp: (i) noninverting on the vertex (typically for the older group) or, if the fontanel was open, midline on the high forehead (typically for the younger group); (ii and iii) inverting on each mastoid; and (iv) ground off-center on the forehead. Impedance between all electrodes was less than 3 kOhms. The EEG was amplified 100,000 times for ASSR and filtered using a bandpass of 30 to 300 Hz (12 dB/octave).

The SmartEP-ASSR system used an analog-to-digital conversion rate of 20 kHz and subsequently down-sampled to 1 kHz. Each sweep lasted 1.024 sec. After each block of 20 sweeps, the results were...
analyzed in the frequency domain by fast Fourier transform. Determination of significance was made by on-line analysis of variance (F-statistic) from the FFT (Picton et al., 1987). The F-ratio was computed between the response amplitude at the modulation frequency and the amplitude of the background EEG noise. The background noise was determined by two means: (i) the mean amplitude in ten sidebins of the FFT, five each on either side of the modulation rate, and (ii) the amplitude at the modulation rate from an FFT of the plus-minus average (Picton et al., 1987; Tlumak et al., 2007a; Mo and Stapells, 2008). To be considered a response, signal-to-noise ratios using both noise estimates both had to be significant \((p < .05)\). Recordings were continued until the mean noise in the sidebins was \(\leq 5\) nV or until a significant response \((p < .05)\) was obtained, whichever occurred first. Although 5 nV is a stringent noise criterion, studies have shown that increasing the recording time can improve the detection of low-amplitude responses close to the EEG noise floor and thus detect responses with lower amplitudes (Picton et al., 2003; John et al., 2004; Luts et al., 2004).³⁴

**PROCEDURES**

The study involved one recording session of one–three hours (testing included tone ABR and ASSR). All ABR testing was conducted prior to ASSR testing using BCEHP (2008) protocols and parameters. All infants had ABR testing carried out at 500, 2000, and 4000 Hz. Participants slept on a bed or in a caregiver’s arms while in a double-walled sound-attenuated booth. For ASSR, both ears were tested simultaneously (with the exception of three subjects, where it was determined after each subject woke up that one of the insert phones was out of the ear canal). Two-channel (ipsilateral and contralateral) EEG recordings were obtained for all subjects (except the subjects noted above). Only ipsilateral EEG channel results were considered when determining normal ASSR presence and threshold, although subsequent analyses compared contralateral EEG results to those for the ipsilateral EEG channel (see Results and Discussion).

ASSR threshold estimation procedures were as follows: the starting intensity was typically 40 dB HL. If, after at least 20 sweeps (the minimum number required for analysis by the Intelligent Hearing Systems), the response was present at all frequencies, the stimulus intensity was decreased in 10 dB steps until a “no response” was obtained at least at one or more frequencies. If there was no response obtained at any frequency for 40 dB HL, the stimulus intensity was increased to 50 dB HL. Intensity was increased or decreased in 10 dB steps in the direction that would most likely provide the greatest number of true ASSR thresholds but did not exceed 70 dB HL. Time permitting, additional intensities above threshold were tested. Thus, multiple ASSRs were recorded (in 10 dB steps) for several intensities for each infant, usually for both ears simultaneously. ASSR threshold for a specific carrier frequency/ear combination was considered the lowest intensity at which a response was present and a “no response” was obtained at 10 dB below that intensity. Responses occurring at levels where there was no response 10 or 20 dB HL higher were considered “false positive.” Responses were considered “false negative” when significant responses were obtained at two consecutive intensities below a “no response.” In this case, the lower of the two intensities was considered as threshold. Actual thresholds could not be obtained for all frequencies for all infants (see below). Therefore, in addition to determination of actual thresholds for a subset of infants (as described above), the results of all infants were combined, and cumulative percent ASSR presence was calculated. These cumulative presence analyses provided threshold estimates from the 50 percent present point on the distribution.

A maximum of 800 sweeps (13.65 minutes) was recorded per intensity. If the subject did not meet the noise criteria after 800 sweeps despite being asleep, the subject was not included in the study. However, no subjects were excluded outright. For two subjects, one–two carrier frequencies for one of the two ears did not meet the noise criteria after 800 sweeps; thus, results for those carrier frequencies were excluded. For two subjects, one channel did not meet noise criteria; thus, results for that ear were excluded. Recording was continued until subjects woke up, and in some subjects we were able to obtain results for up to six intensities. All subjects had all four frequencies tested at least at one intensity in one ear. Due to the limitations of sleep time, a full ASSR threshold search could not be carried out for all infants. Nevertheless, ASSR results were obtained for three to six intensities for about half the infants.

**DATA AND STATISTICAL ANALYSES**

**Thresholds**

As indicated above, threshold results were assessed in two ways. First, thresholds for the group were estimated from the 50 percent present point on the group cumulative response presence results; responses were assumed to be present at intensities higher than the highest present response (e.g., responses for 80 dB HL were assumed to be present if ASSRs were present at lower intensities). Second, thresholds for individual infants were determined where actual thresholds were
obtained through bracketing. Not all infants had actual thresholds obtained, and for some with actual thresholds, not all frequencies or both ears provided actual thresholds. In order to obtain one set of thresholds (i.e., one each for 500, 1000, 2000, and 4000 Hz) for as many infants as possible, results across ears were combined. If thresholds were obtained for only one ear, then those thresholds were used. For carrier frequencies that had thresholds for both ears, the average of the two ears was used.

In addition to descriptive statistics, threshold comparisons were made using a two-way mixed-model analysis of variance (ANOVA), with carrier frequency (500, 1000, 2000, and 4000 Hz) as a repeated-measures factor and age group (≤ six months and > six months) as a group factor. Huynh-Feldt epsilon adjustments for repeated measures were made where appropriate. Newman-Keuls post hoc comparisons were carried out for significant main effects and interactions. The criterion for statistical significance was \( p < .05 \). Additionally, Pearson product-moment correlation coefficients were calculated for age versus ASSR threshold for each carrier frequency.

**Recording Time**

Recording times were determined for the ASSRs using the total number of sweeps (1.024 sec per sweep) recorded to obtain results for four frequencies for both ears. Recording times for each intensity were determined for all infants. Average recording times were compared (\( t \)-test for independent samples, significance = \( p < .05 \)) between the older (> six months) and younger (≤ six months) infants. Recording times were also determined for those infants with significant responses at all carrier frequencies and for both ears at 40–50 dB HL.

**Ipsilateral versus Contralateral EEG Channel Results**

We compared the presence and absence of ASSRs and ASSR amplitudes recorded in the EEG channels ipsilateral and contralateral to the stimulated ear. Ipsilateral and contralateral results from 30 to 60 dB HL for each carrier frequency were compared in two ways: (i) ipsilateral and contralateral data regardless of presence or absence of a response and (ii) contralateral results when ipsilateral responses were present. To investigate the effect of age on the ipsilateral/contralateral amplitude asymmetry, we compared the asymmetry (pooled across frequency: contralateral amplitude divided by ipsilateral amplitude, in percent) for the older and younger groups at 40 dB HL using a \( t \)-test for independent samples (significance = \( p < .05 \)).

**RESULTS**

**Thresholds**

Figure 1 shows the cumulative percent presence of multiple ASSRs for each carrier frequency for all infants. ASSR thresholds were estimated from the 50 percent point on the regression functions. Thresholds were highest for 500 Hz, decreasing as carrier frequency increased. Specifically, the 50 percent thresholds were 36, 30, 24, and 15 dB HL at 500, 1000, 2000, and 4000 Hz, respectively. Most infants (90% or more) had responses to 40–50 dB HL stimuli at all carrier frequencies.

When divided into the older (> six months) and younger (≤ six months) infant groups, the cumulative occurrence was essentially the same between the two groups. For example, at 40 dB HL, pooled across all frequencies, the cumulative percentage for the older group was 83.1 percent, and that for the younger group was 87.5 percent.

Table 1 shows the means and standard deviations for the actual ASSR thresholds as a function of frequency, broken up by age group as well as for both ages combined. The ANOVA revealed a significant effect of carrier frequency (\( F = 19.0; \) df = 3, 60; \( e = 1.0; \) \( p < .0001 \)). Newman-Keuls post hoc analysis revealed that there were significant differences at all frequencies, with significantly improved thresholds with increasing carrier frequency (\( p = .0001 \) to .047). ASSR thresholds at 500 Hz were significantly worse than those for all other carrier frequencies (\( p = .0001 \) to .029), and ASSR thresholds at 4000 Hz were significantly better than those for all other carrier frequencies (\( p = .0001 \) to .0003). Table 1 also suggests that there is no difference in the actual thresholds between
the older and younger groups at any carrier frequency. Pooled across frequencies, there was little difference between groups, with a mean of 35.6 dB HL (CI95% = 31.5–39.7) for the older group and a mean of 33.6 dB HL (CI95% = 30.3–36.9) for the younger group. The ANOVA revealed no significant difference in thresholds between the older and younger groups (F = .0001; df = 1, 20; p = .98) and no group by carrier frequency interaction (F = 0.58; df = 3, 60; ε = 1.0; p = .631). In addition, there was no significant correlation between age and threshold at any carrier frequency, with correlation coefficients ranging from –0.08 to 0.06 for all frequencies.

**Ipsilateral versus Contralateral Responses**

Recording two EEG channels provided data for the ipsilateral and contralateral EEG channels. Figure 3 presents the mean ASSR amplitudes recorded in the
EEG channels ipsilateral and contralateral to the stimulated ears and shows clearly that the contralateral ASSR amplitudes are substantially smaller than those in the ipsilateral channel. Compared to ipsilateral ASSRs, contralateral ASSRs to 500, 1000, and 2000 Hz stimuli were less than 50 percent of the amplitude of the contralateral recordings for all intensities. Results for 4000 Hz stimuli also showed smaller contralateral ASSRs; however, the amplitudes of the contralateral ASSRs were, on average, over 50 percent of the ipsilateral amplitudes at all intensities. On average (pooled across frequency, 40 dB HL), the contralateral response amplitudes were 48 percent (SD = 19, CI95% = 41–55) and 43 percent (SD = 16, CI95% = 35–50) of the ipsilateral responses for the older and younger infant groups, respectively. Essentially the same between groups, this small difference was not significant (t = 1.01, df = 48, p = .319). Even when only results with present ipsilateral ASSRs are considered, the amplitude of the contralateral ASSR remains less than half the amplitude of the ipsilateral responses. Figure 4 shows the percent presence of contralateral ASSRs for those recordings with a present ipsilateral ASSR. Across all intensities, contralateral ASSRs were present least often for 500 Hz and most often for 4000 Hz. Across all frequencies and intensities, the contralateral ASSR was present for only 31 percent of infants.

DISCUSSION

Thresholds

The present study indicates that infants had ASSR thresholds (i.e., 50% present) between 36 (at 500 Hz) and 15 (at 4000 Hz) dB HL. Threshold significantly improved as carrier frequency increased. These thresholds are closest to Lins and colleagues (1996, Ottawa data), who report multiple-stimulus ASSR thresholds at 33, 22, 17, and 21 dB HL at 500, 1000, 2000, and 4000 Hz, respectively. Compared to single-stimulus ASSR studies from the Melbourne group, the present study demonstrated similar ASSR thresholds at 500 Hz and lower (better) ASSR thresholds at 4000 Hz (Rickards et al, 1994; Rance and Tomlin, 2006; Rance et al, 2006). Compared to other ASSR studies, the present study demonstrated better thresholds for all carrier frequencies (Levi et al, 1993; Savio et al, 2001; Cone-Wesson et al, 2002; Rance and Rickards, 2002; Swanepoel and Steyn, 2005; Luts et al, 2006).

The comparable thresholds between the present study and Lins et al (1996, Ottawa data) might be attributable, at least in part, to the similar conditions of both studies. Both studies were carried out in a sound-attenuated suite, and both used multiple stimuli. Most infants in both studies were at least one month of age, and both studies recorded for longer times than most other studies. These two studies likely differ from other studies because of the following: test environment, age of infants, and length of recording time. In contrast to most normative ASSR studies, Savio and colleagues (2001) recorded ASSRs in a room...
with relatively high ambient noise. In their article, Savio and colleagues suggest this as a possible reason for poorer ASSR thresholds relative to other studies. With regard to age of infants, two age effects on ASSR thresholds have been studied: (i) comparison of infants and adults and (ii) comparison of newborns and infants. Rance and Rickards (2002) compared ASSR thresholds of adults and older infants (mean age = three months). They found that adults and older children have lower (better) ASSR thresholds than infants. If we compare the present study’s infant ASSR thresholds to the adult data from Herdman and Stapells (2001), the infants’ ASSR thresholds are higher at all frequencies other than 4000 Hz (which is similar). Rance and Rickards (2002) and the present study indicate that infant thresholds are poorer than those of adults and older children.

Specifically concerning the effects of age on ASSR thresholds in infants, there is some evidence in the literature that very young infants have significantly higher thresholds compared to older infants, children, and adults (Cone-Wesson et al, 2002; John et al, 2004; Luts et al, 2006; Rance and Tomlin, 2006). Several studies examined subjects who were aged less than two weeks and as young as one day (Levi et al, 1993; Rickards et al, 1994; Cone-Wesson et al, 2002; John et al, 2004; Luts et al, 2006). ASSR thresholds were higher for these groups of very young infants compared to the infants in the present study. John et al (2004), Luts et al (2006), and Rance et al (2006) indicate that very young infants and premature infants show elevated ASSR thresholds, which they suggest may be due to neural immaturity, vernix in the ear canal, or changes to the acoustics of the ear canal or middle ear.

In his recent review, Rance (2008) concludes that most of the increase in young infants is due to neural factors. Although we did not study very young infants (i.e., less than two weeks old), our results do not show any substantial differences between infants aged > six months and those aged ≤ six months. In addition, for the range of ages we tested (0.7–66.2 months), there was no significant correlation between age and ASSR threshold at any carrier frequency.

To investigate the age issue further, we can compare the cumulative response present data for the ten very young infants in our study who were aged three months or less (0.7–3 months) to the results of the other infants. In contrast to other studies, cumulative percentages for these ten very young infants were similar to those of the other infants. Pooled across frequency and 40 and 50 dB HL, the ten very young infants showed responses for 89 percent of the tests compared to 91 percent for the rest of the infants. Although this is a limited number of very young infants, our results are consistent with the recommendation of John et al (2004) that ASSR testing is appropriate for confirming normal hearing in infants aged one to three months.

With regard to length of recording time, testing at each intensity in the current study ranged from 1.02 to 13.65 minutes (mean = 6.30 minutes) for all infants. Our test times are similar to those reported by other studies using multiple stimuli (Lins et al, 1996; Savio et al, 2001; John et al, 2004; Swanepoel and Steyn, 2005; Savio et al, 2006). Most single-stimulus studies, including those by Levi et al (1993), Cone-Wesson et al (2002), Rance and Rickards (2002), Rance and Tomlin (2006), and Rance et al (2006), recorded for 20–90 sec per intensity. Short recording times can lead to higher EEG noise levels and as a result, higher ASSR thresholds (Picton et al, 2005). Reduction in the EEG noise is important for finding ASSR thresholds because high EEG noise can obscure small ASSRs (Picton et al, 2005). One way to reduce EEG noise is to increase averaging time, increasing the SNR by lowering the EEG noise. In the present study, we regularly observed that longer averaging times were needed for the carrier frequency of 500 Hz in order to meet our noise criteria. Although longer averaging can increase test time, small responses at all carrier frequencies, and at 500 Hz in particular, might have been missed using less conservative noise criteria. The results of this study, as well as the results from a pilot study in another group of infants, support the assertion of Picton et al (2005) that longer recording times are required to reduce the EEG noise to a level to allow easier recognition of responses and better threshold estimation.

We found significant differences in thresholds (in dB HL) between carrier frequencies, specifically 500 and 4000 Hz. Thresholds for 500 Hz were significantly poorer than those for other carrier frequencies; this finding was the same for both the older and the younger group. Other air-conduction ASSR studies have also reported this (Lins et al, 1996; Cone-Wesson et al, 2002; Swanepoel and Steyn, 2005; Han et al, 2006). John et al (2004) hypothesize that the poorer 500 Hz ASSR thresholds in infants may be due to (i) poor neural synchronization in young babies and/or to (ii) less effective low-frequency transmission due to the presence of vernix, middle-ear pressure variations, or maturational changes of the outer and middle ear. In the present study, which tested infants aged 0.7–66.2 months, vernix would certainly not be an issue for our older group (mean age = over two years), and poor neural synchronization would be unexpected. Middle-ear pressure variations or maturational changes cannot be ruled out as potentially contributing factors. It should be noted that the finding of higher thresholds at 500 Hz is also seen in tone ABR testing (Stapells et al, 1995; Stapells, 2000a, 2000b), and 500 Hz ASSR thresholds are also higher in
Our results indicate that thresholds for 4000 Hz are significantly better (9–19 dB) than for other carrier frequencies. Such a large difference has not been reported in other studies (Lins et al., 1996; Herdman and Stapells, 2001; Dimitrijevic et al., 2002; Luts et al., 2006; Rance and Tomlin, 2006). Although ear canal factors might explain some of the difference, based on published ear canal SPL corrections (e.g., Westwood and Bamford, 1995; Rance and Tomlin, 2006), neither they nor transducer variations can explain all of the difference, especially given the similarity of thresholds between the younger and older infants. It is possible that a trade-off between ear canal SPL and neural synchrony occurs such that in young infants better 4000 Hz thresholds due to higher ear canal SPL are partly offset by poorer neural synchrony, whereas in the older infants the ear canal SPL decrease is offset by better neural synchrony. Further research is required.

**Recording Time**

For all infants in the present study, the average test time was 6.30 minutes per intensity. The 1- to 1.5-minute differences in time between the older and younger groups are of little or no practical or clinical significance, and these differences were not statistically significant. For two ears, four frequencies per ear, normal results (40–50 dB HL) could be confirmed within an average of less than four minutes. Our test times were similar to those reported by other studies using multiple stimuli. Despite the present study using longer recording times than single-stimuli studies, the results indicate that multiple ASSR is a time-efficient method to establish normal hearing in infants.

Tone ABR testing is currently the “gold standard” for threshold estimation in infants (Stapells, 2000a, 2000b; Joint Committee on Infant Hearing, 2007). Although the actual recording times for the tone ABR were not determined in this study, one may estimate a typical ABR test time based upon the following typical scenario in a quietly sleeping normal infant: two replications for 2000 sweeps (rate of 39.1/ sec) for three frequencies: 500, 2000, and 4000 Hz (see BCEHJP, 2008, protocols). This calculation would not take into account time to manipulate waveforms or conduct a third replication at any frequency. Thus, the time required to obtain normal tone ABR results for three frequencies and two ears under these ideal conditions would be 8.97 minutes. Compared to our data from our 18 infants with normal ASSR results at 40–50 dB HL, the ASSR test times are similar and somewhat faster. Over 90 percent of our infants had responses for four frequencies and both ears in eight minutes. Moreover, the mean test time for these infants with present responses at 40–50 dB HL was only 3.9 minutes, and 47 percent of these infants had responses for four frequencies and both ears within three minutes. Given that an infant may (and often does) wake up at any moment, the five-minute faster test times of ASSR may give it an advantage over the tone ABR.

**Ipsilateral versus Contralateral EEG Channel Results**

Only two previous studies have compared ipsilateral and contralateral ASSRs for air-conducted or bone-conducted stimuli (van der Reijden et al., 2005; Small and Stapells, 2008), and neither study provides these results for dichotic stimuli or for several intensities. The present study indicates that for multiple ASSR infant contralateral ASSRs are often absent when ipsilateral ASSRs are present. This is similar to van der Reijden et al. (2005), who found that for 500 and 2000 Hz, infants had smaller or absent ASSRs in the channel contralateral to the stimulus compared to the ipsilateral channel. Thus, absent contralateral ASSRs cannot be used to indicate an elevated threshold.

One ipsilateral/contralateral asymmetry of interest is at 4000 Hz. The results of the present study as well as those of Small and Stapells (2008) indicate a smaller difference between ipsilateral and contralateral channels for 4000 Hz stimuli compared to other carrier frequencies (although 4000 Hz contralateral ASSRs were still much smaller compared to those in the ipsilateral channel). The reasons for the smaller ipsilateral/contralateral asymmetry for 4000 Hz stimuli are not clear. In the present study, the AC stimuli were delivered using insert phones and at relatively low intensities, thus the smaller asymmetry is not likely due to acoustic crossover or stimulus artifact. It is more likely due to a neurophysiological difference for responses to high frequencies.

Relative to ipsilateral responses and in general, the infant’s response in the contralateral EEG channel is smaller and very often absent. Similar results have been shown for the air- and bone-conducted ABR (e.g., Edwards et al., 1985; Stapells and Ruben, 1989; Foxe and Stapells, 1993). As with the ABR, clinicians must ensure a correct recording setup and record from the ipsilateral stimulated ear so as not to mistakenly interpret absent contralateral ASSRs as an elevated threshold. Occasionally, an ASSR may be present in the contralateral EEG channel when absent in the ipsilateral EEG channel. Out of 366 tests at 40 dB HL (i.e., across both ears, four frequencies, all infants), only six significant ASSRs were seen in the contralateral channel when ASSRs were absent in the ipsilateral channel. Thus, when considering infants’ ASSRs to AC stimuli, we recommend that only results from the ipsilateral EEG channel be considered. More study...
is needed to determine whether a contralateral response without the presence of an ipsilateral response should be considered a true response.

**CLINICAL APPLICATION**

The present study is the only multiple ASSR that used tone ABR to confirm normal hearing in infant subjects. Using cumulative percent present data (Figure 1), 90 percent of the infants showed ASSRs at 49, 45, 36, and 32 dB HL at 500, 1000, 2000, and 4000 Hz, respectively. Table 2 shows similar “normal” results from several infant ASSR studies. Considering the results of the present study as well as those from the other studies, we recommend “normal” screening levels of 50, 45, 40, and 40 dB HL for 500, 1000, 2000, and 4000 Hz, respectively.

Converted to dB SPL, recommended “normal” ASSR levels for 500, 1000, 2000, and 4000 Hz are 55.5, 50, 43, and 45.5 dB SPL, respectively. For comparison, recommended tone ASSR levels for 500, 1000, 2000, and 4000 Hz are 52–57, 50–55, 40–50, and 46–51 dB SPL, respectively (Stapells, 2000a; BCEHP, 2008). Thus, when considered in dB SPL, these levels are similar for ASSR and ABR and much higher than those for behavioral measures. This suggests that the 80 Hz ASSR, like the ABR, does not reflect temporal integration seen behaviorally and may lend evidence toward the ABR and 80 Hz ASSR having similar generators.

The present study recommends ASSR levels that are similar to recommended tone ASSR levels (BCEHP, 2008). These “normal” levels might miss a very mild hearing loss; however, most current early hearing programs (including the BCEHP) have made a decision to target only hearing losses greater than 25 dB HL and thus not focus on very mild hearing losses.

Preliminary data from our clinic for a small group (N = 23) of children with hearing loss (determined by tone ABR) suggest that the ASSR screening levels recommended above would not “pass” an infant with hearing loss. In 73 of 80 tests, the ASSR and tone ABR both showed the same category (“normal” vs. “elevated”). In six of 80 tests, the ASSR indicated “elevated” whereas the tone ABR indicated “normal.” Importantly, in only one of 80 tests (1.3%) was ASSR “normal” when the tone ABR was “elevated.” In this latter case, the other ASSR frequencies were elevated, and thus the infant would not have “passed” the ASSR. Although these hearing loss data are from a small group with diverse hearing loss and are thus only preliminary, these results lend support to the above recommended ASSR “normal” screening levels.

We recommend use of a relatively stringent noise criterion for “no response.” In the current study, this criterion was sideband noise of 5 nV or less. Although this might result in longer recording times, the literature (Picton et al, 2005) suggests that longer recording times will result in better threshold estimation. Despite recording longer, we were able to complete recording within a reasonable time frame, such that across all infants, the average test time was 6.3 minutes per intensity, with normal results (40–50 dB HL) being confirmed for most infants in less than five minutes for both ears and four frequencies.

The results in the EEG channel contralateral to the stimulated ear should not be used to determine ASSR thresholds. When considering infants’ responses to AC stimuli, we recommend that only results from the ipsilateral EEG channel be considered. Further, to ensure a correct setup, we recommend routine use of two-channel EEG recordings. This would avoid having to change electrodes between ears if presenting monaural stimuli and would ensure always having results for the ipsilateral channel. This would also allow two-channel recordings for bone-conduction ASSR, where comparison of ipsilateral and contralateral ASSR results appears to indicate which cochlea is responding to the bone-conducted stimuli (Small and Stapells, 2008). As many clinical ASSR systems currently do not provide two-channel EEG recordings, manufacturers will need to alter their systems.

Our results support the use of multiple ASSR for the time-efficient confirmation of normal hearing for

<table>
<thead>
<tr>
<th>Study</th>
<th>Age</th>
<th>N</th>
<th>Stimulus</th>
<th>Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lins et al, 1996 (Ottawa data)</td>
<td>1–10 months</td>
<td>21</td>
<td>M</td>
<td>48 43 41 40</td>
</tr>
<tr>
<td>Cone-Wesson et al, 2002</td>
<td>1–120 days</td>
<td>51</td>
<td>S</td>
<td>&gt;71 &gt;72 50 54</td>
</tr>
<tr>
<td>John et al, 2004 (older group)</td>
<td>3–15 weeks</td>
<td>20</td>
<td>M</td>
<td>&gt;46 &gt;50 47 44</td>
</tr>
<tr>
<td>Swanepoel and Steyn, 2005</td>
<td>3–8 weeks</td>
<td>5</td>
<td>M</td>
<td>50 &gt;50 &gt;50 40</td>
</tr>
<tr>
<td>Rance and Tomin, 2006</td>
<td>6 weeks</td>
<td>20</td>
<td>S</td>
<td>50</td>
</tr>
<tr>
<td>Luts et al, 2006</td>
<td>1–10 months</td>
<td>10</td>
<td>M</td>
<td>&gt;44 &gt;50 42 44</td>
</tr>
<tr>
<td>Present study</td>
<td>1–66 months</td>
<td>54</td>
<td>M</td>
<td>49 45 36 32</td>
</tr>
</tbody>
</table>

Note: S = single-stimulus auditory steady-state response (ASSR); M = multiple-stimulus ASSR.
air-conduction stimuli in young infants. We continue to collect data for children with hearing loss; however, more data are needed for infants with hearing loss before we would recommend the use of ASSR alone for threshold estimation in infants and children with hearing loss (Stapells et al, 2005).

Acknowledgment. We wish to thank Laurie Usher and Renée Janssen for their assistance in and support of this research.

NOTES

1. All infants were assessed using a two-channel EEG recording montage for comparison of ASSRs in the ipsilateral and contralateral channels. Although the location of the vertex electrode varied depending on whether the fontanel was open or closed, research on infants and adults would suggest that this difference in placement would have no effect on the results (van der Reijden et al, 2005; Thumak et al, 2007b; Small and Stapells, 2008).

2. The Intelligent Hearing Systems (IHS) SmartEP-ASSR system labels a response present (Yes) or absent (No) based upon the p value and the amplitude of the response (response amplitude must be ≥13 nV to be considered a response). For the present study, however, we also considered responses <13 nV as being present if p < .05.

3. In a pilot study, we compared the number of present responses on the IHS SmartEP-ASSR at 40–50 dB HL for 10 nV versus 5 nV noise criteria in 21 infants with normal tone ABR. Responses (results for right and left ears combined) were more often present for a 5 nV criterion compared to the 10 nV criterion. Responses were present 56, 90, 95, and 100 percent of the time for 500, 1000, 2000, and 4000 Hz, respectively, using the 5 nV criterion, as opposed to responses being present 28, 60, 85, and 88 percent of the time for the 10 nV criterion.

4. Our recent studies using MASTER-based systems have used a mean sidebin noise of 10 nV when recording the 80 Hz ASSR (e.g., Herdman and Stapells, 2003; Van Maanen and Stapells, 2005; Small and Stapells, 2006). EEG noise calculations are likely different for different ASSR systems using different analyses. For example, the IHS SmartEP-ASSR uses two measures of noise, one from the mean of ten sidebins around the modulation frequency and another at the modulation frequency from the FFT of the plus-minus average. The MASTER system calculates the noise from the mean of 120 sidebins around the modulation frequency.

REFERENCES


