INTRODUCTION

Hearing deficits are more common among neonates in neonatal intensive care units [1] and preterm neonates [2]. Good hearing is necessary for normal language development [3]. It is recommended that all infants should undergo hearing screening by the age of one month in order to enable early rehabilitation [4]. After initial screening, the diagnosis of a hearing deficit should be conducted using a reliable method and the results should be analysed using reference values calculated for preterm infants. In addition, the stimulation method should be taken into consideration. Thus, the aim of this study was to gather reference values for the recordings of brainstem auditory evoked potential (BAEP) and brainstem audiometry (BA) performed with Tubal Insert phone (TIP) stimulation.

BAEP and BA are considered the most accurate hearing evaluation methods because they can reliably detect and dissect peripheral and central (cochlear and brainstem) abnormalities [5]. There are also automated devices, for example, an a-ABR (automated auditory brainstem response), but they only give a rough pass or fail result, and the diagnostic reliability attained with these devices is not as accurate as that of a proper clinical neurophysiological evaluation. BA is also considered the most reliable test for hearing screening in high-risk neonates [6]. Small or preterm neonates have the greatest risk of hearing loss, especially as regards central defects, thus they are the category most in need of BAEP and BA examinations. For good clinical practice, normative reference values for neonatal BAEP and BA recordings are required with a sufficiently wide age distribution, including preterm babies. In previous reference value studies, most of the infants have been full term [7] or all the material on preterm infants has been excluded from the study [8].

BAEP and BA recordings can be made using either traditional headphones or tubal insert phones (TIPs) (Figure 1). Several studies have concluded that insert phones are a useful method for delivering the stimuli [9,10,11,12]. Headphones may cause several problems in neonatal recording. Large headphones often cause restlessness in the child, which can lead to artefacts in the BAEP recordings. Headphones usually fit poorly on the child's head, which can result in leakage of the stimuli around the ear cushions [10]. In neonates especially, the pressure of the earphone can cause the ear canal to collapse [9]. With TIPs, the stimuli are delivered to the child's ear through a silicon tube and, as a consequence of this, the air conduction time prolongs the BAEP latencies. This simultaneously diminishes the effect of stimulation artefacts [11] that often obscure or distort the early BAEP waveforms I and II, when traditional headphones are used.

Because TIPs give a better definition of BAEP components I and II, and are more comfortable to use in small neonates (Figure 1), the application of TIPs instead of headphones for neonatal BAEP and BA recordings was started 2002 in Turku University Hospital. However, specific reference values for neonatal BAEP or BA recordings with TIPs have not been published, although they are a prerequisite for achieving the full clinical benefit of the improved quality of the BAEP and BA responses. This is especially the case regarding the analysis of the function of the peripheral part of the auditory pathways reflected in BAEP components I and II. The aim of this study was to calculate novel and accurate BAEP and BA reference values specifically for TIP stimulation technique in a large group of neonates consisting mainly of preterm infants.

MATERIALS AND METHODS

Participants

At Turku University Hospital, 263 BAEP recordings were made in the department of clinical neurophysiology of infants born between 2002 and 2006 with tubal insert phones. This retrospective cross-sectional study was approved by the Hospital District of Southwest Finland Ethics Review Committee in 2010; as this was a register study, an undersigned consent from the parents was not required by the Committee.

For the calculation of the reference values, we included recordings of 177 neonates who had bilaterally repeatable BAEP and BA responses, which were normal according to the literature reference values [13]. The infants had to show normal hearing in later controls until the age of two years when their hearing was tested with a miniature audiometer (intensity 45 dB, frequency 3-4 kHz). The parameters in this device are constant and in this test, the infant sits in the parent's lap and the examiner stands behind the parent. A sound is then produced for both ears separately from a distance of 50 cm. If the infant turns his/her head or in other ways clearly reacts to the sound, the test is considered normal. The miniature audiometer test was done in free field stimulation settings, such as a doctor's office, during the 2-year follow-up visit. In addition to hearing, the neonatologist also evaluated whether or not the children could understand small, context-bound requests, could understand or use words, and had begun to use word combinations. The formation of the reference value database is presented in Figure 2. The clinical characteristics of the infants are described in Table 1. Most neonates (82 %) were preterm (birth weight ≤1500 g or GA ≤32 weeks) and most infants (89 %) also underwent a BA recording, and their data were used to calculate reference values for the BA.

Procedure and equipment

BAEP and BA recordings were conducted in a quiet room by an expert in electrophysiological audiological testing at the Department of Clinical Neurophysiology. A paediatrician checked the eardrums and, if necessary, cleaned the ear canals before the recordings. All the children were either in a natural sleep condition or peacefully awake, usually after feeding. If the child was restless, a 20 % glucose solution was given orally to soothe the infant. Each ear was tested separately. The BAEPs were recorded using an eight-channel Nicolet Viking IV instrument (Nicolet Biomedical Instruments, Madison, Wisconsin, USA). The recording electrodes were placed on the mastoids, the reference electrode at the vertex (Cz) anterior to the fontanel and the ground electrode on the forehead (Fp2'). Tubal insert earphones (Nicolet model TIP 300 Ohm) were used to deliver the air-conducted stimuli to the outer auditory canal, and were selected according to the size of the canal (Figure 1). The length of the silicon tube was 153 mm.

First, the infants underwent a BAEP recording. Broadband rarefaction click stimuli were given at the intensity of 85 dB nHL (repetition rate: 10.3 Hz). Simultaneously, the non-stimulated ear received masking white noise at 45 dB nHL. The earphones were calibrated using Sound Technology Spectra Pro software, an artificial ear of type B&K TYPE 4152 and a 2 cc coupler. We used ±3 dB as the accepted tolerance range, which is in accordance with the NHSP (Newborn hearing screening programme, England) recommendation. Calibration of the equipment is done yearly according to the accredited quality control system of our laboratory and the measurements have never exceeded the acceptable limits and have remained stable over the whole period. We set the low frequency filter (LFF; high pass filter) to 150 Hz and the high frequency filter

(HFF; low pass filter) to 3 kHz. The amplifier sensitivity was 10 µV. 2000 responses were averaged at least twice. If a discernible BAEP response was not elicited, the stimulus level was raised to 95 dB nHL. After the recordings, an experienced technologist noted the peaks and the clinical neurophysiologist (SKJ) checked the peaks I, II, III, IV and V, and the troughs following peaks I and V.

After a successful BAEP recording, a BA was conducted in order to determine the click threshold of each ear separately. The recording was started at the intensity of 35 dB nHL (33.3 Hz) with a masking noise of 15 dB nHL in the non-stimulated ear. The normal procedure for BA testing in our hospital is that the intensity is increased in 10 dB steps from 35 dB, and if no clear responses are seen at the level of 65 dB nHL, the recording is stopped. In this study, we only included infants with normal hearing at 2-years of age, so the higher intensity levels (>45 dB nHL) were not needed nor applied in this reference group. The evaluation of the BA results was based on the presence of waves III and V, because wave I has a higher threshold than components III and V, and it is mostly not discernible when III and V are already well visible at lower levels of stimulation (35 dB and 45 dB nHL).

Statistical analysis

To calculate the reference values for latencies I, III, V, amplitude ratios I/V, interpeak latencies (IPLs) I-III, I-V, III-V, and interside differences for latencies and IPLs, we used SPSS 17 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, 2008) and Microsoft Office Excel 2003 software. Because the latencies were age dependent, we divided the data into two groups according to the infants' post term age at the time of the recording. Based on a visual estimation of the raw data plots, the two groups

consisted of infants ≤1.5 (n=137) and >1.5 months (n=40). The reference values were calculated for different age groups starting from -0.5 to 6 months. We found that most of the data was normally distributed. The only variables not normally distributed were the interside differences of latencies in the >1.5 months group. Using Student's t-tests, we investigated the differences in the post term recordings between boys and girls, and between preterm and infants born full term. Bonferroni correction was applied to adjust the p-values at the 0.05 alpha level for multiple comparisons. We used SPSS to make a linear regression model in order to obtain upper (for latencies and inter-peak intervals) or lower (for amplitudes) prediction limits for the determination of the reference values. Age was the only independent variable in the regression models, as gender differences were minimal or absent. Regression diagnostics were used to assess the adequacy of the model. The reference values, i.e. the upper or lower reference limits for each variable, were calculated using the equation "limit = predicted value ± 1.96 x SD". Thus, there is a 97.5 % probability that the measured latency values are longer than the upper reference limit or that the amplitude values are lower than the lower reference limit in healthy neonates.

RESULTS

Reference values for BAEP recording

In Figure 3 A, a normal BAEP recording for a preterm baby is shown. The BAEP reference values (upper limits) are shown in Table 2. The reference values and standard deviations of waves I, III and V are also presented in Figure 4. We found that latencies were post term age dependent (all $p<0.001$, except for latency III with $p=0.001$ and for IPL I-III $p=0.11$; the corresponding R-values for latencies I, III, V and for IPLs I-

V, I-III, III-V were -0.28, -0.25, -0.51, -0.43, -0.12 and -0.40, respectively). Therefore, the reference values are presented separately for 10 different age groups.

There were no statistically significant differences between boys and girls in 9 out of the 18 variables, so common reference values were calculated for both genders. In cases with a significant difference, boys usually had only 0.1 ms longer mean latencies, on average. Girls had slightly higher amplitudes and boys had a higher I/V amplitude ratio. The variables with significant gender differences after the Bonferroni correction were, on the right side, latency III ($p = 0.005$), amplitude V ($p = 0.001$), amplitude ratio I/V ($p = 0.012$), and on the left side, latency III ($p = 0.0007$), IPL I-III ($p = 0.002$), and amplitude ratio I/V ($p = 0.017$). Further, there were no differences between preterm and full term infants for any of the measured variables when the Bonferroni correction was applied to adjust the p-values for multiple comparisons. The interside differences between boys and girls or between preterm and full term babies were not found to be significant either.

Reference values for BA recording

On the right side, at an intensity of 35 dB nHL, peak III was found in 97.5 % and peak V in 98.1 % of the healthy neonates. On the left side, at an intensity of 35 dB nHL, peak III was found in 95.6 % and peak V in 99.4 % of neonates. On both sides, at an intensity of 45 dB nHL, peaks III and V were found in 100 % of neonates. The normal threshold level, i.e. the upper reference limit for the occurrence of a BA click response was thus 35 dB nHL. Figure 3 B shows a normal BA recording in a baby born preterm.

DISCUSSION

BAEP and BA recordings are objective methods for diagnosing hearing loss in infants, however, appropriate reference values taking into account the various stimulation parameters and headphones used are needed to accurately interpret and fully utilise the results. As far as we are aware, this is the first report on reference values for neonatal BAEP and BA using tubal insert phones for stimulation. Furthermore, it is one of the largest reported BAEP and BA databases predominantly including preterm infants (146 babies, 82 %).

There are several undisputable advantages when tubal insert phones are used for stimulation. TIPs give a better discernible BAEP I and II waveform, because there is less interference from the stimulation artefact. This enables the utilisation of an IPL I-V and an amplitude ratio I/V in the analysis, even in neonates. With clearly defined early waveforms I and II, it is easier to localise the defect in the auditory pathway correctly as the peripheral part of the pathway can also be analysed. With proper reference values that also take into account the amplitude and latency of BAEP component I, the TIP stimulation technique allows a precise level of diagnostics to be made from the most distal parts of the auditory pathway in cochlea to the superior colliculus, even in small-for-date preterm babies. In addition, TIPs fit better and are significantly better tolerated by the babies than traditional large headphones, which often cause restlessness leading to artefacts in the recordings, as illustrated in Figure 1.

The largest previous study so far conducted on neonatal BAEP [14] included 179 preterm and 465 term babies and they reported that in matching age groups, peak latencies were longer in the preterm group than in the full term group. Eggermont et al. 1988 [14] used standard earphones, whereas we needed reference values for BAEP

recordings performed with TIPs. The reference values obtained with TIPs can be considered more reliable because the problems with headphones (air leakage, ear canal collapse, poor suitability for the child's ear, waveform artefacts), which create additional variability in the measurements can be avoided. In addition, Eggermont et al. 1988 [14] conducted the BAEP recordings in the ICU environment of the preterm babies soon after birth; the main interest of the study being the possible developmental changes of the different BAEP components. In contrast, we gathered this new reference material for BAEP and BA for applications in the routine clinical setting of our hospital. In our study, the measurements were performed at a mean age of 1.1 months post term age. The noisy and artefact-prone ICU environment may have led to some of the discrepancies between the present study and that by Eggermont et al. 1988 [14].

As we found no significant differences between preterm and full term infants as regards most of the BAEP variables in our study, common reference values could be calculated for clinical practice. This is in line with the study of Jiang et al. 2008 [15], which showed no appreciable differences in BAEP variables for infants born at 33–36 GA and infants born at term. However, it has also been reported that very preterm infants have an advanced peripheral development but a retarded central development of the auditory pathway [16]. Due to maturational differences in the brainstem between preterm and term infants, we thus presume that it may be important to obtain reference values that are calculated from a database including a representative sample of preterm born infants.

The longer peak latencies in our study compared to earlier reports [7,8] are at least partly due to differences in the stimulation technique, as we used TIPs for stimulation

instead of headphones; this leads to slightly longer peak latencies. However, IPL should not be influenced by the type of headphones used for stimulation. Another explanation for the longer latency values is the larger proportion of preterm infants in our study compared to the earlier studies [7,8]. There have also been a few studies using insert earphones for stimulation, which have reported mean latencies and standard deviations for different BAEP components [11,17]. However, in these studies the size of the data is significantly smaller than in ours (n=34 and 86). In our study, the mean latencies for waves I, III and V were shorter compared to the study of Cornacchia et al. 1998 [11], which can be due to a slower click rate and a higher GA at the time of the recording in our study.

The majority of the children in our study also underwent a BA recording, so that we could determine the threshold level for the BA as 35 dB nHL. Previously in our hospital, the BA limit for normality had been set, as a rule of thumb, at 45 dB nHL in order to avoid false positive findings. With these novel reference values for the BAEP and BA recordings, we can increase the diagnostic sensitivity of the laboratory tests and, consequently, separate children with a higher risk from a large group of neonates.

Limitations of the study

A BAEP recording can be difficult due to restlessness or poor cooperation, which may cause artefacts in the recordings. In our group, there were only six infants whose post-term age was over four months, thus the reference values for the age group of 4 to 6 months are not as accurate as for the younger babies. The clinical hearing status at the time of the BAEP recording was not obtainable because there is no gold standard for hearing ability in infants. However, the hearing evaluations at the age of two years were

normal, when tested with a miniature audiometer, as was the production and comprehension of speech. However, it is possible that more children could have developed late onset hearing loss after the age of 2 years. In a recent study, it was found that the rate of significant hearing loss at 10.5 years of age (2.17/1000) was twice the rate found in infants in neonatal hearing screening (0.99/1000) [18]. In our data of 177 neonates, this means that one of the children could have developed a hearing loss later. Late onset hearing loss is caused, for example, by frequent ear infections, head injuries, exposure to very loud noises (www.asha.org). These reasons do not usually play a role in the neonatal period. In our study, the ears of the infants were checked before the recording and no infections were diagnosed. Thus, we concluded that this matter was not a problem in our study. Although this study has limitations, the large number of preterm infants in our database and the use of TIPs can be considered a definite advantage when interpreting both BAEP and BA recordings.

Conclusions

It has been shown that hearing screening of newborn infants is associated with benefits to language development in deaf children [19]. Early detection of hearing loss also leads to better overall development, social development, and quality of life [20]. BAEP and BA recordings offer the most sensitive method of diagnosing possible hearing defects. However, different stimulation techniques affect the quality of the recordings as well as the amplitudes and latencies of the responses. In consequence, separate reference values are required for TIPs and standard headphone stimulation techniques. Due to the differences in auditory maturation in preterm and term babies, BAEP and BA recordings should be analysed based on reference values which are specifically calculated for preterm infants. The TIP technique enables the best quality and most

reliable BAEP and BA recordings, thus facilitating a sensitive and precise level of diagnostics for disorders within the auditory pathways. The possible association between abnormalities in early BAEP and BA recordings and delayed auditory maturation, as well as delayed language development, requires more research; this can be facilitated by using these new, comprehensive, and accurate reference values for preterm babies acquired with a TIP stimulation technique optimal for preterm babies. In the future, more accurate, quantitative BAEP and BA measures will, hopefully, enhance the scientific research on auditory maturation and language development.

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CONFLICT OF INTEREST STATEMENT

None declared.

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Table 1. Clinical characteristics of the infants forming the reference group.

	Reference Value Data
	n = 177
Male/Female (n)	101/76
Mean birth weight (g) (SD)	1570 (878)
Birth weight range (g)	565-4370
Birth weight ≤1500 g (n)	120 (68 %)
Mean gestational age (weeks) (SD)	30.6 (4.4)
Gestational age range (weeks)	23-42
Gestational age ≤32 weeks at birth (n)	124 (70 %)
≤1500 g or ≤32 weeks at birth (n)	146 (82 %)
Small for gestational age (n)	53 (30 %)
Mean post-term age at the time of BAEP recordings (months) (range)	1.1 (-1.2-6.6)

Table 2. Reference values for BAEP component I, III and V latencies, inter-peak latencies , and I/V amplitude ratios calculated for each side separately as well as for the inter-side differences of these variables.

	Post-Term Age At The Time Of BAEP Recordings (months)											
	-0.5	0	0.5	1	1.5	0.5-1.5	2.0	3	4	5	6	2-6
Latency (ms), Upper Limit												
I (R)	2.4	2.4	2.3	2.3	2.3		2.2	2.2	2.2	2.2	2.2	
I (L)	2.4	2.3	2.3	2.3	2.3		2.3	2.2	2.2	2.2	2.2	
ISD I	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
III (R)	5.3	5.3	5.3	5.3	5.2		5.2	5.2	5.2	5.1	5.1	
III (L)	5.4	5.3	5.3	5.3	5.3		5.1	5.1	5.0	4.9	4.9	
ISD III	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.4
V (R)	7.8	7.7	7.6	7.5	7.4		7.4	7.3	7.2	7.1	7.0	
V (L)	7.8	7.7	7.6	7.5	7.4		7.3	7.3	7.2	7.1	7.0	
ISD V	0.4	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.5	0.5	0.5
IPL I-III (R)	3.1	3.1	3.1	3.2	3.2		3.2	3.2	3.1	3.1	3.1	
IPL I-III (L)	3.2	3.2	3.2	3.2	3.2		3.1	3.0	3.0	2.9	2.9	
ISD IPL I-III	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.4
IPL I-V (R)	5.7	5.6	5.5	5.4	5.3		5.4	5.3	5.2	5.1	5.0	
IPL I-V (L)	5.7	5.6	5.5	5.4	5.3		5.3	5.2	5.2	5.1	5.1	
ISD IPL I-V	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5
IPL III-V (R)	2.9	2.8	2.7	2.6	2.5		2.7	2.6	2.6	2.5	2.4	
IPL III-V (L)	2.8	2.8	2.7	2.6	2.5		2.5	2.5	2.5	2.5	2.5	
ISD IPL III-V	0.4	0.4	0.4	0.4	0.4	0.4	0.6	0.5	0.6	0.7	0.7	0.7

Amplitude I/V, Upper Limit

I/V (R)	2.9	3.0	3.2	3.3	3.4	3.3	3.2	3.0	2.9	2.8
I/V (L)	2.9	3.0	3.0	3.1	3.1	3.4	3.4	3.4	3.4	3.4

BAEP	Brainstem auditory evoked potential
IPL	Inter-Peak Latencies
ISD	Interside difference between right and left ear
L	Left ear
R	Right ear

FIGURE LEGENDS

Figure 1. Set-up for neonatal BAEP and BA recording performed with traditional headphones (A) and tubal insert earphones (B). Headphones and TIPs at a closer view (C). The length of the silicon tube in TIPs is 153 mm and in our department the length is monitored regularly.

Figure 2. The formation of the reference value database.

Figure 3. Normal BAEP recording showing waves I, II, III and V (A). Click stimuli was given at the intensity of 85 dB nHL. This infant's gestational age was 26+5 (birth weight 980 g) and the recording was taken at the age of one month from term age. Before component I, fast early oscillations (that may represent e.g. cochlear microphonic potentials) are visible. Normal BA recording in the same infant (B) showing clearly discernible and repeatable waveforms III and V on both sides with 35 dB nHL stimulus intensity.

Figure 4. Upper prediction limits (diamonds) and ±1 standard deviation bars for latencies of BAEP components I, III and V in ten different age groups.