

Developmental changes in refractoriness of the cortical auditory evoked potential

Phillip M. Gilley^{a,*}, Anu Sharma^a, Michael Dorman^b, Kathryn Martin^a

^aCallier Advanced Hearing Research Center, School of Behavioral and Brain Sciences,
University of Texas at Dallas, 1966 Inwood Road, Dallas, TX 75235, USA

^bDepartment of Speech and Hearing Science, Arizona State University, Tempe, AZ 85287, USA

Accepted 16 September 2004
Available online 5 November 2004

Abstract

Objective: This study examined morphological changes in the cortical auditory evoked potential (CAEP) waveform as a function of varying stimulation rate. Stimuli were presented in a paradigm which indirectly assesses the refractory properties of the underlying neuronal generators.

Methods: CAEPs were recorded in 50 normal-hearing children (3–12 years) and 10 young adults (24–26 years). A speech sound was presented in a stimulus train with sequentially decreasing inter-stimulus intervals (ISIs) of 2000, 1000, 560, and 360 ms. Latencies and amplitudes of the P1, N1, and P2 components at the Cz electrode were examined as a function of stimulus rate and age.

Results: Results revealed significant changes in the CAEP as a function of age and stimulation rate. At younger ages the N1–P2 component was elicited only at the slowest stimulation rates, and was more clearly apparent at successively faster stimulation rates as age increased.

Conclusions: We have described a stimulus paradigm that allows examination of the development of refractoriness by highlighting the interaction between age and rate on CAEP morphology.

Significance: Complex maturational patterns of CAEP components are best understood when the effects of both age and stimulus rate on the CAEP waveform are considered.

© 2004 International Federation of Clinical Neurophysiology. Published by Elsevier Ireland Ltd. All rights reserved.

Keywords: Refractoriness; Development; Auditory evoked potentials; Central auditory system; Stimulation rate

1. Introduction

In a series of papers, we have investigated aspects of maturation of the human central auditory pathways (Sharma et al., 1997, 2002a–c). Our measures of central auditory system maturation are the age-related changes in the morphology, latency, and amplitude of the P1, N1, and P2 components of the cortical auditory evoked potential (CAEP). P1, N1, and P2 are obligatory components of the CAEP that are generated with input from auditory thalamo-cortical and cortico-cortical pathways, primary auditory

cortex, and various association cortices (Ceponiene et al., 1998; Naatanen and Picton, 1987; Ponton et al., 2002).

Several studies have examined the development of the P1, N1, P2 CAEP components with widely varying results (Albrecht et al., 2000; Ceponiene et al., 2002; Eggermont and Ponton, 2003; Musiek et al., 1988; Ponton et al., 1996b, 2000, 2002; Sharma et al., 1997; Surwillo, 1981). For example, the development of P1 latency has been shown to vary anywhere from 14 to 26 years and beyond (Eggermont, 1988; Ponton et al., 1996b, 2000; Sharma et al., 1997, 2002a). Similarly, the age of first appearance of the N1 and P2 components in young children is debated with some authors noting that it first appears around 3–8 years, while others have suggested that the N1 component is absent in young children

* Corresponding author. Tel.: +1 214 905 3185; fax: +1 214 905 3146.
E-mail address: pgilley@utdallas.edu (P.M. Gilley).

(Ceponiene et al., 1998; Pang and Taylor, 2000; Sharma et al., 1997; Tonnquist-Uhlen et al., 2003).

It is likely that variations in stimulation rate underpin some of the differences in outcome. Only a handful of studies have examined the development of the P1, N1, and P2 responses in childhood as a function of stimulation rate (Ceponiene et al., 1998, 2002; Surwillo, 1981; Wible et al., 2002). Surwillo reported that a systematic decrease in the latency of the N1 component occurred with an increase in inter-stimulus interval (ISI) (from 250 to 1000 ms) for children aged 9–13 years, but not for adults. Surwillo suggested that the refractory properties of the underlying neural components involved in the N1 response may not be fully developed in children since cortical processing of stimuli at faster rates revealed a less robust CAEP response in children.

Ceponiene et al. (1998) examined CAEPs in 7–9 year old children at 3 different ISIs of 1400, 700, and 350 ms. As the ISI was decreased, the latency of the P1 and N1 increased. Interestingly, the authors observed that the N1 component was not present at the fastest stimulation rate (350 ms). The N1 component began to emerge as the ISI was slowed from 350 to 700 ms, and was more robust at the slowest rate (1400 ms ISI). Based on this finding, the authors suggested that the indiscernible N1 response at rapid stimulation rates indicates that the neural generators of the N1 response undergo significant developmental changes in refractoriness in early childhood.

As described by Naatanen and Picton (1987), the N1 wave of the CAEP has at least 3 distinct generators giving rise to 3 obligatory components. Component 1 of the N1 wave is thought to be most sensitive to amplitude changes as a result of differences in stimulation rate, particularly when the ISI is relatively short (Naatanen and Picton, 1987). Components 2 and 3 of the N1 wave are less likely to be affected by changes in ISI, and are thought to represent processes of attention (component 2) or an orienting response (component 3) (Naatanen and Picton, 1987). The changes in the CAEP waveform described above are likely driven by changes in refractoriness of component 1 generators.

Only a few studies in adults have attempted to differentiate the effects of refractoriness (the time needed for a neural population to recover after generating a response to a stimulus) from long-term habituation (a decrease in waveform amplitude with continuous, repeated stimulation) on CAEP waveform morphology (Budd et al., 1998; Roeser and Price, 1969; Roth et al., 1976). Roth et al. (1976) examined CAEP responses to stimuli presented in different combinations of ISIs (3, 1.5, and 0.75 s) and showed that amplitude changes were dependent only on the stimulus *immediately preceding* the stimulus used to elicit the CAEP response and not other preceding stimuli. The amplitude changes were attributed to refractoriness in the N1 and P2 components rather than to effects of long-term habituation. Habituation would have

resulted in continuous amplitude decrements over the course of all the stimuli preceding the one used to elicit the CAEP response (Roeser and Price, 1969). These results were later replicated by Budd et al. (1998) who used stimulus blocks differing in ISI and containing a randomly interleaved deviant sound. They compared the amplitude of responses immediately following the deviant sound to the responses not following the deviant. The authors reported that the changes in N1 amplitude were specific to the ISI condition and were not affected by the deviant stimulus. In their study, the authors concluded that N1 amplitude decrements reflect a refractory process from the preceding stimulus rather than habituation. Taken together, these studies suggest that a stimulation paradigm in which the ISI just preceding the stimulus used to elicit the CAEP response is varied (e.g. a ‘stimulus train’) can be used to study the effects of neuronal refractoriness on CAEP morphology separate from the effects of long-term habituation.

We examined the development of the CAEP response morphology in a paradigm which examines the effects of refractoriness separate from long-term habituation. We used a train of brief vowels [uh] presented at sequentially decreasing inter-stimulus intervals (2000, 1000, 510, and 360 ms) to elicit CAEPs. In this manner, the ISI just preceding the stimulus used to elicit the CAEP was varied to better examine the effects of refractoriness on CAEP components (Budd et al., 1998; Roth et al., 1976). Responses were recorded from Cz to highlight refractory changes in component 1 of the N1 response. Our aim was to examine the developmental pattern of changes in CAEP morphology as a function of age and stimulation rate in normal-hearing children aged 3–12 years and young adults.

2. Methods

2.1. Subjects

CAEPs were recorded in 50 normal-hearing children ranging in age from 3 to 12 years, and from 10 normal-hearing young adults ranging from 24 to 26 years of age. All subjects and parents of subjects under the age of 18 years received informed consent prior to participation in any of the experimental procedures. All procedures and protocols, including informed consent procedures used in the present study, received prior approval by the University of Texas at Dallas and its Institutional Review Board. Subjects had no reported history of neurological pathology or severe head injury, and no reported speech, language, or learning impairments. Subjects were divided into 6 groups based on age. Table 1 provides a summary of these age groups.

2.2. Stimulus paradigm

Cortical auditory evoked responses were recorded in response to a natural speech syllable [uh]. The duration of

Table 1
Summary of subjects per age group

Age group	Mean age	Age range	No. of M/F	Total <i>N</i>
3–4	3.81	3.2–4.9	7/3	10
5–6	6.07	5.1–6.8	4/4	8
7–8	7.46	7.1–8.8	5/6	11
9–10	9.75	9.0–10.8	6/3	9
11–12	11.8	11.0–12.3	3/9	12
24–26	24.77	24.3–26.7	0/10	10

Note. No. of M/F indicates the number of males and females.

the speech sound was 23 ms. We used the vowel [uh] to elicit an N1 response without the possible confounds of a ‘double-on’ N1 response, which may result when using a consonant–vowel stimulus (Sharma and Dorman, 2000; Sharma et al., 2000). The sound was presented in a stimulus train sequence of 4 presentations with sequentially decreasing ISIs (offset-to-onset) of 2000, 1000, 560, and 360 ms preceding each presentation within the stimulus train (Fig. 1). In this manner, the ISI just preceding the stimulus eliciting the CAEP response was varied. The stimulus was delivered via a loudspeaker placed at an angle of 45° to the right of the subjects. The stimulus was presented at a constant level of 70 dB SPL measured at the head location in the sound booth.

2.3. Evoked response recording procedures

Subjects were seated comfortably in a reclining chair placed in an attenuated sound booth. Subjects watched a DVD movie or cartoon of their choice on a TV monitor placed in the sound booth. Audio levels from the TV monitor were kept below 40 dB SPL (Kraus et al., 1995). This method has been found to be an effective way of engaging young subjects without interfering with the time-locked stimulus used to elicit the CAEP (Cunningham et al., 2000; Kraus et al., 1995; Sharma et al., 1997, 2000, 2002a–c). Evoked potentials were collected using Cz as the active electrode referenced to the right mastoid. This recording montage was used in order to best represent component 1 of the N1 response (Naatanen and Picton, 1987). The ground electrode was placed on the forehead. Eye movements were monitored using a bipolar electrode montage (lateral outer canthus referenced to superior orbital).

Averaging was automatically suspended by the recording computer when eye blinks were detected. The recording window included a 5 ms pre-stimulus and 365 ms

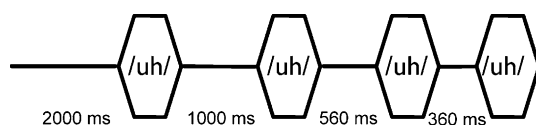


Fig. 1. Schematic representation of the stimulus paradigm. Each box represents the 23 ms speech sound [uh]. Each sound in the stimulus train is separated by a sequentially decreasing ISI (from offset to onset of the speech sound) represented by the numbers between the speech sound.

post-stimulus time. This recording window was determined to be the most efficient for displaying CAEPs from short ISIs without interference from preceding responses (Wible et al., 2002). Incoming evoked responses were analog filtered from 0.1 to 100 Hz (12 dB/octave). The recording session was stopped when the number of EEG epochs acceptable for averaging reached approximately 1200. The test session, including electrode application and evoked response recording, lasted about 45 min.

2.4. Data analysis

EEG epochs greater than $\pm 100 \mu\text{V}$ were rejected offline, and the remaining epochs were averaged according to the preceding ISI to compute an averaged waveform. Individual subjects had 4 averaged AEP waveforms, one for each ISI condition, with approximately 300 epochs per condition. We were unable to render averaged waveforms for the 1000 ms condition for two of the subjects, but did have results for the remaining 3 conditions (2000, 560, and 360 ms). Each averaged waveform was digitally bandpass filtered offline from 4 to 30 Hz (FIR, zero phase shift, 12 dB/oct, 60 ms filter width) in order to enhance detection of the CAEP components (Ceponiene et al., 2002; Kavanagh and Franks, 1989; Sharma and Dorman, 2000a). The filters used for analysis in the present study accurately represent the morphology and scalp distribution of the AEP peak components without distortion from the filtering process (Ceponiene et al., 2002; Gilley, unpublished data; Kavanagh and Franks, 1989). Peaks for each component of the CAEP were identified visually and independently by two experienced testers (authors PG and KM), and were in agreement for 96% of the identified peaks. P1 was defined as the first robust positivity in the waveform. N1 was defined as the first negativity occurring after the P1 response, and in the range of about 80–130 ms after stimulation. An additional criterion for the identification of an N1 component was the presence of a positive peak immediately following the negativity, defined as P2. The N1 and P2 peaks were not labeled if agreement between the two independent testers was not met, or if the components were not discernable from extraneous EEG noise. Within subject comparisons of the waveforms for each ISI condition were compared to differentiate peak components from possible noise. Latency and amplitude values were determined for each component (P1, N1, and P2) when present without regard to subject, age, or ISI condition.

3. Results

3.1. Statistical analysis

Latency and amplitude were treated as dependent variables in separate, partially repeated measures analyses of variance (ANOVA). In this design, age was treated as

the between-subject condition and ISI was treated as the within-subject condition. In addition, if an N1 component was present, then the N1–P2 peak-to-peak amplitude values were computed for each subject in each ISI condition.

3.2. Waveform morphology

Grand average waveforms for each age group in each ISI condition are shown in Fig. 2. For the two youngest age

groups (3–4 and 5–6 years) P1 dominates the CAEP waveform and peaks at about 100 ms in all ISI conditions. An N1 or P2 component is not seen in averaged waveforms from the two youngest age groups. However, in the 7–8 year age group a slight invagination in the waveform, labeled N1, begins to emerge in the slowest ISI condition. In the 11–12 year old group, the N1–P2 complex is apparent in all conditions, but is most robust in the slower ISI conditions. In the 24–26 year age group the N1–P2 complex is

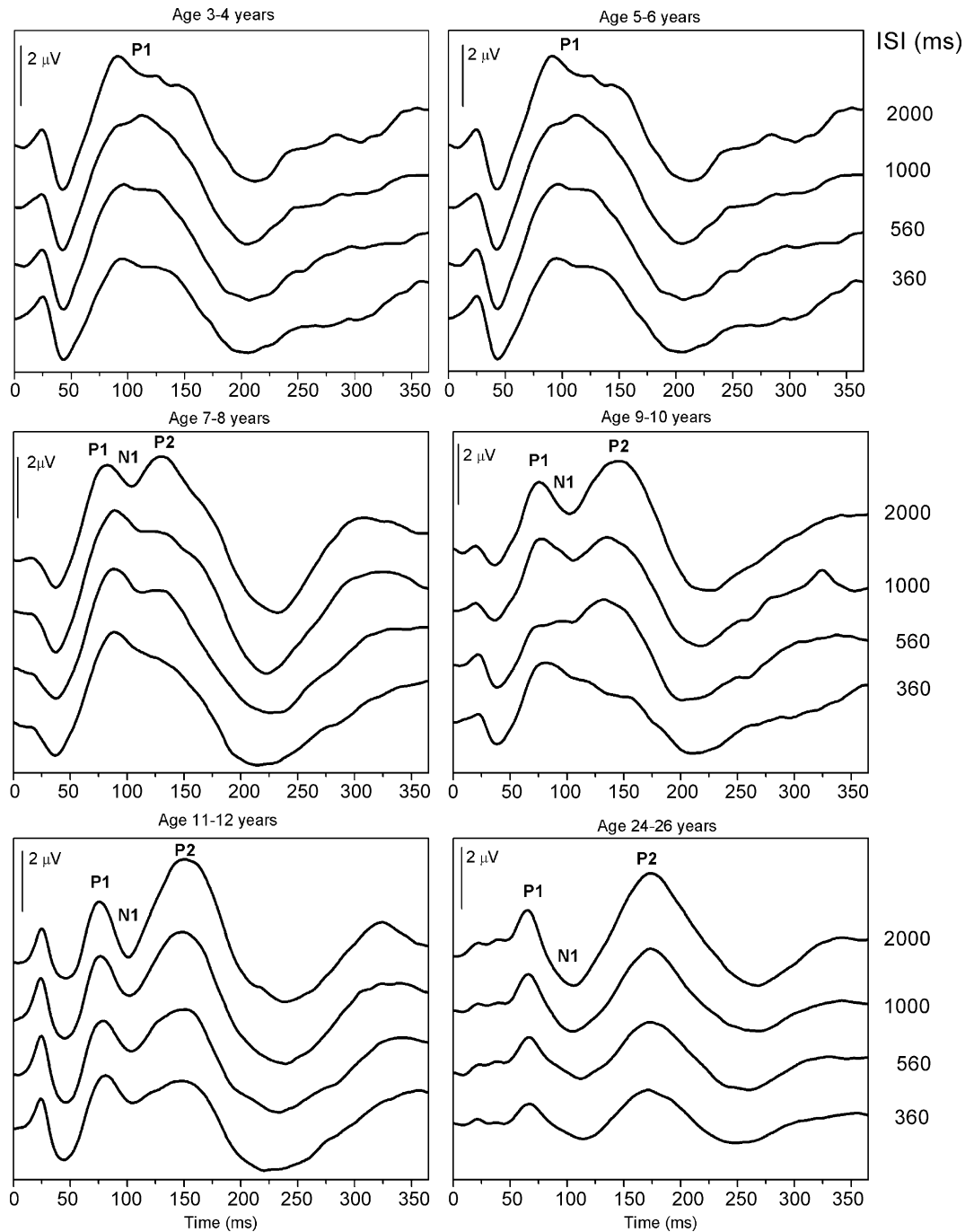


Fig. 2. Grand average auditory evoked responses for each age group and ISI condition. Grand average waveforms are shown for the 4 ISI conditions: 2000, 1000, 560, and 360 ms ISI.

Table 2
Means and standard deviations (parentheses) for latency and amplitude of CAEP components

Age (years) by condition	P1 latency	P1 amplitude	N1 latency	N1 amplitude	P2 latency	P2 amplitude	N1–P2 amplitude
<i>2000 ms ISI</i>							
3–4	96 (10)	2.6 (1.1)	119 (13)	0.9 (1.0)	145 (14)	2.6 (1.2)	0.7 (1.0)
5–6	92 (13)	3.4 (1.4)	111 (10)	1.3 (1.1)	150 (24)	2.8 (1.9)	0.6 (0.9)
7–8	93 (20)	3.5 (0.9)	114 (18)	0.6 (2.1)	142 (23)	3.3 (2.1)	1.5 (3.2)
9–10	79 (16)	1.8 (0.6)	107 (15)	0.0 (1.0)	147 (19)	2.5 (1.4)	2.7 (1.7)
11–12	76 (4)	1.9 (1.2)	106 (8)	–0.5 (1.3)	150 (15)	2.9 (0.8)	3.5 (1.6)
22–24	66 (5)	1.39 (0.7)	105 (15)	–1.9 (0.7)	176 (13)	2.8 (1.1)	4.7 (1.8)
<i>1000 ms ISI</i>							
3–4	102 (13)	2.9 (1.1)	119 (18)	1.2 (0.8)	145 (19)	2.6 (0.9)	0.4 (0.7)
5–6	107 (14)	3.4 (1.8)	137 (–)	0.2 (–)	160 (–)	0.7 (–)	0.1 (0.2)
7–8	97 (14)	3.6 (0.9)	112 (5)	1.4 (1.0)	145 (13)	2.7 (0.5)	0.4 (0.7)
9–10	91 (23)	2.4 (0.5)	109 (13)	0.4 (0.7)	145 (16)	2.1 (0.6)	1.3 (1.0)
11–12	77 (4)	1.9 (1.0)	109 (11)	0 (0.9)	148 (15)	2.4 (0.9)	2.4 (1.3)
22–24	67 (6)	1.2 (0.6)	106 (15)	–1.4 (0.6)	175 (12)	2.1 (1)	3.5 (1.4)
<i>560 ms ISI</i>							
3–4	107 (10)	2.3 (0.9)	121 (21)	0.8 (1.0)	140 (16)	1.9 (0.7)	0.2 (0.5)
5–6	99 (21)	3.2 (1.6)	106 (16)	0.6 (0.9)	138 (9)	1.9 (0.3)	0.5 (0.8)
7–8	100 (16)	3.6 (1.4)	118 (11)	1.4 (1.3)	137 (19)	1.9 (0.5)	0.3 (0.5)
9–10	89 (17)	1.6 (0.7)	102 (11)	0.4 (0.3)	135 (9)	1.9 (0.8)	1.2 (1.0)
11–12	82 (8)	1.9 (1.0)	110 (8)	0.2 (1.0)	152 (14)	2.1 (1.0)	1.6 (1.6)
22–24	66 (8)	1.1 (0.6)	108 (16)	–1 (0.7)	176 (14)	1.6 (0.8)	2.6 (1.3)
<i>360 ms ISI</i>							
3–4	106 (18)	2.0 (0.5)	115 (11)	0.7 (0.6)	138 (22)	2.3 (0.7)	0.3 (0.7)
5–6	101 (22)	2.6 (0.9)	112 (22)	0.6 (0.1)	132 (16)	1.4 (0.1)	0.2 (0.4)
7–8	95 (11)	2.8 (1.1)	121 (16)	0.7 (0.7)	149 (13)	1.6 (1.1)	0.3 (0.6)
9–10	90 (17)	2.1 (0.9)	114 (23)	0.0 (0.9)	154 (27)	1.2 (0.7)	0.8 (1.0)
11–12	82 (8)	1.9 (1.1)	113 (18)	0.1 (0.4)	150 (16)	1.9 (0.9)	1.0 (1.1)
22–24	65 (11)	0.9 (0.4)	107 (18)	–0.9 (0.6)	182 (21)	1.5 (0.8)	2.4 (1.1)

Note: (–) indicates no standard deviation due to only one data point for the condition.

the dominant waveform in all ISI conditions. Means and standard deviations for latency and amplitude for each age group and ISI condition are provided in Table 2.

3.3. P1

P1 was detected in all individuals and in all ISI conditions. In the youngest age group (3–4 years) P1 appeared at about 95 ms in the 2000 ms ISI condition and increased in latency to 105 ms in the 360 ms ISI condition. P1 latency decreased to 75 ms in the oldest group of children (11–12 years) for the 2000 ms ISI condition and to 82 ms for the 360 ms ISI condition. The adult group showed stable P1 latencies at 66 ms for all ISI conditions.

The amplitude of P1 in the youngest age group was about 2.6 μ V from baseline for the 2000 ms ISI condition and 2.01 μ V for the 360 ms ISI condition. P1 amplitude in the adult group was about 1.4 μ V for the 2000 ms ISI condition and about 0.9 μ V for the 360 ms ISI condition.

The ANOVA indicated a main effect of age for P1 latency [$F(5,52)=17.07$, $P<0.0001$] and for P1 absolute amplitude [$F(5,52)=9.04$, $P<0.0001$]. An ANOVA also indicated a main effect of ISI for P1 latency [$F(3,153)=5.7$, $P=0.001$]. The interaction between age and ISI for P1 latency was not significant [$F(15,153)=0.94$, $P=0.527$]. The absence of an

interaction between age and ISI was due, most likely, to the slow change in morphology across the 3 younger age groups. Therefore, a post hoc analysis using pair wise comparisons (detailed below) were performed to better understand the developmental trends. There was also a main effect of ISI for P1 absolute amplitude [$F(3,153)=8.62$, $P<0.0001$] and an interaction between age and ISI [$F(15,153)=1.7$, $P=0.05$].

A post hoc analysis of all possible pair wise comparisons (Bonferroni correction for multiple comparisons) between age groups and ISI conditions was used to assess the interactions of age and ISI on waveform morphology. The results of this analysis revealed no significant differences in P1 latency between the two youngest age groups (3–4 and 5–6 years) for any of the ISI conditions. However, there were small differences in P1 amplitude between the two youngest age groups. P1 latency began to show minor changes between the slow 2000 ms ISI condition in the 7–8 year age group and the faster 360 ms ISI condition in the two youngest age groups ($P<0.01$). The 9–10 year age group shows the first sign of major differences in P1 latency and amplitude between the 3 younger age groups (3–4, 5–6, and 7–8 years) with large differences between the two slower ISI conditions of 2000 ms and 1000 ms and all conditions from the younger age groups ($P<0.0001$). The oldest age group of children (11–12 years) showed

significant differences in both P1 latency and P1 amplitude for all ISI conditions relative to all other conditions from the younger age groups with the exception of the 2000 ms condition in the 9–10 year age group ($P < 0.0001$). The adult group showed significant differences for P1 latency and P1 amplitude compared to all of the child age groups ($P < 0.0001$).

3.4. N1/P2

Fig. 3A shows the percent detectability of the N1–P2 complex for all age groups and ISI conditions. Most generally, detectability increased with age and longer ISIs. For the 3–4 year old group, an N1/P2 complex was seen in 40% of the children at the longest ISI. The complex was seen in 100% of the 11–12 year olds at the longest ISI. At the shortest ISI for the 3–4 year old group, an N1/P2 complex was seen in 20% of the children. The complex was seen in 63% of the 11–12 year olds at the shortest ISI. The complex was present in all of the adult subjects for all ISI conditions.

When present in the younger subjects, N1 appeared as a very small negative deflection at about 120 ms. P1 appeared as a positive deflection at about 145 ms. Both responses were embedded within a broad positivity. N1 latencies decreased to 105 ms in both the oldest group of children (11–12 years) and the adult group. N1 latencies in these two oldest age groups remained stable between ISI conditions. With increasing age, P2 latencies increased to about 150 ms for the 11–12 year old group and to 176 ms for the adult group.

ANOVA results revealed no significant effect of age or ISI for N1 latency [$F(5,38) = 0.89$, $P = 0.500$ and $F(15,79) = 1.59$, $P = 0.197$, respectively]. Results for N1 absolute amplitude show only a main effect of age [$F(5,38) = 9.59$,

$P < 0.0001$], but no effect of ISI. Post hoc analysis of all possible pair wise comparisons (Bonferroni correction for multiple comparisons) reveals that the only large, significant differences for N1 latency and N1 absolute amplitude, and for P2 latency and P2 absolute amplitude are found between the adult age group and all other age groups ($P < 0.0001$). However, these results should be interpreted very carefully as they may be influenced by the small percentage of subjects in the younger age groups for which an N1–P2 component could be identified, or was considered present.

Because we were specifically interested in the refractory properties of the CAEP, we measured combined N1–P2 peak-to-peak amplitudes, which is considered a stable indication of change in the magnitude of CAEP response components evoked by trains of stimuli (Prosser et al., 1981). Upon visual inspection of the individual data, it seemed apparent that the relative amplitudes of the N1 and P2 components were affected by the various ISI conditions. When present, there appeared to be a clear decrease in the combined N1–P2 peak-to-peak amplitudes as the stimulation rate increased from the 2000 ms ISI condition to the 360 ms condition. Fig. 4B shows means and standard errors for N1–P2 peak-to-peak amplitude. As can be seen in Fig. 4, there is virtually no change in peak-to-peak amplitude for the two youngest age groups between ISI conditions. However, the N1–P2 peak-to-peak amplitudes show a more rapid increase in the slowest ISI condition as age increases.

ANOVA results for N1–P2 peak-to-peak amplitude revealed a clear effect of age [$F(5,52) = 14.3$, $P < 0.0001$] as well as a clear effect of ISI [$F(3,156) = 25.55$, $P < 0.0001$]. Additionally, there is an interaction of age and ISI for N1–P2 peak-to-peak amplitudes [$F(15,156) = 2.11$, $P = 0.0117$]. Post hoc analysis of all possible pair wise comparisons (Bonferroni correction for multiple comparisons) showed no

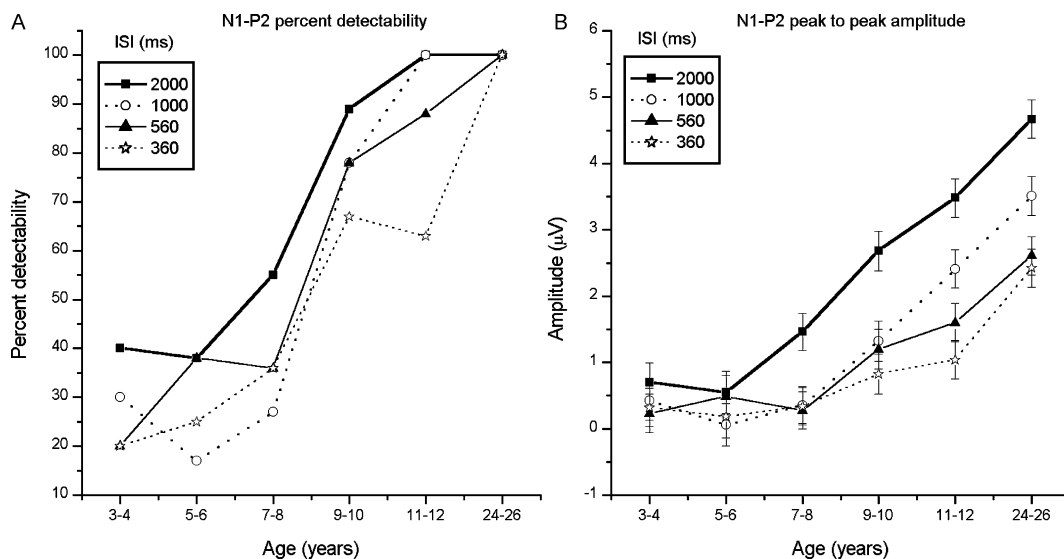


Fig. 3. (A) Percentage detectability of N1–P2 components and (B) mean N1–P2 peak-to-peak amplitudes are shown as a function of age and ISI. Error bars represent standard error.

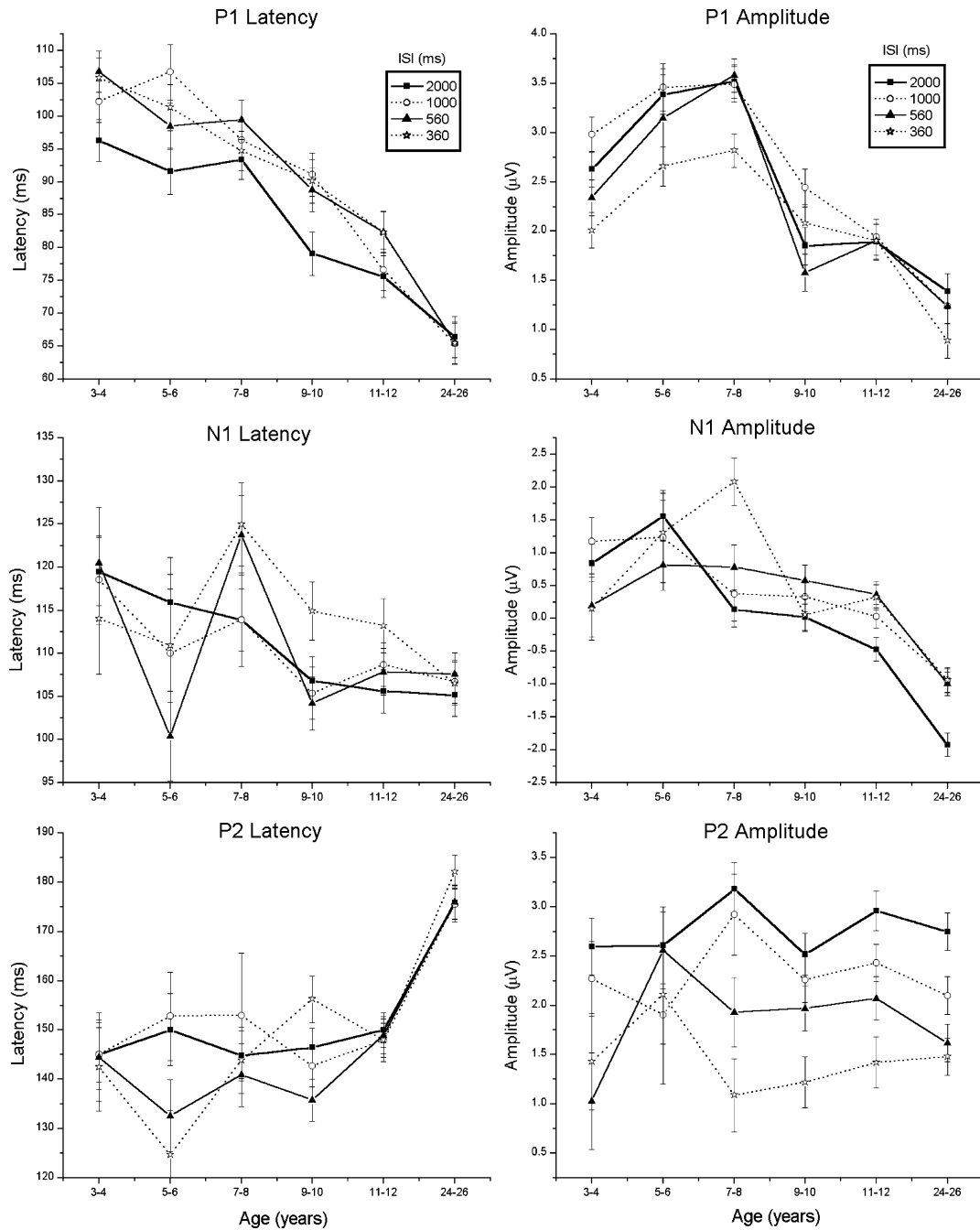


Fig. 4. Mean latencies and amplitudes for the P1, N1 and P2 components of the CAEP as a function of age and ISI. Error bars represent standard error.

significant differences in the two youngest age groups for any of the ISI conditions. The 7–8 year age group showed the first indication of a difference between the younger age groups for the slowest ISI condition of 2000 ms, although this difference was not significant. The 9–10 year age group showed significant differences for all ISI conditions compared to the 3 younger age groups, with the exception of the 2000 ms condition in the 7–8 year age group. The oldest age group (11–12 years) also showed significant differences for all ISI conditions compared to the 4 younger age groups, with the exception of the 2000 ms condition in the 9–10 year age group. The adult group showed significant differences for all

ISI conditions compared to all of the child groups, with the exception of the 2000 ms condition in the oldest child age group (11–12 years). Table 3 provides a complete summary of the pair wise comparisons for N1–P2 peak-to-peak amplitude.

4. Discussion

In the research reported here, we used a train of brief vowels presented at sequentially decreasing inter-stimulus intervals to probe the effects of stimulus rate and age on

Table 3
N1–P2 peak to peak amplitude

		2000 ms ISI						1000 ms ISI					
		24–26	11–12	9–10	7–8	5–6	3–4	24–26	11–12	9–10	7–8	5–6	3–4
3–4		***	***	**	ns	ns		***	**	ns	ns	ns	
5–6		***	***	**	ns			***	***	ns	ns		
7–8		***	**	ns				***	**	ns			
9–10		**	ns					**	ns				
11–12		ns						ns					
24–26													
		560 ms ISI						360 ms ISI					
		24–26	11–12	9–10	7–8	5–6	3–4	24–26	11–12	9–10	7–8	5–6	3–4
3–4		***	ns	ns	ns	ns		**	ns	ns	ns	ns	
5–6		**	ns	ns	ns			**	ns	ns	ns		
7–8		***	ns	ns				**	ns	ns			
9–10		ns	ns					*	ns				
11–12		ns						ns					
24–26													
Age	ISI	24–26				11–12				9–10			
		360	560	1000	2000	360	560	1000	2000	360	560	1000	2000
3–4	2000	*	**	***	***			*	***				**
	1000	**	***	***	***			**	***				**
	560	***	***	***	***			***	***				***
	360	**	***	***	***			**	***				***
5–6	2000	*	**	***	***			*	***				**
	1000	***	***	***	***			***	***				***
	560	**	**	***	***			**	***				**
	360	**	***	***	***			**	***				***
7–8	2000			**	***				**				
	1000	**	***	***	***			**	***				***
	560	***	***	***	***			***	***				***
	360	**	***	***	***			**	***				***
9–10	2000				**	*				*			
	1000			**	***				**				
	560			***	***				***				
	360		*	***	***				***				**
11–12	2000					***	**						
	1000					***							
	560			**	***				**				
	360			***	***				***				
24–26	2000	***	**										
	1000												
	560				**								

Significant post hoc analyses are shown (* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$). Data shown are symmetrical about the diagonal. Axes indicate ISI condition (ms) by age group (years).

the development of CAEP morphology. Our work is distinct from previous work that has explored development of the CAEP components, in part, because our stimulation paradigm allows us to examine the development of refractoriness of underlying neural generators separate from effects of long-term habituation (typically associated with stimulus paradigms in which the stimulation rate remains constant within a presentation block).

As shown in Fig. 2, we find distinct changes in CAEP morphology during the childhood years. CAEP waveforms in the two youngest age groups (3–4 and 5–6 years)

showed a robust positivity (P1) at all 4 stimulation rates. Many children in the youngest age groups did have clear N1/P2 responses. Low-amplitude N1 and P2 components were detected in only a few children in these two age groups. The most obvious age-related change in the morphology of the CAEP waveform is the appearance of the N1 response which bifurcates the broad, early positivity into the P1/N1/P2 components. As seen in Fig. 2 for children aged 7–8 years, the N1/P2 complex appears as an invagination of the waveform at the slowest ISI condition but not at faster stimulation rates. By age 11–12 years,

the P1, N1, and P2 components are clearly apparent for all ISIs, but the N1 and P2 components are most robust in the slowest ISI conditions. In the 24–26 year old group, the N1–P2 complex (as opposed to the P1 component) is the dominant waveform at all stimulation rates. This overall pattern of development of CAEP morphology is consistent with previous studies (Ceponiene et al., 1998; Cunningham et al., 2000; Ponton et al., 1996b, 2000; Sharma et al., 1997).

Our results provide some insight into apparent disagreements in previous findings. For example, prior studies have reported that P1 latencies reach adult-like values at ages ranging anywhere from 14 to 20 years and beyond (Cunningham et al., 2000; Ponton et al., 1996a,b; Sharma et al., 1997, 2002b). However, the stimulation rates varied substantially across those studies and, in light of the present results, which show a clear dependence of P1 latency on stimulation rate, we suggest that the exact developmental trajectory for P1 latency will be influenced by stimulation rate. We emphasize the importance of examining the interaction between stimulation rate and age-related development when charting the developmental trajectories of CAEP components.

As was the case for P1, previous studies provide conflicting reports about the detectability of the N1 response in school-aged children. For example, Sharma et al. (1997) reported N1 responses in 61% of children aged 6–7 years and 69% of children aged 10–12 years using an ISI of 510 ms. Cunningham et al. (2000) reported N1 responses in 45% of children aged 5–7 years and 55% of children aged 11–12 years using an ISI of 490 ms. We find increasing detectability of N1 with increasing age but also find a significant interaction with stimulation rate. For example, in the 360 ms ISI condition the N1–P2 was detected in 25% of children aged 5–6 years and in 65% of children aged 11–12 years. In the 560 ms ISI condition the N1–P2 was detected in 38% of children aged 5–6 years and in 85% of children aged 11–12 years. Only in the young adult group was the N1 present in 100% of the cases and did not vary as a function of stimulation rate.

It is reasonable to assume that age-related changes in myelination, synaptic refinement and cortical fiber density underlie the age-related changes in latency, amplitude and refractoriness of CAEP components that we have found (Huttenlocher and Dabholkar, 1997; Moore and Guan, 2001; Salamy, 1978). The formation of myelin along the axon increases the conduction velocity of a signal in transmission, and consequently affects the timing of subsequent signal propagation (Sabatini and Regehr, 1999; Salamy, 1978; Sanes et al., 2000). Because the latency and synchrony of the neuronal signal are affected by myelination, the evoked potentials will reveal shorter latencies, increased amplitude, and a more defined waveform morphology with maturation (Musiek et al., 1988). The amount of activity incurred from signal transmission influences the degree to which synaptogenesis occurs (Huttenlocher et al., 1997; Rakic et al., 1986). The organization of synapses is refined with

maturation of the central auditory system, and thus increases the neuronal synchrony of the generators, which underlie the CAEP components. Refinement of synaptic organization also increases the amount of neuronal information transferred resulting in a faster rise in postsynaptic potentials (Eggermont, 1988). Thus, in the immature central auditory system, incomplete myelination and synaptogenesis will lead to longer neuronal refractory periods and lower cortical excitability (Surwillo, 1981). Both factors may contribute to greater refractoriness of the CAEP.

In particular, the time line for synaptic refinement bears a broad similarity to the time line for changes in refractoriness. Huttenlocher and Dabholkar (1997) reported that after age 4 synaptic refinement begins to take place by means of synaptic elimination and is relatively complete by about 12 years of age. Synaptic refinement continues at much slower rates through adolescence and into adulthood. One explanation for our finding of an increase in refractoriness over time is that synaptic refinement increases efficiency of the synaptic mechanisms that underlie the CAEP.

Changes in axonal density in the auditory cortex also follow a time line similar to that for changes in refractoriness. Moore and Guan (2001) reported a steady increase in axonal density until about 5 years of age. After age 5, the auditory cortex begins to develop a more complex network of axons throughout the superficial layers, which reaches adult like density by about age 11 years. The axonal density at ages 11–12 years closely resembles that of young adults up to at least age 27 years. The increased axonal density would increase the efficiency of signal transmission by providing a more mature network of connections within the auditory cortical layers. As the efficiency of these timing mechanisms is increased, we would expect to see components of the CAEP recover at faster rates.

In summary, our results show that the complex maturational patterns of the CAEP components are best understood when the effects of both age and rate on waveform morphology are considered. We have described a stimulation paradigm that allows us to examine the development of refractoriness by highlighting the interaction between age and rate on CAEP morphology. Future studies should assess the value of this stimulus paradigm in diagnosing children who are at risk for abnormal central auditory development such as children with hearing impairment or children with auditory processing problems.

Acknowledgements

We gratefully acknowledge the following persons for their input and assistance during the preparation of this manuscript: Philip Loizou, James Jerger, Herve Abdi, Erin Schafer, William Cooper, Paul Dybala, and Kristi Buckley. We also wish to thank the children and their families for their enthusiastic participation in this study.

References

- Albrecht R, Suchodoletz W, Uwer R. The development of auditory evoked dipole source activity from childhood to adulthood. *Clin Neurophysiol* 2000;111(12):2268–76.
- Budd TW, Barry RJ, Gordon E, Rennie C, Michie PT. Decrement of the N1 auditory event-related potential with stimulus repetition: habituation vs. refractoriness. *Int J Psychophysiol* 1998;31(1):51–68.
- Ceponiene R, Cheour M, Naatanen R. Interstimulus interval and auditory event-related potentials in children: evidence for multiple generators. *Electroencephalogr Clin Neurophysiol* 1998;108(4):345–54.
- Ceponiene R, Rinne T, Naatanen R. Maturation of cortical sound processing as indexed by event-related potentials. *Clin Neurophysiol* 2002;113(6):870–82.
- Cunningham J, Nicol T, Zecker S, Kraus N. Speech-evoked neurophysiologic responses in children with learning problems: development and behavioral correlates of perception. *Ear Hear* 2000;21(6):554–68.
- Eggermont JJ. On the rate of maturation of sensory evoked potentials. *Electroencephalogr Clin Neurophysiol* 1988;70(4):293–305.
- Eggermont JJ, Ponton CW. Auditory-evoked potential studies of cortical maturation in normal hearing and implanted children: correlations with changes in structure and speech perception. *Acta Otolaryngologica* 2003;123(2):249–52.
- Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol* 1997;387(2):167–78.
- Kavanagh KT, Franks R. Analog and digital filtering of the brain stem auditory evoked response. *Ann Otol Rhinol Laryngol* 1989;98(7 Pt 1):508–14.
- Kraus N, McGee T, Carrell TD, Sharma A. Neurophysiologic bases of speech discrimination. *Ear Hear* 1995;16(1):19–37.
- Moore JK, Guan YL. Cytoarchitectural and axonal maturation in human auditory cortex. *J Assoc Res Otolaryngol* 2001;2(4):297–311.
- Musiek FE, Verkest SB, Gollegly KM. Effects of neuromaturation on auditory-evoked potentials. *Semin Hear* 1988;9(1):1–13.
- Naatanen R, Picton T. The N1 wave of the human electric and magnetic response to sound: a review and an analysis of the component structure. *Psychophysiology* 1987;24(4):375–425.
- Pang EW, Taylor MJ. Tracking the development of the N1 from age 3 to adulthood: an examination of speech and non-speech stimuli. *Clin Neurophysiol* 2000;111(3):388–97.
- Ponton CW, Don M, Eggermont JJ, Waring MD, Kwong B, Masuda A. Auditory system plasticity in children after long periods of complete deafness. *Neuroreport* 1996a;8(1):61–5.
- Ponton CW, Don M, Eggermont JJ, Waring MD, Masuda A. Maturation of human cortical auditory function: differences between normal-hearing children and children with cochlear implants. *Ear Hear* 1996b;17(5):430–7.
- Ponton CW, Eggermont JJ, Kwong B, Don M. Maturation of human central auditory system activity: evidence from multi-channel evoked potentials. *Clin Neurophysiol* 2000;111(2):220–36.
- Ponton CW, Eggermont JJ, Khosla D, Kwong B, Don M. Maturation of human central auditory system activity: separating auditory evoked potentials by dipole source modeling. *Clin Neurophysiol* 2002;113(3):407–20.
- Prosser S, Arslan E, Michelini S. Habituation and rate effect in the auditory cortical potentials evoked by trains of stimuli. *Arch Otorhinolaryngol* 1981;233(2):179–87.
- Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science* 1986;232(4747):232–5.
- Roeser RJ, Price LL. Effects of habituation on the auditory evoked response. *J Auditory Res* 1969;9:306–13.
- Roth WT, Krainz PL, Ford JM, Tinklenberg JR, Rothbart RM, Kopell BS. Parameters of temporal recovery of the human auditory evoked potential. *Electroencephalogr Clin Neurophysiol* 1976;40(6):623–32.
- Sabatini BL, Regehr WG. Timing of synaptic transmission. *Annu Rev Physiol* 1999;61:521–42.
- Salamy A. Commissural transmission: maturational changes in humans. *Science* 1978;200(4348):1409–11.
- Sanes DH, Reh TA, Harris WA. Development of the nervous system. San Diego, CA: Academic Press; 2000.
- Sharma A, Dorman MF. Neurophysiologic correlates of cross-language phonetic perception. *J Acoust Soc Am* 2000;107(5 Pt 1):2697–703.
- Sharma A, Kraus N, McGee TJ, Nicol TG. Developmental changes in P1 and N1 central auditory responses elicited by consonant–vowel syllables. *Electroencephalogr Clin Neurophysiol* 1997;104(6):540–5.
- Sharma A, Marsh CM, Dorman MF. Relationship between N1 evoked potential morphology and the perception of voicing. *J Acoust Soc Am* 2000;108(6):3030–5.
- Sharma A, Dorman MF, Spahr AJ. A sensitive period for the development of the central auditory system in children with cochlear implants: implications for age of implantation. *Ear Hear* 2002a;23(6):532–9.
- Sharma A, Dorman MF, Spahr A, Todd NW. Early cochlear implantation in children allows normal development of central auditory pathways. *Ann Otol Rhinol Laryngol Suppl* 2002b;189:38–41.
- Sharma A, Dorman MF, Spahr AJ. Rapid development of cortical auditory evoked potentials after early cochlear implantation. *Neuroreport* 2002c;13(10):1365–8.
- Surwillo WW. Recovery of the cortical evoked potential from auditory stimulation in children and adults. *Dev Psychobiol* 1981;14(1):1–12.
- Tonnquist-Uhlen I, Ponton CW, Eggermont JJ, Kwong B, Don M. Maturation of human central auditory system activity: the T-complex. *Clin Neurophysiol* 2003;114(4):685–701.
- Wible B, Nicol T, Kraus N. Abnormal neural encoding of repeated speech stimuli in noise in children with learning problems. *Clin Neurophysiol* 2002;113(4):485–94.