EFFICIENCY OF THE SINGLE- VERSUS MULTIPLE-STIMULUS AUDITORY STEADY-STATE RESPONSES IN INFANTS

by

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Abstract

Multiple Auditory Steady-State Responses (ASSRs) will likely be included in the diagnostic test battery for estimating infant auditory thresholds in the near future; however, the effects of single versus multiple stimulus presentation in infants has never been investigated. There are known maturational differences between infants and adults and these differences may lead to greater interactions between responses to multiply-presented stimuli in infants. Thus, it is unknown whether or not interactions between responses to multiple stimuli exist, and if they do, whether or not a single-stimulus or multiple-stimulus presentation method is more efficient for testing infants. In the present thesis, two studies were carried out to address this issue. All infants in Study A participated in three stimulus conditions which differed in the number of stimuli presented simultaneously. The monotic single (MS) condition consisted of 500, 1000, 2000, and 4000 Hz tones which were presented singly to one ear. The monotic multiple (MM) condition was composed of four tones (500, 1000, 2000, and 4000 Hz) presented to one ear simultaneously. The dichotic multiple (DM) condition consisted of eight tones presented simultaneously to both ears (four tones to each ear). ASSR amplitudes were obtained from 15 normal infants (mean age: 23.1 weeks) in response to multiple (MM, DM) and single (MS) air-conduction AM tones [77-105 Hz modulation rates; 60 dBSPL]. Mean single-stimulus amplitudes for 500, 1000, 2000, and 4000 Hz were 30, 39, 45 and 43 nV, respectively. Presentation of multiple AM tones (i.e., 4 octave-spaced frequencies) to one ear resulted in ASSR amplitudes that were 97%, 87%, 82%, and 70% (for 500, 1000, 2000 and 4000 Hz, respectively) of the single–stimulus ASSR amplitudes. Results for the dichotic presentation of eight AM

tones show ASSR amplitudes that were 70%, 77%, 67%, and 67% relative to the MS condition. Although decreases in amplitude occurred using multiple stimuli in infants, the multiple ASSR remained more efficient than the single-stimulus ASSR (i.e., multiple-stimulus amplitudes were greater than single-stimulus amplitudes divided by $M^{0.5}$, where M is the number of stimuli). The amplitude reductions seen in the multiple-stimulus conditions in infants could have origins in the ear canal, middle ear, cochlea and/or brainstem. Because greater interactions occur in the dichotic multiple-stimulus condition compared to the monotic multiple-stimulus condition and baseline single-stimulus condition, brainstem origins of these interactions are likely. In study B, ASSR thresholds were determined for 500-Hz in the single- and dichotic multiple-stimulus conditions (14 infants; mean age: 20.2 weeks). Results indicate that ASSR thresholds for 500 Hz presented in the dichotic-multiple condition were elevated 3 dB compared to that obtained in the 500-Hz single-stimulus condition. This statistically non-significant difference is within the range of acceptable test-retest variability, and is thus not of clinical significance. Results from both studies revealed that the multiple-stimulus conditions resulted in shorter time-to-criteria, but this did not quite reach significance. In summary, as with adults, multiple-stimulus presentation in infants is more efficient (in terms of amplitude and time-to-criteria) than single AM tones.

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List of Abbreviations

Abbreviation	Definition
ABR	Auditory brainstem response
AM	Amplitude modulation
AM/FM	Mixed modulation, combination of AM/FM
AN	Auditory nerve
ANOVA	Analysis of variance
ASSR	Auditory steady-state response
CR	Circle radius
DM	Dichotic multiple, eight stimuli
EEG	Electroencephalogram
FFT	Fast Fourier transform
FM	Frequency modulation
fMRI	Functional magnetic resonance imaging
MF	Modulation frequency
MS	Monotic single, baseline
MM	Monotic multiple, four stimuli
SAM	Sinusiodal amplitude modulation
VRA	Visual reinforcement audiometry

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Chapter 1

Literature review: Auditory Steady-State Responses

Auditory Steady-State Responses

Early determination of infant auditory status

The importance of early identification and treatment of hearing loss has been well established with studies demonstrating the benefits of intervention by the first few months of life in terms of subsequent speech and language outcomes (Kennedy et al., 2006; Moeller, 2000; Yoshinaga-Itano, Sedey, Coulter, & Mehl, 1998). Specifically, children with hearing loss identified before six months of age have significantly better receptive and expressive language skills compared to children identified later (Yoshinaga-Itano et al., 1998). These findings set the foundation for many early intervention programs, including the British Columbia Early Hearing Program (BCEHP), whose goal is the identification of permanent hearing loss by three months of age and the initiation of treatment (e.g., amplification) by six months of age (Joint Committee on Infant Hearing, 2007). Early identification of hearing loss in young infants (< 6 months of age) using behavioural methods poses a challenge to clinicians because behavioural responses in young normally-developing infants and many older infants and children with developmental delays are unreliable (American Speech-Language-Hearing Association, 2004), if not impossible.

Visual reinforcement audiometry (VRA) is a commonly used behavioural technique for assessing infant auditory status and works well for infants six months through two years of age (Moore, Wilson, & Thompson, 1977; Thompson & Wilson, 1984). VRA involves a visual reinforcer (e.g., animated toy) used to maintain or reinforce a head-turn response to repeated auditory stimulation. Moore, Wilson, and Thompson (1977) investigated the likelihood of eliciting a head-turn response to broadband noise in three groups of infants ranging between four and 11 months of age (group 1: four month olds; group 2: five- and six-month-old infants; group 3: seven through 11 months of age) either in the presence or absence of visual reinforcement. Results indicated that the presence of a reinforcer significantly increased the number of head-turn responses to auditory stimuli in the two older groups (five to 11 months of age), but no increases were seen for the four-month-old infants. In response to 30 presentations of broadband noise, head-turn responses were elicited 87% of the time in the older groups, but only 21% of the time in the four-month olds. The success with VRA for the older infants, but not younger infants is likely related to the development of localizing towards a sound source, which appears around age 4-6 months and improves throughout the first year of life (Murphy, 1962).

The inconsistency of the VRA method for testing infants under six months of age was further demonstrated in a cross-sectional study conducted by Muir and colleagues (Muir, Clifton, & Clarkson, 1989). Results indicated that head turning capabilities followed a U-shaped pattern as a function of age in response to rattle sounds. Specifically, neonates responded slowly but reliably to sounds, but responses decreased in frequency and strength between one to three months of age, and increased again by four to five months of age (Muir et al., 1989). The authors hypothesized that the decreased orientation ability between one to three months reflected a shift in locus of control from subcortical to cortical structures (Muir et al., 1989). This hypothesis is supported by human and animal studies on the precedence effect, which is thought to be mediated by the auditory cortex (Clifton, Morrongiello, Kulig, & Dowd, 1981; Cranford, Ravizza, Diamond, & Whitfield, 1971). The absence of the precedence effect in infants younger than five months of age is consistent with immaturities in the central auditory system (Clifton, Morrongiello, Kulig, & Dowd, 1980; Clifton et al., 1981).

Clearly, behavioural responses are inconsistent up to age four to five months, therefore precluding the clinical use of behavioural methods for these young infants. Because identification of hearing loss is critical before six months of age, alternative assessment techniques for determining threshold which do not rely on behavioural responses are necessary.

Electrophysiological techniques, such as the Auditory Brainstem Response (ABR) and Auditory Steady-State Response (ASSR), involve recording brain waves in response to auditory stimuli, require no subjective responses from the listener and thus provide an alternative to behavioural testing for threshold determination. These responses are particularly useful when patients are either unable or unwilling to give accurate or reliable behavioural responses (Dobie, 1993).

The tone-evoked ABR is currently the clinical gold-standard for estimating frequency-specific hearing thresholds in young infants under five to six months of age (Stapells, 2000a; Stapells, Herdman, Small, Dimitrijevic, & Hatton, 2005) and can be recorded in infants as young as 29 weeks postconceptional age (Ponton, Moore, Eggermont, Wu, & Huang, 1994). The major disadvantages associated with the ABR is that it currently requires the interpretation of waveforms by an expert clinician (Stapells, 2000a) and it can be time-consuming as only one stimulus and one ear can be assessed at a time. Evoked otoacoustic emissions are also useful for detecting hearing loss, but do not provide information regarding the degree (e.g., mild, moderate, severe) or type of hearing loss (e.g., mixed, conductive, sensorineural), and are thus not suitable for threshold purposes (Kemp, Ryan, & Bray, 1990; Lonsbury-Martin, Whitehead, & Martin, 1991). Much research has been devoted to establishing an auditory evoked potential that is able to overcome the limitations of the tone-evoked ABR. For reasons discussed below, ASSRs to stimuli modulated in the 70-110 Hz range (the "80-Hz" ASSR) have received much recent attention from audiologists and researchers as an alternative to the ABR for estimating hearing thresholds in infants.

The ASSR technique has several advantages over the tone-evoked ABR. ASSRs are more objective because statistical measures determine the presence/absence of responses and thereby eliminate the need for a clinician to interpret waveforms as is currently required for the tone-ABR (Stapells et al., 2005). Further, the "multiple" ASSR technique has the potential to gather more information in a shorter amount of time, thus speeding up test time (Herdman & Stapells, 2001; John, Lins, Boucher, & Picton, 1998). This reduction in test time is accomplished by presenting multiple stimuli simultaneously at different modulation rates to one or both ears (Herdman & Stapells, 2001; John et al., 1998).

The following sections provide an overview of ASSRs with a specific focus on the 80-Hz ASSR for the estimation of infant auditory status. As the studies of the current thesis were conducted in infants, maturational factors related to auditory development will also be reviewed, and the potential impact of maturation on ASSR results considered.

ASSR definition and history

A steady-state response is a repetitive evoked potential that can be recorded from the human scalp that is best thought of in terms of its frequency components rather than its time-domain waveform (Regan, 1989). Steady-state responses were first used to assess the visual modality (Regan, 1966); auditory steady-state responses, are steady-state responses evoked by regularly repeating auditory stimuli (Picton, Stapells, Perrault, Baribeau-Braun, & Stuss, 1984). In evoking the ASSR, the stimulus presentation rates employed are fast enough to result in the overlap between the transient response of one stimulus with the responses to later stimuli (Picton, John, Dimitrijevic, & Purcell, 2003). This is unlike transient auditory evoked potentials (e.g., the ABR), where responses do not overlap due to the use of slower stimulus presentation rates (Picton et al., 1984). By manipulating stimulus rate, the functioning of different levels within the auditory pathway can be assessed; thus, there are several different "ASSRs" (Stapells et al., 2005).

Early studies primarily focused on ASSRs elicited using stimulus rates in the 30-50 Hz range due to their large response amplitude (Galambos, Makeig, & Talmachoff, 1981; Stapells, Linden, Suffield, Hamel, & Picton, 1984) and large signal-to-noise ratio (SNR) which aided response detection (Ross et al., 2000). Although response amplitude is largest at MFs in the 40-Hz range in adult subjects, the 40-Hz ASSR was shown to be unreliable in infants (Aoyagi et al., 1993; Stapells, Galambos, Costello, & Makeig, 1988; Suzuki & Kobayashi, 1984). On the other hand, ASSRs evoked by stimulus rates in the 70-110 Hz range show considerable promise as an accurate method for establishing auditory thresholds in infants and adults (Stapells et al., 2005). For the purposes of this review, the main focus will be on the research pertaining to the 80-Hz ASSR as this was approximately the stimulation rate used in the studies of the current thesis.

Stimulus Factors

Stimulus types

There are numerous types of stimuli used to evoke ASSRs, each with advantages and disadvantages. Some of the more common examples include clicks (Galambos et al., 1981; John, Dimitrijevic, & Picton, 2003), brief-tones (Mo & Stapells, 2008; Stapells et al., 1984; Stapells, Makeig, & Galambos, 1987), sinusoidally amplitude-modulated (AM) tones (Campbell, Atkinson, Francis, & Green, 1977; Herdman & Stapells, 2001; Lins & Picton, 1995; Lins, Picton, Picton, Champagne, & Durieux-Smith, 1995; Lins et al., 1996), frequency-modulated tones (Picton, Skinner, Champagne, Kellett, & Maiste, 1987), mixed-modulation tones (a combination of AM and FM stimuli) (Dimitrijevic, John, van Roon, & Picton, 2001; John, Dimitrijevic, van

Roon, & Picton, 2001; Rance & Tomlin, 2006; Rance, Tomlin, & Rickards, 2006), and exponential AM stimuli (John, Dimitrijevic, & Picton, 2002).

In evoked potential testing there is a tradeoff between stimulus frequency specificity and the size of the resulting evoked potential, such that less frequency-specific stimuli (e.g., clicks) elicit larger responses than do more frequency-specific stimuli (e.g., AM tones) (Picton et al., 2003). Although not appropriate for diagnostic threshold audiometry, these less frequency-specific stimuli which elicit larger responses (and thus aid in faster detection of response presence/absence) have been proposed for screening purposes (John, Brown, Muir, & Picton, 2003). The studies of the present thesis used sinusoidally AM tones because these stimuli have been shown to have good frequency specificity and are thus appropriate for the estimation of frequency-specific thresholds. Sinusoidally AM tones contain spectral energy at the carrier frequency and at two sidebands located above and below, separated from the carrier frequency by the modulation frequency (Picton et al., 2003). For example, a 1000-Hz carrier with 100% amplitude modulation at 80 Hz would have energy at 1000 Hz (carrier frequency), 920 Hz and 1080 Hz (two sidebands).

Modulation rate

The effect of modulation rate on the ASSR in humans has been studied by many and over a broad rate range spanning from around 2 Hz up to 600 Hz (Cohen, Rickards, & Clark, 1991; Lins et al., 1995; Picton et al., 2003; Purcell, John, Schneider, & Picton, 2004; Stapells et al., 1988; Stapells et al., 1984; Stapells et al., 1987). The aforementioned studies, as well as others, have demonstrated that different modulation rates have different effects on ASSR amplitude and phase, as well as EEG noise levels (a more detailed description of EEG noise can be found in the "EEG noise" section).

The largest ASSR amplitudes are elicited by amplitude modulation at low rates (i.e., below 60 Hz) for adults (Galambos et al., 1981; Stapells et al., 1984). Above 40 Hz, response amplitudes drop off, although another smaller amplitude peak is seen in the 80 to 100 Hz range (Cohen et al., 1991; Lins et al., 1995). At even higher modulation rates beyond 100 Hz, response amplitudes decrease further (Aoyagi et al., 1999) and cannot be distinguished from background noise levels above about 485 Hz (Purcell et al., 2004). Unlike adults, infants and young children do not show an amplitude peak for modulation rates around 40 Hz (Aoyagi et al., 1994; Levi, Folsom, & Dobie, 1993; Stapells et al., 1988; Suzuki & Kobayashi, 1984), which suggests immaturities at the level of the infant auditory cortex which limit its ability to follow these rates.

Carrier frequency

ASSRs are typically elicited by carrier frequencies between 500 to 4000 Hz. The 80-Hz ASSR evoked using air-conduction stimuli usually reveals larger response amplitudes in the mid-frequency range (e.g., 1000-2000 Hz) versus the higher (4000-6000 Hz) and lower frequency (500-750 Hz) ranges (John, Dimitrijevic, van Roon et al., 2001). This band-pass shape in terms of amplitude (i.e., larger responses in the mid-frequencies versus the extremes) does not appear related to the resonances of the human ear which amplify mid-frequency sounds, because these differences in amplitude remain when stimuli are presented at a constant dB HL value (Dimitrijevic et al., 2002).

Stimulus intensity

In general, as intensity increases, ASSR amplitudes increase and latencies decrease (Lins et al., 1995; Picton et al., 2003; Stapells et al., 1984). For ASSRs in the 80-Hz range, amplitude increases are more rapid at high intensities (i.e., >70 dB SPL) versus lower intensities (i.e., <70 dB SPL). Between 20 to 70 dB SPL amplitude increases for a 1000-Hz AM tone were found to be 2 nV/dB versus 8 nV/dB between 70 to 90 dB SPL (Lins et al., 1995). Response amplitudes saturate at high stimulus intensities (90 dB HL) (Picton et al., 2003).

EEG noise

Another factor critical in *detecting* ASSRs is the level of background EEG noise. In order to estimate ASSR amplitude and phase, the noise levels in the frequency range surrounding the response must be considered. It has been well established that EEG noise decreases with increasing frequency (Picton et al., 2003). *Figure 1* demonstrates this change in EEG noise with modulation rate. Below 10 Hz EEG noise is largest, which makes responses within this range difficult to detect despite their large amplitudes. Between 10 to 40 Hz, noise levels drop rapidly and then continue to decease at a slower rate above 40 Hz (Picton, Dimitrijevic, John, & Van Roon, 2001). Because EEG noise level and response amplitude vary as a function of rate, and both

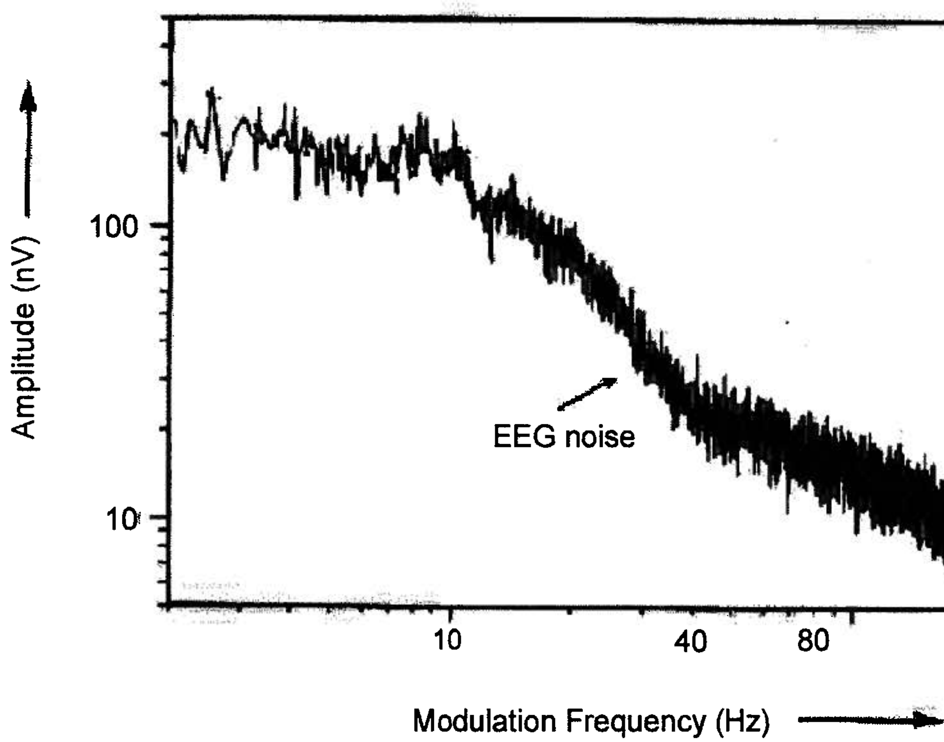

Figure 1. EEG noise as a function of modulation frequency (Hz).

are critical for response detection, most ASSR statistical measures rely on the SNR for response detection. High background noise levels combined with small response amplitudes (small SNR) mean that long averaging times would be necessary in order to determine response presence or absence. Compared to the 40-Hz ASSR, the 80-Hz ASSR reveals larger SNRs in infants (Pethe et al., 2004).

Subject factors

Age

As mentioned previously, the 40-Hz ASSR cannot be reliably recorded in infants (Levi et al., 1993; Stapells et al., 1988; Suzuki & Kobayashi, 1984). Infant ASSR amplitudes do not show the normal adult enhancement in ASSR amplitude near 40 Hz (Levi et al., 1993; Stapells et al., 1988; Suzuki & Kobayashi, 1984). The amplitude of the 40-Hz ASSR becomes adultlike by 14 years of age (Pethe, Muhler, Siewert, & von Specht, 2004). The 80-Hz ASSR, on the other hand, does not change much with age (Pethe, Muhler et al., 2004) and is reliably recorded in infants (Lins et al., 1996; Rickards et al., 1994).

Research on the ASSR at the other end of the age continuum is somewhat limited. The ASSR in adulthood does not change significantly with advancing age (Boettcher, Poth, Mills, & Dubno, 2001; Dimitrijevic, John, & Picton, 2004; Johnson, Weinberg, Ribary, Cheyne, & Ancill, 1988; Muchnik, Katz-Putter, Rubinstein, & Hildesheimer, 1993). Johnson and colleagues (Johnson et al., 1988) and Boettcher and colleagues (Boettcher et al., 2001) demonstrated no differences in the 40-Hz ASSR between young and elderly adults. However, recent work by Dimitrijevic and colleagues (Dimitrijevic et al., 2004) showed that 40-Hz ASSR response amplitudes were slightly larger in older, compared to younger adults. Picton and colleagues (Picton, Dimitrijevic, Perez-Abalo, & Van Roon, 2005) found no differences in 80-Hz ASSR thresholds between young and older normal-hearing listeners.

Arousal state

The effects of sleep versus wakefulness on the ASSR is seen predominately from responses elicited in the higher auditory pathways (i.e., auditory cortex) rather than in the lower pathways (i.e., brainstem) (Picton et al., 2003). Sleep has been shown to significantly reduce the amplitude of the 40-Hz ASSR (Cohen et al., 1991; Dobie & Wilson, 1998; Linden, Campbell, Hamel, & Picton, 1985), whereas 80-Hz ASSRs are less affected by arousal state (Cohen et al., 1991; Lins & Picton, 1995). Cohen, Rickards, and Clark (1991) conducted a study in which they looked at the characteristics of ASSRs to stimuli modulated at 30-60 Hz and 90-125 Hz from awake and sleeping adults. Results of their study showed that sleep reduced response amplitude when modulation rates of 40 Hz were used, whereas there was no effect of sleep on responses elicited using rates in the 80-Hz range. Because sleep also decreases background EEG noise, however, perhaps the 40-Hz SNR does not decrease.

The effects of arousal state (i.e., sleep versus wakefulness) on the ASSR in infants has never been directly studied and all infant ASSR studies to date have been conducted during sleep, either naturally or under sedation (Cone-Wesson, 2008). Because 40-Hz ASSR amplitudes are significantly attenuated by sleep (Linden et al., 1985), and because infants show no prominent ASSR peaks at rates between 9 to 59 Hz (Stapells et al., 1988), ASSRs elicited between 9 to 59 Hz are inappropriate for the assessment of auditory threshold in infants during sleep. Many studies have shown that the 80-Hz ASSR is still recordable in sleeping infants (e.g., Aoyagi et al., 1994; Levi et al., 1993; Rickards et al., 1994). Using AM tones (500 and 2000 Hz) at various modulation rates Levi and colleagues (Levi et al., 1993) measured response coherence, a measure similar to the SNR. Results indicated that the largest response coherence values were obtained in the region of 80 Hz. Consistent with these findings, Rickards, Tan, Cohen, Wilson, Drew, and Clark (1994) conducted a large scale study of newborns demonstrating that the 80-Hz ASSR is recordable in sleeping infants. Modulation frequencies in the 80 Hz range (between 55 to 110 Hz) yielded the highest levels of detection for sleeping newborns, whereas response detection decreased when lower (<55 Hz) and higher (>110 Hz) rates were used (Rickards et al., 1994). Thus, research to date shows that sleep does not preclude the ability to record the 80-Hz ASSR in infants.

ASSR measurement

Several clinical ASSR systems are currently being marketed and sold for the purposes of estimating infant behavioural thresholds (Stapells et al., 2005). The following discussion will focus on the recording and analysis of ASSRs in general, although specific details will be given in regards to the multiMASTER research system (John & Picton, 2000) as this was the system used in the studies of the current thesis.

Analysis of the responses

ASSRs can be measured in the time domain or in the frequency domain (Picton et al., 2003). In the time domain, peaks and troughs within a waveform are selected and measurements of amplitude and phase are calculated (Picton et al., 2003). Difficulties in peak selection by even an expert clinician make time-domain analysis difficult. This can be overcome by analysing ASSRs in the frequency domain using methods such as a Fourier analyzer (Regan, 1966,1989) or a fast Fourier transform (FFT) (Rickards & Clark, 1984) so that subjective peak selection by a clinician is not required. Time-domain and Fourier analyses of ASSR amplitudes have been shown to yield similar results, and either can be used for the analyses of ASSRs (Stapells et al., 1984). Although earlier studies conducted analyses strictly in the frequency domain using analog Fourier analyzers, most current systems employ the FFT. Unlike analog Fourier analyzers which provides amplitude and phase information at only one modulation rate, the FFT does an analysis in the time domain so that one is able to look at the amplitude spectra for many discrete frequencies. The amplitude of the response(s) can be seen at each modulation frequency and compared to adjacent frequency bins which provide an estimate of background noise level (Picton et al., 2003). The multiMaster system measures ASSRs in the time domain and then analyses these recordings online in the frequency domain using a FFT, allowing for easy determination of response presence/absence (Picton et al., 2003).

Signal averaging, artifact rejection, and weighted averaging

Scalp-recorded potentials reflect EEG activity related to the presentation of an auditory signal as well as activity unrelated to the signal. "Background noise" may be related to non-physiological interference in the surrounding environment (e.g., electrical interference from other equipment) and/or physiological activity unrelated to the signal (e.g., muscle activity, eye blinks, crying). Objective ASSR detection relies on the ability to distinguish the response to the signal from the "background noise", and high levels of background EEG noise are one of the major sources of unreliability in recording averaged evoked potentials (Picton, Linden, Hamel, & Maru, 1983), particularly at levels near threshold where the signal strength is relatively small and can be easily obscured.

An EEG noise criterion level of 11 nV is commonly employed and is in fact recommended in order to determine ASSR response absence when no significant result ($p > .05$) is present (Cone-Wesson & Dimitrijevic, in press; Herdman & Stapells, 2003; Picton et al., 2003; Van Maanen & Stapells, 2005). In general, mean EEG noise should be about half the size of the smallest response you want to detect. For example, if one wishes to be able to detect a 20 nV response, a typical near-threshold ASSR amplitude, mean EEG noise levels less than approximately 11 nV must be obtained. The multiMaster system records EEG noise and circle radius, the latter of which represents the 95% confidence interval for response detection rather than background noise level per se. In other words, if response amplitude(s) are larger than the circle radius value(s), one can be 95% certain that a response at the modulation rate of interest differs significantly than the surrounding background noise. Recently, several studies in our laboratory have used a criterion circle radius value of 20 nV in order to determine a no response ($p > .05$) using multiMaster (equivalent to a mean EEG noise level of 11 nV) (Herdman & Stapells, 2003; Small, Hatton, & Stapells, 2007; Van Maanen & Stapells, 2005), although others have used a less strict noise criterion of 30 nV (e.g., John, Brown, Muir, & Picton, 2004). When a circle radius of 20 nV is used, any response amplitudes larger than 20 nV are deemed significantly different from the background noise levels.

Several techniques exist to decrease the effects of noise levels on the detection of ASSRs. To increase the signal-to-noise ratio (SNR), ASSR amplitudes must be increased and/or background noise decreased. To increase ASSR amplitude, stimulus intensity and/or modulation depth can be increased. Increasing recording time (or the number of averages in a recording) is the most effective way to decrease noise (John et al., 1998; Luts & Wouters, 2004; Picton et al., 2003). Assuming the background noise is random, and not related to the stimulus, averaging the response over time will reduce the amplitude of the noise by the square root of the number of sweeps in the average (Picton et al., 1983). For the multiMaster system, recording sweeps are averaged together. Each sweep is formed by a group of smaller segments ("epochs") which typically last around one to two seconds. For the studies of the current thesis, each EEG recording sweep lasted 16.384 seconds, and was composed of 16 epochs each of 1.024 second duration. The rate of each stimulus was modified so that a precise integer number of the stimulus modulation cycles fit into a sweep (Picton et al., 2003). For example, for a 500-Hz tone amplitude modulated at 81.055 Hz (rounded off to 3 decimal places), there would be exactly 83 modulation cycles within an epoch. This allowed the individual epochs to be linked together without artifact caused by edge effects, which occur when non-zero amplitude offsets are present at the beginning and/or the end of an epoch. The longer sweep formed by joining the 16 epochs allowed for an FFT with more precise frequency resolution. Having the sweep broken up into epochs allowed the program to reject individual noisy epochs (1.024 seconds), rather than losing an entire sweep, which is 16.384 seconds. One of the limitations of averaging is that it becomes less effective in reducing noise levels when noise varies from one trial to the next. The multiMaster system uses artifact rejection and weighted averaging to improve the SNR when fluctuating noise levels are present (John, Dimitrijevic, & Picton, 2001). Artifact rejection involves rejecting any epoch(s), rather than an entire sweep, that contain noise levels above a criterion noise level, for example, ±40 μV. Epochs rejected within a recording sweep are filled with the next recorded data. In some cases, however, this may result in too few accepted trials. Weighted averaging, on the other hand, is a newer technique that, rather than using artifact rejection, assigns more emphasis to epochs with lower noise levels compared to those that contain more noise (John, Dimitrijevic et al., 2003).

Objective response detection

Several statistical methods have been developed to objectively determine the absence or presence of ASSRs amongst background EEG noise. These methods include phase coherence (Jerger, Chmiel, Frost, & Coker, 1986; Stapells et al., 1987) and the F-test, the latter of which is the most common (Picton et al., 2003). The multiMaster system determines objective response presence or absence using the F-test, which measures whether a response (plus noise) at the modulation frequency is significantly larger than the noise in the adjacent 120 frequency bins (60 bins above and 60 bins below the ASSR frequency) (John & Picton, 2000). If the amplitude of the signal at its modulation frequency is the same as the amplitude in adjacent frequencies in the spectrum, multiMaster determines no response to be present. If the amplitude at the modulation rate (signal) exceeds that at other frequencies (noise) within 120 frequency bins with a probability of $p < .05$, multiMaster determines that a response is present. When multiple stimuli are presented simultaneously, bins at the modulation frequencies of AM tones are excluded in the 120 bins used to estimate the noise level. Otherwise, the statistical methods outlined in this section also apply to the multiple-stimulus ASSR technique. *Figure 2* demonstrates ASSR amplitude/frequency spectra subsequent to FFT analysis using the single-stimulus ASSR ("2000-Hz Single") and multiple-stimulus ("Dichotic Multiple") ASSR techniques. As can be seen in this figure, responses to each carrier frequency are seen as vertical spikes at their corresponding modulation rate(s). For example, 2000 Hz was modulated at 92.773 Hz for the left ear in the MS and DM conditions and at 96.680 Hz for the right ear in the DM condition. The 2000-Hz response amplitudes are designated by filled squares. Response amplitudes for 2000 Hz in the MS and DM conditions (test ear only) were 43 and 37 nV, respectively. Average circle radius in the surrounding 120 frequency bins of the 2000-Hz carrier were 16 nV for both the MS and DM conditions.

Response absence/presence criteria

In the studies of the present thesis, ASSR response absence/presence was determined using criteria of recent studies in our laboratory (Armstrong & Stapells, 2007; Fontaine, 2006; Small & Stapells, 2006). ASSRs were recorded either until a response was detected *($p < .05$)* for a minimum of two consecutive sweeps or until the EEG noise criterion was reached *(mean noise $\leq$ 11 nV)* and no response detected *($p \geq .05$)*.

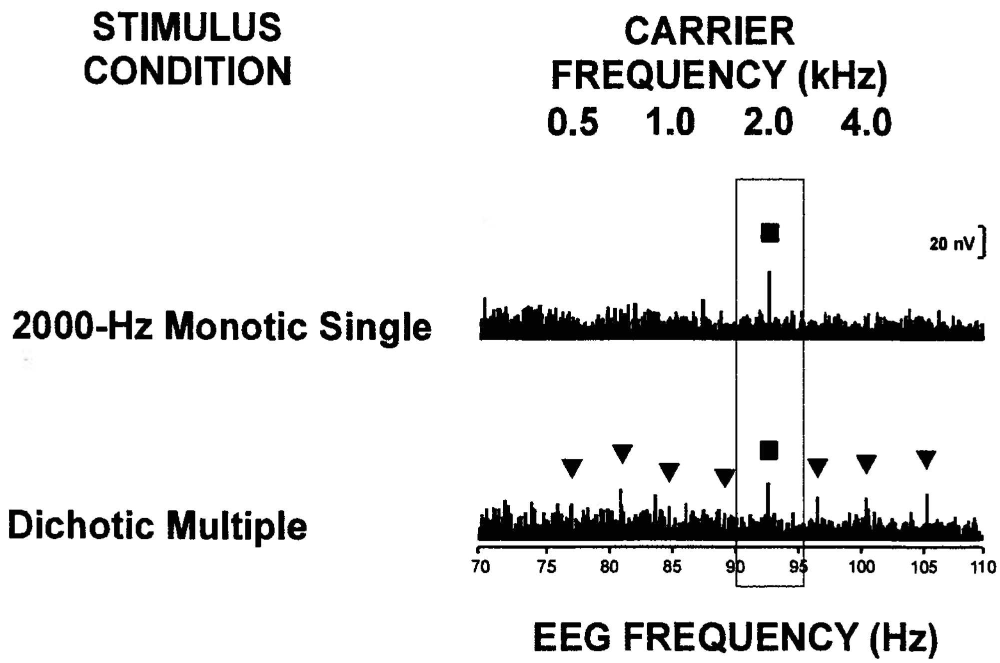

***Figure* 2** ASSR amplitude spectra obtained using Fast Fourier Transform (FFT) for the single stimulus (2000-Hz Monotic Single) and multiple-stimulus (eight simultaneous tones, dichotic multiple) ASSR. Filled triangles and squares indicate that the amplitude at the modulation rate was significantly different from the background EEG noise (i.e., response was present).

Electrode montage

Most ASSR recordings are conducted using a single channel, although multiple-channel recordings may be beneficial in some cases (Small & Stapells, 2008; van der Reijden, Mens, & Snik, 2005; Van Maanen & Stapells, in press). For single-channel recordings, a minimum of three electrodes must be used. For threshold assessment in adults, Lins and colleagues (Lins et al., 1996) recommend recording the EEG using the vertex, nape, and forehead locations for the non-inverting, inverting, and ground electrodes, respectively. This placement is recommended when recording responses to multiple stimuli from two ears in adults in order to avoid response bias to one side (Herdman & Stapells, 2001; Lins et al., 1996). In infants under 6 months of age, the best signal-to-noise ratio is obtained using electrodes at the mastoid ipsilateral to the stimulation and Cz locations rather than at the nape of the neck and Cz locations for adults (van der Reijden et al., 2005). Ipsilateral recording channels are generally recommended in infants because ASSRs recorded contrallaterally have been found to be smaller than ipsilaterally recorded ASSRs (Small & Stapells, 2008; van der Reijden et al., 2005; Van Maanen & Stapells, in press). The studies of the current thesis used a midline (nape) recording channel because when two ears are stimulated simultaneously, a midline (single-channel) recording channel reflects responses from each ear equally, and two channel-recordings are not available on MultiMASTER.

ASSR generators

Determining the neural origins of ASSRs to a variety of modulation rates should contribute to our understanding of the processes involved in normal and impaired hearing (Herdman et al., 2002). Functioning of different levels of the auditory pathway can be inferred because ASSRs at different modulation rates likely reflect activity from different levels of the auditory pathways. Neurons throughout the auditory pathways are capable of responding with a discharge rate corresponding to the rate of a modulated auditory signal, although the range of modulation frequencies over which auditory neurons respond best decreases as one goes from the periphery to the cortex. (Rees & Møller, 1983; Schreiner & Langner, 1988). For example, the auditory nerve, cochlear nucleus, inferior colliculus, and cortex respond best to modulation rates of approximately 1000 Hz, 240-700 Hz, 200 Hz, and 20 Hz, respectively (Frisina, Smith, & Chamberlin, 1990; Langner & Schreiner, 1988; Rees & Møller, 1983). Thus, as one ascends the auditory system the range of modulation frequencies that neurons are responsive to decreases (Frisina et al., 1990).

Kuwada and colleagues (Kuwada et al., 2002), studying the unanaesthetized rabbit, provided evidence supporting the notion that surface-recorded ASSRs reflect the activity of multiple generator sites. In their study, ASSRs were recorded from the superior olivary complex, inferior colliculus, and auditory cortex using either electrodes located on the surface of the scalp or local electrodes inserted at several places in the auditory pathways. Amplitude and delay data were consistent with the idea of multiple ASSR generators as expressed by shifts in amplitude and delay as a function of modulation rate; different generators dominated the responses, depending on the modulation rate utilized. The dominant response at low (<80 Hz) and high (>80 Hz) modulation rates appeared to have cortical and subcortical origins, respectively (Kuwada et al., 2002). Subsequent testing using behavioural stimulation and a depressant drug provided further evidence that the cortex is the primary contributor to responses at low modulation rates. The finding that stimulation (tactile input or cocaine injection) increased amplitude at low modulation rates, but had relatively little effect on amplitude at high modulation rates, suggests the cortex primarily underlies the response at low modulation rates (<80 Hz). When the cortex was reversibly inactivated using a drug that induces cortical depression [potassium chloride (KCl)], responses contralateral to the KCl injection site at low rates were significantly attenuated, although ipsilateral responses were not, nor were the responses at high modulation rates, providing further support that the cortex underlies responses at low modulation rates (Kuwada et al., 2002).

Herdman and colleagues (Herdman et al., 2002), using brain electric source analysis to estimate the generator sites of the ASSR in humans, found results similar to those obtained in animal studies. In their study, AM rates of 12, 39, and 88 Hz were employed. Their responses revealed that the 88-Hz ASSR was composed mainly of responses from the brainstem with some activation of more central structures; the 39-Hz ASSR largely reflected responses from cortical sources, although a small contribution from brainstem generators was also present (Herdman et al., 2002). Megnetoencephalography (MEG) studies have localized the 40-Hz ASSR to the Sylvian fissure in the primary auditory cortex (Romani, Williamson, Kaufman, & Brenner, 1982). Thus, similar to the animal studies, the cortex does not respond as well as the brainstem to fast modulation rates. The 12-Hz ASSR showed activation in both the cortex and brainstem (Herdman et al., 2002).

Findings from functional magnetic resonance imaging (fMRI) are consistent with electrophysiological data. Giraud and colleagues (Giraud et al., 2000) compared fMRI results for sinusoidally amplitude-modulated white noise at rates between 4 to 256 Hz to white noise with no amplitude modulation in order to observe brain responses reflecting temporal processing in five normal-hearing adults. All stimuli were presented to the right ear at 75 to 85 dB SPL. Results indicated that the right lower brainstem (superior olivary complex), the right inferior colliculus, the left medial geniculate body, Heschl's gyrus, the superior temporal gyrus, the superior temporal sulcus, and the inferior parietal lobule were responsive to AM stimuli. Further investigation revealed that each level of the auditory system responded preferentially to a given modulation rate. Specifically, maximum blood flow was seen in the lower brainstem regions when high stimulus rates were used (256 Hz), in the inferior colliculus for AM rates of 32-256 Hz, in the medial geniculate body for AM of 16 Hz, the primary auditory cortex for 8 Hz, and secondary cortical areas for AM of 4-8 Hz (Giraud et al., 2000).

Wong and Stapells (2004) investigated the binaural masking level difference (BMLD) in humans for the 80-Hz ASSR and 13-Hz ASSR. Results showed no BMLD for the 80-Hz ASSR, with BMLDs present for 13-Hz ASSRs. Because the ABR does not show the BMLD and the NI-P2 complex of the slow cortical potential does show the BMLD, and we know the origins of these transient potentials, it seems likely that the 80-Hz and 13-Hz ASSR also have brainstem and cortical origins, respectively.

To summarize, animal and human studies support the notion that the ASSRs to higher modulation rates (~80 Hz) are generated predominately within subcortical structures whereas the ASSRs to lower modulation rates (~40 Hz) are generated predominately by the cortex.

80-Hz ASSR thresholds

Bone-conduction thresholds

There are only a handful of studies that have recorded BC ASSR thresholds in normal-hearing adults (Dimitrijevic et al., 2002; Lins et al., 1996; Small & Stapells, 2005, 2008). Small and Stapells (2008) pooled adult BC-ASSR thresholds from two of their studies (Small & Stapells, 2005, 2008) in order to form a relatively large (N = 18) and representative adult sample for BC ASSR. BC-ASSR thresholds in adults were 30, 25, 20, and 15 dB HL for 500, 1000, 2000, and 4000 Hz, respectively (Small & Stapells, 2008). Adults have significantly poorer thresholds at 500 Hz, although no significant differences in BC-ASSR thresholds were found at 1000, 2000, and 4000 Hz. This is opposite to what is seen in infants (Small & Stapells, 2008).

In the same study just described for adults, Small and Stapells (2008) combined infant BC-ASSR thresholds from several studies to form a larger group of infants encompassing a large age range (Small et al., 2007; Small & Stapells, 2005, 2006). BC ASSRs were obtained from 35 young infants (mean age: 16.0 weeks), and 13 "older" infants (mean age: 18.3 months). In general, results showed that young infants have better (i.e., lower) BC-ASSR thresholds in the low frequencies compared to the high frequencies whereas older infants have better thresholds for 1000 Hz compared to 2000 Hz, although no differences were seen between BC-ASSR thresholds at 500, 1000, and 4000 Hz. Mean BC-ASSR thresholds for the young group were 14, 5, 26, and 14 dB HL for the 500, 1000, 2000, and 4000 Hz, respectively. Mean BC-ASSR thresholds for the "older" infant group were 22, 13, 26, and 13 dB HL for the 500, 1000, 2000, and 4000 Hz, respectively. Comparisons with adult data show that BC-ASSR thresholds in the low frequencies worsen with age (Small & Stapells, 2008). These changes are likely related to skull maturation (Small & Stapells, 2006, 2008).

Air-conduction thresholds

Herdman and Stapells (2001) provide representative adult 80-Hz ASSR thresholds to AC stimuli for the single stimulus and multiple-stimulus ASSR using stimulus conditions identical to those used in the studies of the current thesis. The monotic-single (MS) condition consisted of 500, 1000, 2000, and 4000 Hz tones which were presented singly to the test ear. The monotic multiple (MM) condition was composed of four tones (500, 1000, 2000, and 4000 Hz) presented to the test ear simultaneously. The dichotic multiple (DM) condition consisted of eight tones presented simultaneously to both ears (four frequencies to each ear). Herdman and Stapells (2001) threshold results revealed no significant differences between ASSR thresholds for the three stimulus conditions and the four carrier frequencies. Mean ASSR thresholds averaged across the three stimulus conditions were 22, 18, 18, and 21 dB SPL for 500, 1000, 2000, and 4000 Hz, respectively.

AC-ASSR thresholds in infants have been investigated by many (Cone-Wesson, Dowell, Tomlin, Rance, & Ming, 2002; Han, Mo, Lui, Chen, & Huang, 2006; John et al., 2004; Lins et al., 1996; Luts, Desloovere, & Wouters, 2006; Rance et al., 2005; Rance & Rickards, 2002; Rance et al., 2006; Savio, Cardenas, Perez Abalo, Gonzales, & Valdes, 2001; Swanepoel & Steyn, 2005; van der Reijden et al., 2005; Van Maanen &

Stapells, in press; Vander Werff, Brown, Gienapp, & Schmidt Clay, 2002), yet relatively few normative data for the 80-Hz ASSR have been established for infants (Van Maanen & Stapells, in press). *Table 1* includes 80-Hz ASSR normal-hearing infant threshold results to date, converted to SPL in a coupler for ease of comparison among the various threshold studies and the studies of the current thesis (study B), whose stimuli were calibrated in dB SPL. As evident in *Table 1*, much variability exists in the literature for 80-Hz ASSR thresholds in infants. Sources contributing to the variability between studies include the type of stimuli used (single versus multiple), subject age, equipment, monaural versus binaural testing, test environment, and response/noise criterion levels. Threshold results ranged from a low of 26 dB SPL to a high of 62.5 dB SPL.

The multiple-stimulus ASSR

ASSRs can be recorded to single or multiply-presented stimuli. Responses may be obtained from up to eight stimuli simultaneously with four carrier frequencies presented to each ear. It has been demonstrated that interactions between responses to several stimuli may occur whenever more than one stimulus is presented simultaneously. In adults, interactions cause reductions in response amplitude relative to a single-stimulus baseline at high intensities, although amplitude reductions are not present at lower intensities (Lins & Picton, 1995; John et al., 1998).

One of the benefits of the multiple-stimulus ASSR is the ability to present multiple carrier frequencies simultaneously to one or both ears (Herdman & Stapells, 2001; John, Dimitrijevic et al., 2003; John et al., 1998; Lins & Picton, 1995). Multiple

Table 1: 80-Hz AC-ASSR thresholds in dB SPL (standard deviation in parenthesis) in infants with normal-hearing.

Study	N	Age (Mos)	Stimulus	Thresholds (SD) 500	1000	2000	4000
Rickards et al., 1994	164-177	< 1	Single AM/FM	46.9 (10.4)			40 (11.3)
Levi et al., 1995	35	1	Single SAM	42 (16)	42 (11)	37 (15)	
Lins et al,. 1996 (Ottawa data)	21	1-10	MM SAM	45 (13)	29 (10)	26 (8)	29 (10)
Lins et al., 1996 (Havana data)	30	3-11	DM SAM	58 (12)	43 (14)	39 (12)	40 (12)
Savio et al., 2001**	25	0-1	MM SAM	62.5 (11)	55 (12)	54 (12)	53.5 (13)
Savio et al. 2001**	13	7-12	MM SAM	51.5 (9)	44 (10)	40 (8)	38.5 (9)
Cone-Wesson, Rickards et al., 2002	85	0-79	Single AM/FM	44.5 (8.3)	34 (9.6)	29 (9.5)	44.5 (12.0)
John et al., 2004	50	< 1	Multiple SAM^2	50			
Rance et al., 2005	285	0.5-3	Single AM/FM	37.8 (7.5)	32.5 (6.6)	27.3 (6.3)	33.6 (7.5)
Swanepoel & Steyn, 2005	5	1-2	DM AM/FM	42.5 (8.0)	34 (10.0)	37 (11.0)	35.5 (11.0)
Luts et al., 2006	30 ears	< 3	DM AM/FM	42 (10)	35 (10)	32 (10)	36 (9)
Rance & Tomlin, 2006	20	< 2	Single AM/FM	46.8 (7.1)			49.1 (7.5)
Rance et al., 2006	17	< 2	Single AM/FM	45.2 (6.0)			37.6 (8.5)
Van Maanen & Stapells, in press	10	3.1	DM Cos^2	44.5 (7.4)	33.1 (4.6)	32.3 (7.3)	29.8 (10.1)
Van Maanen & Stapells, in press	19	28.8	DM Cos^2	46.6 (7.4)	37 (10.5)	34 (7.8)	28.5 (10.3)

Single = Singly presented tone
MM = Monotic multiple; multiple tones presented monaurally
DM = Dichotic multiple; multiple tones presented dichotically
**Thresholds presented in John et al. (2004)
SAM^2 = Exponential amplitude modulation
Cos^2 = 12 ms duration, Cos^2 window
SAM = Sinusoidal amplitude-modulation

stimulus presentation is advantageous because it has the potential to decrease test time (relative to a single-stimulus method) assuming no significant interactions between responses to stimuli occur. Even if the multiple-stimulus technique takes the same amount of time as a single-stimulus technique, it probably remains advantageous to obtain information from both ears simultaneously in case the infant wakes up during testing. Both these factors are particularly critical when testing infants. Few studies have directly compared the single-stimulus and multiple-stimulus ASSR and all of these studies have been conducted in normal-hearing adult listeners. Thus, what we know about how efficient the multiple stimulus technique is relative to a single-stimulus technique has been gathered from adults.

Relative efficiency

As mentioned previously, one limitation associated with the multiple-ASSR is the potential for interactions among responses to multiply presented stimuli. Whenever more than one stimulus is presented simultaneously, interactions among the responses to these stimuli along the auditory pathways may occur, which reduce response amplitudes relative to the response had each carrier frequency been presented singly (Lins et al., 1995). "Relative efficiency" is a measure that considers the increase in information relative to the decrease in amplitude due to interactions when going from single- to multiple-stimulus conditions (Armstrong & Stapells, 2007; Fontaine & Stapells, 2007; Herdman & Stapells, 2001; John et al., 1998). In general, background EEG noise decreases as a function of the square root of the number of sweeps averaged (John, Purcell, Dimitrijevic, & Picton, 2002). The multiple ASSR allows one to record information from multiple frequencies simultaneously, thus increasing the amount of information available for a given recording period, while the noise continues to decrease at the same rate. If no amplitude reductions (or EEG noise increases) occur due to the simultaneous presentation of multiple stimuli, the multiple technique is more efficient than a single stimulus presentation method by a factor equal to the number of stimuli presented at one time. For example, four and eight simultaneously presented stimuli would result in tests that were four and eight times more efficient, respectively. However, if amplitude reductions are present in the multiple ASSR, there is a tradeoff between the amplitude reductions and the level of EEG noise required to detect the responses. For example, if the response amplitude for a singly presented stimulus is larger than the amplitude in response to the same stimulus presented in multiple, the larger response to the singly presented stimulus could be detected with fewer sweeps and higher EEG noise levels. For the multiple-stimulus ASSR technique to be more efficient than a single stimulus technique, amplitude reductions can be no greater than $1/\sqrt{N}$, where N is the number of stimuli presented simultaneously (John et al., 1998). In other words, if four and eight stimuli are presented simultaneously, averaging can take up to 2 and 2.8 times longer than a single stimulus technique, respectively, and the multiple technique would still be as efficient as a single-stimulus presentation method. For example, if four stimuli are presented to one ear at the same time, amplitude reductions in the multiple condition cannot be more than 50% from the single stimulus amplitude for the multiple ASSR to be more efficient. If eight stimuli are presented concurrently, amplitude reductions in the multiple condition cannot be more than 65% down from that of the single stimulus amplitude and be as efficient.

The first study to assess the multiple 80-Hz ASSR in adults was conducted by Lins and Picton (1995). In their study, no significant differences were found in the amplitude of the responses from simultaneously presented tones to one ear (500, 1000, 2000, and 4000 Hz) at 60 dB SPL versus these same tones presented separately. They also reported no differences in amplitude relative to the single stimulus comparison group when eight tones were presented (four in each ear) and the carrier frequencies were separated by at least an octave. These results demonstrated that recording multiple stimuli (tones) simultaneously at suprathreshold levels is more efficient than recording each stimulus (tone) individually in normal hearing adults (Lins & Picton, 1995).

John and colleagues (John et al., 1998) compared amplitudes when stimuli were presented alone (responses to a singly presented 1000 Hz and 2000 Hz tone served as baseline), or in multiple (500, 1000, 2000, 4000 Hz presented to one ear or to two ears) in normal-hearing adults. Similar to the Lins and Picton study (1995), their results indicated no significant differences in amplitude between the single and multiple ASSR technique for levels of 60 dB SPL and lower when the carriers were separated by an octave. Herdman and Stapells (2001) investigated the effects of single versus multiple stimulus presentation on the 80-Hz ASSR in normal hearing adults. Comparisons of response amplitudes and thresholds were made between three stimulus conditions at 60 dB SPL and 30 dB SPL: a monotic single condition (500, 1000, 2000, and 4000 Hz tones presented singly), a monotic multiple condition (four tones presented to one ear), and a dichotic multiple condition (four tones presented dichotically to two ears). Results revealed no significant differences in response amplitudes and thresholds between the three stimulus presentation conditions, hence no interactions (Herdman & Stapells, 2001). Thus, the multiple-stimulus ASSR is more efficient at low to moderate intensities.

Importantly, at higher stimulus intensities (e.g., 75 dB SPL) John and colleagues (John et al., 1998) found statistically significant interactions between responses to stimuli which reduced amplitudes in the multiple condition. Specifically, when they presented four tones to one ear simultaneously at 75 dB SPL (500, 1000, 2000, and 4000 Hz), amplitudes for the 1000 Hz and 2000 Hz carrier frequencies decreased to 56% and 49% of their amplitude in the single-stimulus condition, respectively (John et al., 1998). Despite the presence of interactions at higher stimulus intensities, when compared to the single-stimulus technique, the multiple-ASSR technique remains slightly more efficient at 1000 Hz, and only slightly less efficient at 2000 Hz, at least in adults (John et al., 1998). Thus, at high stimulus intensities, the efficiency of the multiple-stimulus ASSR is not better than the single-stimulus technique (John et al., 1998). Recent results from our laboratory also indicate that ASSRs to multiple tones (four to one ear) are no more efficient than ASSRs to single tones when presented at 80 dB SPL (Armstrong & Stapells, 2007).

The efficiency of the single versus multiple-stimulus ASSR has never been directly assessed in adults with hearing loss. Herdman and Stapells (2003) determined multiple-ASSR thresholds and place specificity in a large group of adults with hearing loss. Subjects included 31 adults with sensorineural hearing loss who were chosen based on behavioural pure-tone results to cover a wide range of hearing loss configurations. Two stimulus conditions were included: (1) a single stimulus condition composed of either a 500, 1000, 2000, or 4000 Hz carrier, and (2) a monotic-multiple stimulus condition composed of these same carriers presented simultaneously to one ear. Threshold for only one frequency was found in the single stimulus condition due to time limitations. Threshold results revealed that the multiple ASSR accurately predicted the degree and configuration of hearing loss in adults with hearing loss. In addition, results suggested that *if* interactions are present, they are not changing thresholds. Whether or not the multiple ASSR is more time efficient remains unknown in the population and further investigation is required.

Physiology and mechanisms underlying interactions

For the multiple-ASSR in adults, interactions decrease response amplitude relative to a single-stimulus baseline comparison group at high intensities (≥75 dB SPL), when tones are presented within half an octave of each other, or when the same carrier frequency is modulated at several different rates (Lins & Picton, 1995; John et al., 1998). These reductions in amplitude in normal-hearing adults may occur due to cochlear interactions as a result of the broadening of excitation patterns along the basilar membrane that occurs with intense stimuli, and/or through neural interactions occurring higher in the auditory pathways (Lins & Picton, 1995).

Cochlear interactions

If more than one stimulus activates an area on the basilar membrane, the hair cells located within this region of activation will respond to the combined effect produced by the multiple stimuli (John et al., 1998). Thus, cochlear interactions could occur due to an overlap of excitation patterns on the basilar membrane and this could result in constructive and/or destructive interference. The finding that adults show no interactions between responses to multiply presented stimuli at moderate intensities with octave separations between carriers suggests that there is no overlap of excitation patterns under these moderate-level conditions. A visual representation of this is provided in the top of Figure 3. As can be seen in the top of *Figure 3*, at moderate intensities (e.g., 60 dB SPL), each stimulus activates a different area along the basilar membrane and there is no interference between responses to these stimuli. In contrast, the interactions between responses to multiple stimuli at high intensities and/or half-octave separations (John et al., 1998; Lins & Picton, 1995) suggest that under these conditions there is an overlap of excitation patterns along the basilar membrane that was not present at lower intensities. This is represented in the bottom of *Figure 3* where the shaded areas represent overlapping excitation patterns along the basilar membrane which result in interactions at high intensities. The reductions in ASSR amplitudes seen in adults are consistent with interactions causing destructive interference along the basilar membrane.

Two-tone suppression is one paradigm that has been used to study interactions in the auditory system. A cochlear phenomenon, two-tone suppression occurs when the response produced by one tone (probe tone) is attenuated by the presence of a second tone (suppressor tone) in close proximity to the probe tone (Ruggero, Robles, & Rich, 1992). Studies have revealed that the greatest reduction in firing rate occurs when the suppressor tone becomes close in frequency to the probe/characteristic frequency. Two-tone suppression can occur with suppressor tone frequencies that are both above and below the probe tone (Ruggero et al., 1992; Sachs & Kiang, 1968). These results are not what would be expected from masking, where low-frequency tones exert attenuating effects on high-frequency tones and not vice versa, and demonstrate that 2-tone suppression is not the same as masking (see for a review, Moore, 1985). Less suppression occurs when the frequency difference between the suppressor tone and probe tone increases and it takes less intensity to cause suppression of the probe tone when the suppressor is higher in frequency than the probe tone (high-side suppression) versus when the suppressor is lower in frequency than the probe tone (Sachs & Kiang, 1968). Results from two-tone suppression studies are consistent with the ASSR findings from John and colleagues, who found greater attenuation of the 80-Hz ASSR response to a low carrier frequency in the presence of a higher carrier frequency (John et al., 1998). Assuming ASSR interactions are similar to two-tone suppression, it is reasonable to assume that the interactions between responses to multiply presented stimuli may, in part, reflect cochlear interactions.

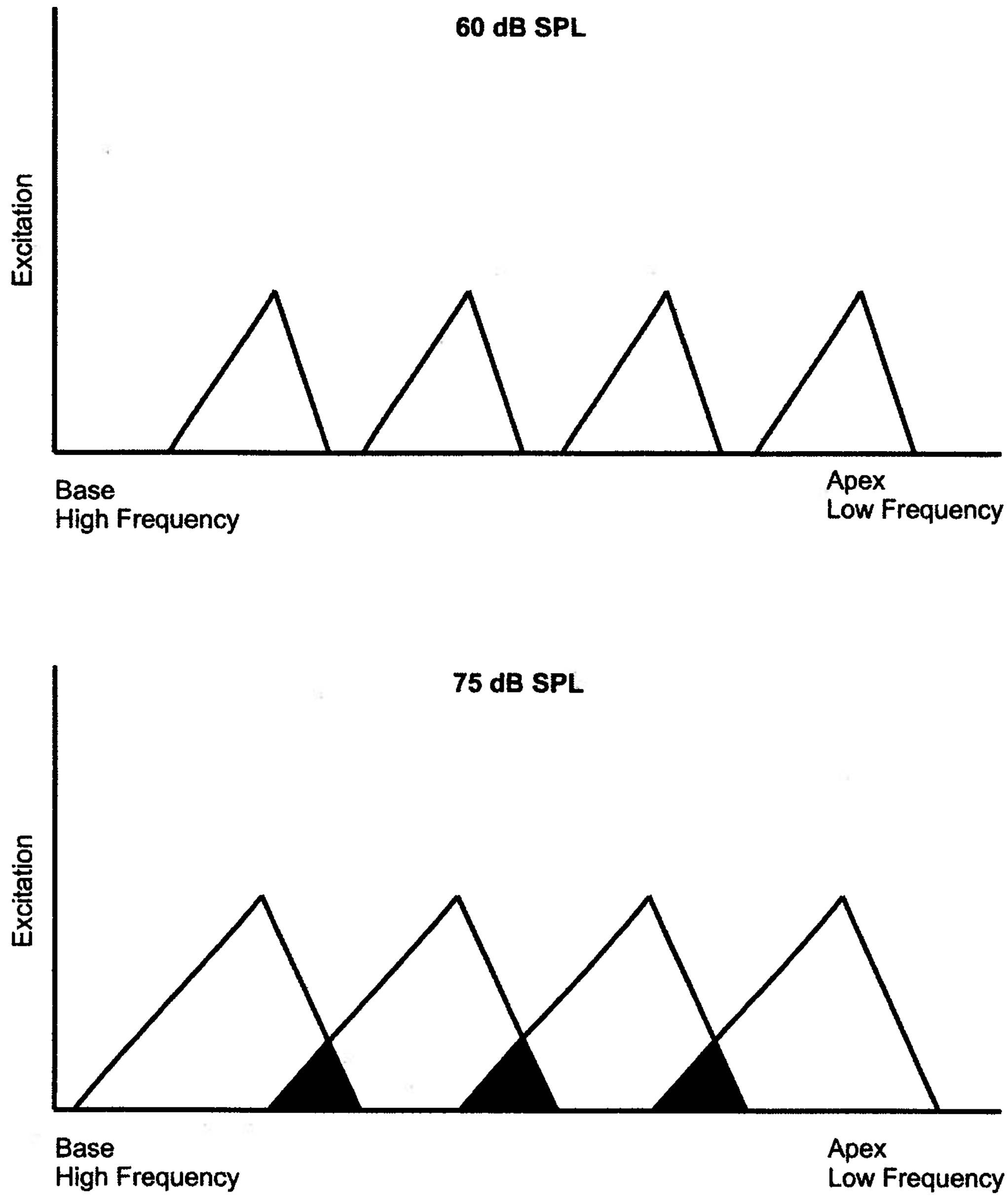

Figure 3 Schematic representation of the changes in excitation patterns that occur as intensity is increased from 60 to 75 dB SPL and multiple stimuli (four tones) presented simultaneously. An overlap of excitation patterns (shaded areas) along the basilar membrane is evident at high intensities (e.g., 75 dB SPL).

Neural interactions

Another possibility is that the interactions seen with the multiple-stimulus ASSR are due to neural mechanisms at the level of the brainstem and/or cortex, rather than cochlear mechanisms. Comparisons of ASSRs at different rates provide one way to assess neural mechanisms. John and colleagues (John et al., 1998) found a decrease in 40-Hz ASSR amplitude at 60 dB SPL when multiple stimuli were presented simultaneously. This contrasts with the 80-Hz ASSR where no interactions are seen at 60 dB SPL, and is consistent with non-cochlear origins of these interactions.

Binaural stimulation provides another means of assessing neural interactions. John and colleagues (John et al., 1998) found greater amplitude reductions when four tones were presented to two ears (for a total of eight tones) compared to when four tones were presented to one ear alone. These decreases can only result from binaural interactions at the level of the brainstem or higher and cannot be explained solely by cochlear interactions. It thus seems likely that both cochlear and neural mechanisms play a role in the interactions seen in the multiple-ASSR in adults.

Research is required to determine the effects of single versus multiple-stimulus presentation in infants as no studies to date have assessed this issue in infants. Everything we know in regards to interactions has been obtained from normal-hearing human adults or in animals. There are, however, known maturational differences in hearing throughout the auditory system. Anatomical and physiological differences between infants and adults may lead to greater interactions between responses to multiply presented ASSR stimuli in infants. If present, these interactions may render the multiple ASSR less efficient than the single-stimulus ASSR. A brief overview of the maturation of the auditory system is needed in order to develop the rationale for the studies of the current thesis.

Maturation of the auditory system

Although much of the development of the auditory system occurs prenatally, development is not fully complete at birth (Moore & Linthicum, 2008). Anatomical data indicate that the human cochlea has reached adult size at birth (Eby & Nadol, 1986) and that the cochlea is anatomically and functionally mature well before birth. Other structures, including the outer ear, middle ear, brainstem, and auditory cortex continue to develop well beyond the neonatal period (Moore & Linthicum, 2008). Behavioural thresholds (refer to "Early identification of infant auditory status" section), admittance and reflectance tympanometry, DPOAE, and ABR measures have demonstrated maturational differences between infants and adults. Comparisons of different methodologies, such as those just mentioned, provide insight into the structures contributing to immaturities and there influences on the transmission of auditory information. The potential impact of these immaturities on auditory evoked potentials must be considered.

The external ear canal and middle ear show changes over the first two years following birth which affect the conduction of sound to the cochlea (Keefe, Bulen, Arehart, & Burns, 1993). There is an increase in the size of the external ear (Anson &

Donaldson, 1981), middle-ear cavity and mastoid with age (Eby & Nadol, 1986; Ikui, Sando, Haginomori, & Sudo, 2000) with the volume of the middle-ear cavity increasing into the teenage years (Eby & Nadol, 1986). Typical ear-canal diameters for a 1-month-old infant and an adult are 4.4mm and 8mm, respectively (Keefe et al., 1993). The length of the ear canal increases with age (Bonaldi, do Lago, Crema, Fukuda, & Smith, 2004). The orientation of the tympanic membrane also changes with age and does not become adultlike until approximately three years of age (Eby & Nadol, 1986; Ikui, Sando, Sudo, & Fujita, 1997). There is a decrease in the mass of the middle-ear system (Anson & Donaldson, 1981; Paparella, Shea, & Meyerhoff, 1980), a tightening of the joints connecting the middle-ear bones (Anson & Donaldson, 1981), and the formation of the bony ear-canal wall (Saunders, Doan, & Cohen, 1993). As previously mentioned, these changes influence the sound level available to stimulate the cochlea. Studies investigating infant-adult differences in SPL output at the eardrum using insert earphones have consistently shown a larger SPL in the infant ear than in the adult ear, with the difference increasing with frequency up to at least 6000 Hz (Bagatto et al., 2005; Rance & Tomlin, 2006; Sininger, Abdala, & Cone-Wesson, 1997). For example, the intensity reaching an infant's eardrum (<10 months of age) is approximately 3 and 13 dB higher than that reaching the adult eardrum at 500 Hz and 4000 Hz (Bagatto et al., 2005; Bagatto, Scollie, Seewald, Moodie, & Hoover, 2002).

Keefe and Levi (1996) investigated maturation-related changes in the external and middle ear using admittance and reflectance measurements from normal-hearing infants (1, and 6-month-old infants) and adults. The responses were measured at atmospheric pressure, a moderate intensity, and over a large frequency range (500 to 16000 Hz). Admittance measurements indicate how easily sound pressure energy is transmitted into the middle-ear space. High levels of admittance are indicative of high levels of transmission into the middle ear whereas low levels of admittance suggest minimal transmission of sound energy into the middle ear. Reflectance measurements provide an alternative way to look at middle-ear transmission characteristics. Low levels of reflectance are consistent with a high level of transmission of energy into the middle ear (Keefe & Levi, 1996). Keefe and Levi (1996) found large infant-adult differences in admittance level (infants had lower admittance levels compared to adults) although these differences disappeared when ear canal size was accounted for. Even when ear-canal volume was accounted for, infants had higher admittance levels than adults below 500 Hz. Thus, this low-frequency difference cannot be explained by ear-canal changes with age (Keefe et al., 1993; Keefe & Levi, 1996), and the mechanism responsible remains unclear. Comparisons of energy reflectance results between infants and adults show that energy reflectance decreases with age, a finding consistent with larger energy losses in the infant ear canal (Keefe et al., 1993). Whether or not this loss in energy is due to greater transmission of energy to the cochlea or due to greater absorption of energy into the soft tissues of the infant ear canal (and thus less energy available to stimulate the cochlea) remains unclear, although the latter is likely. Greater absorption of energy by the infant (6-24 months of age) middle ear relative to the adult middle ear leads to a 2-4 dB reduction, relative to adults, in the intensity available to stimulate the cochlea in the infant (Keefe et al., 1993). Therefore, there appears to be less transmission of energy to the cochlea in the infant (Keefe et al., 1993; Keefe & Levi, 1996). These differences will influence the results of tests involving structures beyond the middle ear.

To assess the status of the cochlea at different ages, Abdala and colleagues (Abdala, Sininger, Ekelid, & Zeng, 1996) looked at DPOAE suppression in normal hearing adults and infants (36-41 weeks gestation). DPOAE suppression occurs when a tone (suppressor tone) is presented simultaneously with the two stimulating tones. A DPOAE iso-suppression curve is obtained by varying the level of the suppressor tone until the DPOAE amplitude is decreased by a criterion amount. Suppression tuning curves (STC) reflect cochlear frequency resolution in the area surrounding the stimulating tone (Abdala et al., 1996). The results of Abdala et al. (1996) results indicate that infant and adult DPOAE STCs are similar in shape, width, slope, and tip frequency, indicating that the cochlea is adultlike at birth for at least the middle and high frequencies (3000 Hz and 6000 Hz; low frequencies were not assessed) (Abdala et al., 1996). Based on these findings, the authors concluded that it is likely that the active mechanisms in the cochlea responsible for the regulation and sharpening of frequency resolution are mature at birth (Abdala et al., 1996).

The auditory neural pathways are not mature in the full-term infant. There is much evidence from evoked potential studies and postmortem fetal tissue sample studies indicating that the auditory brainstem is functionally and structurally immature at birth (Moore, Perazzo, & Braun, 1995; Ponton et al., 1994; Salamy & McKean, 1976). Around the time of birth there is rapid growth and change in the brainstem structures.

Auditory neurons continue to grow in size, reaching 50-60% of their adultlike size by birth, and an increase in myelin density is seen in the cochlear nerve and brainstem, with myelin density becoming adult-like by six to 12 months of age (Moore et al., 1995). The rapid increase in myelin during this time frame is consistent with studies that have shown decreases in ABR latency up to five years of age (Mochizuki, Go, Ohkubo, & Motomura, 1983; Salamy & McKean, 1976). ABR threshold studies also are consistent with auditory immaturities in the neural pathways (Folsom & Wynne, 1986; Klein, 1984).

Klein (1984) investigated auditory evoked potential thresholds to 500, 4000, and 10000 Hz tones in a group of normal-hearing infants (2-28 weeks of age) and adults. Overall results indicated that infants had elevated air-conduction thresholds relative to adults, and this difference decreased with age. The largest infant-adult ABR threshold differences were seen for 4000 Hz, where infant-adult differences in threshold remained at 28 weeks of age (infants were not tested beyond this age). No significant differences in threshold as a function of age were found for 500 Hz, and only the youngest age group (2-4 week olds) had thresholds that were significantly elevated with respect to adult thresholds for 10000 Hz. Subsequently, Werner and colleagues (Werner, Folsom, & Mancl, 1993) compared ABR and behavioural thresholds for a large group of subjects divided into three age groups: 3-month-olds, 6-month-olds, and adults, using identical stimuli for ABR and behavioural threshold estimation (brief tones of 1000, 4000, and 8000 Hz). Relative to adults, behavioural thresholds for infants were elevated, although the pattern of elevation differed as a function of age. Larger infant-adult differences in threshold were seen with increasing frequency for the 3-month-olds

(25 dB and 40 dB differences for 1000 and 8000 Hz, respectively) whereas the 6-month-olds displayed the largest infant-adult differences in threshold for the mid-frequencies (35 dB at 4000 Hz versus 20 dB at 8000 Hz). In contrast to the results of Klein (1984), ABR thresholds for the 3-month-old and 6-month-old groups were similar to adult ABR thresholds and there were no significant differences in thresholds at different frequencies. These results suggest that ABR thresholds are mature and behavioural thresholds immature (particularly for the high frequencies) at 3 months of age, and high-frequency behavioural thresholds improve between 3 to 6 months of age (Werner et al., 1993). Taking a different approach, Folsom and Wynne (1987) assessed frequency-dependent differences in ABR wave V tuning curves in young infants (3 month olds) and adults. Results demonstrated adultlike tuning curves for a 1000-Hz probe tone, but clear infant-adult differences were seen for higher frequency probe tones (4000 and 8000 Hz), findings consistent with poorer frequency resolution for higher frequency stimuli (Folsom & Wynne, 1987). Overall, ABR studies suggest that responses to low-frequency stimulation are mature in infants with responses to high-frequency stimulation remaining immature in infancy. Contributions from outer and middle ear maturation make interpretation of the exact origins of these immaturities difficult (Folsom & Wynne, 1987). However, DPOAE results suggest that the cochlea is mature at birth. Thus, immaturities due to neural mechanisms seem likely.

In addition to immaturities at the level of the auditory brainstem, the cortex remains immature in structure until later childhood. By 11 to 12 years of age, the density of cortical axons appears adult-like (Moore & Guan, 2001). This coincides with the appearance of wave N1 of the slow cortical evoked potential. Specifically, N1 is difficult to obtain in young children, but appears around age nine years, with a latency around 100 ms (Ponton, Eggermont, Don et al., 2000).

To summarize, these findings indicate that the perinatal period is a time characterized by structural and functional maturity of the cochlea, although the outer ear, middle ear, the brainstem pathways and the cortex (in particular) remain relatively immature at birth.

Maturation of the ASSR

The 40-Hz ASSR can be detected close to behavioural threshold in adults, although it is not reliably recorded in sleeping infants. Furthermore, 40-Hz response amplitudes are attenuated by sleep and are unsuitable for testing infant auditory status as they remain immature throughout the first decade of life. Aoyagi and colleagues (Aoyagi et al., 1994) showed that 40-Hz ASSR detectability improved over the first 15 years of life. Pethe and colleagues (Pethe, Mühler R, Siewert, & von Specht, 2004) showed that 40-Hz ASSR amplitudes increased with age and did not reach adult-like size until around 14 years of age. As mentioned previously, infants and children do not show an amplitude peak for modulation rates near 40-Hz (Stapells et al., 1988; Suzuki & Kobayashi, 1984), a finding consistent with an immature auditory cortex in this age group.

The 80-Hz ASSR on the other hand can be recorded in infants and children, as well as adults (e.g., Aoyagi et al., 1993; Cone-Wesson, Parker, Swiderski, & Rickards, 2002; John et al., 2004; Levi et al., 1993; Lins & Picton, 1995; Luts et al., 2006; Rance et al., 2005; Rance et al., 2006; Rickards et al., 1994; Savio et al., 2001; Van Maanen & Stapells, in press). The finding that the 80-Hz ASSR is minimally affected by subject state (Cohen et al., 1991; Levi et al., 1993) and is less affected by maturation factors than the 40-Hz ASSR has led to a widespread interest in using the 80-Hz ASSR for infant auditory assessment. However, maturational factors do seem to play a role in these responses within the first year of life.

Maturation of amplitude

With age, 80-Hz ASSR amplitudes increase, although the exact age at which ASSR amplitudes become adult-like has yet to be established (Rance, 2008). Lins and colleagues (Lins et al., 1996) measured the 80-Hz ASSR in a group of infants (1 to 10 months) and a group of normal-hearing adults. The infants in their study had ASSR amplitudes that were one-third to one-half the size of the adult amplitudes. More detailed studies have suggested that the majority of the amplitude increases occur within the neonatal period (John et al., 2004; Savio et al., 2001). For example, in the Savio and colleagues study (Savio et al., 2001) previously mentioned (under "maturation of the ASSR"), clear differences in responses were seen between the neonatal group (0 to 29 days) versus the older groups (1 to 6 months, 7 to 13 months) such that the neonates had smaller response amplitudes than the older groups. This finding combined with the fact that there were no differences in amplitudes between the two older groups suggests that the amplitude increases occur within the first few weeks of life (Savio et al., 2001). John and colleagues (John et al., 2004) also found differences in response amplitude size when they compared two groups of infants: amplitudes in a group of full-term infants younger than 3 days old were 22% smaller than those obtained from a group of infants between the ages of 3 to 15 weeks. Both of these studies (John et al., 2004; Savio et al., 2001) revealed that amplitudes were smaller and the developmental time course longer for low-frequency stimuli relative to the higher frequencies.

Maturation of ASSR threshold

ASSR thresholds decrease (improve) with increasing age, although the exact time line for threshold development in infants is unknown (Rance, 2008). Savio and colleagues (Savio et al., 2001) found threshold improvements with age at frequencies between 500 to 4000 Hz. Specifically, multiple-stimulus (four tones monaurally presented) ASSR thresholds at a chronological age of 0 to 29 weeks and 7 to 12 months revealed threshold improvements of 7, 10, 12, and 14 dB for 500, 1000, 2000, and 4000 Hz, respectively. Rance and Tomlin (2006) conducted a longitudinal study in which they tracked single-stimulus ASSR thresholds at 500 Hz and 4000 Hz in healthy full-term infants from 3 to 6 days to 6 weeks of age at four time intervals (0, 2, 4, and 6 weeks of age). Overall ASSR threshold improvements of 5 dB over 6 weeks were observed with increasing age for the 500 Hz and 4000 Hz carriers used in this study. These authors concluded that neonates and infants have different (i.e., higher) thresholds than older subjects, indicative of the presence of auditory immaturities in infants at 6 weeks of age. At 6 weeks of age, ASSR thresholds were still about 10 dB higher than adult ASSR thresholds (Rance & Tomlin, 2006). Van Maanen and Stapells (In press) found no differences in thresholds between 3-month-old and 6-month-old infants. This contrasts with the earlier work of Savio and colleagues (2001) and Rance and Tomlin (2006), but may relate to the fact that Van Maanen and Stapells did not test very young infants (i.e., less than two weeks old). Table 1 provides a review of infant 80-Hz ASSR threshold results to date.

Rationale for thesis studies

Mutliple-stimulus ASSR systems are currently being marketed for the purpose of infant auditory threshold assessment (Stapells et al., 2005). This is problematic because no studies in infants have directly compared ASSRs to single versus multiple simultaneous stimuli; thus it is not known whether or not the multiple-stimulus technique is more efficient in infants. Few studies have directly compared the single versus multiple-stimulus ASSR technique in adults. These studies have demonstrated that (1) response amplitudes do not change as a function of the number of stimuli presented simultaneously in adults provided the stimulus is ≤60 dB SPL, and (2) the multiple-stimulus 80-Hz ASSR technique is more efficient than presenting stimuli singly in adults.

Due to the lack of information pertaining to the efficiency of the multiple-stimulus ASSR in infants, the primary aim of the current thesis will be to assess the efficiency of the multiple ASSR in infants. This will be accomplished in Study A through comparisons of response amplitudes and test time between three stimulus conditions which differ in the number of stimuli presented simultaneously. The stimuli (SAM tones) and conditions (single versus multiple) used in the studies of the current thesis were designed similar to previous adult studies which assessed interactions between responses to multiply presented stimuli (Herdman & Stapells, 2001; John et al., 1998; Lins & Picton, 1995) in order to allow easy comparison to adult data. Study B investigated whether infant ASSR thresholds (at 500 Hz) differ between single and multiple ASSR conditions.

Chapter 2:

Efficiency of single- versus multiple-stimulus auditory steady-state responses in infants

Introduction

Auditory steady state responses (ASSRs) to stimuli modulated in the 70-110 Hz range (the "80-Hz" ASSR) have received much recent attention and will likely be recommended for routine clinical assessment of auditory threshold in young infants in the near future, especially once appropriate normative and clinical data in infants are available (Stapells et al., 2005). The ASSR technique has advantages over the current gold-standard, the tone-evoked auditory brainstem response (ABR). ASSRs are more objective because statistical measures determine the presence/absence of responses and thereby eliminate the need for a clinician to interpret waveforms as is currently required for the tone-ABR (Stapells, 2000b). Further, the "multiple" ASSR technique has the potential to gather more information in a shorter amount of time, thus speeding up test time (Herdman & Stapells, 2001; John et al., 1998). This reduction in test time is accomplished by presenting multiple stimuli simultaneously to one or both ears. If no interactions between responses to multiple stimuli occur, such that amplitudes are not smaller when stimuli are presented together, the time to record responses is reduced by the number of stimuli presented simultaneously (Picton et al., 2003). Even if some interactions between responses to multiple stimuli exist, the presentation of multiple simultaneous stimuli will still be more efficient than a single-stimulus technique if the amplitude reduction is less than $1/\sqrt{N}$, where N is the number of stimuli presented at the same time (John et al., 1998).

In adults, the presentation of multiple stimuli simultaneously does not cause a decrease in 80-Hz ASSR amplitude when stimuli are presented at 60 dB SPL or less, provided that the carrier frequencies are separated by at least an octave (Herdman & Stapells, 2001; John et al., 1998; Lins & Picton, 1995). However, for 75 dB SPL stimuli, statistically significant interactions between responses to stimuli are seen such that amplitude for the 1000-Hz and 2000-Hz carrier frequencies in the multiple-stimulus condition (4 frequencies to one ear) decrease to 56% and 49% of their amplitude in the single-stimulus condition, respectively (John et al., 1998). Despite this, when compared to the single-stimulus technique, the multiple-ASSR technique remains more efficient at least for 1000 Hz and at least in adults (Herdman & Stapells, 2001; John et al., 1998).

ASSRs evoked by amplitude-modulated (AM) tones with modulation frequencies between 70 and 110 Hz have been used in the assessment of infant hearing using either single stimuli (e.g., Rance et al., 2005) or multiple stimuli (e.g., Lins, Picton et al. 1996; John, Brown et al. 2004; Savio, Cardenas et al. 2001; Luts 2005; Van Maanen and Stapells, in press) and have shown great promise. However, only a few studies have directly compared ASSRs to single versus multiple simultaneous stimuli (Armstrong & Stapells, 2007; Herdman & Stapells, 2001; John et al., 1998; Lins et al., 1995), and none of these studies have assessed this issue in infants; that is, there are no published studies comparing infant ASSRs for single versus multiple stimuli. Thus, the effects of presenting multiple stimuli simultaneously in infants are unknown, as is whether the multiple-stimulus technique is more efficient for infants than the single-stimulus technique. These questions are of particular interest because multiple-stimulus ASSR systems are currently being marketed and sold to clinicians for the purpose of infant auditory threshold assessment. The purpose of the following studies is to assess, in infants, the efficiency of single versus multiple stimulus presentation by assessing changes in amplitude, time-to-criteria, and threshold.

Methods

Participants

Twenty-nine infants participated in this study. Fifteen infants participated in Study A (mean age: 23.1 weeks; age range: 6-38 weeks) and 14 participated in Study B (mean age: 20.2 weeks; age range: 4-38 weeks). Infants who passed a distortion-product otoacoustic emissions (DPOAE) screening test in both ears on the day of ASSR testing were considered to be at low risk for hearing loss and were included in the study. DPOAE F2 and F1 levels were 65 and 55 dB SPL, respectively. To be considered "normal", the DPOAE signal-to-noise ratio (SNR) was required to be at least 6 dB for at least 2/3 frequencies tested within the 2-4kHz range. Seventy-two percent of the infant ears tested in this study passed at all 3 frequencies assessed; 100% of the infant ears passed 2/3 frequencies assessed.

Auditory Stimuli

For studies A and B, air-conduction stimuli were presented to one or both ears using ER-3A insert earphones. Individual stimuli were calibrated in dB SPL using a Quest Electronics model 1800 sound level meter with a Bruel & Kjaer DB0138 2-cc adapter.

Stimuli consisted of sinusoidal tones with carrier frequencies of 500, 1000, 2000, and 4000 Hz that were 100% sinusoidally amplitude modulated at 77.148, 84.961, 92.773, and 100.586 Hz, respectively for the test ear, and AM at frequencies of 81.055, 88.867, 96.680, and 105.469 for the non-test ear for the dichotic multiple condition. Air-conduction stimuli were generated by the Rotman multiMaster research system (John & Picton, 2000) and attenuated through Tucker-Davis Technologies HB6 and SM3 modules.

The primary purpose of study A was to directly compare response amplitude, time-to-criteria, and relative efficiency results as a function of the number of ASSR stimuli presented. All infants in study A took part in three stimulus conditions which differed in terms of the number of stimuli presented simultaneously. The Monotic Single (MS) condition consisted of 500, 1000, 2000, or 4000 Hz tones which were presented singly to the test ear. The Monotic Multiple (MM) condition was composed of four tones (500, 1000, 2000, and 4000 Hz) presented to the test ear simultaneously. The Dichotic Multiple (DM) condition consisted of eight tones presented simultaneously to both ears (four frequencies to each ear, each with their own modulation rate). In study A, all stimuli were presented at 60 dB SPL (i.e., the levels of the individual stimuli were set to 60 dB SPL).

The purpose of study B was to compare 500-Hz threshold, EEG noise, response amplitude, and time-to-criteria in the single-stimulus condition with that obtained in the dichotic multiple condition. For study B, all infants took part in two conditions: the 500-

Hz MS stimulus condition and the DM stimulus condition. These conditions were identical to those outlined for study A above. Threshold was found for the 500-Hz stimuli in the test ear in the MS and DM conditions using an intensity range between 30 to 75 dB SPL.

Recording

The following information pertains to both studies except when otherwise indicated. ASSRs were recorded using the Rotman multiMaster research system. Three gold-plated electrodes were used to record the electrophysiologic responses: the non-inverting and ground electrode were placed on the high forehead with the non-inverting electrode placed in a high and medial position on the forehead and the ground electrode placed in a high and lateral forehead position. The inverting electrode was placed on the nape just below the hairline in the mid-sagittal plane. The skin beneath the electrodes was lightly abraded in order to obtain inter-electrode impedances less than 5 kOhms at 10 Hz (mean: 2.1 kOhms).

The EEG was amplified 80,000 times (×8000 in Nicolet HGA-200A and Nic501A; ×10 in Labview) and filtered using a 30-250 Hz (12 dB/octave) filter. The amplified analog EEG was digitized using an A/D conversion rate of 1000 Hz and with a clipping level of 62 µV. For study A, non-weighted averaging in the multiMaster system was used. In order to maximize the likelihood of obtaining complete threshold results for study B, weighted averaging was used (John, Dimitrijevic, & Picton, 2001). Additionally, data for two infants in study A were obtained using weighted averaging. Each EEG recording sweep lasted 16.384 seconds (consisting of 16 epochs each of 1.024 seconds duration). Any epochs in which the EEG amplitude exceeded either ±40 μV (Study A) or ±62 μV (Study B) were rejected so that electric potentials due to muscle or movement artifact were minimized.

ASSRs were averaged in the time domain and converted online into amplitude spectra using a fast Fourier transform (FFT) thereby allowing for analyses of responses to the carrier frequencies at their corresponding modulation rates. Amplitudes were measured baseline-to-peak in nanovolts (nV). ASSR presence required a significant signal-to-noise ratio *($p < .05$)* using the F-Statistic (Picton et al., 2003) which compared the amplitude of response at each modulation frequency to the background noise in adjacent frequencies within ±60 bins of the modulation frequency (John & Picton, 2000).

Stopping Criteria

ASSRs were recorded either until a response was detected *($p < .05$)* for a minimum of two consecutive sweeps or until the EEG noise criterion was reached (*mean noise $\leq$ 11 nV*) and no response detected *($p \geq .05$)* (Small & Stapells, 2006). Testing was considered complete if either of these criteria were met for each carrier frequency. For study A, ASSRs were recorded for a minimum of 11 sweeps regardless of response presence/absence. For study B, ASSRs were recorded for a minimum of 5 sweeps. The reduction in the minimum number of sweeps recorded in study B was done to maximize the likelihood of obtaining threshold results for both stimulus conditions.

Procedure

Study A and B each involved one or two recording session(s) which lasted approximately 2 hours each. Parent(s) of the participants signed a consent form before initiation of testing and were paid an honorarium at the end of each session; procedures were approved by the UBC Behavioural Research Ethics Board. All ASSR measurements took place in a double-walled sound-attenuated booth. Infants slept naturally during the testing. The DPOAE hearing screening was first carried out and results (pass/refer) pertaining to the DPOAE screen were given to the parents and explained. If passes from both ears were found using the DPOAE screener, the ASSR test session commenced.

For study A, we attempted to counterbalance the order of the MS 500-Hz, MS-2000 Hz and the multiple-stimulus conditions (MM, DM) if the infant remained sleeping for all the stimulus conditions. When either the 500-Hz MS or 2000-Hz MS conditions were run first, an insert earphone was first placed in only the test ear. The second insert earphone was placed in the non-test ear just prior to testing in the DM condition. After this, the MS 1000-Hz and MS 4000-Hz conditions were tested in random order. If an infant woke up in a particular condition, testing was stopped until the infant fell back asleep and a different condition was initiated in case the previous condition contributed to the infant waking up. In Study A, infants tended to wake up more frequently during the DM condition, thus compared to all other conditions, the DM condition was more frequently presented last for study A.

For study B, the order of the MS and DM conditions was randomized and insert earphones were always placed in both ears at the start of testing. Threshold, amplitude, EEG noise, and time-to-criterion information were determined for the 500-Hz carrier frequency in the MS and DM stimulus conditions. Once the order of the conditions had been established, the condition was completed (i.e., threshold found) before moving onto the next condition. Threshold search always started at 60 dB SPL and intensity increased or decreased using 10-dB steps, depending on which direction yielded the most information. The exception to this was one infant began to wake up with stimulation at 60 dB SPL, so the threshold search for this infant started at 40 dB SPL. Final step size was 5 dB.

Data Analysis

In general, higher EEG levels result in an overestimation of response amplitude (Picton et al., 2005). Thus, the amplitudes we obtained were corrected for EEG noise level using the formula $1 + 0.965e^{-1.348X} + 0.078e^{-0.285X}$ where X represents the ratio between the measured amplitude of the signal and the noise amplitude (Picton et al., 2005). This amplitude correction factor was applied to minimize any effects of EEG noise levels differing between the conditions.

Study A: Descriptive measures of ASSR amplitude, final EEG noise, relative efficiency, and time-to-criteria for all carrier frequencies were determined for all infants. Two-way repeated-measures analyses of variance (ANOVA) with four levels of carrier frequency and three stimulus presentation conditions were conducted each for ASSR amplitude, EEG noise levels, and relative efficiency. For the time-to-criteria results, for the time required to obtain results for all frequencies in one ear, dependent samples t-tests were conducted to compare the MS and DM conditions. To compare time-to-criteria across eight stimuli/both ears for the MS, MM, and DM conditions, a 1-way repeated measures ANOVA was utilized. For all ANOVAs, Huynt-Feldt epsilon degrees of freedom correction factors for repeated measures were used when appropriate and Newman Keuls *post hoc* comparisons were carried out to assess significant main effects and interactions. Results for all analyses were considered statistically significant if $p < .05$.

Relative efficiency is a measure that considers the increase in information relative to the decrease in amplitude when going from single to multiple stimulus conditions (Fontaine & Stapells, 2007). Using the mean (across subjects) MS condition as a baseline amplitude for each frequency, the relative efficiency for each stimulus condition was calculated, where relative efficiency equals (amplitude/mean amplitude of MS condition) x $M^{0.5}$ and M = the number of stimuli (Fontaine & Stapells, 2007; John et al., 1998).

Study B: Measures of 500-Hz ASSR threshold and response amplitude, EEG noise, and time-to-criteria (all in response to 60-dB SPL stimuli) were obtained for the MS and DM conditions. The statistical significance of differences in the measures between the MS and DM conditions were determined using t-tests for dependent samples. Results for these analyses were considered statistically significant if $p < .05$.

Results

Study A:

Response amplitude: Figure 4 presents ASSR amplitude spectra for a typical infant (age: 21 weeks) in the MS, MM, and DM conditions. Filled triangles indicate that the amplitude at the modulation rate was significantly different from the residual EEG noise (i.e., response present), whereas the open triangle indicates no statistically significant response at its corresponding frequency of modulation. Typical of the group results, this infant's ASSR amplitudes were larger in the MS conditions relative to the multiple-stimulus conditions, regardless of frequency. Although smaller in amplitude, responses in the multiple-stimulus conditions (MM, DM) were present for all the carrier frequencies, with the exception of 500 Hz in the MM condition.

As would be expected from previous studies, results for lower carrier frequencies, presented at lower modulation rates, show greater EEG noise than the higher carrier frequencies (Picton, Dimitrijevic, Perez-Abalo, & Van Roon, 2005). Although additional sweeps were often obtained, we compared EEG noise after 11 sweeps as all conditions were obtained at a minimum of 11 sweeps. Mean EEG noise

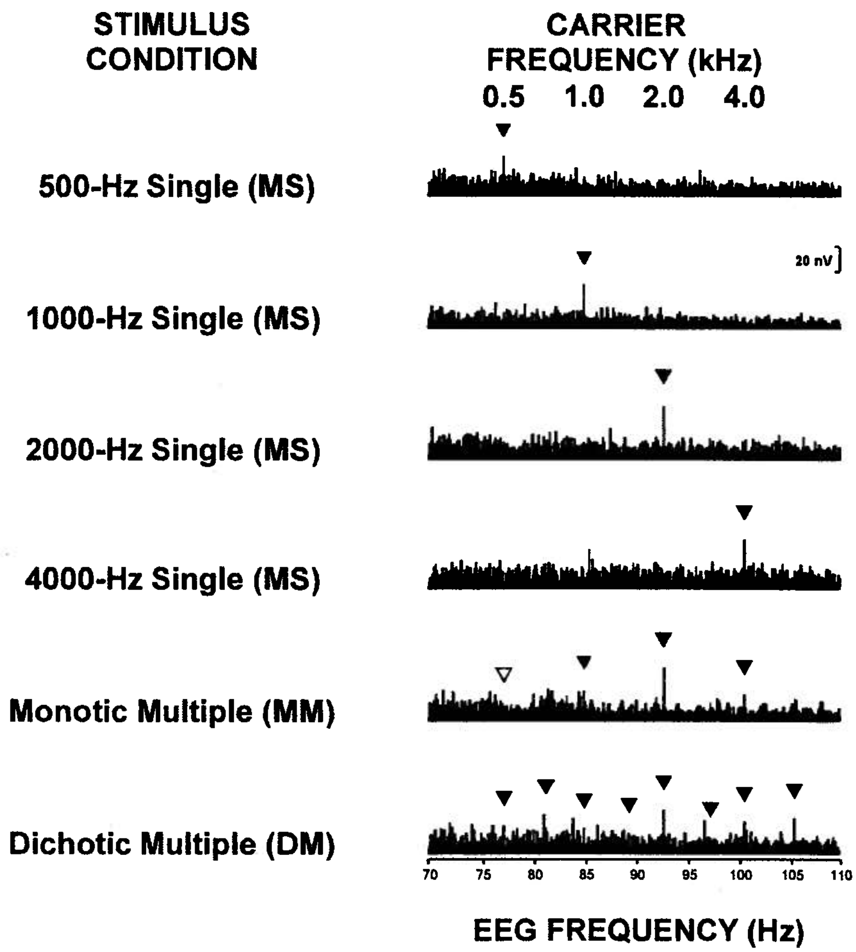

Figure 4 One representative subject's ASSR amplitude spectra for the MS, MM, and DM stimulus conditions presented at 60 dB SPL.

obtained after 11 sweeps (pooled across condition) were 15.4, 14.1, 12.6, and 11.6 nV for the 500, 1000, 2000, and 4000 Hz carrier frequencies. An ANOVA revealed a statistically significant difference in EEG noise level across the carrier frequencies ($F=9.0$; $df=3, 42$; $\varepsilon=1.0$; $p = .0001$). *Post hoc* analyses revealed that EEG noise for 500 Hz was significantly higher than 2000 Hz ($p = .003$) and 4000 Hz ($p < .001$). Although not quite statistically significant, 500 Hz tended to be noisier than 1000 Hz ($p = .098$).

Comparison across stimulus conditions revealed that the DM condition tended to be noisier than the MM and MS conditions. Mean EEG noise pooled across frequency in the MS, MM, and DM conditions were 11.8, 12.1, and 16.3 nV, respectively. The ANOVA revealed that these differences in EEG noise across conditions did not quite reach significance ($F=2.3$; $df=2, 28$; $\varepsilon=.80$; $p = .116$). Because the ANOVA revealed significantly greater noise for the lower frequencies (i.e., 500 Hz) relative to the higher frequencies (i.e., 2000-4000 Hz) as well as a trend for multiple conditions to be noisier, all ASSR amplitudes were corrected for EEG noise. These corrections, however, resulted in only small decreases in amplitudes (pooled across frequency and condition, mean uncorrected amplitude was 32.3 nV compared to a mean corrected amplitude of 30.9 nV).

Amplitude: Figure 5 shows the mean (corrected) ASSR amplitudes for the 15 infants who participated in study A for the three stimulus conditions (MS, MM, and DM) and four carrier frequencies (500, 1000, 2000, and 4000 Hz). Mean amplitudes were largest in the MS condition relative to the MM and DM conditions, with means (pooled across frequency) of 39.5, 32.4, and 27.5 nV for the MS, MM, and DM stimulus conditions, respectively. The ANOVA revealed a significant main effect of condition ($F=.0003$; $df=2, 28$; $\varepsilon=1.0$; $p < .001$). *Post hoc* analysis revealed that amplitudes for the MS condition were significantly larger than those in the MM ($p = .010$) and DM ($p < .001$) conditions. Although not quite reaching statistical significance, there was a trend for ASSR amplitudes in the MM condition to be slightly larger compared to those in the DM condition ($p = .066$). Figure 5 also shows that amplitude at 500 Hz was smaller relative

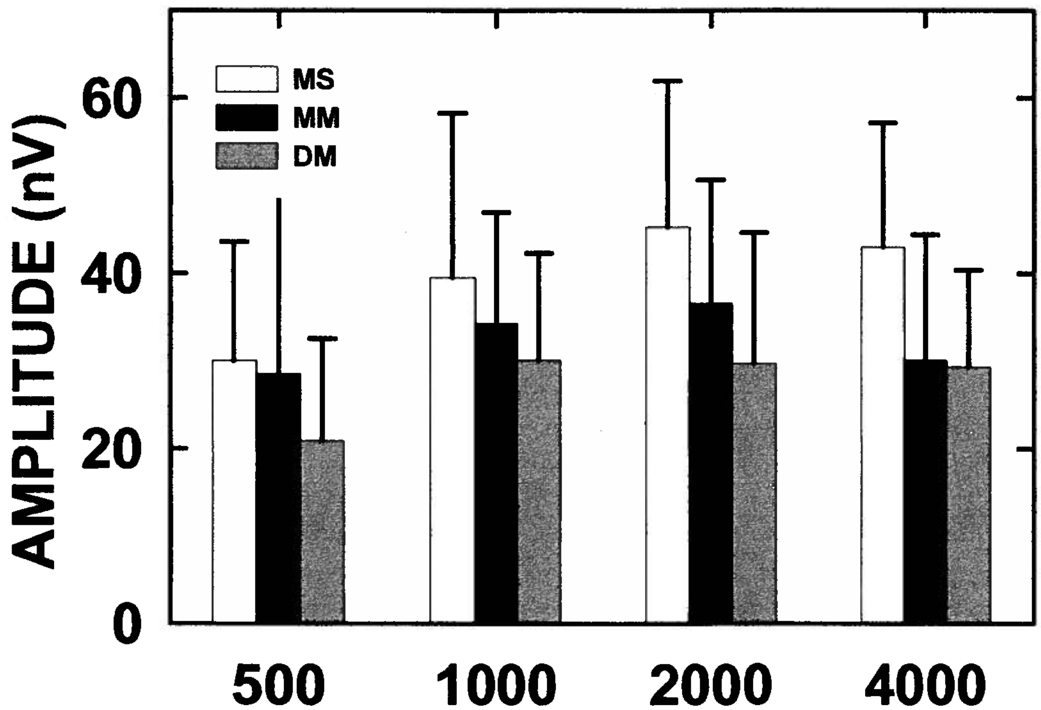

Figure 5 Mean ASSR amplitudes (corrected for EEG noise) and standard deviations for the 500, 1000, 2000, and 4000 Hz carrier frequencies in the MS, MM, and DM stimulus conditions at 60 dB SPL.

to the higher frequencies. Pooled across conditions, mean amplitudes were 26.5, 34.6, 37.2, and 34.1 nV for the 500-, 1000-, 2000-, and 4000-Hz carrier frequencies. The ANOVA revealed a significant main effect of frequency (F=5.0; df=3, 42; ε=0.8; p < .005). *Post hoc* analysis revealed that 500 Hz was significantly smaller than 1000 Hz (p=.022), 2000 Hz (p=.004), and 4000 Hz (p=.013). No significant differences in amplitude were seen for the higher frequencies (i.e., 1000-4000 Hz).

Drop-out rate: The percentage of dropped-out recordings (i.e., non-significant recording in the MM/DM conditions) increased as a function of the number of simultaneous stimuli presented. Overall drop-out rates for the MS, MM, and DM conditions (pooled across frequency) were 1.7% (1/60 recordings), 6.7% (4/60 recordings), and 11.7% (7/60 recordings), respectively. The 500-Hz carrier accounted for the majority (75%; 9/12 dropped-out recordings) of these non-significant recordings.

Relative efficiency: Figure 6, which presents relative efficiency results from study A for the three stimulus conditions and four carrier frequencies, indicates that the MM and DM stimulus conditions have higher efficiency relative to the single-stimulus condition for all carrier frequencies. Mean relative efficiency values (pooled across carrier frequency) for the MS, MM, and DM conditions were 1.0, 2.0, and 1.87. The results of the ANOVA revealed that these differences were significant (Condition main effect: $F=25.1$; $df=2, 28$; $\varepsilon=1.0$; $p < .0001$). *Post hoc* analyses revealed that the multiple stimulus conditions were significantly more efficient than the MS condition ($p < .001$); however, the relative efficiency of the MM and DM multiple stimulus conditions did not differ significantly from each other ($p=.399$). There was no significant effect of carrier frequency ($F=.815$; $df=3, 42$; $\varepsilon=.705$; $p=.493$), and no significant interaction between condition and carrier frequency ($F=1.07$ $df=6, 84$; $\varepsilon=.596$; $p=.310$).

Time-to-Criteria: Time-to-criteria was obtained in the MS condition by summing the number of sweeps required to meet stopping criteria for each carrier frequency in order to determine the time required to obtain information for the four carrier frequencies in one ear using the single-stimulus presentation method. In the MM condition, the number of sweeps required to reach criteria for four frequencies in the test ear when multiple stimuli were presented simultaneously corresponded to the sweep number representing the response that took the longest to meet the stopping criteria in the test ear, as described in the Methods section. Table 2 displays the average amount of time to reach criteria (either significant response or noise criteria) for the MS and MM conditions for the four frequencies in the test ear. Table 2 shows that the MM condition required less time than the MS condition to find information regarding four frequencies in the test-ear alone, although results of a dependent samples t-test revealed these differences did not quite reach significance ($t=1.57$; $df=14$, $p=.139$). The MM condition took 3.4 minutes on average to obtain information for four frequencies at 60 dBSPL whereas the MS condition required, on average, 5.5 minutes to obtain the equivalent amount of information.

The above analysis only considered the time required to reach criterion when testing one ear. However, the dichotic multiple technique allows one to test both ears simultaneously. Thus, in a second analysis of time-to-criteria, we estimated the time to reach criteria for both ears by doubling the time for the four frequencies in the MS and MM conditions, and compared these to the actual time required in the DM condition for both the test ear and non-test ear. Time-to-criteria for the DM condition corresponded to the number representing the response that took the longest to meet stopping criteria in both the test ear and non-test ear. As shown in Table 3, on average, it took 6 minutes to obtain information for 8 frequencies in the multiple-stimulus conditions compared to

11 minutes in the MS condition. Although not quite significant, the results of a repeated measures ANOVA revealed a trend for the multiple conditions to be faster than the MS condition for eight frequencies ($F=2.7$; $df=2, 28$; $\varepsilon=.9$; $p = .081$).

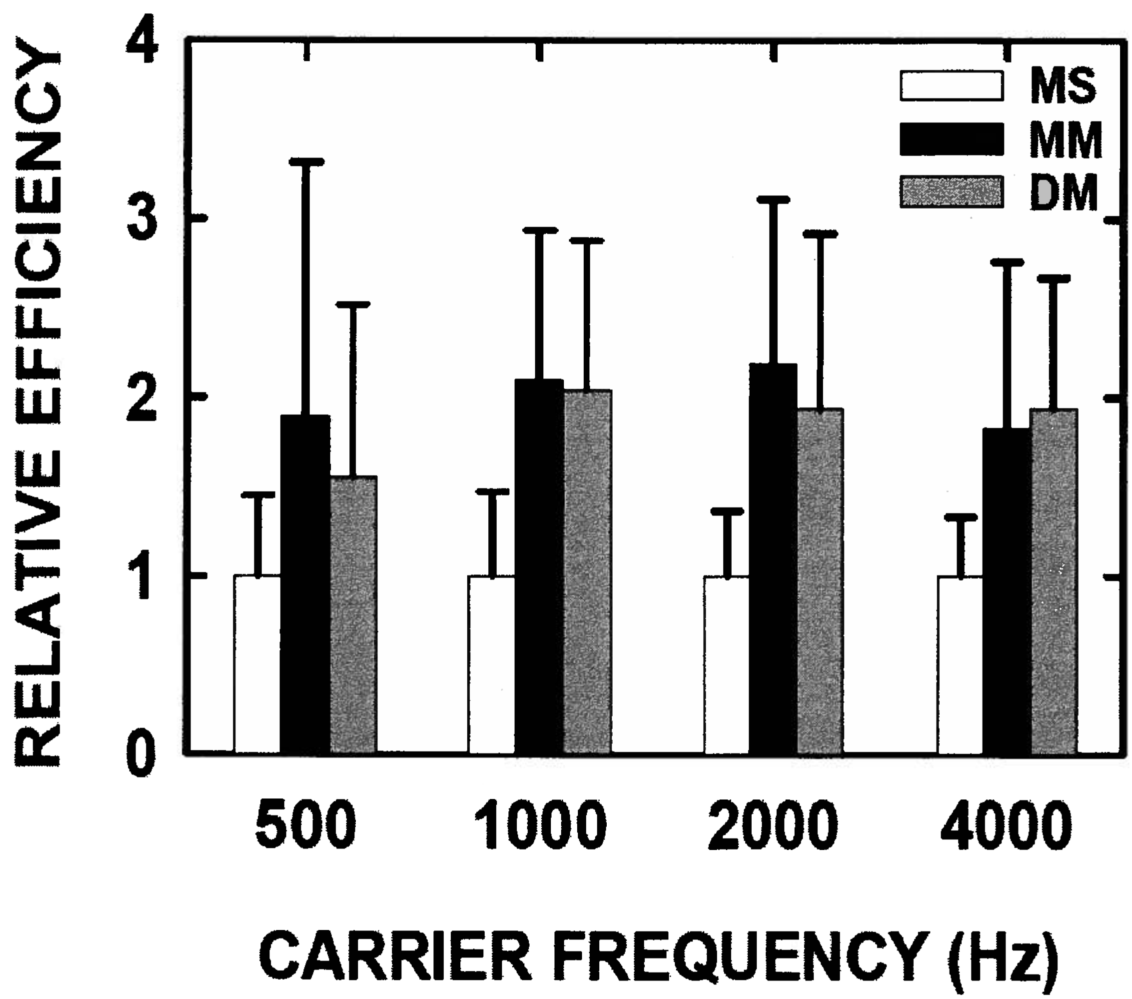

Figure 6 Mean relative efficiency results and standard deviations for carrier frequencies of 500, 1000, 2000, and 4000 Hz in the MS, MM, and DM stimulus conditions at 60 dB SPL.

Table 2. Air-conduction ASSR mean number of sweeps and test time (1 SD) required to obtain responses and or noise criteria for the four carrier frequencies (1 ear) at 60 dB SPL for the MS and MM stimulus conditions in infants with normal hearing.

Condition (test ear)	Sweeps	Test Time (minutes)
MS mean	20.1	5.5
SD	15.3	
MM mean	12.3	3.4
SD	10.65	

Test time = (sweeps*16.384/60 seconds).
MS = the summed number of sweeps required to meet stopping criteria for each carrier frequency.
MM = the sweep number representing the response that took the longest to meet the stopping criteria in the test ear.

Table 3: Air-conduction ASSR mean number of sweeps and test time (1 SD) required to obtain responses and or noise criteria for the eight carrier frequencies (4 per ear) at 60 dB SPL for the three stimulus conditions in infants with normal hearing

CONDITION (8 frequencies)	Sweeps	Test Time (minutes)
MS mean	40.1	11
SD	30.67	
MM mean	24.5	6.8
SD	21.3	
DM mean	22.1	6
SD	11.4	

Test time = (sweeps*16.384/60 seconds)
MS = the summed number of sweeps required to meet stopping criteria for each carrier frequency multiplied by two.
MM = the sweep number representing the response that took the longest to meet the stopping criteria in the test ear multiplied by two.
DM = the sweep number representing the response that took the longest to meet the stopping criteria in the test ear and the non-test ear.

Study B:

Study B was conducted to assess differences, if any, in ASSR thresholds for the MS and DM conditions. Due to sleep-time limitations, only thresholds for 500 Hz in the MS and DM conditions were evaluated. We concentrated on the frequency with the smallest amplitude, 500 Hz, and the condition with the largest interactions, the DM condition. We also focussed on these conditions and this frequency due to the finding in study A that 500 Hz in the DM condition tended to drop-out, or lose significance more often than 500 Hz in the MS and MM conditions. Study B also addressed the potential order effect in study A by ensuring insert earphones were placed in both ears at the beginning of all testing and the order of conditions randomized.

Threshold: Results for the 500-Hz MS and 500-Hz DM conditions from study B revealed mean (1 SD) thresholds of 51.8 (8.9) and 55.0 (11.4) dB SPL for the MS and DM stimulus conditions, respectively. The slightly higher (3.2 dB) mean thresholds obtained in the DM condition did not quite reach statistical significance (dependent samples t-test: $t=-1.55$; $df=13$; $p=.14$).

The mean EEG noise levels (after five sweeps, at 60 dBSPL) in study B were 19.8 and 30.5 nV for the 500-Hz MS and DM conditions, respectively. A dependent samples t-test showed that this difference between conditions was significant ($t=-2.61$; $df=12$; $p=.02$). In order to compare amplitudes between conditions similar to Study A, response amplitudes were again corrected for EEG noise level. Corrected ASSR amplitude at 60 dB SPL for 500 Hz in the MS condition (28.1 ± 10.7 nV) was larger than that obtained in the DM condition (24.5 ± 14.7 nV), however, this difference was not statistically significant (t=.60; df=12; p=.56).

Time-to-criteria: In study B we were able to compare time-to-criteria for the MS and DM conditions for 500 Hz. Time-to-criteria was determined identically to that in study A, with the exception that only 500 Hz was assessed for study B. We also estimated the 500-Hz time to reach criteria for both ears by doubling the time for the 500-Hz carrier frequency in the MS condition, and compared this to the actual time required to find results for 500 Hz in the DM condition for both the test ear and non test ear. Time-to-criteria for the DM condition corresponded to the number of sweeps for 500 Hz (which usually took the longest to meet stopping criteria in either the test or non-test ear). Results for 500-Hz in the MS and DM conditions (at 60 dBSPL) show that the DM condition was slightly faster at obtaining information for 500 Hz for two ears than the MS condition. On average, it took 3.4 minutes to complete testing in two ears for 500 Hz in the DM condition compared to 4.0 minutes required to complete testing in the MS condition. Results of a dependent samples t-test, however, revealed that this difference in test time was not significant (t=.50; df=12; p=.62).

Discussion

ASSR amplitudes

This is the first infant ASSR study to directly compare amplitude across single versus multiple stimulus conditions allowing for the assessment of interactions. The infant ASSR amplitudes in this study show interactions between responses to multiple stimuli presented at 60 dB SPL, such that amplitudes decrease as the number of simultaneous stimuli increase. In study A, amplitudes in the MS condition were significantly larger than those in the multiple stimulus conditions; there was no significant difference in amplitude between the MM and DM stimulus conditions. These results differ from those seen in adults, where interactions are not seen at 60 dB SPL (Herdman & Stapells, 2001; John et al., 1998). Potential contributors to these interactions are discussed below.

Ear-canal changes can affect the level of the stimulus reaching the cochlea. Studies investigating infant-adult differences in stimulus SPL at the eardrum using insert earphones have consistently shown larger SPL in the infant ear than in the adult ear, with the difference increasing with frequency up to at least 6000 Hz (Bagatto et al., 2005; Rance et al., 2006; Sininger et al., 1997; Voss & Herrmann, 2005). For example, the intensity reaching an infant's eardrum (<10 months of age) is approximately 3 and 13 dB higher at 500 Hz and 4000 Hz than that reaching the adult eardrum (e.g., (Bagatto et al., 2005; Bagatto et al., 2002). In adults, ASSR interactions between responses to multiply presented stimuli are seen at higher stimulus intensities (John et al., 1998), thus interactions might be expected in the high frequencies in infants. In the current study, however, we did not find a significant frequency x condition interaction (that is, in Study A, the amplitude reductions were present for all carrier frequencies). Therefore, it is not likely that a higher stimulus intensity at the infant's eardrum can explain our findings. Also, ear-canal acoustics cannot explain the interactions at 500 Hz, as there is only about a 3-dB increase for the average intensity at the infant ear canal at this frequency (Bagatto et al., 2005; Bagatto et al., 2002).

Development of the middle-ear system is also unlikely to account for the interactions present in the current study. Although maturational changes in the middle ear may alter the power transmission and therefore the sound level reaching the cochlea, the magnitude of this effect is small and thus unlikely to change ASSR results significantly (Keefe & Levi, 1996; Sininger et al., 1997). Keefe and Levi (1996) investigated maturation-related changes in the external and middle ear using admittance and reflectance measurements from normal-hearing infants (1-, and 6-month-old infants). Comparisons of energy reflectance results between infants and adults demonstrated that energy reflectance decreases with increasing age, a finding consistent with larger energy losses in the infant ear canal (Keefe et al., 1993). This loss in energy may be due to greater absorption of energy into the soft tissues of the infant ear canal and thus less energy available to stimulate the cochlea, although the exact mechanism is unknown. Therefore, there appears to be 2-4 dB less transmission of energy to the cochlea in the infant (Keefe et al., 1993; Keefe & Levi, 1996). This lower intensity does not explain the interactions seen in Study A, which occur with higher stimulus intensities.

If the increased interactions seen with increased intensity in adults occur because stimuli reach a specific sensation level (SL) above threshold (i.e., rather than absolute intensity), then this intensity effect is not likely to be present in infants. For example, Herdman and Stapells (2001) found ASSR thresholds in adults of 25, 17, 15, and 22 dB SPL for 500, 1000, 2000, and 4000 Hz, respectively; Van Maanen and Stapells (in press) report thresholds in infants of 46, 36, 34, and 27 dB SPL for the same carrier frequencies. As mentioned previously, interactions, which reduce amplitude, are present at 75 dB SPL in adults; this level corresponds to an average of 55 dB SL for the adults, but would only be 39 dB SL for the infants (and, indeed, only 20 dB SL for 500 Hz in the DM condition in Study B). Sensation level is thus lower in infants than adults, providing further evidence against the higher intensity (at the ear canal) hypothesis as explaining the interactions seen in the multiple stimulus conditions for infants.

The finding that amplitudes are reduced in the multiple-stimulus conditions relative to the MS condition may be due to cochlear and/or neural immaturities. In the cochlea, interactions between multiply presented stimuli may occur. If there is an overlap of activation patterns along the basilar membrane, the hair cells located within these areas of overlap will respond according to the interference on the basilar membrane for that particular area (John, Lins, Boucher, & Picton, 1998). The most common interactions involve either an attenuation of low-frequency responses in the presence of a higher frequency or the enhancement of responses to high-frequency stimuli when presented with lower frequency stimuli (John, Purcell, Dimitrijevic, & Picton, 2002). The interactions in the current study are consistent with destructive interactions on the basilar membrane and greater overlap between travelling waves along the basilar membrane, possibly due to a broadening of cochlear filters. As indicated above, it is not likely that a higher stimulus intensity can explain the interactions seen in the current study. Thus, broader filters due to cochlear immaturity appear to be a possibility. Most studies suggest that the cochlea is mature at birth (e.g., Abdala et al., 1996), although some recent data in neonates (mean age: 38.6 weeks) suggest some cochlear immaturities exist (Dhar and Abdala, 2007). The current study's interactions in babies are not likely to be of cochlear origin, but cochlear immaturities cannot be ruled out.

The reduction in amplitudes in the multiple conditions relative to the MS condition could result from interactions between the brainstem neuronal pools related to each characteristic frequency. The finding that amplitudes reduced further, albeit not quite significantly, from the MM condition to the two-ear (DM) condition suggests that maturation factors related to brainstem immaturities play a role in the interactions present in the current study. There is much evidence from evoked potential studies and postmortem fetal tissue sample studies indicating that the auditory brainstem is functionally and structurally immature at birth (Moore et al., 1995; Ponton et al., 1994; Salamy & McKean, 1976). The ABR exhibits amplitude and morphological changes up to 5 years of age (Mochizuki et al., 1983; Salamy, 1984) and maturation of the later auditory evoked potentials is not complete until adolescence (Moore & Guan, 2001; Ponton, Eggermont, Kwong, & Don, 2000). Infant ABR studies suggest that infants have higher sensitivity to rapidly presented signals (Lasky, 1984; Lasky & Rupert, 1982). ASSR assessment in sleeping infants requires modulation frequencies between 70 to 100 Hz in order to obtain reliable responses. Because the 80-Hz ASSR employs fast rates of stimulus presentation and multiple stimuli, it is possible that interactions at the level of the brainstem play a role in the interactions present in infants.

In summary, the results from the current study suggest that the ASSR amplitude decreases in the multiple stimulus conditions relative to the single stimulus baseline condition are the result of immaturity of cochlear development or more likely of neural development in the auditory brainstem, rather than more peripheral factors, such as infant-adult differences in intensity at the eardrum and middle ear changes, although the exact mechanisms are not fully understood.

ASSR 500-Hz threshold

Our 500-Hz threshold results are within, but at the higher end of, the range of those reported previously (e.g., Lins, Picton et al. 1996; John, Brown et al. 2004; Swanepoel and Steyn 2005; Van Maanen and Stapells, in press; refer to Table 1). Our thresholds were higher than those recently reported by Van Maanen and Stapells (in press); these differences may be due to higher noise EEG in our 500-Hz infant ASSR results.

Many other authors have found 500-Hz ASSR threshold to be elevated relative to the other carrier frequencies (Cone-Wesson, Dowell et al., 2002; Han et al., 2006; Lins et al., 1996; Swanepoel & Steyn, 2005; Van Maanen & Stapells, in press). Elevated threshold at 500 Hz may be related to poor neural synchronization in infants or less-effective low-frequency transmission related to maturational changes of the outer and middle ear (John et al., 2004). Sininger and colleagues (Sininger et al., 1997) and Rance and Tomlin (2006) found infant ABR and ASSR thresholds (in dB SPL in the ear canal) to be elevated with respect to adult thresholds and concluded that the elevated thresholds in infants were the result of auditory brainstem (neural) development (ASSR: Rance et al., 2006; ABR: Sininger et al., 1997). Auditory brainstem immaturities are also consistent with the ABR work done by Ponton and Colleagues (1992), for example, which showed infants to have prolonged synaptic transmission time compared to adults and that synaptic transmission times continues to shorten up to three years of age (e.g., Ponton et al., 1994). Thus, it seems likely that neural immaturities play a role in ASSR threshold elevations.

ASSR relative efficiency

The present study provides the first direct evidence in infants of the efficiency of the multiple-stimulus technique for recording the 80-Hz ASSR . Despite the finding that amplitudes decrease when going from single to multiple stimuli, multiple-stimulus presentation in infants is still more efficient than presenting single AM tones. Although there are no previous reports in infants on the efficiency of the multiple ASSR, the findings of the current study are in partial agreement with adult studies which also found the multiple 80-Hz ASSR technique to be more efficient than a single-stimulus technique at moderate levels and lower (Herdman & Stapells, 2001; John et al., 1998; Lins et al., 1995). Relative efficiency values calculated from the published adult data of Herdman and Stapells (2001) were 1.9 and 2.6 for the MM and DM conditions, respectively. Thus, in adults, an increase in relative efficiency is seen when going from the MM to the DM condition. The relative efficiency results in the current study clearly show the multiple ASSR to be more efficient than the single stimulus ASSR, although the MM and DM multiple-stimulus conditions were similar in efficiency. Nevertheless, the DM method of stimulus presentation may be more appropriate for infant testing because it is able to record information from two ears simultaneously whereas the MM condition only records information for one ear. Thus, if an infant were to wake up during testing, one would still obtain some information regarding both ears if using the DM condition, but not if using the MM condition.

ASSR time-to-criteria

Another factor that would influence which technique (single versus multiple) is better is that of test time. In the present study, the average time-to-criteria for four carrier frequencies in one ear for the MS condition was 5.5 minutes, almost 40% longer than the time required to obtain the equivalent amount of information in the MM condition, which took on average 3.4 minutes. The test time required to obtain information for two ears for four carrier frequencies (eight frequencies in total) was shorter in the multiple conditions than in the single condition, although there was no significant difference in test time between the MM and DM conditions. However, it must be noted that these test times underestimate the time-advantage of the DM condition because, unlike the DM condition, the MS and MM conditions require extra time to switch between ears and stimulus files. These test times also underestimate the time-advantage of the MM condition relative to the MS condition. Thus, similar to our conclusions from the relative efficiency findings, the DM condition would appear to be the recommended stimulus presentation method.

Drop-out rate

Although the multiple-stimulus ASSR is more efficient than a single stimulus technique, some responses dropped-out, or lost significance, when going from single to multiple stimulus conditions. Overall drop-out rates in Study A were 2%, 7% and 12% for the MS, MM, and DM conditions, respectively, with 500 Hz accounting for the majority of the dropped-out responses. One possible reason for the drop-out of responses in the DM condition relative to the MS condition may be related to the stimulus intensity used in study A, which was 60 dB SPL. It is possible that this intensity was too close to the infants' 500-Hz ASSR threshold, particularly for the DM condition (i.e., thresholds were only 8 dB and 5 dB below the stimulus intensity for the MS and DM conditions, respectively; see Study B). This most likely accounts for the increased drop-out rate at this frequency, in this condition. Although not assessed in this study, it is likely that drop-out of responses would not be an issue if stimuli were to be presented at least 10-15 dB above threshold.

EEG noise

In the current study, EEG noise in the DM condition tended to be noisier than the one-ear stimulus conditions (i.e., MS and MM). Although an order effect in study A might account for this because the DM was most often carried out at the end of the study, this was accounted for in study B where the order of conditions was randomized and both inserts in place upon test initiation. Nevertheless, even in Study B, the EEG noise in the DM condition remained significantly higher. Thus, the tendency for EEG noise to be higher in the DM condition in study A most likely represents a true effect as a function of stimulus condition.

The higher noise levels in the DM condition, but not the MM condition, suggest that increased noise levels are the result of testing two ears vs one ear rather than the result of presenting multiple stimuli simultaneously. It is possible that these findings represent a binaural summation effect occurring above the 80-Hz generators, resulting in noisier EEG that is not reflected in the 80-Hz ASSR amplitude and threshold data. Although central mechanisms may explain our findings, the exact mechanism of this finding in not known.

Conclusions

To conclude, infants show significant ASSR interactions – amplitude reductions – for multiple ASSR stimuli presented at 60 dBSPL. This contrasts with adult data, where no interactions are seen at this intensity. The interactions present in the current study in the multiple stimulus conditions appear to be related to cochlear and/or brainstem immaturites rather than external or middle ear maturational factors, although further research is required to clarify this. Despite these interactions, the multiple-stimulus ASSR remains more efficient than a single-stimulus technique in terms of both relative efficiency and time-to-criteria. The fact that several measures show the multiple-stimulus ASSR to be better than the single-stimulus ASSR (either statistically significant or at least a trend) supports the overall conclusion that the multiple ASSR is more efficient than the single-stimulus ASSR.

Study B revealed small and non-significant threshold differences for 500 Hz as a result of using multiple versus single stimuli. The increased drop-out rate for the dichotic multiple condition in study A most likely resulted from using a stimulus intensity too close to 500-Hz threshold in the dichotic multiple condition. Thus, even small amplitude decreases in the dichotic multiple condition were enough to cause responses to drop out at this intensity and frequency.

The higher EEG noise levels seen in the DM condition appear to be related to a two-ear phenomenon. One possibility is that the increased noise in the DM condition is the result of an increase in loudness that increased the likelihood of an infant waking up in this condition.

Importantly, although our results show that the multiple-stimulus ASSR technique is more efficient that a single-stimulus technique in normal hearing infants, it is not known if this remains the case in infants with hearing loss, particularly with non-flat hearing losses. Therefore, similar studies in infants with hearing loss are in need before the multiple ASSR can be deemed more efficient in this group of the population.

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Study A

Appendix A: Corrected amplitude (nV) data by condition and subject

Appendix A: Corrected amplitude (nV) data by condition and subject

	MS				MM				DM (Test ear)				DM (Non-Test ear)			
N	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**
1	24.1	16.4	19	21.8	12.7	14.8	19	**3.6**	**3.9**	**11.9**	18.3	14.2	29.7	16.2	18.5	42.3
2	23.4	26.7	69.2	57.3	**5.6**	49	53	36.2	**10.3**	17.1	13	16	**1.9**	**7.7**	24.3	35.5
6	32.2	38	58.6	40.9	19.7	39.9	22.5	22.5	24.8	42.9	39.9	37	**10.3**	32.6	31.7	31.1
8	29.7	25.8	30.6	30.5	26.2	20.3	32.6	28.6	31.6	27.7	50.1	36	18.1	30.8	19.2	**10.9**
10	69.6	56.4	65.9	49.8	46.3	52.5	39.5	40.6	22.8	48.2	37.3	42.3	16.4	39.2	23.9	12.5
11	40.6	70.8	64	67.8	79.7	46.8	47.5	51.1	26.7	39.6	41.1	33.4	65.1	36.8	32.9	38.5
14	**6.1**	35.6	64.6	58.7	20	36.5	54.6	44.5	**8.4**	25.2	52.9	48	**11.4**	27.4	24.1	25.3
15	34.1	50.7	29.9	30.3	26.7	30.2	17.9	19.1	16.2	23.3	9.8	16.3	14.6	25.4	19.3	17.3
16	31.4	38.1	42.1	41.5	**10**	14.2	47.4	13.9	19.7	19.9	36	23.7	29	19.2	24.8	29
20	33.8	30.7	39.2	29.7	20.6	30.8	26.9	16.4	15.4	34.7	14	20.9	34.6	33.8	24.8	26.3
21	32.9	58.4	55.6	42	28.8	44.8	38.7	36.8	37.3	45.6	39.7	35.8	24.2	38.5	32.7	32.8
22	23.3	13.5	23.7	25.5	10	24.8	17.7	16.7	**7.1**	20.9	**8.4**	13.9	15.7	29.2	23.1	11.8
23	18.8	22.7	31.9	37.4	63.7	23.6	29.7	36.1	33.4	18.1	21.6	35.2	**11.4**	**1.1**	14.4	24.9
24	29	33.8	40.1	48.3	46.9	47.3	59.6	33.8	42.1	48.4	40.7	39	23.4	26.9	31.7	23.9
25	24.1	74.7	45.5	62.8	**10.9**	39	42.3	51.7	**13.5**	27.7	22.7	28	**7.6**	**10.9**	32.9	28.2

Conditions: monotic single (MS), monotic multiple (MM), dichotic multiple (DM).

Bold values specify amplitudes that met noise criterion but did not differ significantly from the background EEG noise.

Study A

Appendix B: EEG noise (nV) at 11 sweeps by condition and subject

Appendix B: EEG noise (nV) at 11 sweeps by condition and subject

	MS				MM				DM (Test ear)				DM (Non-Test ear)			
N	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**
1	31.6	10.3	16.1	14.9	6.9	6.9	6.9	6.3	16.7	15.5	14.9	13.8	15.5	15.5	14.9	13.2
2	13.8	12.6	10.3	19.5	29.3	28.7	27.6	27	9.2	8.6	7.5	8	8.6	8	7.5	7.5
6	15.5	13.8	6.9	14.4	19.5	16.1	16.7	15.5	14.9	13.8	12.6	10.9	14.9	13.2	12.1	10.3
8	14.4	8.6	5.2	5.7	6.9	5.7	5.2	4.6	13.8	12.6	13.8	12.6	13.2	12.6	13.2	13.2
10	7.5	33.9	12.1	5.2	8	7.5	6.3	5.7	8.6	9.2	8	8	9.2	8.6	8	7.5
11	22.4	13.2	17.2	5.7	20.1	19.5	17.2	14.9	34.4	29.3	31	26.4	32.7	30.4	30.4	28.1
14	8	5.2	7.5	6.3	6.9	6.3	6.3	6.9	30.4	31	33.9	29.3	31	31.6	32.2	31.6
15	8	12.1	8.6	11.5	9.2	7.5	6.9	6.3	16.1	11.5	10.9	10.3	13.8	10.9	10.9	9.8
16	10.9	8.6	9.2	16.1	12.1	12.6	11.5	9.2	12.1	11.5	9.8	9.2	12.6	10.3	8.6	8
20	13.8	20.1	13.2	17.8	17.2	16.7	14.9	11.5	22.4	19.5	17.8	15.5	20.1	18.4	17.8	12.6
21	9.2	8.1	6.9	9.8	21.2	19.5	20.1	18.9	7.5	6.3	5.2	4	6.9	6.3	4.6	4
22	6.3	7.5	3.4	5.2	5.2	3.4	3.4	3.4	12.6	12.1	11.5	10.3	12.1	12.1	11.5	10.9
23	19.5	12.1	9.2	6.9	21.2	15.5	11.5	9.2	25.3	24.7	22.4	21.8	25.3	21.8	22.4	22.4
24	12.1	13.8	23	8	15.5	13.8	13.2	9.8	20.7	18.9	14.9	12.1	18.9	17.2	13.2	11.5
25	14.4	14.4	6.9	5.2	9.2	9.2	7.5	5.7	31.6	25.2	23	23.5	28.1	24.1	23.5	20.7

Conditions: monotic single (MS), monotic multiple (MM), dichotic multiple (DM).

Study A

Appendix C: Relative efficiency data by condition and subject

Appendix C: Relative efficiency data by condition and subject

	MS				MM				DM (Test ear)			
N	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**
1	0.797	0.414	0.419	0.508	0.838	0.749	0.838	0.167	0.368	0.855	1.143	0.938
2	0.774	0.678	1.527	1.334	0.368	3.241	3.509	2.394	0.685	1.129	0.862	1.06
6	1.066	0.962	1.293	0.954	1.303	2.643	1.491	1.491	1.643	2.838	2.643	2.447
8	0.985	0.654	0.674	0.709	1.734	1.344	2.156	1.894	2.092	1.836	3.314	2.38
10	2.304	1.429	1.454	1.158	3.064	3.471	2.613	2.686	1.512	3.192	2.467	2.802
11	1.345	1.792	1.412	1.579	5.278	2.371	2.095	2.382	2.5	2.836	2.565	2.2
14	0.202	0.902	1.425	1.366	1.326	2.412	3.616	2.942	0.558	1.666	3.503	3.174
15	1.13	1.284	0.661	0.705	1.768	1.528	0.792	0.891	1.514	1.665	0.614	1.075
16	1.041	0.965	0.928	0.967	0.663	0.942	3.137	0.921	1.303	1.316	2.38	1.571
20	1.118	0.778	0.864	0.691	1.361	2.039	1.783	1.083	1.022	2.296	0.93	1.385
21	1.089	1.479	1.226	0.977	1.909	2.966	2.562	2.434	2.467	3.015	2.625	2.368
22	0.77	0.343	0.524	0.594	0.664	1.253	0.78	0.776	0.664	1.498	0.526	0.916
23	0.623	0.575	0.703	0.87	4.218	1.56	1.964	2.387	2.21	1.199	1.433	2.237
24	0.961	0.856	0.885	1.125	3.103	2.395	2.632	1.574	3.944	3.469	2.542	2.569
25	0.797	1.89	1.003	1.463	0.72	2.582	2.802	3.421	0.895	1.836	1.502	1.854

Conditions: monotic single (MS), monotic multiple (MM), dichotic multiple (DM).

Study A

Appendix D: Sweeps-to-criteria by condition and subject

Appendix D: Sweeps-to-criteria by condition and subject

N	MS	MM	DM (Test ear)	DM (Both ears)
1	64	11	20	20
2	15	41	15	15
6	15	28	8	17
8	22	5	9	15
10	16	1	3	11
11	15	7	30	30
14	14	7	38	38
15	18	3	23	23
16	16	18	11	11
20	15	16	29	29
21	4	14	3	4
22	7	3	19	19
23	41	14	38	40
24	32	5	4	18
25	7	11	42	42

MS = Sweeps-to-criteria in the MS condition was obtained by summing the number of sweeps required to meet stopping criteria for each carrier frequency.

MM = The sweep number representing the response that took the longest to meet stopping criteria in the "test ear" when four frequencies were presented simultaneously.

DM = The sweep number representing the response that took the longest to meet stopping criteria in the test ear(DM test ear) and non-test ear (DM both ears) when eight frequencies were presented simultaneously to two ears.

Study B

Appendix E: 500-Hz threshold (dB SPL) by condition and subject

Appendix E: 500-Hz threshold (dB SPL) by condition and subject

N	MS	DM (Test ear)
1	45	60
2	40	45
3	60	60
4	60	70
6	50	45
7	55	70
8	55	50
9	60	75
10	50	50
12	35	35
13	65	60
15	40	45
17	55	50
19	55	55

Study B

Appendix F: 500-Hz corrected amplitude (nV) at 60 dB by condition and subject

Appendix F: 500-Hz corrected amplitude (nV) at 60 dB by condition and subject

N	MS	DM (Test ear)
1	22.5	41.9
2	37.8	31.6
3	35.1	26.2
4	21.6	2.0
6	29.7	20.7
7	41.1	3.1
8	17.4	40.0
9	24.6	11.4
10	31.1	14.2
13	56.2	49.3
15	30.6	23.2
17	45.9	20.1
19	23.2	34.8

Study B

Appendix G: 500-Hz EEG noise (nV) at 60 dB (5 sweeps) by condition and subject

Appendix G: 500-Hz EEG noise (nV) at 60 dB (5 sweeps) by condition and subject

N	MS	DM (Test ear)
1	29	56
2	19	19
3	24	25
4	86	71
6	38	52
7	24	86
8	41	85
9	33	38
10	15	12
13	27	103
15	20	20
17	41	31
19	33	94

Study B

Appendix H: Sweeps-to-criteria at 60 dB by condition and subject

Appendix H: Sweeps-to-criteria at 60 dB by condition and subject

N	MS	DM (Test ear)
1	9	8
2	1	3
3	4	5
4	13	17
6	2	27
7	1	27
8	19	12
9	10	16
10	3	6
13	9	13
15	1	2
17	5	13
19	17	13

Appendix I: Behavioural Research Ethics Board (BREB) approval

The University of British Columbia
Office of Research Services and Administration
Behavioural Research Ethics Board

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
Stapells, D.R.	Audiology & Speech Sciences	B06-0672

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

UBC Campus ,

CO-INVESTIGATORS:

Hatton, Jennifer L., Audiology & Speech Sciences

SPONSORING AGENCIES

Unfunded Research

TITLE :

Efficiency of the Single- versus Multiple-Stimulus Auditory Steady-State Response in Infants: Amplitude, Time to Significance and Threshold

APPROVAL DATE	TERM (YEARS)	DOCUMENTS INCLUDED IN THIS APPROVAL:
SEP 14 2006	1	Aug. 13, 2006, Advertisement / Consent form

CERTIFICATION:

The application for ethical review of the above-named project has been reviewed and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

Approved on behalf of the Behavioural Research Ethics Board
by one of the following:
Dr. Peter Suedfeld, Chair,
Dr. Jim Rupert, Associate Chair
Dr. Arminee Kazanjian, Associate Chair
Dr. M. Judith Lynam, Associate Chair

This Certificate of Approval is valid for the above term provided there is no change in the experimental procedures