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BRAINSTEM AUDITORY FUNCTION IN VERY PRETERM INFANTS

Reference values, risk factors and relation to future hearing and language outcome

Jaana Antinmaa (nee Saranto)

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To my family

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ABSTRACT

Very preterm infants have a higher risk of suffering from hearing loss, auditory neuropathy and delayed auditory maturation compared to term born infants. As a normal auditory function is the prerequisite for normal language development, any abnormalities in auditory pathway development should be detected as early as possible to enable prompt intervention. Click-evoked brainstem auditory evoked potentials (BAEP) and brainstem audiometry (BA) recordings are reliable and objective, quantitative methods for evaluation of the auditory function and the pathway from inner ear to brainstem and thus, they are commonly used in very preterm infants. However, laboratory specific reference values are needed for correct interpretation of the BAEP and BA results. Neonatal BAEP and BA methods aid in identifying preterm infants at risk for delayed language development, and in the study of possible risk factors for abnormal auditory pathway development.

The aim of this thesis was to calculate reference values for neonatal BAEP and BA recordings at the Department of Clinical Neurophysiology of Turku University Hospital. The reliability of the new reference limit for BA was evaluated against pure-tone audiometry results at the age of five years. Other aims were to study the association between neonatal BAEP and BA recordings and later language development. The harmful effects of different risk factors in neonatal intensive care unit (NICU) on the hearing of very preterm infants were analysed as well. There is a lack of information and contradictory finding in these areas of research.

The reference values for BAEP and BA recordings were determined to facilitate the interpretation of the results and they are currently in clinical use. The reference limit of 35 decibel normalized hearing level for BA can be considered reliable as it showed good sensitivity in relation to pure-tone audiometry results at the age of five years. We also found that slower auditory conduction in the neonatal BAEP recording of very preterm infants associated with poorer acquisition of receptive language at the corrected age of one year. In addition, this thesis discovered that continuous positive airway pressure (CPAP) treatment during the NICU management may have adversely influenced the developing auditory pathway in very preterm infants.

KEYWORDS: Brainstem auditory evoked potentials, brainstem audiometry, preterm infant, auditory pathway, reference values, language development, CPAP

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Kliininen neurofysiologia
JAANA ANTINMAA: Aivorungon kuuloherätevastetutkimus vastasyntyneillä – viitearvot, riskitekijät ja kuuloradan toiminnan vaikutus puheen kehitykseen
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TIIVISTELMÄ

Hyvin ennenaikaisesti syntyneillä lapsilla on suurempi riski kuuloradan toiminnan poikkeavuuksiin, kuten kuulovikaan tai kuuloradan hitaaseen kypsymiseen, verrattuna täysaikaisesti syntyneisiin. Koska normaali kuuloradan toiminta on edellytys normaalille puheen kehitykselle, kuuloradan toiminnan poikkeavuudet tulisi havaita mahdollisimman varhain. Äänespurskeita hyödyntävät aivorunko-herätevastetutkimus (BAEP) ja aivorunkoaudiometria (BA) ovat luotettavia ja objektiivisia kuulontutkimusmenetelmiä, jotka eivät vaadi potilaan ko-operaatiota ja joita voidaan käyttää kaikenikäisillä tutkittavilla. BAEP ja BA antavat tarkkaa ja objektiivista tietoa kuuloradan toiminnasta ja siksi niitä käytetään erityisesti hyvin ennenaikaisesti syntyneillä vastasyntyneillä. BAEP- ja BA-rekisteröintien tuloksien tulkintaa varten tarvitaan laboratoriokohtaiset viitearvot. Lisäksi BAEP- ja BA-tutkimuksia voidaan hyödyntää kuuloradan toimintaan vaikuttavien riskitekijöiden kartoittamisessa sekä viivästyneen puheen kehityksen ennakoinnissa.

Tämän väitöskirjatyön tavoitteena oli laskea viitearvot Kliinisen neurofysiologian osastolla tehtäville BAEP- ja BA-rekisteröinneille. Myöhemmin viitearvojen luotettavuutta arvioitiin BA menetelmän osalta suhteessa noin viisivuotiaana tehtyyn audiogrammiin. Lisäksi tavoitteena oli tutkia BAEP- ja BA-tulosten yhteyttä myöhempään puheen kehitykseen sekä kartoittaa kuuloradan kehitykseen haitallisesti vaikuttavia riskitekijöitä hyvin ennenaikaisesti syntyneillä. Erityisesti riskitekijöiden osalta aiemmat tutkimukset ovat antaneet osin ristiriitaisia tuloksia.

Viitearvot BAEP- ja BA-tutkimuksille määriteltiin, jotta tulosten tulkinta helpottuisi. Viitearvot ovat tällä hetkellä kliinisessä käytössä Turun yliopistollisessa keskussairaalassa. BA-rekisteröinnin viiterajaksi laskettiin 35 desibeliä, mikä osoittautui myöhemmissä analyyseissä luotettavaksi vastaten viisivuotiaana tehdyn audiogrammin tuloksia. Lisäksi havaitsimme ennenaikaisesti syntyneillä, että viivästyneet kuuloherätevasteet vastasyntyneisyyskaudella ovat yhteydessä niukempaan ymmärrettyjen sanojen määrään vuoden korjatussa iässä. Riskitekijöitä tutkittaessa ylipainehengityshoito (CPAP) ennenaikaisesti syntyneillä näytti olevan yhteydessä hitaampaan informaation kulkuun aivorungon kuuloradastossa.

AVAINSANAT: Aivorunkoherätevaste, aivorunkoaudiometria, hyvin ennen-aikaisesti syntynyt, kuulorata, viitearvot, kielen kehitys, CPAP-hoito

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Abbreviations

µmol	Micromole
µV	Microvolt
aABR	Automated auditory brainstem response
ABR	Auditory brainstem response
aOAE	Automated otoacoustic emissions
BA	Brainstem audiometry
BAEP	Brainstem auditory evoked potentials
BPD	Bronchopulmonary dysplasia
CDI	The MacArthur-Bates Communicative Development Inventories
CI	Confidence interval
cm	Centimetre
CMV	Cytomegalovirus
CPAP	Continuous positive airway pressure treatment
CRP	C-reactive protein
dB	Decibel
dB(A)	A-weighted decibel
dB nHL	Decibel normalized hearing level
DPOAE	Distortion-product otoacoustic emissions
EEG	Electroencephalogram
ELBW	Extremely low birth weight
ERP	Event-related potentials
FinCDI	The Finnish version of CDI
g	Gram
GA	Gestational age
HFNC	High-flow nasal cannulae
HL	Hearing level
Hz	Hertz
ICD	International Classification of Diseases
IPL	Interpeak latency
IQ	Intelligence quotient
JCIH	The Joint Committee on Infant Hearing

kHz	Kilohertz
l	Litre
MD	Medical doctor
min	Minute
MMN	Auditory mismatch negativity
MRI	Magnetic resonance imaging
mg	Milligram
ms	Millisecond
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
OAE	Otoacoustic emissions
OR	Odds ratio
PCA	Postconceptional age
PDA	Patent ductus arteriosus
PMA	Postmenstrual age
PTA	Pure-tone average
RDLS	The Reynell Developmental Language Scales
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SD	Standard deviation
SGA	Small for gestational age
SNHL	Sensorineural hearing loss
TEOAE	Transient-evoked otoacoustic emissions
THL	Terveyden ja hyvinvoinnin laitos
TIP	Tubal insert earphone
VLBW	Very low birth weight
WHO	The World Health Organization

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Saranto J, Lapinleimu H, Kärpijoki EL, Matomäki J, Björkqvist M, Jääskeläinen SK. Reference values for neonatal BAEP and BA recordings using tubal insert phones. *Early Hum Dev*, 2016; 103:113–118.

II Antinmaa J, Lapinleimu H, Salonen J, Stolt S, Kaljonen A, Jääskeläinen SK. Neonatal brainstem auditory function associates with early receptive language development in preterm children. *Acta Paediatr*, 2020; 109:1387–1393.

III Antinmaa J, Salonen J, Jääskeläinen SK, Kaljonen A, Lapinleimu H. Continuous positive airway pressure treatment may negatively affect auditory maturation in preterm infants. *Acta Paediatr*, 2021; 110:2976–2983.

IV Antinmaa J, Jääskeläinen SK, Salonen J., Koivisto M, Lapinleimu H. Normal neonatal brainstem audiometry in preterm infants predicted normal hearing later in childhood. *Acta Paediatr,* 2023 (Published online 3.6.2023).

1 Introduction

In very preterm infants the normal development of the auditory pathway is compromised as they are exposed to an extrauterine environment at a time when the brain and auditory pathway structures are still developing (Moore and Linthicum 2007, McMahon et al. 2012, Ortinau and Neil 2015). In consequence, very preterm infants are at great risk for an abnormal development of the auditory pathway such as hearing loss (van Dommelen et al. 2015), auditory neuropathy (Xoinis et al. 2007) and delayed auditory maturation (Stipdonk et al. 2016). Each of these conditions has been found to associate with problems in acquiring communication and language skills (Wang et al. 2020, Grey et al. 2022, Ramanathan et al. 2023). Thus, it is important to already recognise abnormal auditory function in the neonatal period to enable early treatment.

Neonatal morbidities and treatments related to prematurity can damage any part of the auditory pathway (Jiang et al. 2010, 2022, Stipdonk et al. 2016, Bureš et al. 2017, Raposo et al. 2021). In consequence, the hearing and auditory pathway function of very preterm infants should be examined with a method which gives information, not only on the inner ear, but also about the more central parts of the auditory pathway. This requirement is met by click-evoked brainstem auditory evoked potentials (BAEP) and brainstem audiometry (BA, hearing threshold estimate for click stimuli) recordings which are considered to be reliable tools for hearing screening in critically ill neonates (Suppiej et al. 2007). BA thresholds have also been found to associate well with future hearing status (Cheng et al. 2021) but previous studies only included a few very preterm infants (Baldwin and Watkin 2013). In Turku University Hospital, all very preterm infants are examined with BAEP and BA, but to accurately interpret BAEP and BA results, laboratory specific reference values are needed, and their reliability should be evaluated.

In addition to traditional hearing status assessment, BAEP and BA have been explored more widely in clinical practise and auditory pathway research. Studies have shown that BAEP and BA recordings could help to recognise preterm infants at risk for future cognitive problems as slower auditory pathway conduction indicates, for example, lower language scores later in childhood (Amin et al. 2014). Very preterm infants especially would benefit from early recognition of possible

future problems in language development, and thus, more research in this area is needed including larger populations of very preterm infants. The risk factors for abnormal auditory pathway development can also be studied in detail by utilising BAEP and BA recordings (Raposo et al. 2021, Borenstein-Levin et al. 2022). For example, it has been discovered that gentamicin and bronchopulmonary dysplasia (BPD) can slow down central auditory pathway conduction (Jiang et al. 2010, Raposo et al. 2021), but contradictory findings exist regarding many of the neonatal risk factors. In addition, there are risk factors that have been less studied for example continuous positive airway pressure treatment (CPAP), noise and closure of patent ductus arteriosus (PDA).

2 Review of the Literature

2.1 Preterm infants

2.1.1 Definitions and incidences for preterm birth

Preterm birth is usually defined based on gestational age (GA). According to the World Health Organization (WHO), an infant is preterm if the birth occurs before 37 complete gestational weeks. Preterm birth can be further divided into moderate to late preterm infants who are born between 32 and 37 gestational weeks, very preterm infants who are born between 28 and 32 weeks and extremely preterm infants who are born before 28 weeks are completed. (WHO 2022a) In addition, the following criteria based on birth weight are also used in Finland: an infant is preterm if the birth weight is under 2500 grams (g), and very preterm if birth occurs ≤32 gestational weeks or birth weight is ≤1500 g (very low birth weight, VLBW). If the birth weight is under 1000 g, infant is classified as extremely low birth weight (ELBW). (Preterm birth: Current Care Guidelines, 2011).

GA is a commonly used term in the literature, and it means the time between the first day of the last menstrual period and the day of delivery. Postmenstrual age (PMA) describes the time from the first day of the last menstrual period plus the time elapsed from birth in perinatal period. Corrected age means the time elapsed from the expected day of delivery and it is recommended for use with preterm children until the age of two years. The term postconceptional age (PCA) is not recommended but it is sometimes used in the literature. It describes the time elapsed from the conception. (Blackmon et al. 2004)

Every year worldwide, approximately 15 million infants are born preterm, and one million die due to prematurity (Cao et al. 2022, WHO 2022a). Deaths could be prevented in 75 % of cases by counselling and a comprehensive follow-up routine during pregnancy (WHO 2022a). In Finland in 2021, 49 700 children were born of which 2910 (5.9 %) were born prematurely. The proportion of preterm birth has remained stable since the 1990 varying between 5.4-6.3 % (THL 2022) which is a little lower when compared to preterm birth rate of 8.7 % in Europe and of 11.2 % in North America (Chawanpaiboon et al. 2019). Generally in high-income countries, the incidence of preterm birth is increasing, which is thought to be a result of increase

in maternal age and infertility treatments (Cao et al. 2022). At the same time, the mortality rates are decreasing due to improvements in neonatal care, such as the use of prenatal corticosteroids (Cao et al. 2022, WHO 2022b). The survival rates of extremely preterm infants can be seen to be especially improving. For example, 10.9 % of infants born at 22 gestational weeks survive although only 36 % are actively treated (Bell et al. 2022). In consequence, there is a growing population of preterm infants requiring intensive follow-up throughout the neonatal period and childhood.

2.1.2 Neonatal morbidity and treatments in preterm infants

Most immature preterm infants suffer more often from morbidities than the preterm infants born at later gestational ages. For example, 38.2 % of infants born at 22 gestational weeks and 5.3 % of infants born at 28 gestational weeks are diagnosed with severe intracranial hemorrhage. (Bell et al. 2022) In recent years due to improved care such as prenatal corticosteroids (Sweet et al. 2019, WHO 2022b), the incidence of some morbidities have decreased such as necrotizing enterocolitis (NEC), late-onset sepsis or meningitis, severe intracranial hemorrhage and retinopathy of prematurity (ROP) (Bell et al. 2022). However, the incidence of BPD has increased (Stoll et al. 2015, Bell et al. 2022) most likely due to improved survival rates of the most immature infants (Stoll et al. 2015). BPD has been found to associate with adverse neurodevelopmental outcome (Oluwole et al. 2023) and lower intelligence quotient (IQ) in extremely and very preterm infants later in childhood (Twilhaar et al. 2018).

The treatments of neonatal morbidities may also induce problems during the child´s hospital stay or later in life. For example, breathing difficulties can be treated with mechanical ventilation, CPAP treatment and high-flow nasal cannulae (HFNC), however, these methods can have harmful effects. Mechanical ventilation is sometimes necessary for the survival of the infant and in the treatment of respiratory distress syndrome (RDS) but it associates with the increased risk for BPD, pneumothorax and emphysema (Rutkowska et al. 2018, Sweet et al. 2019). Mechanical ventilation for a preterm infant has also been found to associate with an adverse neurodevelopmental outcome at the corrected age of two years (Oluwole et al. 2023) and hearing loss (Yu, Lin, et al. 2022).

To avoid BPD, CPAP treatment has been increasingly used instead of mechanical ventilation as CPAP treatment is gentler on the lungs (Sweet et al. 2019). However, CPAP treatment may also have harmful effects such as pneumothorax (Ho et al. 2020), increased middle ear pressure, otic barotrauma (McCormick et al. 2016), nasal irritation (McCoskey 2008), swallowing of air and abdominal distension (Bonner and Mainous 2008, Bjorklund et al. 2019). In addition, both CPAP devices and HFNC produce high noise intensities (Kirchner et al. 2012, Roberts et al. 2014).

In older CPAP devices high noise intensities up to 89 A-weighted decibels (dB(A)) have been measured (Kirchner et al. 2012) and thus, an effort has been made to develop devices that are quieter. In a more recent study, lower noise levels of 50-58 dB were found (Singh and Fusch 2021) but these levels are still above the recommended 45 dB limit in the neonatal intensive care unit (NICU) (Committee on Environmental Health 1997).

Several neonatal morbidities and treatments have been found to associate with unfavourable development of auditory pathway (Chapter 2.4.2.4). However, there is limited information on the possible adverse effects of CPAP treatment on hearing and auditory pathway function. Furthermore, several other neonatal morbidities and treatments show contradictory results of the effect on auditory pathway.

2.1.3 Neurodevelopmental outcomes in preterm infants

Preterm birth increases the risk of an adverse neurodevelopmental outcome which is most likely due to a too early exposure to an extrauterine environment (Ortinau and Neil 2015, Schmidt Mellado et al. 2022). Important processes of brain development such as neuronal migration, cortical folding, synaptogenesis, and the development of cells that produce myelin are occurring during the time when very and extremely preterm infants are born (Ortinau and Neil 2015). The developing brain is vulnerable to the harmful effects of hypoxia and inflammation that can lead to white matter injury (periventricular leukomalacia and hypomyelination), grey matter injury (neuronal loss in the brainstem, thalamus and cerebral cortex) (Ortinau and Neil 2015), and intra-ventricular hemorrhage (IVH) (Bassan 2009).

In consequence, preterm infants are at a great risk for a neurodevelopmental impairment including poor motor (Bell et al. 2022) and cognitive outcome (Munck et al. 2010, Bell et al. 2022), delayed language development (van Noort-van der Spek et al. 2012), abnormal visual development (Leung et al. 2018) and hearing loss (Chapter 2.4.2.1.). Preterm children are especially at risk for requiring special assistance in school than full term children (Twilhaar et al. 2017) and very preterm children can have problems in executive function and maintaining attention (Aarnoudse-Moens et al. 2009). Preterm born children consistently show worse skills in later academic performance, for example, in reading and mathematics (Twilhaar et al. 2017, McBryde et al. 2020) as well as language function (van Noort-van der Spek et al. 2012, Taskila et al. 2022). The IQ of preterm children is on average 0.86 standard deviations (SDs) lower than the IQ of full term born children at the age of 5-20 years (Twilhaar et al. 2018). The most immature infants are at a greater risk for lower cognitive scores compared to more mature infants (Bell et al. 2022). In very preterm babies, the risk factors for a poor cognitive outcome are BPD (Twilhaar et al. 2018, Oluwole et al. 2023), surgically treated NEC (Matei et al. 2020,

Lu et al. 2022), a low birth weight, Stage III-IV ROP, prolonged hospitalization (Li et al. 2022), major brain pathology (Munck et al. 2010, Li et al. 2022), low maternal education (Sentenac et al. 2021), postnatal corticosteroid treatment and intestinal perforation (Munck et al. 2010).

Although more preterm infants are surviving, than before, this has not led to an improvement in neurodevelopmental outcome (Twilhaar et al. 2018, Marlow et al. 2021). Thus, there is a need for accurate measures for predicting neurodevelopment, language development and hearing for preterm infants. Studies have tried to find methods to identify those prematurely born children that are at the greatest risk for developing neurological problems. There is evidence that the total brain volumes and the volumes of several different brain structures (the white matter, the grey matter, the hippocampus, the cerebellum and the corpus callosum) are relatively smaller in very preterm and VLBW infants compared to full term infants (de Kieviet et al. 2012). These changes persist in adolescence (de Kieviet et al. 2012) and adulthood (Kuula et al. 2022). The smaller brain volumes have been found to associate with an adverse cognitive outcome such as poorer language abilities and executive functioning (Taylor et al. 2011). In addition to brain magnetic resonance imaging (MRI), brain ultrasound, neurologic examination (Frisone et al. 2002, Setänen et al. 2014), brain metabolites (Gire et al. 2022), cerebrospinal fluid proteins (Leifsdottir et al. 2022) and neurophysiological measurements, such as electroencephalogram (EEG) and BAEP, have been studied to identify those infants at risk for neurological problems (Cox et al. 1992, Majnemer and Rosenblatt 1996, Wang et al. 2020, Nordvik et al. 2022). Normal neurologic examinations, brain MRIs and brain ultrasounds at term age have especially been shown to predict normal neurological outcome two years later (Setänen et al. 2014). In contrast to brain imaging and neurologic examinations, BAEP and BA could reveal more subtle abnormalities at a functional level. Thus, more research is still needed especially regarding the feasibility of BAEP and BA recordings with a focus on very preterm infants.

2.2 Methods for hearing screening and diagnostics in neonates

The Joint Committee on Infant Hearing (JCIH) recommends that the hearing of all infants should be screened before the age of one month, preferably before hospital discharge, and the hearing status should be confirmed before the age of three months. Possible intervention should be started before the age of six months. (The Joint Committee on Infant Hearing 2019) Children who meet this 1-3-6 month recommendation for early hearing detection show better language outcomes compared to children, who do not meet this guideline (Grey et al. 2022). After hospital discharge, the hearing should be regularly monitored in child health centres

and schools, and hearing should always be evaluated if the parents suspect a problem with their child´s hearing or there is a delay in child´s communication skills (The Joint Committee on Infant Hearing 2019).

Newborn screening programs using automated methods before the age of one month have been found to improve the age of hearing loss diagnosis by 13 months (Edmond et al. 2022). However, the early identification of hearing loss must lead to early intervention with hearing aids, cochlear implants, assistive hearing technologies and parent counselling to ensure auditory pathway development and the acquisition of spoken language (The Joint Committee on Infant Hearing 2019). Early intervention for the hearing impaired can lead to language and psychosocial development similar to normal hearing children (Moeller 2000, Stika et al. 2021).

The initial hearing screening is usually performed in hospitals after birth by using automated methods (Chapter 2.2.1.1) which are simple devices to identify the infants with hearing loss. From eight months onwards in child health centres in Finland, a miniature audiometer is used for screening (Sahlberg et al. 2011) (Chapter 2.2.1.2). However, these devices are not suitable for accurate diagnostics of a hearing loss. If abnormal hearing status is suspected or if the child has possible risk factors for hearing loss, e.g. preterm birth and NICU treatment, a more thorough hearing evaluation is needed. BAEP and BA and otoacoustic emissions (OAE) are used in non-cooperative patients such as infants to investigate the function of the auditory pathway from the inner ear to the brainstem (Chapter 2.2.2.1 and 2.2.2.2). Later, from four to five years of age when the child can cooperate, a psychoacoustic audiogram (2.2.2.3) is used to evaluate hearing thresholds at different frequencies.

2.2.1 Hearing screening

2.2.1.1 Automated methods

The automated methods are simple, non-invasive, and quick to use as the test signal production and analysis of the response are automatic (Katz 2002, Khaimook et al. 2019). Thus, these methods are suitable for hearing screening in hospitals where many infants must be examined. Both ears are analysed separately. In automated otoacoustic emission (aOAE) measurement, a stimulus is given to the infant´s ear. In the cochlea the stimulus causes vibration in the basilar membrane which moves the outer hair cells. This generates OAEs which can be recorded by microphone attached to the probe in the ear canal. (Katz 2002). Either transient-evoked OAEs (TEOAE) or distortion-product OAEs (DPOAE) can be used (The Joint Committee on Infant Hearing 2019). OAEs can detect responses only from the outer hair cells and it does not give information of the status of the inner hair cells or auditory nerves, nor of the central auditory pathways (Katz 2002, Khaimook et al. 2019).

Infants who have been treated in the NICU are at higher risk for auditory neuropathy which cannot be detected by using OAE. The JCIH recommends that these infants should be screened with automated auditory brainstem response (aABR) (The Joint Committee on Infant Hearing 2019). In aABR, a click stimulus is given creating a response in the auditory pathway which can be measured with electrodes on the scalp. This method identifies also defects that are located centrally in the auditory pathway. (Katz 2002, The Joint Committee on Infant Hearing 2019) In neonates admitted to the NICU, the sensitivity for aTEOAE is 79-100 % and for aABR 89-92 %. The specificity is 78-88 % and 71-92 %, respectively. (Suppiej et al. 2007, Khaimook et al. 2019) Both aOAE and aABR give either a pass or refer the result, which limits their usability in scientific research when the possible defect in auditory pathway needs to be localized more accurately.

2.2.1.2 Miniature audiometer

From the age of eight months on, a miniature audiometer is used in child health centres in Finland to evaluate hearing. The miniature audiometer is a device that produces a standardized frequency modulated sound at the intensity of 45 dB at 50 centimetres (cm) distance and at the frequency of 3-4 kilohertz (kHz). During the examination the child sits in the parent´s lap and the sound is given to both ears separately 50 cm behind the ear. If the child localizes the sound by for example turning the head to the direction of the sound, the test is considered normal. If the child does not localize the sound the test can be renewed at the age of nine months when the sound localization should have been developed. At this age, if the test is still abnormal, the child should be referred to a more detailed hearing evaluation. (Mäki et al. 2017)

2.2.2 Diagnostics

2.2.2.1 Brainstem auditory evoked potentials (BAEP) and brainstem audiometry (BA)

The present thesis utilises BAEP and BA to assess the function of the auditory pathway. In the literature, the term auditory brainstem response (ABR) and ABR threshold are also used (Eggermont 2019). BAEP and BA have several advantages. BAEP and BA are objective methods generating quantitative results, and reference values can be applied to interpret the findings (Eggermont and Salamy 1988, Chiappa 1997). The BAEP result accurately indicate the location of the possible defect in the auditory pathway (Chiappa 1997, Davies 2016). In addition, BAEP and BA examinations are non-invasive, painless and they do not require co-operation from the patient, which is especially useful in neonates and small children (Chiappa 1997).

The five main components of the BAEPs are elicited in the auditory system from the cochlear nerve to the brainstem up to midbrain level when an acoustic stimulus causes momentary neuronal activation (Katz 2002). This leads to electrical currents in the nervous system that travel through the tissues, and can be recorded with electrodes on the scalp (Chiappa 1997). After a single stimulus, the change in the voltage of electrical brain activity is mostly too small to be distinguished from the normal background EEG activity, and electric background noise. Thus, several hundreds or even thousands of single recordings have to be averaged, to clarify the BAEP waveform, that is time-locked to the stimulus, from random noise that is averaged towards 0 microvolts (μV) with increasing number of averaged trials. (Sininger 1993, Chiappa 1997) In addition, patients should be relaxed to avoid muscle activity that causes artifacts and excess noise in the recording. Infants are preferably examined while sleeping. (Chiappa 1997) In BAEP, the neural activity along the auditory pathway produces waveforms with a series of positive peaks and negative troughs within 10 milliseconds (ms) from the onset of acoustic stimulus (Katz 2002). Latency (ms) is the time from the beginning of the stimulus to a certain point of the composite waveform (usually a peak or a trough). Amplitude (μV) is the height of the component, measured either from baseline to peak, as usual, or between the peaks and the troughs of the BAEP waveform.

Figure 1. An infant with traditional headphones (A) and TIPs (B). Headphones and TIPs presented more closely (C). Abbreviations: tubal insert earphones (TIPs). With parents´ permission. Modified with permission from Saranto et al. 2016.

The auditory stimulus is delivered unilaterally to each ear, either through traditional headphones or tubal insert earphones (TIPs) (**Figure 1**). The TIPs are inserted into the ear canal. The volume under the TIPs is more uniform compared to the on-the-ear headphones, and there is no risk of collapse of the ear canal with the TIPs (Clemis et al. 1986). Either clicks or tonebursts can be used as an auditory stimulus (Katz 2002). Broadband click stimulus is commonly used in the clinical diagnostics, and it was also applied in the recordings of this thesis work. The polarity of the click can be positive (a condensation click), negative (a rarefaction click) or alternating polarity. To avoid misinterpretation of the results due to bone conduction

of the stimuli to the contralateral ear, the non-stimulated ear should receive a masking white noise of lower intensity. In infants, the BAEPs and BA are recorded with scalp EEG electrodes where the recording electrodes are usually placed on both mastoids, and the reference electrode is placed on the vertex, while the ground electrode is on the forehead. (Chiappa 1997)

The parameters used in auditory click stimulation varies between studies. This can make it difficult to compare different studies, as these parameters affect BAEP latencies and amplitudes. The stimulus intensity used in pediatric BAEP recordings varies from 60 decibels normalized hearing level (dB nHL) (Jiang et al. 2009) to 105 dB nHL (Chhajed et al. 2021). An intensity over 95 dB nHL is not recommended due to the risk of noise induced hearing loss (Chiappa 1997). The prefix "nHL" conventionally refers to the sound level (dB) of a signal referenced to the thresholds for a group of otologically normal hearing people. nHL is often associated with a short duration signal that has been calibrated using the peak-to-peak equivalent sound pressure level method. Short duration signals such as click stimuli or frequency specific tonebursts are used in objective hearing assessment via evoked potentials like BAEP and BA.

When click intensity is increased, the latencies decrease, and the amplitudes increase. However, as all latencies decrease similarly, there is no change in the interpeak latencies (IPL). (Chiappa 1997) The click rates vary from 10 hertz (Hz) (Chiappa 1997) to 91 Hz (Jiang et al. 2009). When the click rate is increased, the latencies increase and the amplitudes of the earliest components diminish while the component V may become more prominent, and is better discernible (Chiappa 1997, Katz 2002). Muscle activity and surrounding electrical equipment produce background noise, which must be filtered. A desired frequency range is chosen, and frequencies above and under this range are filtered away (low-pass filter and high-pass filter). (Katz 2002) In literature, the frequency range is usually 100-3000 Hz (Jiang et al. 2002, Chhajed et al. 2021).

In a BA recording, the purpose is to find an intensity level at which the response is still just detectable (Sininger 1993, Davies 2016). The threshold is determined in 5 or 10 dB nHL steps, and usually the BA threshold is the intensity level where the wave V is still recognizable (Sininger 1993, Baldwin and Watkin 2013, Ping and Jiang 2013, Cheng et al. 2021). Normal BAEP and BA recordings are shown in Figure 3 in the original publication I and an abnormal BA is shown in **Figure 2**.

One difficulty in click evoked BAEP is that the broadband click stimulus activates several regions in the cochlea. First the high-frequency regions at the base of the cochlea are activated, and later the low-frequency regions, as the stimulus travels from the basal part to the apical part of the cochlea. If there is a damage in the high- frequency (basal) region of the cochlea, then the BAEP response is elicited in the apical part of the cochlea. This leads to longer latencies, because it takes more

time for the stimulus to travel to the apex. (Katz 2002, Eggermont 2019) To avoid this problem, and to have better understanding of the configuration of the hearing loss, tonebursts may be used for stimulation (The Joint Committee on Infant Hearing 2019). However, studies have shown that click evoked BA thresholds associate with behavioural pure-tone audiometry thresholds later in life (Katz 2002, Baldwin and Watkin 2013, Cheng et al. 2021) but there are only a limited number of studies that include very preterm infants

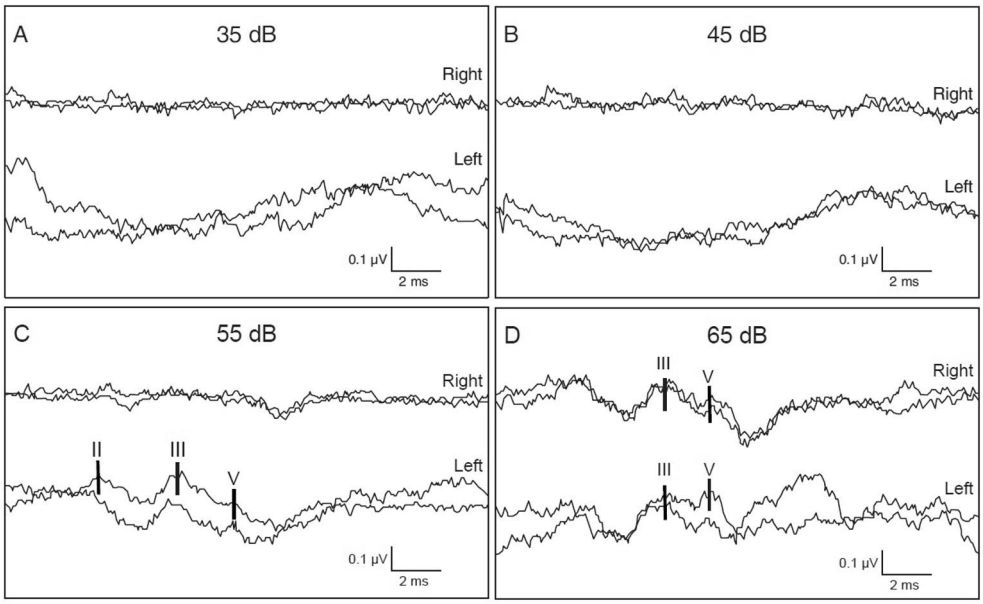


Figure 2. An abnormal BA recording. The gestational age was under 32 weeks, and the birth weight was 1300 g. **A.** The recording was started at the intensity of 35 dB nHL, at which no response could be seen. **B.** The intensity is raised to 45 dB nHL and there are still no responses. **C.** The intensity is raised to 55 dB nHL, and now the waves III and V are visible on the left. Component V is small, but repeatable. **D.** The intensity is raised to 65 dB nHL and waves III and V are now visible on both sides. In later hearing investigations, the hearing was considered normal and adequate for language development. Middle ear effusion due to a nasogastric tube is quite common reason for temporary hearing loss. Abbreviations: brainstem audiometry (BA), decibel (dB), gram (g), microvolt (µV), millisecond (ms), normalized hearing level (nHL). Retrieved and modified from patient files with patient´s permission.

The BAEP response consists of up to seven components or waves that originate from different parts of the auditory pathway (**Figure 3**). Wave I originates from the distal part of the cochlear nerve at its exit from the petrous bone. Wave II originates from the intracranial part of the cochlear nerve when the stimulus leaves the petrous bone. Wave II can sometimes be missing from normal subjects. Wave III originates from the cochlear and superior olivary nuclei in the lower pons. From the cochlear

nucleus some of the axons cross over to the contralateral side and travel via the lateral lemniscus to the colliculus inferior. The majority of the axons leaving the cochlear nucleus form synapses in the superior olivary nucleus and continue then through the lateral lemniscus to the inferior colliculus. Wave IV is thought to be generated in the lateral lemniscus. This wave can be missing or can merge with wave V. Wave V originates in the colliculus inferior bilaterally and is normally visible in both ipsi- and contralateral BAEP responses. Wave VI is thought to originate from the medial geniculate body in the thalamus. Sometimes even a wave VII can be observed, probably originating within the thalamo-cortical pathway. Waves VI and VII are not normally used in clinical diagnostics due to the wide variability in their occurrence and latencies. (Chiappa 1997, Eggermont 2019)

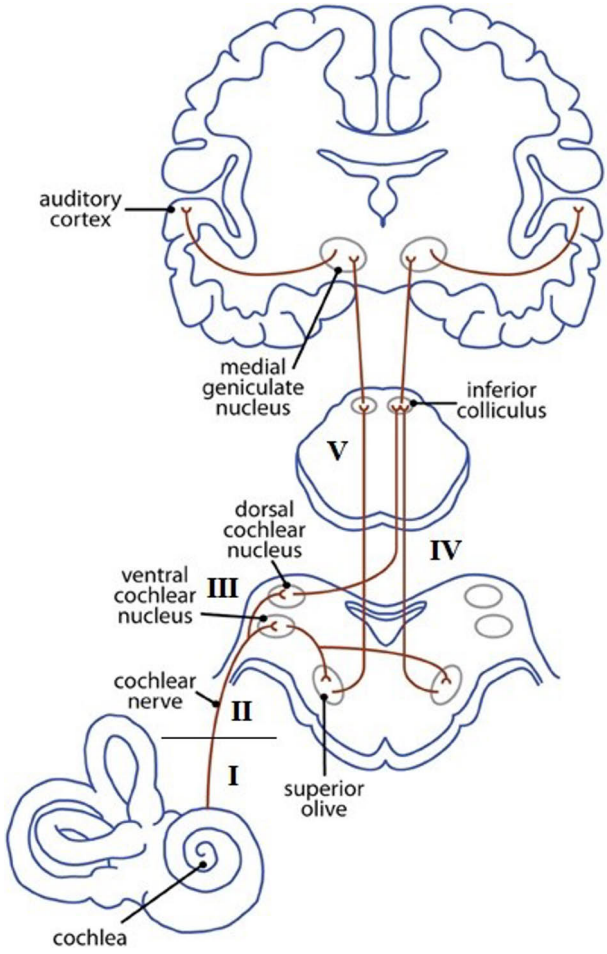


Figure 3. Auditory pathway and the origins of the BAEP responses. The line between responses I and II represents the petrous bone. Abbreviations: brainstem auditory evoked potentials (BAEP). Retrieved and modified from http://neurones.co.uk.

The most utilised BAEP variables are the latencies of BAEP components I, III and V. In addition, IPLs I-III, III-V and I-V are used in the diagnostics. IPL I-V represents the whole brainstem auditory pathway. Interval between waves I and III represents conduction in the peripheral part of the auditory pathway (between the cochlear nerve and the lower brainstem) and the interval between III and V represents the conduction in the central part of the brainstem auditory pathway (from the pons to the midbrain). (Chiappa 1997, Jiang et al. 2002) Due to the crossing of the auditory nerve fibers, contralateral responses with waves III and V are normally seen in children and adults, and can often be recorded even in the neonates and preterm babies from 30 weeks PCA (32 gestational weeks) onward (Coenraad et al. 2011).

The latencies consist of synaptic transmission time and axonal conduction time, and both are influenced by age. With increasing age synaptic efficiency improves and myelination increases conduction velocity which causes a decrease in latencies and IPLs during early childhood. (Chiappa 1997, Eggermont 2019) In consequence, the age of the children at the time of the recording should be considered in scientific research and when calculating normative values. In healthy children, the latency of BAEP component I reaches values similar to adults approximately at the age of two weeks to two months, and IPL I-V at the age of two to five years (Mochizuki et al. 1983, Jiang et al. 1991, Chiappa 1997, Coenraad, Van Immerzeel, et al. 2010). Synaptic efficacy and myelination can be disturbed by different risk factors for hearing loss (Chapter 2.4.2.4.).

BAEP can be utilised for the localization of a defect in the auditory pathway of neonates with an accuracy of 1-2 cm (Chiappa 1997). For example, in peripheral hearing loss (defect in the inner ear or in the cochlear nerve), all waves can be missing or low in amplitude or all absolute latencies may be delayed while IPLs remain normal (**Figure 4A**). In upper brainstem injuries (problem between the lower pons and the midbrain), IPL I-III remain normal while waves IV and V are delayed and IPLs III-V and I-V are prolonged, or wave V can even be missing (**Figure 4B**). (Chiappa 1997, Mervaala et al. 2018)

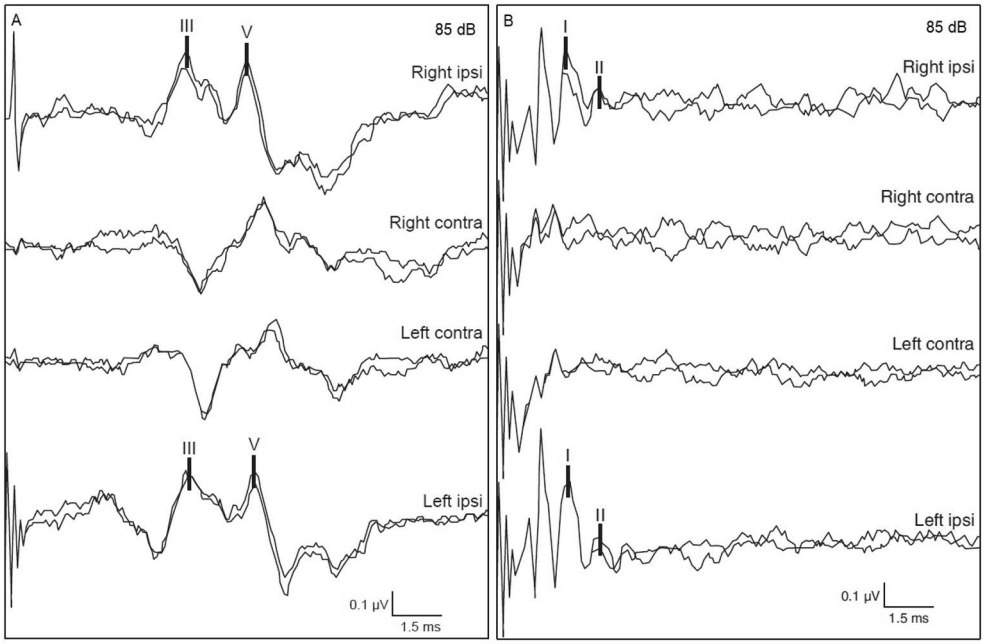


Figure 4. **A.** In this BAEP recording conducted at the intensity of 85 dB nHL, only the waves III and V are visible. In the right ear, absolute latency of BAEP component III is prolonged, and latency of BAEP component V is normal. In the left ear, latencies of BAEP components III and V are prolonged. IPL III-V is normal on both sides. The infant's gestational age was under 26 weeks (birth weight 650 g) and the recording was obtained at the corrected age of one month. In later investigations, the child had almost normal hearing in the left ear and a mild hearing loss in the right ear. The BAEP recording suggests a peripheral auditory pathway injury as BAEP waves I and II are missing and IPL III-V is normal. **B.** Here only the waves I and II are visible and the later peaks are missing which suggests a lower brainstem injury. Latency of BAEP component I is normal on both sides. The infant's gestational age was under 30 weeks (birth weight 1500 g), and the recording was taken at the corrected age of one month. Later, the child was diagnosed with severe bilateral sensorineural hearing loss and received a hearing aid for both ears before the age of six months. Abbreviations: brainstem auditory evoked potentials (BAEP), decibel (dB), gram (g), interpeak latency (IPL), microvolt (µV), millisecond (ms), normalized hearing level (nHL). Retrieved and modified from patient files with patient´s permission.

2.2.2.2 Otoacoustic emissions (OAE)

In OAE, the stimulation technique is similar to aOAE, but the results are analysed more thoroughly. During OAE measurement subjects must be still and quiet and environmental noise must be minimized. Different types of stimuli can be used to evoke OAEs. In TEOAEs, the stimulus is transient or brief and it can be a click or a toneburst. The level (amplitude) of TEOAE is evaluated. TEOAEs are not seen if the level of hearing loss is more severe than 30-35 dB HL. (Katz 2002, The Joint Committee on Infant Hearing 2019) DPOAEs are measured after two pure-tone stimuli (primaries). The interaction between these two stimuli creates responses in

the cochlea areas representing other frequencies. DPOAE responses are then evaluated and compared to the background noise. (Katz 2002)

OAE only measures the function of the outer hair cells in the inner ear. OAEs can be seen even when the cochlear nerve is damaged. In auditory neuropathy, OAEs remain normal although auditory brainstem responses are abnormal. (Katz 2002, Davies 2016, The Joint Committee on Infant Hearing 2019). The OAE responses may be abnormal if the outer hair cells are damaged due to hypoxia (Leite et al. 2016), and ototoxic medication (Vella-Brincat et al. 2011).

2.2.2.3 Psychoacoustic audiogram

The psychoacoustic audiogram or pure-tone audiometry is a subjective method used for hearing screening and diagnostics, and is the gold standard for examining hearing status in co-operating patients (The Joint Committee on Infant Hearing 2019). An audiogram is a graph presenting audiometric thresholds in dB hearing level (HL) for different frequencies (**Figure 5**). The tested frequencies are usually 250, 500, 1000, 2000, 4000 and 8000 Hz. (Davies 2016) The 0 dB HL is defined so, that it is the average of the hearing thresholds of normal hearing young adults on every measured frequency (Katz 2002). The hearing is considered normal if the threshold is 20 dB HL or less. The audiometric threshold is the lowest intensity of the pure-tone signal detected by the subject in 50 % of the time.

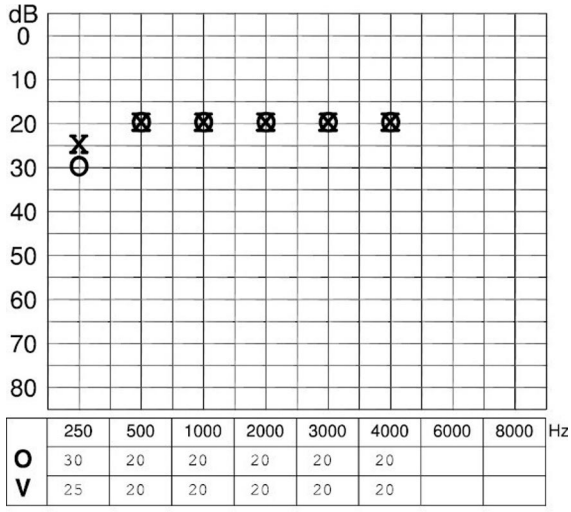


Figure 5. An audiogram of a 5-year-old child born very prematurely. In the right ear, the threshold for 250 Hz frequency was 30 dB HL and for the left ear, 25 dB HL. Thresholds at other frequencies were 20 dB HL which was the screening threshold. The audiogram was considered normal. Abbreviations: decibel (dB), gram (g), hearing level (HL), hertz (Hz), left ear (V), right ear (O). Retrieved from patient files.

Normally the signal is delivered to the subject´s ear through headphones. Masking noise is provided to the non-stimulated ear if needed to prevent the non-stimulated ear from hearing the stimuli. Both air-conduction and bone-conduction thresholds are examined if there is a hearing loss to differentiate between conductive and sensorineural hearing loss (SNHL). Different hearing impairments cause different configurations in the audiogram. Noise induced hearing loss usually causes a notch at the frequency of 4000 Hz. In serous otitis media, the bone-conduction thresholds are normal, but the thresholds in the air-conduction are increased at all frequencies. In SNHL, both air and bone conduction thresholds are increased. (Davies 2016)

2.2.2.4 Auditory mismatch negativity (MMN)

Event-related potentials (ERP) are elicited by a standard auditory stimulus creating a short-term memory trace. A typical ERP consists of two peaks (N1 and P2). When the standard stimulus is interrupted with a deviant stimulus, an additional negative component, the mismatch negativity (MMN), is formed. To create a clearer MMN, the subject´s attention is drawn away from the auditory stimulus. The MMN can, for example, be seen when native-language phonemes are suddenly replaced with deviant non-native phonemes. It has been found that memory traces for native language are present from 6 months onwards. The MMN originates from the supratemporal areas bilaterally and from the frontal areas in the right hemisphere. The MMN can be recorded in preterm infants from 33 gestational weeks onwards. (Luck and Kappenman 2012)

2.2.3 Hearing screening in Turku University Hospital

Figure 6 shows the current hearing screening protocol used in Turku University Hospital based on the study by Sahlberg et al., the hearing screening guideline by the Department of Paediatrics and the Hearing Centre at Turku University Hospital (Sahlberg et al. 2011, Korhonen et al. 2022) and personal communication (Salonen J. and Lapinleimu H, February 27, 2023). This is in concordance with the guidelines of JCIH recommending hearing screening for all newborns with an objective physiologic measure. JCIH recommends that infants should present normal responses from both ears to pass the screening. (The Joint Committee on Infant Hearing 2019) A pass in one ear has been a sufficient result according to the guidelines of Turku University Hospital. However, in the future the aim is to also identify a single sided hearing loss. Rescreening of healthy babies should be done preferably before discharge, but no later than at the age of one month for both ears

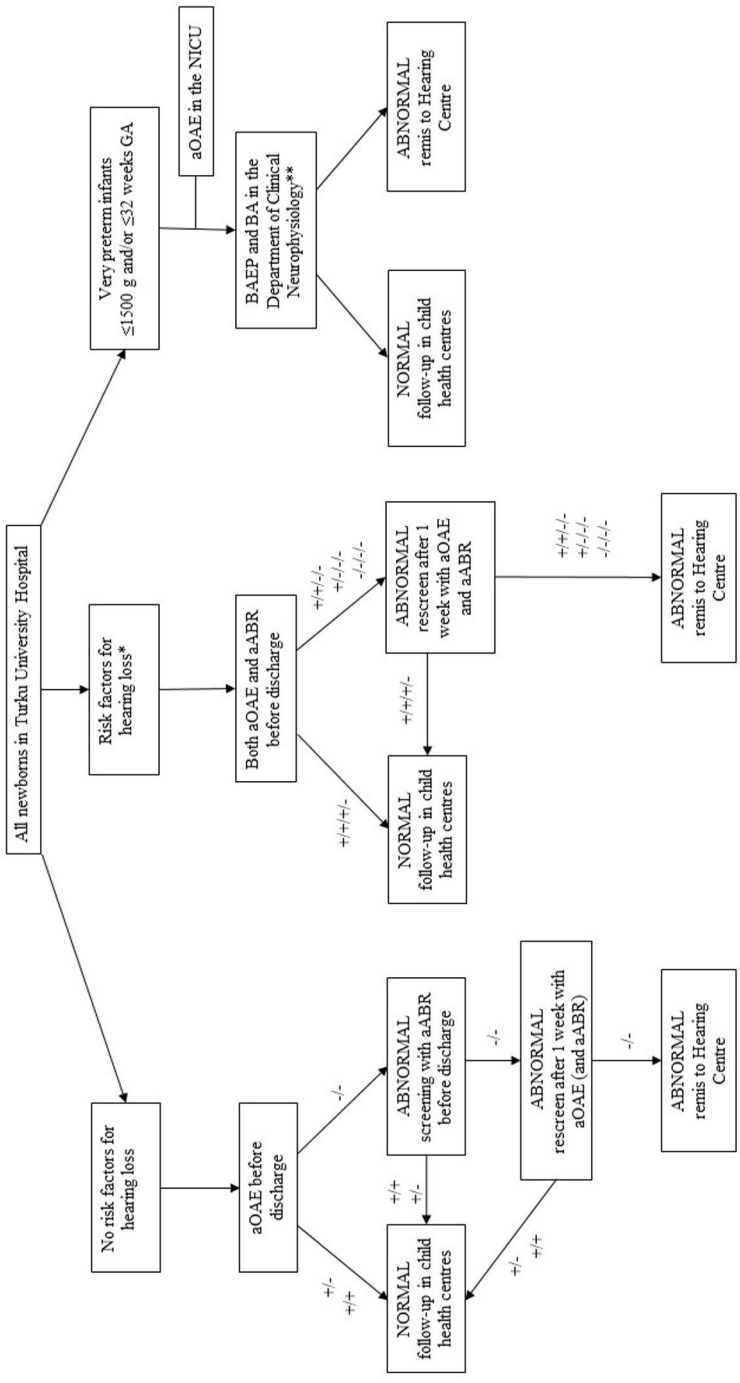


Figure 6. Hearing screening protocol used in Turku University Hospital. Plus indicates a pass screening result and minus indicates a failed result in one ear. *Family history of congenital hearing loss, craniofacial malformations, chromosomal abnormalities, long QT syndrome **If necessary BAEP is conducted in critically ill infants. Abbreviations: automated auditory brainstem response (aABR), automated otoacoustic emission (aOAE), brainstem audiometry (BA), brainstem auditory evoked potentials (BAEP), gestational age (GA), gram (g), neonatal intensive care unit (NICU). The author´s own drawing.

with an aOAE or an aABR (The Joint Committee on Infant Hearing 2019). In Turku, the aim is that the rescreening is done with both aOAE and aABR for both ears, and three of the four measurements should be normal (e.g. normal OAE in both ears and normal aABR in one ear) to pass the screening.

JCIH recommends that infants who are treated in the NICU should be screened with an aABR due to a higher risk for hearing loss and auditory neuropathy compared to healthy newborns, and the rescreening should be done by an audiologist. They conclude that screening in high risk infants with both aOAE and aABR would be the best method, but it is not officially recommended. (The Joint Committee on Infant Hearing 2019) In Turku University Hospital, infants with risk factors for hearing loss, are screened with both aOAE and aABR, and three of the four measurements should be normal. The rescreening is done after one week.

In Turku University Hospital, all very preterm children (birth weight ≤1500 g and/or birth ≤32 gestational weeks) are referred to the Department of Clinical Neurophysiology for BAEP and BA examinations (Sahlberg et al. 2011) which are considered most reliable methods for hearing screening in high risk neonates (Suppiej et al. 2007). The aim is that these children are also screened with aOAE in the NICU. JCIH does not recommend separate hearing screening protocol for preterm infants. It only recommends that the hearing of preterm infants with prolonged stay in hospital should be screened before discharge. (The Joint Committee on Infant Hearing 2019)

2.3 Reference values – calculation and application

To interpret the results of a laboratory test reference values or limits are needed. The reference values should be reliable and validated as they can influence medical decision making. To calculate the reference values a group of healthy subjects is needed. Sometimes it is necessary to divide the data into subgroups according to, for example, age and gender. Parametric, non-parametric and robust methods can be used to determine the reference values. (Henny et al. 2016) In the parametric method, the data should be normally distributed. In this method, the 95 % reference interval is used and thus, 2.5 % of the lowest or highest values are considered abnormal. The 95 % interval corresponds the same area that can be calculated by determining the values that are ±2 SD (usually 1.96) from the mean value. If the data is not normally distributed a transformation can be used. (Horn and Pesce 2003) In the non-parametric method, the data does not have to be normally distributed, but it requires a group of at least 120 subjects. The robust method is convenient when there is limited data. (Henny et al. 2016)

Reference values for latencies, IPLs, amplitudes and thresholds facilitate the accurate interpretation of BAEP and BA recordings. However, as described earlier,

several variables affect the BAEP and BA results. The variables, such as the type of headphones used, the type of equipment, filtering, the stimulation rate, the intensity, and the type of stimulus vary between different laboratories. Thus, laboratory specific reference values are recommended (Chiappa 1997).

2.4 The development of the auditory pathway

2.4.1 Normal development of the auditory pathway

Knowledge about the development of the human auditory system can improve understanding of the BAEP results and its association with language development in infants. The most important events in the development of the auditory system, language development and BAEP evolution are presented in **Figure 7.** During the first 13 weeks of fetal development, all basic structures of the auditory pathway appear. In the beginning, an otocyst emerges continuing its development into a cochlear duct and the cochlear nerve. In the cochlear duct, the organ of Corti appears during the 9th fetal week. By the end of the 8th week, all brainstem structures are already visible: the cochlear nuclei, the superior olivary complex, the lateral lemniscus, the inferior colliculus, and the medial geniculate nucleus. (Moore and Linthicum 2007)

During the second trimester, a rapid maturation of the cochlea and the cochlear nerve occurs. By the end of this period, the cochlea is almost fully developed but with only a few synapses. (Moore and Linthicum 2007) The brainstem has been shown to develop from the periphery towards the central parts (Jiang et al. 2009). At the 15 weeks the Schwann cells, that form myelin, are present in the peripheral cochlear nerve. The myelination begins within the cochlea at the 22nd week and at the 24th week the myelin sheath extends to the nerve exit at the temporal bone. (Moore and Linthicum 2007) By the 29th week, myelination can be observed in all auditory pathway structures (Moore et al. 1995). Myelination is the prerequisite for rapid neural conduction. In consequence, the first reactions to sound in fetuses have been observed at 25th week and consistent reactions at 28th week (Birnholz and Benacerraf 1983). Approximately at the 28th weeks of gestation, the first auditory brainstem responses are also seen (Starr et al. 1977). During this time, very preterm infants are exposed to an NICU environment, and they have been shown to have retarded maturation in the central parts of the brainstem (Jiang et al. 2009).

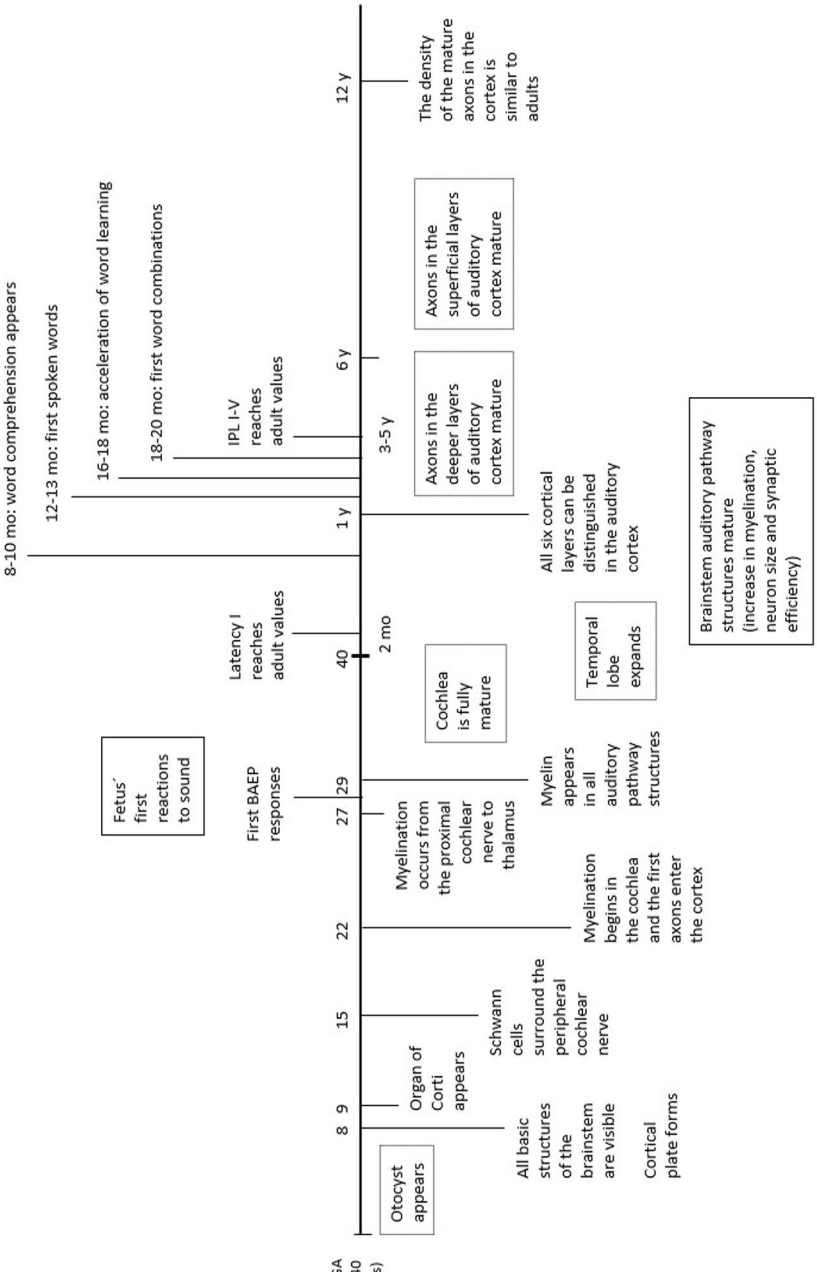


Figure 7. The development of the auditory pathway and language and the evolution of BAEP responses. Abbreviations: brainstem auditory evoked potentials (BAEP), gestational age (GA), interpeak latency (IPL), months (mo), years (y). The author's own drawing based on the articles by Starr et al. 1977, Birnholz and Benacerraf 1983, Mochizuki et al. 1983, Moore et al. 1995, Moore and Linthicum 2007, Coenraad et al. 2010.

When approaching term age, the cochlea reaches full maturity. After birth, the brainstem auditory neurons grow, and the dendrites branch. The myelin density in the auditory nerve and the axons in the brainstem continues to increase. The conduction velocity in the auditory pathway increases and BAEP latencies decrease while the myelin sheath thickens. Based on histological brainstem samples, the auditory pathway myelination continues at least until the age of one year. (Moore et al. 1995, Moore and Linthicum 2007) Based on studies utilising BAEP recordings, the maturation of the central brainstem auditory pathway continues until the age of two years (Inagaki et al. 1987) or even up to the age of five years (Mochizuki et al. 1983).

At the age of one year all six cortical layers can be distinguished, and the axons enter the deeper layers. In early childhood, the maturation and the myelination of the axons occur in the deeper layers in the auditory cortex. This development continues until the age of six years. (Moore and Linthicum 2007) Quantitative MRI studies have shown that the myelination in the language-correlated regions in the cortex (including the auditory cortex) continues into adulthood but is almost fully mature at the age of 18 months which is the time of rapid language development (Su et al. 2008).

Starting from the age of five years, the axons in the superficial layers of the auditory cortex continue to mature and by the age of 12 years, the density of the mature axons is similar to those in adults. During the same time period the intracortical connections and communication between the right and left auditory areas appear leading to more complex auditory processing skills. (Moore and Linthicum 2007)

2.4.2 Deviations from normal development of auditory pathway

Normal hearing is essential for normal acquisition of language and other cognitive skills and social development. However, the normal auditory pathway development can be compromised due to different risk factors, for example NICU treatment, asphyxia, ototoxic medication, all of which may lead to hearing loss, auditory neuropathy or delayed auditory maturation. (The Joint Committee on Infant Hearing 2019) Auditory neuropathy and delayed auditory maturation can also adversely affect language development and communication even when hearing thresholds are normal (Amin et al. 2014, Davies 2016, Wang et al. 2020).

2.4.2.1 Hearing loss

Preterm infants and infants treated in the NICU are at a higher risk for hearing loss. The prevalence of bilateral congenital permanent hearing loss is 0.8 per 1000

newborns in well-baby nurseries and 5.9 per 1000 newborns in the NICU (Butcher et al. 2019). Unilateral hearing loss is found in 0.8-2.7 per 1000 newborns (Krishnan and Van Hyfte 2016). The lower the gestational age or the weight at birth the higher is the risk for hearing loss. In a population of very preterm infants (birth before 32 gestational weeks), the prevalence of hearing loss (unilateral or bilateral) is 7.5 % if the child is born at 24 weeks of gestation and 1.2 % at 31 weeks. The prevalence of hearing loss is 4.8 % if the birth weight is <750 g, and 1.4 % if the birth weight is ≥1500 g. Very preterm neonates who are small for gestational age (SGA) are at a greater risk for hearing loss compared to appropriate for gestational age neonates. (van Dommelen et al. 2015)

Hearing loss can be sensorineural, conductive, or mixed (sensorineural and conductive). In SNHL the defect is in the cochlea or in the auditory nerve. In conductive hearing loss the problem is in the external or middle ear blocking sound transmission. (Deltenre and Van Maldergem 2013, Korver et al. 2017) Newborns can have any type of hearing loss but usually it is sensorineural (Luotonen 2008, Sahlberg et al. 2011). Hearing losses in children can also be divided into acquired and inherited (genetic) based on the etiology of hearing loss (Deltenre and Van Maldergem 2013, Korver et al. 2017). Acquired hearing loss is a result of prenatal or postnatal factors for example infections, ototoxic medication and environmental noise (Kenna 2015). Inherited hearing losses occur as a part of a syndrome or they are non-syndromic (Deltenre and Van Maldergem 2013).

After the hearing loss is confirmed, etiological examinations should be done. These include a physical examination of the child, gathering information of the child´s clinical history and family history, blood samples, radiologic studies, genetic testing and an ophthalmological evaluation. (The Joint Committee on Infant Hearing 2019). However, the etiology of congenital SNHL remains unknown in 40 % of cases (Dietz et al. 2009)

2.4.2.2 Auditory neuropathy

Hearing can be impaired because of auditory nerve dysfunction i.e., auditory neuropathy. In auditory neuropathy, OAEs or cochlear microphonics (auditory evoked potentials of the inner ear) remain normal representing normal outer hair cell function but the auditory brainstem responses are absent or abnormal. In auditory neuropathy, the defect is in the inner hair cells in the cochlea, in the synapses between inner hair cells and auditory nerve or in the auditory nerve. (Starr et al. 1996, Rance and Starr 2015). In 46 % of cases, the auditory neuropathy is caused by auditory nerve abnormalities (Liddle et al. 2022). Results in the psychoacoustic audiogram can vary from normal to severely abnormal (Starr et al. 1996, Boudewyns et al. 2016, Ramanathan et al. 2023). In child health centres, bilateral or unilateral

auditory neuropathy is found in 0.09-0.16 of 1000 newborns but in 6.5-7 % of children with congenital hearing loss (Boudewyns et al. 2016, Liddle et al. 2022). When NICU infants are included, the incidence is higher: 0.27 per 1000 of all newborns (Dowley et al. 2009). In the NICU population, 25 % of infants with congenital hearing loss present auditory neuropathy, and the smallest premature infants are at highest risk for auditory neuropathy (Xoinis et al. 2007). As preterm infants and infants treated in the NICU are at high risk for auditory neuropathy, their hearing should be screened with aABR and/or BAEP and BA. The symptoms of auditory neuropathy include difficulty in sound localization, word discrimination and understanding of speech especially in a noisy environment. Patients usually report that they can hear the speech, but they cannot understand the content of the speech. (Rance and Starr 2015, Ramanathan et al. 2023)

2.4.2.3 Delayed auditory maturation

The maturation of the auditory pathway depends on the myelination and the synaptic efficacy (Chiappa 1997) which can be disrupted by different clinical conditions. Maturation of auditory pathway can be delayed due to for example prematurity (Stipdonk et al. 2016), asphyxia (Zhang and Jiang 1992, Misra et al. 1997), chronic pulmonary disease (Jiang et al. 2006, 2010) and hyperbilirubinemia (Agrawal et al. 1998). This delay can be observed in BAEP recordings as an increase in absolute latencies and interpeak intervals (Jiang et al. 2006, Stipdonk et al. 2016) as well as increased threshold levels in BA (Zhang and Jiang 1992). The delay can appear without a hearing loss, and compared to auditory neuropathy, it disappears with increasing age (Misra et al. 1997, Stipdonk et al. 2016, Liddle et al. 2022).

2.4.2.4 Etiology and risk factors for abnormal auditory pathway development in infants

There are several possible etiologies and risk factors for deviant auditory pathway function. The associations between different risk factors and the presence of hearing loss have been studied quite extensively. In addition, it is equally important to map possible risk factors for delayed auditory maturation as it can also adversely affect cognitive development including acquisition of language skills (Cox et al. 1992, Amin et al. 2014, Wang et al. 2020). By utilising BAEP and BA recordings, the location of the defect caused by different risk factors can be localized. Studies on this area are needed as there are contradictory findings regarding many of the risk factors, and some risk factors have not yet been thoroughly investigated. (**Table 1**)

2.4.2.4.1 Risk factors affecting mainly the peripheral part of the auditory pathway

Prenatal and postnatal infections are risk factors for hearing loss (The Joint Committee on Infant Hearing 2019). Prenatally, a child can be exposed to cytomegalovirus (CMV) which is one of the most common acquired causes of SNHL. Of CMV infected children, 13 % will develop hearing loss which is usually severe or profound. (Goderis et al. 2014) CMV is thought to damage the hair cells and the vessels in the cochlea (Yu, Shi, et al. 2022). Postnatally, especially bacterial meningitis can cause hearing loss due to loss of outer hair cells, damage to spiral ganglion neurons and inner hair cell ribbon synapses (Perny et al. 2016). One study reported a prolonged latency of BAEP components I and V, and IPL I-III after viral meningitis in infants (Kim et al. 2020) suggesting a delay in the peripheral part of the auditory pathway. Another study found that meningitis did not affect BAEP values in preterm infants but the etiology of meningitis was not mentioned (Raposo et al. 2021). CMV infection has been shown to associate with decreased IPL III-V possibly due to earlier environmental exposure to sound or that the injury to auditory function might develop later (Raposo et al. 2021). Both CMV and bacterial meningitis increase BA threshold levels in infected compared to uninfected animal models (Perny et al. 2016, Yu, Shi, et al. 2022). In preterm infants, CMV and meningitis did not affect BA thresholds (Raposo et al. 2021).

Ototoxic medication, for example aminoglycosides, can cause hearing loss in childhood (The Joint Committee on Infant Hearing 2019). Aminoglycosides, such as gentamicin, are widely used in the NICU for treating sepsis. Gentamicin can enter the hair cells in the inner ear, and cause cell death leading to hearing loss. The hair cells at the base of the cochlea and the outer hair cells especially are vulnerable to gentamicin. Noise can potentiate the toxicity of gentamicin by increasing its uptake in the hair cells. (Zimmerman and Lahav 2013) However, there are reports where gentamicin does not associate with hearing screening failure at discharge (Puia-Dumitrescu et al. 2018) especially if the screening is performed with OAE (Vella-Brincat et al. 2011). However, OAE can detect only outer hair cell defects in the inner ear. The administration of gentamicin can lead to BAEP abnormalities, such as BA threshold elevation, prolonged latency of BAEP component I (Raposo et al. 2021) and prolonged IPL I-V (Ito 1984). In addition, gentamicin is regarded as a risk factor for auditory neuropathy (Dowley et al. 2009). These findings could imply that gentamicin damage the inner ear and also the more central auditory pathway.

Table 1. Risk factors for hearing loss based on Chapter 2.4.2.4. The table presents the location at which the risk factor damages the auditory pathway and how this damage can be observed in the BAEP and BA recordings. Abbreviations: brainstem audiometry (BA), brainstem auditory evoked potentials (BAEP), bronchopulmonary dysplasia (BPD), continuous positive airway pressure treatment (CPAP), cytomegalovirus (CMV), interpeak latency (IPL), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA). The author´s own drawing.

RISK FACTOR FOR AUDITORY PATHWAY ABNORMALITY	TARGET IN THE AUDITORY PATHWAY	CHANGES OBSERVED IN BAEP	CHANGES OBSERVED IN BA
	MAINLY THE PERIPHERAL PART		
CMV	Inner ear (inner hair cells, cochlear vessels)	Decreased IPL III-V	Threshold elevation
MENINGITIS	Outer hair cells, spiral ganglion neurons and inner hair cell ribbon synapses	Prolonged latencies I, V, and IPL I-III	Threshold elevation
GENTAMICIN	Base of the cochlea, outer hair cells, central auditory pathway?	Prolonged latency I and IPL I-V	Threshold elevation
MECHANICAL VENTILATION, BPD	Eustachian tube oedema and dysfunction leading to middle ear effusion, central auditory pathway	Prolonged latency V, IPLs I-V and III-V Decreased latency V, IPLs I-V, I-III and III-V	Threshold elevation
	MAINLY THE CENTRAL PART		
PREMATURITY	Central auditory brainstem	Prolonged latencies I and V, IPL III-V	Threshold elevation
NEC	Brainstem	Prolonged latency V and IPL I-V	No change
PDA CLOSURE	Central auditory pathway	?	-
	THE WHOLE AUDITORY PATHWAY		
ASPHYXIA	Central auditory pathway, inner ear	Prolonged latency V and IPLs I-V, I-III, III-V	Threshold elevation
NOISE	Hair cells, central auditory pathway	-	Threshold elevation
CPAP	?	No effect on BAEP variables	-
HYPERBILIRUBINEMIA	Auditory nerve, brainstem	Prolonged latencies III and V and IPLs I-V, I-III and III-V	Threshold elevation
PERI-INTRAVENTRICULAR HEMORRHAGE **IVH**	Whole auditory pathway	Prolonged latencies I, III, V and IPLs I-V and I-III Decreased IPL I-III	-

Assisted ventilation (Hille et al. 2007, Zhou et al. 2022) and especially mechanical ventilation has been found to be a risk factor for hearing loss (Bielecki et al. 2011). Mechanical ventilation increases the risk for BPD (Rutkowska et al. 2018). Preterm infants with BPD are at a greater risk for developing conductive hearing loss compared to preterm infants without BPD. The mechanism is most likely that prolonged intubation causes edema and dysfunction of the Eustachian tube, which can lead to middle ear infection and, in consequence, the risk for conductive hearing loss increases. (Gray et al. 2001) In addition, the central auditory pathway may be damaged due to hypoxia in children with chronic respiratory difficulties. Studies have shown that infants diagnosed with BPD have longer latency of BAEP component V and IPLs I-V and III-V compared to healthy term infants. These children are often treated with supplementary oxygen, which can damage their lungs even further, worsening the hypoxia. (Jiang et al. 2006, 2010). However, contradictory findings also exist showing decreased latencies and IPLs in preterm children treated with mechanical ventilation or diagnosed with BPD (Raposo et al. 2021, Borenstein-Levin et al. 2022). This accelerated maturation of BAEP variables has been explained to be possibly due to early extrauterine exposure to sounds in the most sick and immature infants.

2.4.2.4.2 Risk factors mainly affecting the central part of the auditory pathway

There are several studies utilising BAEP and BA recordings, including one meta-analysis, which show that preterm birth affects the maturation of the auditory pathway (Eggermont and Salamy 1988, Jiang et al. 2002, 2009, Stipdonk et al. 2016). At term age, normal hearing preterm infants have prolonged auditory conduction compared to full term infants. This prolongation is especially observed in the central parts of the brainstem auditory pathway observed in increased IPL III-V in BAEP recordings. (Jiang et al. 2002, 2009, Stipdonk et al. 2016) Eggermont et al. discovered longer absolute latencies I and V, but not IPLs in preterm infants compared to term infants in matching age groups which can be explained by possible mild conductive or high frequency hearing loss (Eggermont and Salamy 1988). There are also studies presenting similar BAEP results for preterm and term infants, especially when moderately preterm infants are included in the analysis (Jiang and Wilkinson 2008, Raposo et al. 2021). The auditory pathway impairment in preterm infants is possibly due to exposure to an extra-uterine environment at the time of critical brain development, and NICU treatment in particular has been found to be a risk factor for abnormal auditory pathway development (Stipdonk et al. 2016).

NEC is a risk factor for hearing impairment (Matei et al. 2020). In addition, NEC and conditions associated with NEC (hypoxia-ischemia, malnutrition, low birth

weight, infections) have been shown to damage the developing brainstem and the auditory pathway. Very preterm infants with NEC show an increase in latency of BAEP component V and IPL I-V but no changes in early components I and III compared to age controlled preterm infants without NEC. This would suggest damage to the more central parts of the auditory pathway above the pons. (Jiang et al. 2022)

Closure of the PDA with indomethacin and/or operatively has been shown to lead to a poorer neurodevelopmental outcome including hearing loss at the age of 2-3 years. It has been suggested that cardiorespiratory instability during surgery of PDA and anaesthetic drugs could damage the immature brain. In addition, indomethacin may cause hypoperfusion in the brain. (Janz-Robinson et al. 2015) Indomethacin treated PDA has been shown to associate with failed hearing screening with aABR before discharge (Leung et al. 2016). However, BAEP in neonates with PDA has been less studied. In one recent study, a PDA closure performed operatively or medically did not affect the BAEP variables (Borenstein-Levin et al. 2022), which contradicts the above cited studies and thus, more research is needed in this area.

2.4.2.4.3 Risk factors affecting the whole auditory pathway

Severe birth asphyxia (Apgar score <5 at 1 min and <7 at 5 min age) increases the risk for hearing loss (Hille et al. 2007). Apgar scores indicate the condition of the newborn and perinatal hypoxia at the ages of 1, 5 and 10 minutes (min). It has been found, that the lower the Apgar scores at 5 min, the longer the latencies of BAEP components III and V and IPLs I-V, I-III, III-V will be three days after birth (Jiang and Wilkinson 2006). In addition, infants who suffer from perinatal asphyxia (Apgar <6 at 5 min age and clinical signs of hypoxia and hypoxic-ischemic encephalopathy) have a longer latency of BAEP component V, IPLs I-V, I-III and III-V compared to healthy term infants, which suggest there is an adverse effect on the whole auditory pathway (Jiang et al. 2010). In term born infants with asphyxia, damage to outer hair cells have also been observed by using TEOAE (Ribeiro et al. 2016). BA threshold elevations have been observed due to hypoxia, which depresses the sodium-potassium pump and endocochlear potential (Sohmer et al. 1989, Jiang 1998). Endocochlear potential is the positive potential between perilymph and endolymph in the cochlea and it is necessary for depolarization of the inner hair cell (Stelma and Bhutta 2014).

Noise can damage the peripheral as well as the more central auditory structures especially during the development of the auditory system (Bureš et al. 2017). Normally, the developing cochlea and the auditory pathway are only exposed to low-frequency sounds in the womb, and the high frequency sounds are attenuated. When

the child is born preterm and placed in an NICU for potentially long period of time, the sound environment changes. The prematurely born infant is deprived of the maternal sounds, and is exposed to the high-frequency sounds and noise created by monitors, alarms and staff in the NICU, all of which can injure the developing auditory pathway. (McMahon et al. 2012, Lahav and Skoe 2014). Based on animal models, noise can damage the inner and the outer hair cells (Gerhardt et al. 1999), cause permanent structural changes in the inferior colliculus, the medial geniculate body and the auditory cortex (Bureš et al. 2010, Ouda et al. 2016). Noise can alter synaptogenesis (Kujawa and Liberman 2009, Kumar et al. 2014, Qi et al. 2022) and the myelination of the auditory nerve (Tagoe et al. 2014). However, there are also studies reporting that the myelin sheath is not affected by noise (Qi et al. 2022). In neonatal guinea pigs, prenatal noise exposure leads to elevated BA thresholds postnatally but latencies are not affected (Morimoto et al. 2017). In one study including preterm infants, the BAEP variables were not affected by the CPAP treatment (Borenstein-Levin et al. 2022) which is one source of noise in the NICU (Kirchner et al. 2012). Nevertheless, studies investigating CPAP treatment and auditory function are limited.

Hyperbilirubinemia requiring exchange transfusion is considered a risk factor for hearing loss (The Joint Committee on Infant Hearing 2019) and hyperbilirubinemia is also a risk factor for auditory neuropathy (Dowley et al. 2009, Liddle et al. 2022). Hyperbilirubinemia usually disturbs the brainstem cochlear nuclei and later the auditory nerve while the cochlea remains intact (Olds and Oghalai 2016). In BAEP recordings, the hyperbilirubinemia causes prolonged latencies of components III and V and IPLs I-V, I-III and III-V (Mandour et al. 2020) and BA threshold elevation (Agrawal et al. 1998). In most cases, BAEP and BA changes revert back to normal after therapy for hyperbilirubinemia (Agrawal et al. 1998).

Brain injury (Chant et al. 2022) including for example a severe intraventricular hemorrhage (IVH) (Yu, Lin, et al. 2022) is considered a risk factor for hearing loss. Contradictory results have been found for the association between brain injury and BAEP and BA results. One study reported that preterm infants with peri-intraventricular hemorrhage had longer latencies of BAEP components I, III and V and longer IPLs I-V and I-III compared to infants without peri-intraventricular hemorrhage (da Silva et al. 2018). These results would imply impairment in the whole auditory pathway. However, Borenstein-Levin et al. found that IVH and periventricular leukomalacia decreased IPL I-III in preterm infants suggesting accelerated maturation of the peripheral part of the auditory pathway (Borenstein-Levin et al. 2022).

2.5 Early language development

Optimal auditory input is necessary for normal auditory development and in consequence, for normal language development (McMahon et al. 2012). In very preterm infants especially this development is compromised (McMahon et al. 2012), and they consistently show weaker language skills compared to term born infants (van Noort-van der Spek et al. 2012). Thus, reliable diagnostic methods for early identification of abnormal auditory pathway development and language skills are needed to enable early intervention.

2.5.1 Normal language development

Normally, children begin to understand words at the age of 8-10 months (**Figure 7**). In Finland, healthy full term infants at the age of 12 months, understand a mean of 89 (63 SD) and a median of 67 (range 6-263) words, and at the age of 14 months, a mean of 164 (83 SD) and a median of 159 (range 16-322) words (Lyytinen 1999). However, there are notable inter individual differences in this development. For example, according to the normative data of Fenson et al., at the age of 12 months the lowest 10 percentile understand 21 words or less, and the highest 10 % understand 199 words or more (Fenson et al. 2007). The early receptive lexicon (at the age of ≤12 months) consists mainly of social terms (for example people and routines) and nouns. At the same time when the receptive lexicon size grows, the percentage of social terms and nouns decrease, and the percentage of verbs and adjectives increase. (Stolt et al. 2008)

The first signs of word comprehension are followed by the appearance of gestures which lead to word production (Fenson et al. 1994, Lyytinen 1999). The acquisition rate of the receptive lexicon is quicker than that of the expressive lexicon (Stolt et al. 2008). The production of words begins at the age of 12-13 months (Fenson et al. 1994). In Finland, at the age of 12 months, children speak a mean of 7 (9 SD) and a median of 4 (range 0-60) words, and at the age of 24 months, a mean of 278 (162 SD) and a median of 269 (range 0-595) words (Lyytinen 1999). The acquisition order of produced and comprehended words of different categories is similar (Stolt et al. 2008). The first spoken words are also usually social terms and nouns (Fenson et al. 1994, Lyytinen 1999, Stolt et al. 2008).

In healthy infants, girls are slightly ahead of boys in word comprehension, word production and the appearance of word combinations (Fenson et al. 1994, 2007). The gap between boys and girls is usually approximately 1-2 months. However, this difference is considerably less than individual differences. (Fenson et al. 1994) In the Finnish normative study, girls were slightly ahead of boys, but the differences in language scores were not significant (Lyytinen 1999).

2.5.2 Language development in very preterm infants compared to healthy infants

Several studies have established, that preterm children suffer from delayed language development throughout childhood compared to full-term children even when they do not have any major disabilities (Foster-Cohen et al. 2007, Stolt et al. 2009, van Noort-van der Spek et al. 2012, Taskila et al. 2022). Extremely preterm born infants are at the greatest risk for delayed language development (Foster-Cohen et al. 2007). Delayed language development may be, at least partially, due to early exposure to an extrauterine environment where preterm infants are surrounded by noise, monitor sounds and silence (Caskey et al. 2011), and they are often deprived of parental talk (Caskey et al. 2011, Rand and Lahav 2014, Ståhlberg-Forsén et al. 2022). This may adversely impact the auditory development and the organization of the language areas in the cortex (Rand and Lahav 2014).

The differences in early language development between preterm and term infants can be studied by comparing vocabulary sizes. In a study of Finnish VLBW children, the size of the receptive lexicon reported by parents was smaller at the ages of 9, 12 and 15 months compared to full-term infants. Full-term infants understood approximately 1.8 times more words than VLBW infants. For example, at the age of 12 months, full-term born infants understood a mean of 94 (68 SD, range 12-262) words, and VLBW infants understood a mean of 56 (44 SD, range 7-193) words. The expressive lexicon reported by parents was smaller in VLBW infants at the age of 24 months compared to full-term infants. The proportion of grammatical function words in the receptive lexicon tended to be smaller in VLBW children compared to full-term infants. In addition, the proportion of nouns was bigger in VLBW children compared to full-term infants when the total expressive lexicon size was still small. (Stolt et al. 2009) Preterm children have also been found to produce more onomatopoetic word, names and routines (usually acquired early) and less nouns, predicates, morphological endings in words, irregular words and word combinations at the age of two years compared to full-term children (Foster-Cohen et al. 2007, Kern and Gayraud 2007). However, similarities also exist as VLBW children acquire words from different categories mostly in the same order as full-term children. This applies to both the receptive and expressive lexicon. (Stolt et al. 2009)

The differences in language development between preterm and full term children can also be observed later in childhood (Taskila et al. 2022). According to a meta-analysis, preterm children (<37 weeks GA) performed worse in simple and complex language tests at the ages of 3-12 years compared to full-term children. In the complex language tests, the difference in scores between the preterm and full term born children increased over time. (van Noort-van der Spek et al. 2012)

2.5.3 Risk factors for deviant language development in preterm infants

In preterm infants, the risk factors for delayed language development are considered to be a low gestational age (Foster-Cohen et al. 2007), the presence of a disability at two years (for example neuromotor, vision and hearing disabilities), a longer time spent in the hospital, male gender and a lower weight SD score at one year of age (Marston et al. 2007). Combining the data from 15 very preterm cohorts in Europe, showed that a low maternal educational level associated with lower cognitive skills especially between the ages of 4 to 15 years (Sentenac et al. 2021). In addition, neonatal diseases such as RDS and IVH have been suggested as risk factors for poor language development in preterm infants (Taskila et al. 2022). Some of these factors might not, however, explain the language delay in preterm children compared to full-term children (Foster-Cohen et al. 2007, Marston et al. 2007, van Noort-van der Spek et al. 2012, Taskila et al. 2022). For example, it has been found that preterm children perform worse in language tests compared to term born children independently from socioeconomic status (van Noort-van der Spek et al. 2012), maternal education (Foster-Cohen et al. 2007, Taskila et al. 2022), parental occupation and education (Marston et al. 2007) as well as gender (Taskila et al. 2022).

2.5.4 Testing language development in children

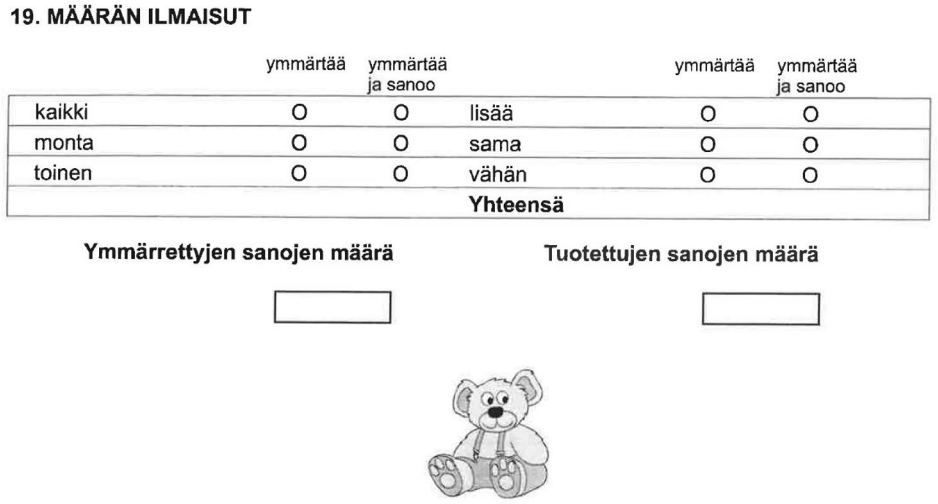
Language development can be evaluated with structured tests, with language samples or by parents. Structured tests must be performed by trained personnel, and they require much time. In addition, to complete structured tests the children must co-operate. In language sample, the goal is to observe the child, and gather information of their language for example during play and conversation. This requires trained experts, a considerable amount of time and the child might still act and speak differently in the presence of a strange adult. (Fenson et al. 2007)

Traditionally, parent reported data has been considered less reliable than the data obtained in clinical settings. Parents can be biased, overestimate their child´s language abilities or misunderstand the given instructions. (Fenson et al. 1994, 2007) In addition, parents from lower education levels tend to overestimate the language skills of their child, especially when the child is 8-12 months old (Fenson et al. 2007). However, there are several advantages to parent reported data especially when children are young, and language is still simple to evaluate. Parents can observe their children for long periods of time and in different situations. In addition, the child usually speaks more freely with the parents than with the health care personnel. The parent report is a cost-effective method, and it does not require much time from the researchers. The effect of any over- or underestimation by the parents can be minimized by using structured questionnaires. (Fenson et al. 1994, 2007)

2.5.4.1 The MacArthur-Bates Communicative Development Inventories (CDI)

One widely used parental report instrument is the MacArthur-Bates Communicative Development Inventory (CDI). It gathers information on the child´s early gestures, word comprehension and spoken words as well as early grammar. There are separate CDI forms for younger and older children. The CDI: Words and gestures Finnish form is for 8-16 months old children (**Figure 8**) and the CDI: Words and sentences form is for 16-30 month old children (Lyytinen 1999, Fenson et al. 2007).

The CDI: Words and gestures form has two parts. In the first part, information about the first signs of understanding words and phrases, and first imitations of language are gathered. In addition, the first part includes a vocabulary checklist, where parents mark the words their child understands (receptive lexicon), and the words their child understands and says (expressive lexicon). (Fenson et al. 2007) The Finnish version of CDI (FinCDI), the Words and gestures form has 19 semantic categories comprising of altogether 380 words. The checklist is comprised social words (for example people, games, and routines), nouns (for example animal names, toys and body parts), verbs, adjectives and grammatical function words (pronouns, question words and quantifiers). In the second part, information on actions and gestures is gathered. These include pointing at an object, dancing and imitating an action of an adult. (Lyytinen 1999, Fenson et al. 2007, Stolt et al. 2008)



19. MÄÄRÄN ILMAISUT

	ymmärtää	ymmärtää ja sanoo		ymmärtää	ymmärtää ja sanoo
kaikki	O	O	lisää	O	O
monta	O	O	sama	O	O
toinen	O	O	vähän	O	O
			Yhteensä		

Ymmärrettyjen sanojen määrä **Tuotettujen sanojen määrä**

Figure 8. Category 19 in the Finnish version of the MacArthur-Bates Communicative Development Inventories Words and gestures form. Retrieved with permission from Niilo Mäki Institute.

In the CDI: Words and sentences form, information on the expressive lexicon and acquisition of grammar is gathered (Fenson et al. 2007). The checklist for the expressive lexicon in the Finnish version comprises of 20 semantic categories and 595 words. In addition, parents indicate whether the child uses the past tense, morphological endings and word combinations. They are also advised to write down the longest phrases their child produces. (Lyytinen 1999) In CDI, the memory mistakes made by parents are small as parents are advised to complete in the form based on the child´s current language skills. The parents are not asked to memorize earlier development. (Lyytinen 1999)

2.5.4.1.1 Comparison of CDI with formal language tests

The CDI method has been found to be a reliable and valid method for studying early lexical development (Lyytinen 1999, Fenson et al. 2007, Stolt et al. 2009). The CDI method has good internal consistency (alpha values of 0.56-0.96) and test-retest reliability (r=0.6-0.95) (Fenson et al. 2007). In addition, the CDI has been found to correlate well with formal language tests (Lyytinen 1999, Fenson et al. 2007, Stolt et al. 2008). In the Finnish normative study, the expressive lexicon sizes evaluated by parents and by specialists at the same time at the ages of 18, 24 and 30 months were in good agreement. For example, at the age of 18 months the expressive lexicon evaluated by the FinCDI and the Reynell Developmental Language Scales (RDLS) showed good correlation ($r=0.85$, $p\leq0.001$). At the age of 24 months the expressive lexicon of FinCDI correlated well with the Bayley mental scale ($r=0.70$, $p\leq0.001$). (Lyytinen 1999) In VLBW children, expressive lexicons evaluated by the FinCDI and RDLS III are in good agreement at the age of 24 months ($r=0.86$, $p<0.001$) (Stolt et al. 2009).

2.5.4.1.2 Predictive value of CDI on later language development

Early gestures and an early expressive lexicon as well as the receptive lexicon evaluated by the CDI associate with later language development (Lyytinen 1999, Fenson et al. 2007, Stolt et al. 2008). In healthy term infants, the receptive lexicon evaluated by the FinCDI at the ages 12 and 14 months correlates with the RDLS receptive score evaluated at the age of 18 months ($r= 0.38$ and 0.43, $p\leq0.001$). The expressive lexicon at the age of 14 months correlates with RDLS scores at the ages of 18 and 30 months ($r=0.58$, $p\leq0.001$ and 0.30, $p\leq0.01$) (Lyytinen 1999). In VLBW children, the receptive and expressive lexicon sizes evaluated by the FinCDI at the age of 12 months predicts the total language score at the age of two years examined with RDLS III ($r=0.67$, $p<0.001$ and $r=0.41$, $p<0.05$). At the ages of 12 and 15 months, the correlation with RDLS III was stronger for the receptive lexicon

compared to expressive lexicon. (Stolt et al. 2009) In very preterm children, the receptive lexicon size at the age of one year has also been found to correlate with cognitive development at the age of two years ($r=0.27-0.33$, $p<0.05$) (Alatalo 2015).

2.5.5 Identifying infants at risk for delayed language development by using neonatal BAEP and BA

Hearing loss, auditory neuropathy and delayed auditory maturation can adversely influence the acquisition of language and communication skills (Cox et al. 1992, Amin et al. 2014, Davies 2016, Wang et al. 2020). Deviant neonatal BAEP and BA results have been found to indicate poorer receptive and expressive language skills (Amin et al. 2014), IQ and academic achievement (Cox et al. 1992) as well as overall cognitive outcome (Wang et al. 2020) in preterm infants. For example, in very preterm infants, longer IPL I-V in a neonatal BAEP recording has been found to associate with poorer language development at the age of three years without hearing loss or auditory neuropathy (Amin et al. 2014). In earlier studies, the most commonly used BAEP variable has been the IPL I-V (Cox et al. 1992, Amin et al. 2014) although the inclusion of other BAEP variables could give more information regarding the association between auditory pathway development and cognitive outcomes. In addition, earlier studies included a limited number of very preterm infants (Cox et al. 1992, Amin et al. 2014, Wang et al. 2020). Regarding this research area, the main goal is to investigate whether BAEP and BA recordings could be used to identify very preterm children at risk for abnormal cognitive or language development already in the neonatal period in order to enable early intervention.

2.5.6 Interventions supporting language development

Early exposure to language is important for preterm infants and language development can also be taken into account in the NICU (McMahon et al. 2012). In the NICU, appropriate auditory stimuli are lacking, as the sound environment consists mainly of monitor sounds and noise, and the proportion of adult or infant language is only 2-5 % of the total sound exposure (Caskey et al. 2011, McMahon et al. 2012). Preterm infants have been found to vocalize more when their parents are present in the NICU, and when they hear adults talk (Caskey et al. 2011). The more the parents talk to their premature child (birth weight ≤1250 g) in the NICU, the better the cognitive and language outcomes are at the ages of 7 and 18 months (Caskey et al. 2014). In addition, close contact between preterm infant and the parent associates with a better language outcome at the corrected age of 18 months (Ståhlberg-Forsén et al. 2022). Thus, in the NICU, attention should be paid to the

noise levels, and the interaction between the parents and the child should be encouraged as much as possible.

After discharge, the parents should be encouraged to create an optimal language environment at home. Studies have shown that the amount of parental talk and the quality of the early language environment is important for language development. The more the preterm infants hear caregiver talk at home, the better their language outcome is at the age of 18 months (Adams et al. 2018). Children whose caregivers provide a richer language environment (use of specific vocabulary and explanations with more complex statements and questions) achieve better language skills compared to children, whose caregivers use a more directive and structured style (use of simple words and brief statements) (Landry et al. 2002). Parents´ sensitive responsiveness, including responding to the child´s initiatives, also associates with better language outcome compared to children with less sensitive parental behaviour (Madigan et al. 2019). Children with hearing loss show better language outcomes, when the family is actively involved in the rehabilitation compared to children with limited support from the family (Moeller 2000).

In addition, language rehabilitation includes school or day care arrangements with smaller groups and minimizing environmental noise. Augmentative and alternative communication methods, such as pictures and baby sign language, can also be utilised. Speech therapy and other rehabilitation methods, such as occupational therapy and neuropsychological rehabilitation should be organized if necessary. According to Finnish guidelines, speech and language therapy should be started at the age of 2-2.5 years if a language delay is suspected. (Developmental Language Disorder: Current Care Guidelines, 2019).

3 Aims

The aims of this study were:

- to calculate reference values for neonatal BAEP and BA recordings conducted at the corrected age of one month to help the hearing loss diagnostics in the Department of Clinical Neurophysiology in Turku University Hospital (Study I)
- to study whether neonatal BAEP and BA recorded in very preterm infants associates with receptive language development at the age of one year (Study II)
- to study how the different risk factors for hearing loss, especially CPAP treatment during the neonatal period, associates with abnormalities in BAEP and BA recordings conducted at the age of one month in very preterm infants (Study III)
- to study the associations between neonatal BA recording and pure-tone audiometry at five years of age in very preterm children (Study IV)

4 Materials and Methods

4.1 Participants

4.1.1 Data formation

In Study I, we included both preterm and term infants, born between 2002 and 2006 who underwent a BAEP and/or BA recording during their first 6 months according to the hearing screening protocol of Turku University Hospital. The exclusion criteria are shown in **Figure 9**. In order to calculate the reference values for BAEP and BA recordings, we only included infants who had bilaterally normal BAEP and BA recordings based on the reference values in the literature (Chiappa 1997). In addition, these infants did not have a hearing loss diagnosis in the patient files at the time of data acquisition, and they showed normal hearing at two years of age examined with miniature audiometer. Consequently, 177 infants were included in the reference value database. In addition to BAEP recording, 89 % of infants underwent a BA recording. These data were used to calculate reference values for the BA.

For Study II, we included all very preterm infants whose birth weight was ≤1500 g and/or who were born at ≤32 gestational weeks in Turku University Hospital between 2007 and 2012. This time period was chosen because of the clinical use of the CDI form. Parents filled in the form of understood or spoken words and used gestures by their child. This form was collected as a part of a clinical follow-up of these preterm infants born in Turku University Hospital. In Study II, the CDI was used to gather data of receptive language development at the age of one year. In addition to the available CDI results, Study II included infants who underwent neonatal BAEP recording before the corrected age of 2.5 months and attended follow-up visits in a preterm follow-up clinic at Turku University Hospital. The children also had to be from a monolingual Finnish speaking family living inside the catchment area of Turku University Hospital (**Figure 10**). In consequence, 155 very preterm infants were included in the study (II), which investigated the association between neonatal BAEP and BA recordings and language abilities at the age of one year.

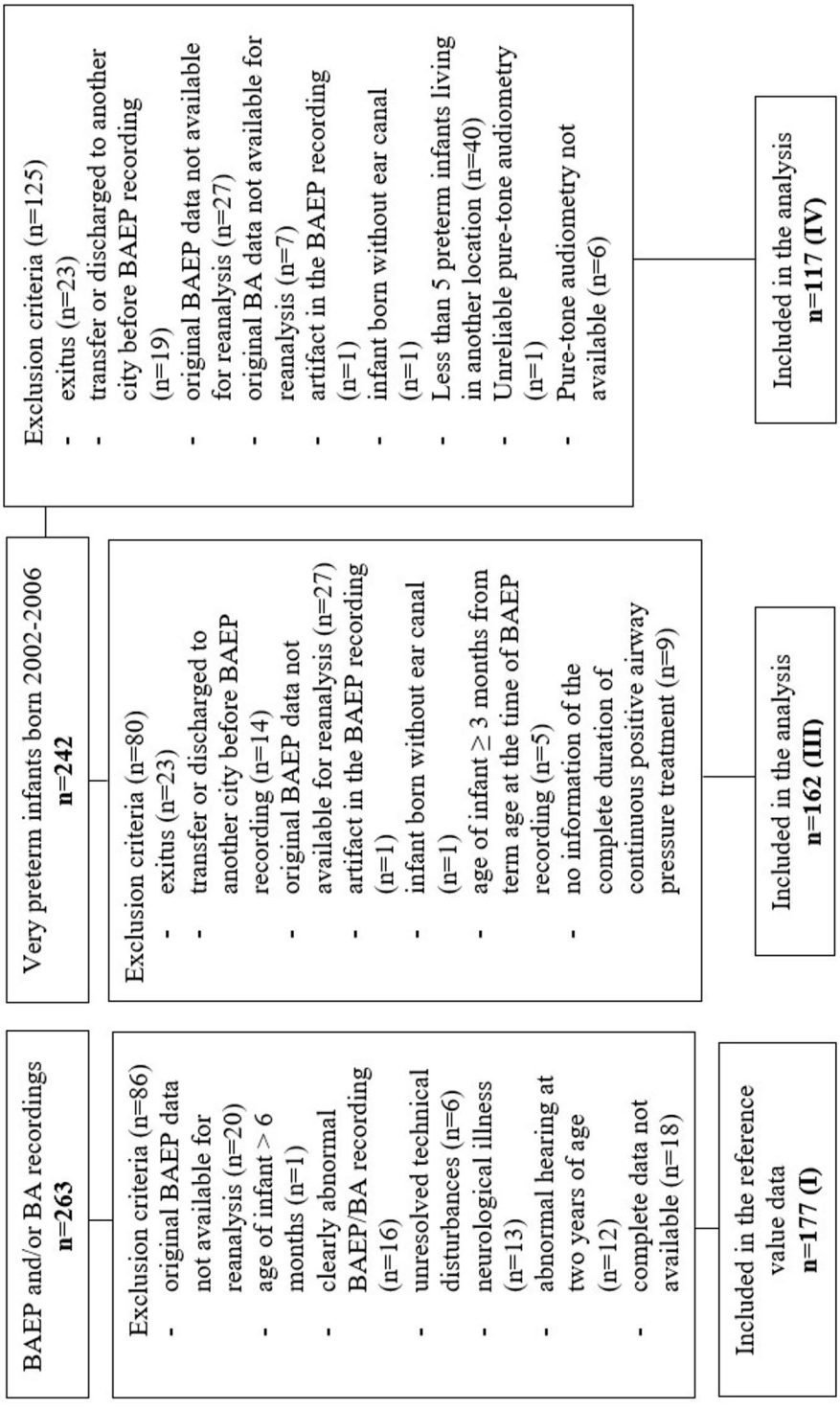


Figure 9. The participants in Studies I, III, IV were born between 2002-2006 in Turku University Hospital. Abbreviations: brainstem audiometry (BA), brainstem auditory evoked potentials (BAEP). Modified with permission from Saranto et al. 2016 and Antinmaa et al. 2021.

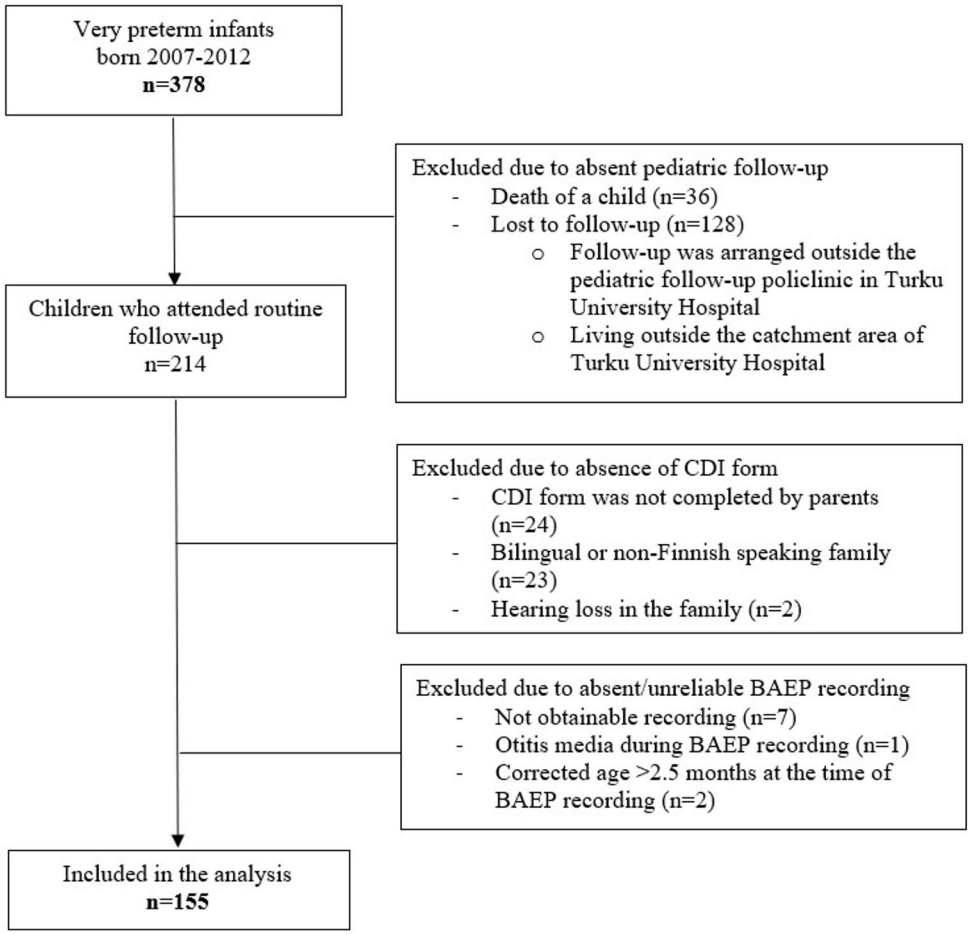


Figure 10. Formation of the data in Study II. Abbreviations: brainstem audiometry (BA), brainstem auditory evoked potentials (BAEP), the MacArthur-Bates Communicative Development Inventory (CDI). Modified with permission from Antinmaa et al. 2020.

For Studies III and IV, we included all very preterm infants who were born between 2002-2006 in Turku University Hospital and whose birth weight was ≤1500 g and/or gestational age was ≤32 weeks. Infants were excluded from the studies due to, for example, the death of the infant, missing clinical data, a transfer or discharge to another city, no available BAEP recordings or technically unsuccessful BAEP recordings. In Study III, we excluded children whose corrected age was three months or more at the time of the BAEP recording. In this study, we wanted to focus on neonatal BAEP and BA recordings to study the effects of CPAP treatment and other risk factors for hearing loss on the auditory pathway function. In Study IV, we

included all children up to six months of age as there was no need to exclude the older children because of longer follow-up period of five years. To study the association between neonatal BA and pure-tone audiometry at around five years of age, the children who did not have available BA or pure-tone audiometry results were excluded from Study IV. Consequently, 162 very preterm infants were included in Study III and 117 very preterm infants in Study IV (**Figure 9**).

4.1.2 Data collection

The data was gathered from patient files in Turku University Hospital (Studies I-IV). The following clinical information was collected for all the studies: gender, date of birth, gestational age, birth weight, SGA status and hearing examined with miniature audiometer at the age of two years. In addition, we conducted a search for children who had their hearing loss diagnosed in the Department of Audiology because that is the only unit providing hearing rehabilitation in the catchment area of Turku University Hospital. Neonatal BAEP and BA recordings were retrieved from the data archives of the Department of Clinical Neurophysiology. BAEP results including latency of BAEP component I, III, V, IPLs I-V, I-III, III-V (ms), amplitude of components I and V (μV), amplitude ratio I/V, and BA thresholds were collected bilaterally. The date when the BAEP and/or BA was conducted was registered. For Study II, we also collected data for the contralateral responses of components III and V of BAEP recordings.

For Study II, the CDI results were collected from the forms the parents had completed. The age of the child at the time of CDI completion was also noted. For Study III, a comprehensive list of different clinical risk factors for hearing loss was gathered (**Table 5**) with special interest in the duration of neonatal CPAP treatment. For Study IV, we collected pure-tone audiometry results from patient files of Turku University Hospital and from patient files of the health care centres of Turku, Salo, Kaarina, Lieto, Pori, Rauma, Parainen, Raisio, Masku and Loimaa as well as Pori Hospital. We requested permission to collect pure-tone-audiometry results from these centres as five or more children had moved there during the follow-up period. We chose the audiometry which was done close to the age of five years. The thresholds for each tested tone were collected, and the date of the audiometry registered. Of the 117 pure-tone audiometry results, the majority (32 cases) were conducted in Turku Health Care Centre and 6 were conducted in Turku University Hospital.

4.2 Measurements and equipment

4.2.1 BAEP and BA (I-IV)

In Turku University Hospital, all very preterm infants were examined with BAEP and BA recordings in the neonatal period at the Department of Clinical Neurophysiology. At the time of the study, term born infants were also sometimes examined, if they presented failed hearing screening results or if they had risk factors for hearing loss. At the time of the Studies I-IV (2006-2012), the recordings were performed around the corrected age of one month.

BAEP and BA were recorded in a quiet room by experienced evoked potential technologists. The ear canals of the children were checked and, if necessary, cleaned by a pediatrician at the Developmental outpatient clinic before the recordings. To obtain a clear and reliable BAEP and BA recordings, the aim was that the infants would be asleep or peacefully awake, after breast feeding. Occasionally, a 20 % glucose solution was given orally if the infants were restless. In Studies I, III and IV, BAEP and BA were recorded using an eight-channel Nicolet Viking IV instrument (Nicolet Biomedical Instruments, Madison, Wisconsin, USA). In Study II, this device was used for 25 infants (recorded mostly in 2007) and a Viking Select (VIASYS Neurocare, Nicolet Biomedical Inc., Madison, Wisconsin, USA) was used for 130 infants. The recording electrodes were placed on both mastoids and the reference electrode at the vertex (Cz) anterior to the fontanel, and the ground electrode on the forehead (Fp2'). The air-conducted stimulus was delivered to the outer ear canal with TIPs (Nicolet model TIP 300 Ohm). The earphones were selected according to the size of the ear canal and the length of the silicon tube was 153 millimetres. Right and left ear were tested separately.

For BAEP, broadband rarefaction click stimuli were used at the intensity of 85 dB nHL with stimulation frequency of 10.3 Hz. The rate is around 10 Hz, with decimal to avoid subharmonic synchrony with electric 50 Hz interference. At the same time, masking white noise was delivered to the non-stimulated ear at the intensity of 45 dB nHL. The low frequency filter (high pass filter) was set to 150 Hz and the high frequency filter (low pass filter) to 3 kHz. The amplifier sensitivity was 10 μV. A sample of 2000 responses were averaged at least twice to ensure good-quality responses and their repeatability. At the time of Studies I-IV, the stimulus level was at times raised to 95 dB nHL, if necessary, to elicit a clear BAEP response. An experienced technologist marked the peaks I, II, III, IV and V, and the troughs following peaks I and V to the recordings. In addition, the clinical neurophysiologist checked the recordings and the reports. In Study II, we also identified BAEP components III and V of the contralateral responses. Contralateral responses were considered present if the waves III and/or V were clear and repeatable.

After the BAEP recording, almost all infants underwent a BA recording to determine the click threshold of the right and left ears separately. First, the stimulation intensity was 35 dB nHL (33.3 Hz), delivered to the ear canal, and a masking white noise of 15 dB nHL was delivered to the non-stimulated ear. The intensity increased in increments of 10 dB nHL up to 65 dB nHL (and masking white noise up to 40 dB nHL), if necessary, until clear waveforms III and V were visible. Components III and V were chosen for evaluation because they can usually be identified already at the levels of 35 dB nHL and 45 dB nHL compared to component I which has a higher threshold. According to the results of Study I, the reference limit for BA is 35 dB nHL, and at the time of Studies I-IV, it was the lowest BA threshold applied. As a consequence, in Studies II-IV we defined the normality of BA recording as follows: the BA threshold was considered normal if waves III and V were visible at the intensity of 35 dB nHL on both sides, and abnormal, if the waves were visible only at the intensity of 45 dB nHL or higher in either ear.

4.2.2 Evaluation of hearing with miniature audiometer (I)

At the 2-year visit in the Developmental follow-up clinic of Turku University Hospital, a paediatrician tested the hearing of preterm infants with a miniature audiometer in free field stimulation settings. The intensity of stimulation was 45 dB and frequency 3-4 kHz. During the test, the infant sits in the parent's lap, and the examiner is behind the parent. From a distance of 50 cm a sound is given separately for the right and left ears. If the infant clearly reacts to the sound, for example by turning the head, the test is considered normal. At the same follow-up visit, the paediatrician also roughly evaluated the language abilities of the child.

4.2.3 Evaluation of language abilities at the age of one year (II)

In Study II, we gathered information on the language abilities of very preterm infants at the corrected age of one year. During the routine follow up, the parents are advised to complete the Finnish version of the CDI: Words and gestures form (Lyytinen 1999). The CDI is a structured instrument including 380 words presented in 19 semantic categories which can be divided in four subcategories: Social-pragmatic words (CDI categories 1, 11 and 12), nouns (2-9), verbs and adjectives (13, 15) and closed class words (16-19). Parents mark the words their child understands but does not say which comprises the receptive lexicon, and the words their child understands and says which comprises the expressive lexicon. The child was considered to understand the word if he/she repeatedly and clearly responded correctly to the word. The parents also indicated the day when the form was completed.

In the analyses of Study II, we only used the receptive lexicon because this lexicon size is larger compared to the expressive lexicon size at the age of one year in preterm children. We also divided the children into two groups using a cut-off value of 17 receptive words: if the child understood ≤17 words the CDI result was considered abnormal and if the child understood >17 words the result was considered normal at the corrected age of one year. The cut-off of 17 receptive words was chosen because it is the weakest 10th percentile at the age of one year according to the normative data of the Finnish CDI and it has been applied in previous research (Lyytinen 1999, Bavin and Bretherton 2013).

4.2.4 CPAP device in the neonatal intensive care unit (III)

Between 2002 and 2006, when the infants of Study III were born, the CPAP device used was an Infant Flow device (Electro Medical Equipment, Brighton, UK).

4.2.5 Pure-tone audiometry (IV)

A pure-tone audiometry was conducted at a primary health care or at the Hearing Centre in Turku University Hospital when the child could co-operate. The HL is determined for each tone beep (250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz) separately. For statistical purposes in Study IV, we calculated the average hearing levels of frequencies 500, 1000, 2000 and 4000 Hz (pure-tone average, $PTA^{0.5\text{-}4\ kHz}$) for each ear separately. In health care centres the screening level was 20 dB HL and thus, lower intensities were not usually applied. In Study IV the $PTA^{0.5\text{-}4\ kHz}$ was considered normal if the hearing level was 0-25 dB HL on both sides and abnormal if the level was over 25 dB HL in one or both ears.

4.3 Ethics

This retrospective register study was approved by the Hospital District of Southwest Finland Ethics Review Committee in 2010 (Study I) and 2016 (Studies I-IV).

4.4 Statistical analysis

4.4.1 Study I

In Study I, we calculated the reference values for the latencies of BAEP components I, III, V, the amplitude ratio I/V, the IPLs I-III, I-V, III-V, and inter-side differences for the latencies and IPLs by using SPSS 17 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, 2008) and Microsoft Office Excel 2003 software. The

latencies depended on the age of the children, and thus for further analysis, we divided the data into two groups based on a visual estimation of the raw preliminary data plots. The two groups were infants whose corrected age was ≤1.5 (n=137) and >1.5 months (n=40) at the time of BAEP recording. The reference values were calculated separately for age groups of -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6 months from term age. Most of the data was normally distributed; the only variable not normally distributed were the inter-side differences of latencies in the >1.5 months group. Using Student's t-tests, we analysed the differences between boys and girls, and between preterm infants and infants born full term for different BAEP variables. Bonferroni correction was applied to adjust the p-values at the 0.05 alpha level for multiple comparisons. SPSS was used to make a linear regression model to determine upper (for latencies and inter-peak intervals) or lower (for amplitudes) limits for different BAEP variables. Age was the only independent variable in the regression models, as gender differences were small. 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 2.5 % 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.

4.4.2 Study II

First, univariate associations were analysed between BAEP parameters including latencies of BAEP components I, III, V, IPLs I-V, I-III, III-V (ms), amplitudes of components I and V (μV), amplitude ratio I/V, contralateral BAEP responses, and BA variables, and the receptive lexicon. Results from each ear were analysed separately. To study the associations between continuous, normally distributed within-subject variables (gestational age, birth weight, age at the time of CDI completion, different BAEP/BA variables, age at the time of BAEP/BA recording) and the size of receptive lexicon, a Pearson's correlation was used. To study the associations between these variables and CDI normality, a t-test for independent samples was used. The t-test was also used to analyse the associations between dichotomous within-subject variables (presence of contralateral responses in BAEP, BA normality, SGA status, gender, presence of hearing loss) and the size of the receptive lexicon. The associations between dichotomous variables and CDI normality were studied using a chi-square test or a Fisher´s exact test when there were only few observations.

Next, two separate regression analyses were made. The BAEP variables were chosen based on their significant associations in the univariate analysis. In the first analysis, the latency of the BAEP component V, the IPL I-V, and the presence of contralateral response were grouped one at a time with gestational age and gender.

In the other analysis, the latency of the BAEP component V, IPL I-V and the presence of contralateral response were analysed in one group and gestational age and gender in one group. Linear regression analysis was used with the receptive lexicon size variable and a binary logistic regression analysis was used to quantify the significant associations with the dichotomous receptive lexicon variable (CDI normality). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated.

4.4.3 Study III

First, an unadjusted (univariate) analysis was made to study the effects of clinical factors and risk factors on hearing loss for different BAEP variables and BA-normality. The following BAEP variables were included: latencies of BAEP components I, III, V, amplitudes of BAEP components I and V, amplitude ratio I/V and IPLs I-III, I-V and III-V. The clinical factors that were included in the analysis are listed in **Table 2** and **Table 5**. Both ears were analysed separately. Pearson's correlation coefficient was used to analyse the associations between continuous, normally distributed clinical factors and BAEP variables. A t-test for independent samples was used to study the effects of dichotomous clinical factors on BAEP variables. A chi-square test, or, in the case of only few observations, Fisher´s exact test was used to study the associations between dichotomous clinical factors and BA normality. A binary logistic regression analysis was used to study the associations between continuous clinical factors and BA normality.

Next, an adjusted regression analysis was made where the clinical factors were chosen based on the univariate analysis and the literature. Gender, gestational age, age at the time of the BAEP recording, administration of gentamicin and/or vancomycin and the duration of mechanical ventilation had several significant associations with BAEP or BA in the univariate analysis; thus these variables were chosen for the regression analysis. Apgar at 5 min age, severe infection, RDS, PDA closure, NEC, BPD and ROP were chosen based on their association with hearing loss in the literature (Kountakis et al. 2002, Zanchetta et al. 2010, Eras et al. 2014, Ping and Jiang 2014, Leung et al. 2016). Before the regression analysis, these variables were divided into three different groups so that similar variables were grouped together. The first group included clinical characteristics describing the clinical state of the infant: gender, gestational age, Apgar score at 5 min and the age at the time of BAEP/BA. Birth weight was not included due to collinearity with gestational age. The second group included variables considered risk factors for hearing loss: the duration of ventilation treatment, sepsis and/or meningitis, administration of ototoxic antibiotics (gentamicin and/or vancomycin), RDS, closure of PDA and NEC. The third group included conditions that can result from

prematurity: BPD, ROP and hearing loss. Each group was analysed one at the time for each BAEP variable by using linear regression analysis, and for BA normality by using binary logistic regression analysis. The effect of vancomycin was analysed by using linear regression analysis because the number of observations was too small for a logistic regression analysis. Afterwards, for each BAEP variable and for BA normality, a final model was generated including all significant variables from the three groups and the length of the CPAP treatment.

We also conducted an additional regression analysis where we excluded the Apgar at 5 min age, retinopathy, RDS and BPD variables as they did not have significant associations in the initial univariate analysis; although they are considered risk factors of hearing loss in the literature. RDS and BPD might also cause a multicollinearity problem with the duration of CPAP treatment. In our study, almost all infants received gentamicin (94%) and thus gentamicin and/or vancomycin administration was not included in the additional analysis. Other dependent and explaining variables were the same as in the initial regression analysis. In this analysis, we formed two groups. The first group included gender, gestational age, and the age at the time of BAEP/BA. From this group all significant variables were chosen to the final model with the duration of ventilation treatment, sepsis and/or meningitis, closure of PDA, NEC, duration of CPAP treatment and hearing loss diagnosis.

In Studies II and III, SAS for Windows version 9.4 (SAS Institute Inc, Cary, NC, USA) was used for statistical analyses and P-values below 0.05 were considered statistically significant.

4.4.4 Study IV

In Study IV we calculated the sensitivity and specificity as well as the positive and negative predictive values of BA recordings conducted during the neonatal period against hearing levels at the age of five years. In addition, we used McNemar´s test to investigate whether the proportion of abnormal results had changed between neonatal BA and later pure-tone audiometry measurements. SPSS Statistics 26 (Armonk, NY: IBM Corp) was used in the analysis.

5 Results

The clinical characteristics of the participants in all four studies are presented in **Table 2**.

5.1 Reference values for neonatal BAEP and BA (I)

The reference values for the BAEP recording are presented in **Table 3** for ten different age groups due to the post-term age dependency of the BAEP latencies and IPLs: the latencies and inter-peak intervals are shorter in older infants. The age dependency was significant for almost all BAEP variables (**Table 4**). Latencies and, to a lesser extent, also the inter-peak intervals become shorter with the increasing age of the infants as also shown in **Figure 11** and **Figure 12**.

To calculate reference values, boys and girls as well as preterm and term infants were grouped together as we found no differences between these groups in most of the BAEP variables. In addition, there were no differences between boys and girls or between preterm and term babies in inter-side differences. After the Bonferroni correction, the boys showed slightly longer latencies of BAEP component III (right ear $p = 0.005$, left ear $p=0.0007$) and IPL I-III (left ear $p = 0.002$) than girls. Although the latencies differed between genders, the boys usually had only approximately 0.1 ms longer mean latencies. Girls had a slightly higher amplitude V (right ear $p = 0.001$) and lower amplitude ratio I/V (right ear $p = 0.012$, left ear $p=0.017$) than boys. After the Bonferroni correction, none of the measured BAEP variables differed between infants born preterm or at term.

Of the reference value database, 158 infants also underwent a BA recording. We evaluated the appearance of the waves III and IV separately for both ears. At an intensity of 35 dB nHL in the right ear, wave III was found in 97.5 % and wave V in 98.1 % of infants. At an intensity of 35 dB nHL in the left ear, wave III was found in 95.6 % and wave V in 99.4 % of infants. At an intensity of 45 dB nHL in both ears, waves III and V were present in all infants. In consequence, the normal threshold for BA is 35 dB nHL, and a threshold of 45 dB nHL can already be considered abnormal.

Table 2. Clinical characteristics of all the Studies. *n= 158 (19 missing values), n**=152 (3 missing values), n***=154 (8 missing values), ****n=133 (22 missing values). BA was considered abnormal if wave III or V was seen at the intensity of 45 dB nHL or higher in the right and/or left ear. Pure-tone audiometry was considered abnormal if the $PTA^{0.5\text{-}4\ kHz}$ was over 25 dB HL in the right and/or left ear. Abbreviations: brainstem audiometry (BA), brainstem auditory evoked potentials (BAEP), centimetre (cm), decibel (dB), gram (g), hearing level (HL), kilohertz (Hz), minute (min), normalized hearing level (nHL), pure-tone average (PTA), standard deviations (SD), the Finnish version of the MacArthur-Bates Communicative Development Inventory: Words and gestures (CDI). Modified with permission from Saranto et al. 2016, Antinmaa et al. 2020, Antinmaa et al. 2021.

	STUDY I (N=177)	**STUDY II (N=155)**	**STUDY III (N=162)**	**STUDY IV (N=117)**
MALE, N (%)	101 (57)	93 (60)	94 (58)	65 (56)
AT BIRTH				
WEIGHT, G, MEAN (SD), RANGE	1570 (878), 565-4370	1245 (366), 535-2180	1204 (352), 560-2120	1184 (360), 560-2120
≤1500 G, N (%)	120 (68)	115 (74)	136 (84)	99 (85)
GESTATIONAL AGE, WEEKS, MEAN (SD), RANGE	30.6 (4.4), 23-42	29 (2.4), 24-35	29.0 (2.7), 23-35	29 (2.7), 23-35
≤32 WEEKS, N (%)	124 (70)	137 (88)	140 (86)	101 (86)
≤1500 G AND/OR ≤32 WEEKS, N (%)	146 (82)	155 (100)	162 (100)	117 (100)
HEIGHT, CM, MEAN (SD)	-	-	38.4 (3.6)	38.2 (3.6)
HEAD CIRCUMFERENCE, CM, MEAN (SD)	-	-	26.9 (2.6)	26.7 (2.7)
APGAR SCORE AT 1, 5 AND 15 MIN, MEAN	-	-	6/7/7	6/7/7
SMALL FOR GESTATIONAL AGE, N (%)	53 (30)	49 (32)	48 (30)	34 (29)
BAEP AND BA RECORDINGS				
CORRECTED AGE (MONTHS) AT THE TIME OF BAEP AND/OR BA RECORDINGS, MEAN (SD), RANGE	1.1 (1.2), -1.2-6.6	1.1 (0.3), 0.3-2.2	0.8 (-1.2-2.8)	1.0 (1.0), -1.0-6.6

	STUDY I (N=177)	STUDY II (N=155)	STUDY III (N=162)	STUDY IV (N=117)
ABNORMAL BA, N (%)	10 (6)*	17 (11)**	32 (21)***	29 (25)
CDI FORMS				
CORRECTED AGE (MONTHS) AT THE TIME OF CDI COMPLETION****, MEAN (SD), RANGE	-	12.1 (0.6), 10.0-15.5	-	-
RECEPTIVE LEXICON (NUMBER OF UNDERSTOOD WORDS) AT THE AGE OF ONE YEAR, MEAN (SD), RANGE	-	71 (67), 0-311	-	-
PURE-TONE AUDIOMETRY				
AGE (YEARS) AT THE TIME OF AUDIOMETRY, MEAN (SD), RANGE	-	-	-	5.3 (0.8), 4.0-7.8
ABNORMAL PURE-TONE AUDIOMETRY, N (%)	-	-	-	5 (4)
HEARING LOSS DIAGNOSIS, N (%)	0	2 (1)	6 (4)	6 (5)

Table 3. Reference values for latencies of BAEP components I, III, V, and IPLs I-III, I-V, III-V, and I/V amplitude ratios as well as for the inter-side differences of these variables calculated separately for the right and left ear. Abbreviations: brainstem auditory evoked potential (BAEP), interpeak latency (IPL), left ear (L), millisecond (ms), right ear (R). Modified with permission from Saranto et al. 2016.

	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	
INTERSIDE DIFFERENCE 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	
INTERSIDE DIFFERENCE 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	
INTERSIDE DIFFERENCE 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	
INTERSIDE DIFFERENCE 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	
INTERSIDE DIFFERENCE 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	
INTERSIDE DIFFERENCE 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	

Table 4. The correlation coefficient (r-value) and p-values for post-term age dependency of the BAEP latencies and IPLs according to the regression analysis in Study I. Abbreviations: brainstem auditory evoked potential (BAEP), interpeak latency (IPL). The author´s own drawing.

BAEP VARIABLE	R-VALUE		P	
	RIGHT EAR	LEFT EAR	RIGHT EAR	LEFT EAR
LATENCY I	-0.28	-0.28	<0.001	<0.001
LATENCY III	-0.25	-0.31	0.001	<0.001
LATENCY V	-0.51	-0.46	<0.001	<0.001
IPL I-V	-0.43	-0.38	<0.001	<0.001
IPL I-III	-0.12	-0.20	0.11	0.01
IPL III-V	-0.40	-0.27	<0.001	<0.001

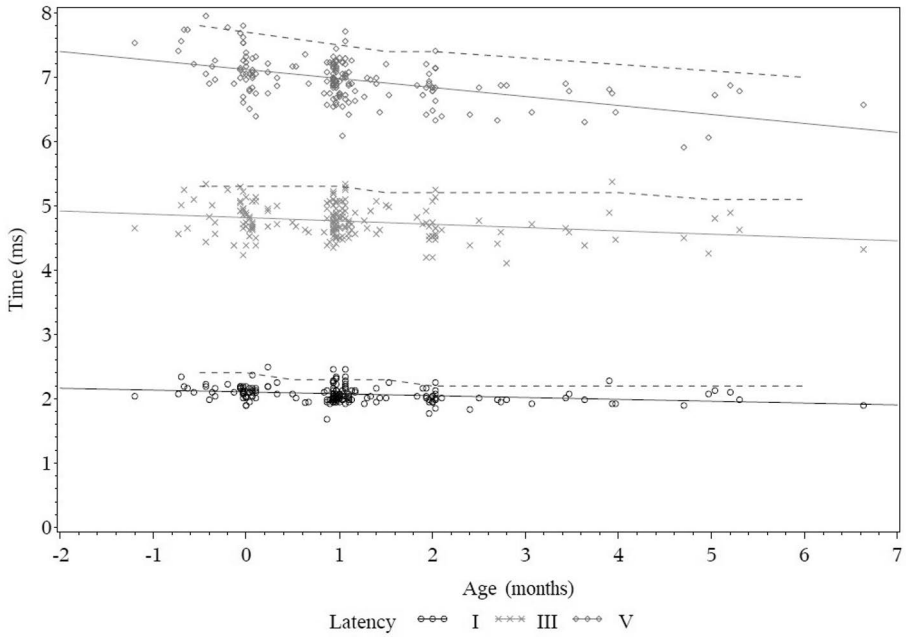


Figure 11. Latencies of peaks I, III and V of the right ear recorded at the intensity of 85 dB nHL for 177 normal hearing neonates. The straight line is the regression line, and the dashed line is the upper 95 % limit of latencies (reference values). Ages are given as months from term age. Abbreviations: decibel (dB), millisecond (ms), normalized hearing level (nHL). The author´s own drawing.

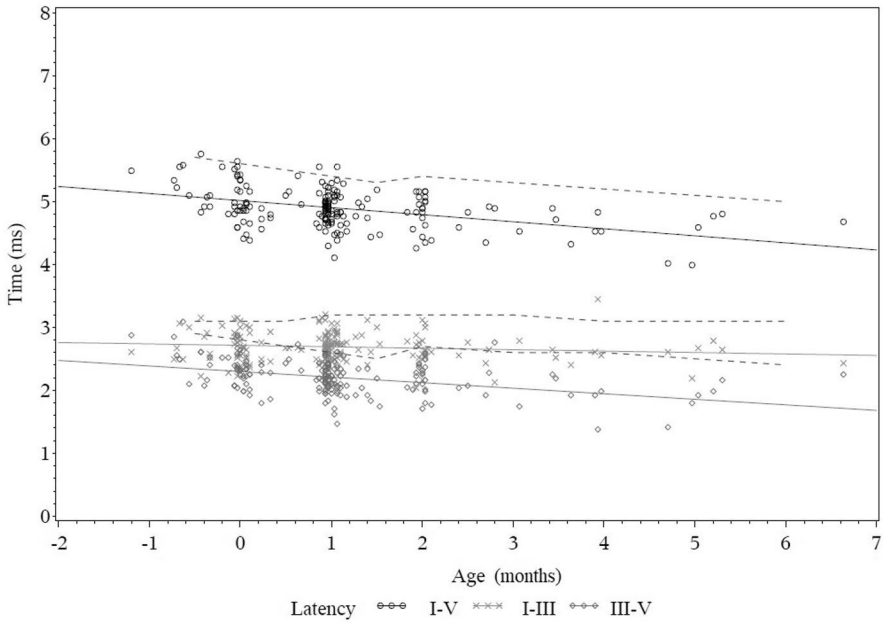


Figure 12. Interpeak latencies I-V, I-III and III-V of the right ear recorded at the intensity of 85 dB nHL for 177 normal hearing neonates. The straight line is the regression line, and the dashed line is the upper 95 % limit of latencies (reference values). Ages are given as months from term age. Abbreviations: decibel (dB), millisecond (ms), normalized hearing level (nHL). The author´s own drawing.

5.2 The association between BAEP and language development (II)

The CDI form was available for 155 children at the mean corrected age of 12.1 months. At this age, the mean receptive lexicon size was 71 words (range 0-311), and the expressive lexicon size was 4 words (range 0-34). An abnormal CDI score of <17 understood words was found in 33 children (21%).

The univariate analysis showed that the latency of the BAEP component V (r=-0.18, p=0.029) and the IPL I-V (r=-0.19, p=0.016) on the right side at the neonatal period were longer and associated to reduced receptive lexicon size at one year of age. The association between IPL I-V and the receptive lexicon size is shown in **Figure 13**. The children with a clear contralateral BAEP response with right ear stimulation understood more closed class words than children without the contralateral response (p=0.009). BAEP variables with left ear stimulation did not associate with the receptive lexicon size at one year. In consequence, the latency of the BAEP component V, the IPL I-V, and the presence of contralateral response to right ear stimulation were chosen for the regression analysis. The confounding factors (gender, gestational age, SGA status, hearing loss, age at the time of CDI completion and

BAEP recording) did not affect the receptive lexicon size. However, as the gender and gestational age can influence auditory function (Stipdonk et al. 2016, Borenstein-Levin et al. 2022), they were included in the regression analysis. The receptive lexicon size did not differ between infants with normal or abnormal BA, and higher BA threshold did not indicate weaker receptive lexicon size.

In the regression analyses, the longer the IPL I-V in the right ear, the smaller the receptive lexicon size at approximately one year later (p=0.043) after controlling for gestational age and gender. When the latency of the BAEP component V, the IPL I-V and the presence of contralateral response were analysed, infants with longer IPL I-V were more likely to have an abnormal receptive lexicon size (OR 29.9; 95% CI 1.3-683.7; p=0.033). The absence of a contralateral response (right ear stimulation) also increased the risk for an abnormal receptive lexicon size (OR 0.2; 95% CI 0.02-0.99; p=0.049). When gender and gestational age were considered, the receptive lexicon size did not differ between boys and girls, and it was not influenced by gestational age.

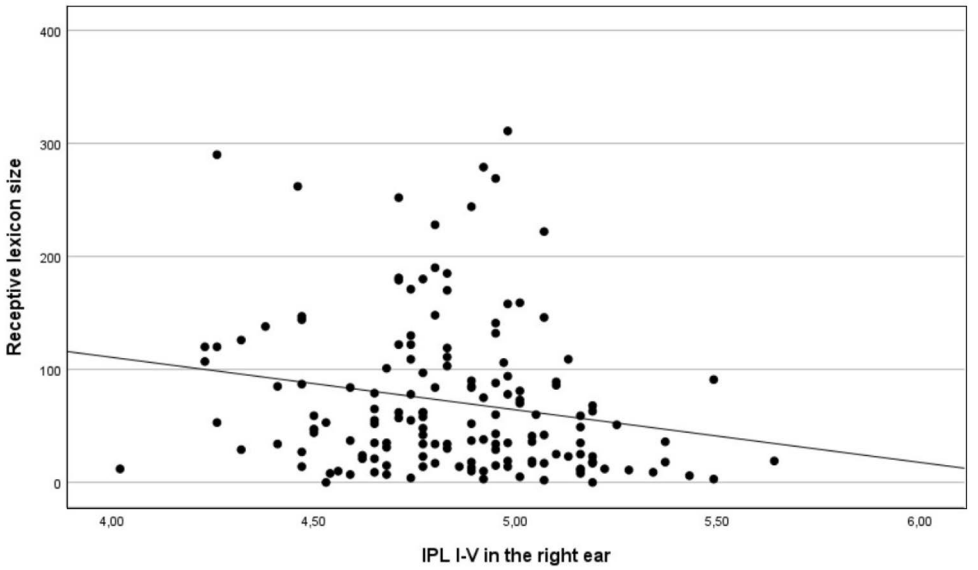


Figure 13. This scatter plot shows that longer IPL I-V (ms) with the right ear stimulation in the neonatal BAEP recording conducted at the intensity of 85 dB nHL associates with a smaller receptive lexicon size (number of understood words) at the corrected age of one year. The straight line is the regression line. Abbreviations: brainstem auditory evoked potential (BAEP), decibel (dB), interpeak latency (IPL), millisecond (ms), normalized hearing level (nHL). The author´s own drawing.

5.3 Risk factors for BAEP abnormalities (III)

In Study III, our focus was on the association between BAEP variables and the duration of CPAP treatment. The data included 162 infants, of whom 151 received CPAP therapy. The mean duration of the CPAP treatment was 17 days, the median was 15 days, and the range was 0-60 days. Of the 11 infants, who did not receive any CPAP therapy (0 days), two were on a ventilator during their treatment period in the NICU. The clinical characteristics are shown in **Table 5**. The BA was recorded for 154 children during the neonatal period and in 32 cases the BA was abnormal. Until the age of eight years, a hearing loss was diagnosed in six children, and they all needed a hearing aid. Of these six children with hearing loss, one had a normal BA, four had an abnormal BA and one did not undergo a BA recording during the neonatal period.

The main results of Study II are presented in Table 2 in the original publication III. The adjusted linear regression analysis showed that with a longer duration of the CPAP treatment, the latencies of BAEP components III and V increased on the right side ($p=0.01$ and $p=0.02$, respectively). In the additional analysis, a longer CPAP treatment duration associated weakly with a longer IPL I-V (right ear $p=0.048$) in addition to the latency of BAEP component V (right ear $p=0.002$). A longer duration of CPAP treatment did not increase the risk of an abnormal BA.

In the regression analyses, when compared to girls, boys had a longer latency of BAEP component III (left ear $p=0.0009$), and longer IPL I-V (left ear $p=0.03$) and IPL I-III (right ear $p=0.02$; left ear $p=0.0006$). A younger corrected age at the time of the BAEP recording indicated a longer latency of the BAEP component V (right ear $p=0.0006$; left ear $p<0.0001$), IPL I-V (right ear $p=0.01$; left ear $p=0.0004$) and IPL III-V (right ear $p=0.007$; left ear $p=0.009$). A lower gestational age was associated with a longer IPL III-V (left ear $p=0.04$). Infants who were treated with gentamicin and/or vancomycin had a longer IPL I-V (right ear $p=0.006$, left ear $p=0.007$) and IPL I-III (right ear $p=0.01$) compared to infants who did not receive any antibiotics. Infants who had their PDA closed with indomethacin and/or operatively had a smaller latency of BAEP component III (right ear $p=0.005$) and longer IPL III-V (right ear $p=0.02$). Children diagnosed with hearing loss had a longer latency of BAEP component V (left ear $p=0.0002$) compared to normal hearing children. Abnormal BA increased the risk for later hearing loss (OR 16.9; 95% CI 1.5-184.9; $p=0.02$).

Table 5. The clinical factors that were collected in Study II and included in the univariate analysis. *Asphyxia criteria were Apgar score ≤7 at the age of 1 min combined with breathing difficulty and asphyxia symptoms (ICD 2016), **NEC was categorized according to Vermont Oxford Network definitions, ***Diuretics were furosemide and/or hydrochlorothiazide and/or spironolactone, ****Gentamicin and vancomycin concentrations were measured before the second dose. Abbreviations: continuous positive airway pressure (CPAP), c-reactive protein (CRP), International Classification of Diseases (ICD), litre (l), micromole (µmol), milligrams (mg), minute (min), necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), standard deviations (SD). Modified with permission from Antinmaa et al. 2021.

STUDY II	**N=162**
RISK FACTORS FOR HEARING LOSS	
RESPIRATORY DISTRESS SYNDROME, N (%)	113 (70)
DURATION OF MECHANICAL VENTILATOR TREATMENT, DAYS, MEAN (SD), RANGE	6 (11), 0-60
DURATION OF CPAP TREATMENT, DAYS, MEAN (SD), RANGE	17 (14), 0-60
NO CPAP THERAPY, N (%)	11 (7)
ASPHYXIA*, N (%)	7 (4)
FETOFETAL TRANSFUSION, N (%)	6 (4)
PDA CLOSURE WITH INDOMETHACIN, N (%)	63 (39)
PDA CLOSURE BY SURGERY, N (%)	20 (12)
NECROTISING ENTEROCOLITIS**, N (%)	9 (6)
BLOOD CULTURE POSITIVE SEPSIS AND/OR MENINGITIS, N (%)	19 (12)
MAXIMUM CRP CONCENTRATION, MG/L, MEAN (SD)	
AGE <1 MONTH FROM BIRTH	15 (26)
AGE >1 MONTH FROM BIRTH	56 (80)
ADMINISTRATION OF DIURETICS***, N (%)	18 (11)
ADMINISTRATION OF GENTAMICIN, N (%)	152 (94)
GENTAMICIN, CONCENTRATION, MG/L****, MEAN (SD)	2.2 (0.5)
ADMINISTRATION OF VANCOMYCIN, N (%)	32 (20)
VANCOMYCIN, CONCENTRATION, MG/L****, MEAN (SD)	8.3 (5.2)
MAXIMUM CONCENTRATION OF BILIRUBIN, µMOL/L, MEAN (SD)	184 (43)
MAXIMUM CONCENTRATION OF BILIRUBIN IN THE FIRST THREE DAYS, µMOL/L, MEAN (SD)	141 (34)
OUTCOME MEASURES	
RETINOPATHY (DIAGNOSED BY AN OPHTHALMOLOGIST), N (%)	33/101(33)
BRONCHOPULMONARY DYSPLASIA, N (%)	21 (13)

5.4 The association between BA and pure-tone audiometry (IV)

Study IV included 117 very preterm infants (birth weight ≤1500 g and/or birth ≤32 gestational weeks) with available BA and pure-tone audiometry results. The BA was recorded during the neonatal period at the mean corrected age of one month, and a pure-tone audiometry was conducted at the mean age of five years. All the children who had a normal BA, also showed normal $PTA^{0.5\text{-}4\ kHz}$ results. Five children had both abnormal BA and $PTA^{0.5\text{-}4\ kHz}$. An abnormal BA but a normal $PTA^{0.5\text{-}4\ kHz}$ was found in 24 children (**Table 6**). The proportion of abnormal results was higher in the BA than in the pure-tone audiometry measurements ($p<0.0001$). Concerning later hearing, the sensitivity and negative predictive value of the neonatal BA were both 100%. The specificity was 79%, but the positive predictive value only 17%.

Table 6. The number of cases for each BA and pure-tone audiometry group. *BA was abnormal if wave III or V was seen at the intensity of 45 dB nHL or higher in the right and/or left ear **Pure-tone audiometry was considered abnormal if the $PTA^{0.5\text{-}4\ kHz}$ was over 25 dB HL in the right and/or left ear. Abbreviations: brainstem audiometry (BA), decibel (dB), hearing level (HL), normalized hearing level (nHL), kilohertz (kHz), pure-tone average (PTA). The author´s own drawing.

	ABNORMAL PURE-TONE AUDIOMETRY**	**NORMAL PURE-TONE AUDIOMETRY**
ABNORMAL BA*	5	24
NORMAL BA	0	88

6 Discussion

Very preterm children have an increased risk for delayed auditory maturation (Stipdonk et al. 2016), hearing loss (van Dommelen et al. 2015) and delayed language development (van Noort-van der Spek et al. 2012) and thus, they are in need of reliable hearing screening and diagnostics during the neonatal period. Automated OAE is commonly used before discharge, but it only provides information on the function of the outer hair cells in the cochlea, and preterm infants are especially at risk for central auditory pathway abnormalities. Central defects can be detected by aABR, but it only delivers a robust pass or refer result. Compared to aOAE and aABR, BAEP and BA give more detailed information about the auditory function, and are considered more reliable for hearing screening in infants admitted to the NICU (Suppiej et al. 2007). However, to optimally utilise BAEP and BA for diagnostics, reliable reference values are needed. As this thesis work shows, BAEP and BA can be utilised in mapping possible risk factors for hearing impairment, and they were useful in recognising very preterm children who are at risk for future language delays and hearing difficulties.

6.1 Reference values for neonatal BAEP and BA recordings and later audiometric evaluation (I, IV)

In Study I, we calculated reference values for BAEP and BA recordings which were taken into clinical practice in 2011 in the Department of Clinical Neurophysiology at Turku University Hospital. Previously, reference values from the literature were applied (Chiappa 1997). However, literature reference values may not be applicable if the measuring equipment and sound conduction methods or recording sites differ. It is recommended, that different laboratories should have their own reference values as the equipment, recording conditions and stimulus parameters (intensity, frequency, shape of the stimulus) can vary affecting the BAEP and BA results. We also wanted to calculate reference values including very preterm infants, as they have the most need for reliable and sensitive BAEP and BA examinations. In several previous studies presenting reference values for neonatal BAEP recording, preterm

infants have been excluded (Jiang et al. 1991, Scaioli et al. 2009, Coenraad, Van Immerzeel, et al. 2010).

In 2006, the need for new, specific reference values emerged when traditional headphones were replaced with TIPs to deliver the stimuli to the ear canal. The TIPs have several advantages. They cause less sound leakage and ear canal collapse and are better suited to infant´s ears than headphones. TIPs are also more comfortable for infants, and better tolerated than headphones (**Figure 1**). As a consequence of using TIPs, the BAEP and BA recordings are of better quality with less artifacts. In addition, the air conduction time caused by silicon tube in TIPs, prolongs BAEP latencies making the early components I and II better discernible. Earlier, most research groups used headphones to record BAEP responses in normal hearing neonates (Eggermont and Salamy 1988, Jiang et al. 1991, Issa and Ross 1995), which may have diminished the reliability of those BAEP results. Later, insert earphones became more commonly used (Cornacchia and Del Prete 1998, Amorim et al. 2009 and Chhajed et al. 2021). However, these studies included only a few preterm infants (n=12-75) compared to our study (n=146). In addition, the infants were only moderately preterm (GA 34-36 weeks) (Chhajed et al. 2021) compared to our GA of 32 weeks or less, or the gestational age range was not mentioned (Cornacchia and Del Prete 1998, Amorim et al. 2009). To our knowledge, our study (I) is one of the largest neonatal BAEP reference value databases including mostly (82 %) very preterm infants whose birth weight was ≤1500 g and/or they were born ≤32 weeks.

Normal-hearing preterm infants have a delayed auditory pathway conduction at term age compared to term born infants (Stipdonk et al. 2016). The central auditory pathway function in particular is still incomplete in very preterm infants (Jiang et al. 2002, Stipdonk et al. 2016). This delay in central auditory maturation was also seen in Study III, where a lower gestational age associated with a longer IPL III-V that reflects conduction in the pontine-midbrain auditory circuits. Consequently, very preterm infants should be included in neonatal reference value databases as they also have the highest risk of hearing loss. Compared to earlier studies presenting reference values for neonatal BAEP variables in very preterm infants (Jiang et al. 2002, Coenraad et al. 2011) (**Table 7**), the BAEP and BA recordings were conducted later in our study (I) at the mean corrected age of one month. This may lead to more reliable results as the risk for confounding factors such as fluid in the middle ear is smaller. In addition, the lower click rate of 10.3 Hz compared to earlier studies (Jiang et al. 2002, Coenraad et al. 2011) may have led to shorter mean absolute latencies in our study (I). As a result, due to several methodological differences, the normative data presented in previous studies are not applicable at our department.

Table 7. Comparison of previous studies presenting reference values for preterm infants. Abbreviations: Brainstem auditory evoked potentials (BAEP), decibel (dB), gestational age (GA), hertz (Hz), normalized hearing level (nHL), postconceptional age (PCA), very low birth weight (VLBW). The author´s own drawing.

	EGGERMONT ET AL. 1988	JIANG ET AL. 2002	COENRAAD ET AL. 2011
N	179	62	28
AT BIRTH	-	24-32 weeks GA	VLBW
AGE AT THE TIME OF BAEP	32 weeks PCA (34 GA) -5 years	37-42 PCA (39-44 GA)	26-34 PCA (28-36 (GA)
CLICK INTENSITY	-	60-80 dB nHL	90-100 dB nHL
CLICK RATE	15 Hz	21-91 Hz	29 Hz
HEADPHONE TYPE	Earphones	Earphones	-

Contrary to the findings of Eggermont et al. 1988, Jiang et al. 2002 and Stipdonk et al. 2011, other studies have found that the BAEP variables did not differ between preterm and term infants (Jiang and Wilkinson 2008, Amorim et al. 2009, Raposo et al. 2021). In our study (I), most of the BAEP variables, recorded at the corrected age of one month did not differ between infants born preterm or term, and thus common reference values were calculated. Due to the representative sample of very preterm infants in our data, we believe that our reference values can also be applied to this population. This is supported by the study of Eggermont et al. where the mean latencies and IPLs of the preterm group were roughly the same as in our study (Eggermont and Salamy 1988).

Common reference values were also calculated for boys and girls as we found no differences between genders in most of the BAEP variables, which is in line with other studies (Cornacchia and Del Prete 1998, Scaioli et al. 2009, Coenraad, Van Immerzeel, et al. 2010). In our reference value data (I), boys had only approximately 0.1 ms longer mean latencies. However, it should be kept in mind that some evidence for gender differences exist, where boys may present longer conduction times compared to girls (Mochizuki et al. 1983, Borenstein-Levin et al. 2022).

Separate reference values were calculated for right and left ear because it has been suggested that the right ear is the dominant ear in most neonates (Ari-Even Roth et al. 2016). Scaioli et al. also reported data separately for the right and left ears, as they found that BAEP variables differed between the ears (Scaioli et al. 2009). In other earlier studies, results from the best ear (Coenraad et al. 2011) or results only from the right ear (Coenraad, Van Immerzeel, et al. 2010) or left ear (Jiang et al. 2002) were included.

Most of the infants in our study completed a BA recording, and in this sample, the normal BA threshold was determined to be 35 dB nHL (Study I). This is in line with previous literature, as thresholds between 30-40 dB nHL have been considered normal (Jiang and Wilkinson 2008, Coenraad, Goedegebure, et al. 2010, Chhajed et al. 2021). However, Jiang et al. determined the normal upper limit for the BA threshold to be 20 dB HL for healthy term-born infants (Jiang et al. 1991). According to the results of Study IV, the threshold of 35 dB nHL can be considered appropriate as it showed a 100 % sensitivity and a negative predictive value when the BA results were compared to pure-tone audiometry results five years later (Study IV). In previous studies, click evoked BA has been found to associate well with later pure-tone audiometry showing good correlation coefficients of r=0.62-0.98 (Marttila and Karikoski 2006, Baldwin and Watkin 2013, Lu et al. 2017, Cheng et al. 2021). The best correlations have been seen with PTA over the frequencies of 2-4 kHz, 1-4 kHz and 500 Hz-4 kHz, and the lowest correlations with PTA over the frequencies of 500 Hz-1 kHz (Baldwin and Watkin 2013, Lu et al. 2017, Cheng et al. 2021). Our study (IV) included only very preterm born children with normal and abnormal hearing, whereas previous studies included only hearing impaired children with only a few who were born very prematurely (Marttila and Karikoski 2006, Baldwin and Watkin 2013, Lu et al. 2017, Cheng et al. 2021).

In Study IV, all the infants who had a normal BA (threshold of 35 dB nHL in both ears) in the neonatal period, had a normal pure-tone audiometry result approximately five years later. Of the 29 infants who had abnormal neonatal BA, only five had abnormal pure-tone audiometry at the age of five years. This discrepancy between elevated BA thresholds and normal audiometry is probably due to transitory changes during the neonatal period in the auditory pathway, such as middle ear effusion or delayed auditory maturation, that do not cause permanent hearing loss. All previous studies also reported that click evoked BA may overestimate future risk of hearing loss (Marttila and Karikoski 2006, Baldwin and Watkin 2013, Lu et al. 2017, Cheng et al. 2021). Especially very preterm infants born before 35 gestational weeks, have greater inconsistencies between neonatal BA and future audiometric results compared to those children born after 35 gestational weeks (Baldwin and Watkin 2013). The type of hearing loss seems to diminish the predictive value of click evoked neonatal BA. However, when neonatal BA underestimates the future hearing loss, it is mostly due to progressive disorders leading to hearing loss and not the type of hearing loss which supports the use of click evoked BA in hearing screening. (Baldwin and Watkin 2013) Despite the overestimation of hearing loss by neonatal BA, our study (IV) indicates that with perfect sensitivity and negative predictive value, BA threshold of 35 dB nHL can be considered a reliable reference limit.

6.2 Detecting infants at risk for delayed language development (II)

According to our results, abnormalities in a BAEP recording in the neonatal period indicates a poorer receptive lexicon size one year later in preterm born infants. The BAEP abnormalities were prolonged IPL I-V and absent contralateral responses with right ear stimulation which are most likely due to poorer synaptic efficacy and delayed myelination within the auditory brainstem circuitry. The results suggest that even these milder auditory pathway abnormalities can disturb language development. Only two children in Study II were diagnosed with unilateral hearing loss at the age of two months and at two years.

Our results are in line with previous studies reporting associations between auditory pathway function and language development (Cox et al. 1992, Amin et al. 2014, Wang et al. 2020). At the age of eight years, VLBW children with bilateral neonatal BAEP abnormalities (BA threshold over 30 dB nHL or prolonged IPL I-V) had lower scores on IQ test, language and academic achievement, and they were more likely to repeat a grade compared to children with normal BAEP (Cox et al. 1992). As in our study, longer IPL I-V indicated poorer language scores at the age of three years in children born before 33 gestational weeks in a study of Amin et al. 2014. In both of these studies, the BAEP recordings might have been vulnerable to conditions related to prematurity, as they were conducted before discharge or at 35 weeks PMA (Cox et al. 1992, Amin et al. 2014). In a more recent study on 95 preterm children with GA range of 28-36 weeks, the BAEP recordings were conducted at the age of six months (Wang et al. 2020). At this age, children are clinically stable which may lead to more reliable BAEP results. The study found that abnormal BAEP (BA threshold over 40 dB or prolonged latencies or IPLs) indicated lower scores on cognitive tests including language assessment at the age of two years. Compared to these earlier comparative studies on older children, we found that deviant language scores can already be found at the corrected age of one year (II). In addition, the number of children in these previous similar studies were smaller (n=56-95) compared to our study (n=155).

Previous studies have utilised for example the Wechsler Intelligence Scale for Children, the Token Test for Children (Cox et al. 1992), the Preschool Language Scale-4 (Amin et al. 2014) and the Bayley Scales of Infant Development (BSID II) (Wang et al. 2020) to study language development. These structured tests are performed by professionals in laboratory setting, whereas the CDI used in our study, is performed by parents. However, parental evaluation can also have several advantages as described in Chapter 2.5.4. In addition, studies have shown that CDI is a valid method to evaluate language development in children (Lyytinen 1999, Fenson et al. 2007).

Very preterm children especially are in need of reliable tests for language evaluation, as they are at a greater risk of having delayed language development throughout childhood compared to term born infants (Stolt et al. 2009, van Noort-van der Spek et al. 2012, Putnick et al. 2017). In VLBW children, the receptive lexicon size at the age of one year has been found to predict language development at the age of two years (Stolt et al. 2009). Our study suggests that a weak early receptive lexicon can be due to slower auditory processing reflected in neonatal BAEP and BA recordings. Hence, BAEP recordings conducted at the corrected age of one month could help professionals to reliably identify those preterm infants who are at risk of delayed language development.

In addition to BAEP and BA, other methods have been investigated to identify preterm infants at risk for adverse neurodevelopmental outcomes. Abnormal findings in brain MRI and ultrasound performed at term age, that show, for example, white matter lesions, have been found to predict later cognitive outcome including delayed language development (Chau et al. 2013, Burkitt et al. 2019, de Bruijn et al. 2022). A normal brain MRI at term age is particularly thought to indicate normal cognitive outcome at the age of five years (Setänen et al. 2013). Contradictory findings of the predictive value of brain MRI at term age can also be found (Burkitt et al. 2019). The predictive value of brain MRI or ultrasound on neurosensory outcome can be improved by performing neurologic examination at term age (Setänen et al. 2014). However, MRI and ultrasound can only detect visible lesions, and they do not reveal abnormalities at the functional or cellular levels. Brain metabolites have been studied by proton magnetic resonance spectroscopy to detect abnormalities at a molecular level. Reduced N-acetylaspartate/choline ratio especially has been found to associate with neurodevelopmental problems including poorer language scores at the corrected age of 18-24 months in preterm infants (Cebeci et al. 2022, Gire et al. 2022). In addition, levels of cerebrospinal fluid proteins, that are important for brain development, have been studied. For example, lower levels of vascular endothelial growth factor C, have been found in preterm infants with adverse neurodevelopmental outcome compared to infants with normal neurodevelopment (Leifsdottir et al. 2022). Early postnatal EEG representing brain activity may also predict cognitive outcome in extremely preterm infants (Nordvik et al. 2022) which can be improved by other neurophysiological methods (somatosensory evoked potentials) especially in infants with hypoxic-ischaemic encephalopathy (Nevalainen et al. 2017). While presenting interesting possibilities for predicting neurodevelopment in preterm infants, especially the brain MRIs and cerebrospinal fluid samples are not as feasible in every day practice compared to BAEP and BA recordings that are non-invasive, quick to administer and relatively easy to analyse. At the moment, BAEP and BA combined may be the most practical

and cost-effective method to identify infants at risk for delayed language development already in the neonatal period, enabling early intervention.

Early intervention for delayed language development can be started already in the NICU. Studies have shown that preterm infants present better cognitive and language outcome at the corrected age of 7 and 18 months after exposure to adult talk during their stay in the hospital (Caskey et al. 2014). Interactive talk with the infant seems to particularly lead to better language development in very preterm infants. In the NICU, attention should also be paid to the amount of close contact between parents and the child as the closeness has been shown to be beneficial for language development. (Ståhlberg-Forsén et al. 2022) After discharge, caregivers should be informed and supported to provide rich language environment at home including conversations with the child (Landry et al. 2002, Tulviste and Tamm 2021). Based on the results of Study II, these interventions should be targeted especially to preterm infants who present BAEP abnormalities in the neonatal period. To begin interventions already in the NICU, it would require that BAEP and BA recordings are conducted during the hospital stay. It should be kept in mind that examining very immature and possibly sick infants may lead to unreliable BAEP and BA results compared to the results of the present study (II) where clinically stable infants were examined at the corrected age of one month. Due to ongoing morphological changes in BAEP response, the recording should not be performed before 34 weeks PCA (36 gestational weeks) (Coenraad et al. 2011).

6.3 Risk factors for abnormal auditory pathway development in preterm infants (III)

Preterm birth is considered one of the risk factors for hearing loss as it seems to adversely influence development of the auditory system, especially the auditory pathways in the brainstem (Stipdonk et al. 2016, Jiang et al. 2020). This may be due to the exposure to an extrauterine environment during a time when the brain is still critically developing (Moore and Linthicum 2007, Lahav and Skoe 2014, Schmidt Mellado et al. 2022). Preterm infants often have long treatment periods in the NICU, at which time they are exposed to different risk factors for hearing loss. Often risk factors are studied by using robust hearing screening results (pass/refer) or the presence of later clinical hearing loss (Chant et al. 2022, Yu, Lin, et al. 2022). For clarity, the harmful effects of different risk factors should be investigated by using sensitive measures such as BAEP and BA, to obtain more detailed information on the functioning of the auditory pathway. In addition, these methods aid localizing possible defects. Furthermore, as Study II shows, compromised auditory function elucidated in abnormal neonatal BAEP recordings may indicate problems in

language development even without clinically evident hearing loss in later childhood.

In Study III, we found that CPAP treatment for very preterm infants in the NICU is a novel independent risk factor for abnormal auditory development. A longer duration of CPAP treatment independently associated with longer latencies of BAEP component III and V, and longer IPL I-V on the right side. Earlier studies have concluded that the harmful effects of CPAP treatment include pneumothorax (Ho et al. 2020), increased middle ear pressure, otic barotrauma (McCormick et al. 2016), nasal irritation and abdominal distension (Bjorklund et al. 2019). Besides this scarce and indirect evidence, there is very limited information of the possible effects of CPAP treatment on hearing and auditory function. Our results indicate that CPAP has an adverse effect on the central auditory function reflected in the prolonged IPL I-V of the BAEP recording. This might be caused by loud noise generated by CPAP devices.

It has been reported, that CPAP devices can generate noise levels of 54-89 dB(A) (Kirchner et al. 2012) and 50-58 dB (Singh and Fusch 2021) that are clearly above recommendations (Committee on Environmental Health 1997). Despite the efforts to reduce noise levels, studies still report intensities up to 47-83 dB in the NICU (Mayhew et al. 2022) and roughly 30 % of the sound environment of a preterm infant in the NICU is noisy (Caskey et al. 2011). Loud noise can damage any part of the developing auditory pathway from the hair cells in the cochlea to the auditory cortex (Bureš et al. 2017). Noise may disrupt the myelination and synaptogenesis in developing auditory pathway (Kujawa and Liberman 2009, Tagoe et al. 2014). However, these findings are based on animal models and there is limited information of the effect of noise on the developing human auditory pathway. Noise exposure during pregnancy does not increase the risk for hearing screening failure in the neonatal period, but this may be explained by attenuation of noise by maternal tissue (Guven et al. 2019). The sound environment of a foetus is very different from the noisy NICU that preterm infants in our study were exposed to.

To our knowledge, Study III was the first study investigating the association between CPAP treatment and neonatal BAEP results. Since then, one study including infants born before 37 gestational weeks, found that CPAP treatment did not associate with IPLs in BAEP recording (Borenstein-Levin et al. 2022). Our study (III) included only very preterm infants and rather old and perhaps noisier CPAP equipment, which may partly explain the opposite results. Another study (Rastogi et al. 2013), showed that very preterm infants treated with CPAP are not at increased risk for hearing loss compared to children treated with mechanical ventilation. However, in that study, hearing was assessed with an aABR that does not give as detailed results as the BAEP used in the present thesis work. Due to these

contradictory findings, further study on this area is needed, controlling for general noise levels in the NICU in addition to CPAP treatment and its sound level.

Our study (III) did not find associations between mechanical ventilation or BPD and BAEP variables. Earlier studies have reported that mechanical ventilation did not affect IPLs in BAEP recording (Borenstein-Levin et al. 2022). However, it has also been found that mechanical ventilation (Raposo et al. 2021) and BPD (Borenstein-Levin et al. 2022) might accelerate auditory pathway maturation. This might be due to earlier exposure to an extrauterine environment of the most immature infants who are also at risk for requiring mechanical ventilation. Opposite results exist showing that preterm children with BDP and term children with asphyxia have increased latencies and IPLs compared to healthy term infants (Jiang et al. 2010). This could be explained by the fact that the auditory pathway is vulnerable to hypoxia.

NEC did not associate with the BAEP results in our study (III), contrary to a report on preterm infants in which the NEC showed longer latency V and IPL I-V compared to children without NEC (Jiang et al. 2022). In our study (III), infants who had their PDA closed with indomethacin and/or operatively had a smaller latency of BAEP component III, but longer IPL III-V indicating delayed central auditory pathway development and accelerated development of the peripheral part of the auditory pathway. Another study found no association between the PDA closure and BAEP results (Borenstein-Levin et al. 2022).

In our study (III), the infants who were administrated with gentamicin and/or vancomycin had longer IPLs I-V and I-III compared to infants who did not receive antibiotics, which indicates, that the central auditory structures may be involved. Another study reported increased latency of BAEP component I and an increased BA threshold in preterm born infants, who received gentamicin (Raposo et al. 2021) indicating more peripheral involvement. Other studies show similar findings of the detrimental effects of aminoglycosides to both peripheral and central auditory structures (Ito 1984, Zimmerman and Lahav 2013). Thus, it is important to monitor the concentrations of gentamicin and vancomycin especially in infants with renal problems and to adjust the dose of these antibiotics. In our study (III), sepsis and/or meningitis and ROP did not associate with the BAEP variables which is also supported by recent studies (Raposo et al. 2021, Borenstein-Levin et al. 2022).

In summary, there are contradictory findings regarding many of the risk factors for neonatal auditory abnormalities. It appears that the maturation of the auditory system in preterm infants is affected by conflicting conditions of the accelerating effects of extrauterine overexposure and the harmful effects of preterm birth, as well as related clinical conditions and treatments.

6.4 Strengths and limitations of the study

All BAEP and BA recordings in this thesis work were conducted by professionals using quality-controlled standardized methods. TIPs were used to deliver the auditory stimuli, further improving the reliability and quality of the recordings. Occasionally, BAEP and BA recordings can be difficult to perform due to the restlessness of the infant. However, only eight infants had to be excluded from the databases (I-IV) due to this kind of technical disturbances. The recordings were carried out at the mean corrected ages of 0.8-1.1 months at which time infants no longer had any acute conditions such as hyperbilirubinemia or infections. The ears of all infants were checked for middle ear effusion by a paediatrician before the recordings which diminished the possibility of confounding effects on the BAEP and BA variables. The sample sizes were large which is also a strength of this thesis work.

Despite the large data set in Study I, only six infants were over four months, and six infants were under 38 gestational weeks at the time of the BAEP recording. Thus, the reference values of these age groups might not be as reliable as for babies tested near term age. The most common exclusion criteria were that the original BAEP data was not available for reanalysis (n=7-27). In these cases, especially in the years 2002-2006, the original BAEP recording was no longer obtainable from the archives. We believe that this did not affect our results, as there was no consistency regarding the missing recordings. In Study II, 24 infants had to be excluded because the parents did not return the CDI form. However, these 24 infants had a similar gestational age and birth weight to the children who were included in the study.

All studies were retrospective and thus, some important information is missing. In Study II, we did not have information on the educational level of the parents which is known to influence language development of the child (Sentenac et al. 2021). In Study III, more detailed information of CPAP treatment would have been informative. For example, flow rates, which are known to affect noise intensities (Karam et al. 2008), were not obtainable. In addition, the actual noise intensities of the CPAP devices were not measured due to the retrospective study design, and we did not have information on the distance between the infant and the CPAP device, or where the infants were treated (crib or incubator). However, these issues may have a minor role as the loudest noise made by the CPAP is produced in the nasal prongs and by air turbulence in the upper airways (Karam et al. 2008, Kirchner et al. 2012, Singh and Fusch 2021). The oxygen levels of infants during the CPAP treatment would also have been informative, as hyperoxia can adversely affect the immature brain (Reich et al. 2016).

In Study III, the prolonged latencies of BAEP components and IPLs, which were associated with longer CPAP treatment, may have normalized with advancing age. We did not conduct control BAEP and BA recordings to investigate this matter due

to the retrospective study design. In addition, fluid in the middle ear can temporarily cause prolonged latencies. The ears were checked by a paediatrician before the recordings, but a tympanometry would have given more reliable information concerning the inner ear status. However, although the changes might have been transitory and resolved over time, the changes at the corrected age of one month still appear to associate, for example, with language development as seen in Study II.

The data in all the studies is rather old so the results should be interpreted with caution. For example, the CPAP device used for infants born during the study period 2002-2006 is not used anymore. In a more recent study, CPAP treatment did not have an adverse effect on auditory pathway conduction in preterm infants (Borenstein-Levin et al. 2022). However, changing to more modern equipment might not lower the sound intensities produced by nasal prongs. In addition, high noise levels are an ongoing problem in the busy NICUs (Mayhew et al. 2022), and our results indicate that loud noise may disturb auditory pathway development.

6.5 Future lines of research

In the future, the reference value database should be expanded so that the results for older infants would be more reliable. There is a need to conduct BAEP and BA recordings at term age or even earlier so the reference value database can be expanded to also cover younger neonates. The association between abnormal neonatal BAEP and BA results and later hearing should be further investigated in a prospective study design to confirm the reliability of these reference values in the neonatal diagnostics of hearing; in addition to their ability to predict delays in language development as well as hearing impairments in later childhood. In Study II, whether the child had an abnormal or normal BA did not affect the later receptive lexicon. However, it would be interesting to know whether the same would be found with different BAEP variables, or with a lower BA limit of 20 dB nHL that is also currently used in our department. These could give professionals and parents more precise information on the possible risk for delayed language development.

In Study IV, information on the aOAE and aABR results was not gathered due to missing data. Currently, it is recommended that aOAE should be conducted in the NICU at Turku University Hospital for all very preterm infants before the BAEP and BA. Ideally, all newborns should be screened with methods that identify both inner ear and central auditory pathway abnormalities (**Figure 14**). However, this might be difficult to implement due to, for example, financial restrictions, the lack of trained personnel and reliable hearing screening equipment. In the future, it would be beneficial to compare the reliability of aOAE, aABR, BAEP and BA in very preterm infants to obtain the best possible protocol for hearing screening, as there is a lack of research in this area.

As discussed earlier, there are contradictory findings regarding several different risk factors, for example preterm birth and BPD, and their association with auditory function. In the future, more research on this subject is needed with larger databases including good representation of all possible risk factors for hearing loss. Previously, CPAP treatment has not been included in the regression models, but based on the results of this thesis work, it can be concluded that a long duration of CPAP treatment may disrupt auditory development. Future studies should include modern CPAP devices with noise intensity measurements. The associations between CPAP treatment duration and psychoacoustic and neurophysiological findings later in childhood require more research.

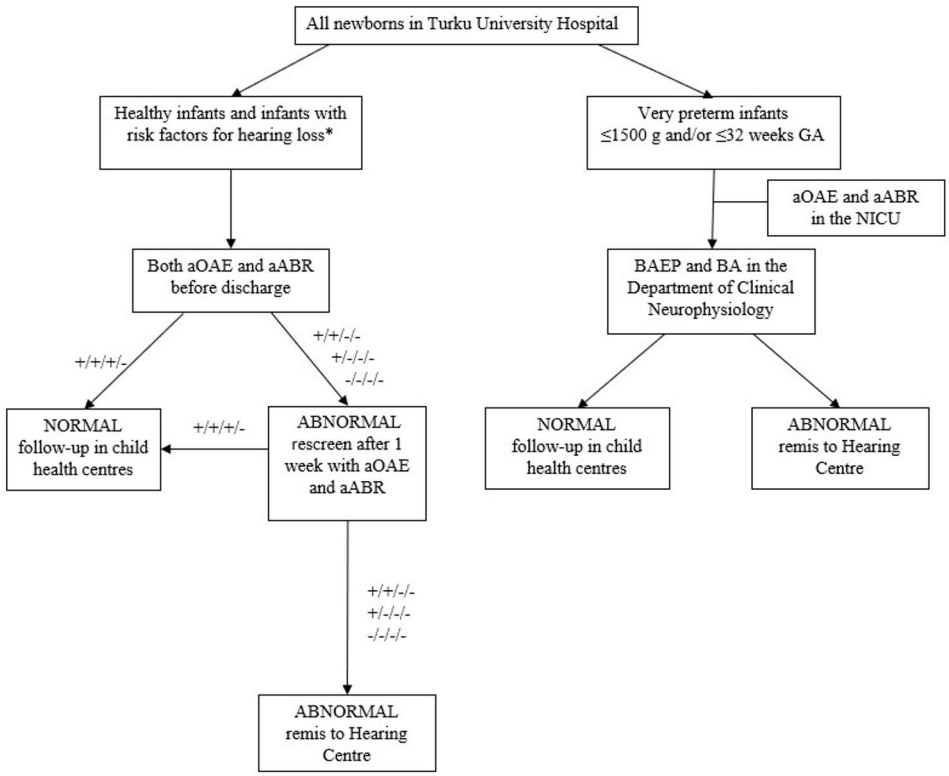


Figure 14. In the future, this would be the preferred hearing screening protocol for newborns. Plus indicates a pass screening result and minus indicates a failed result in one ear. *Family history of congenital hearing loss, craniofacial malformations, chromosomal abnormalities, long QT syndrome, NICU treatment. Abbreviations: automated auditory brainstem response (aABR), automated otoacoustic emission (aOAE), brainstem audiometry (BA), brainstem auditory evoked potentials (BAEP), gestational age (GA), gram (g), neonatal intensive care unit (NICU). The author´s own drawing.

In the future, reliable methods should be found to identify as early as possible very preterm infants at risk for later neurodevelopmental problems. This would enable early intervention giving very preterm infants the best possible environment to achieve their full potential. So far in preterm infants, an abnormal brain MRI (Chau et al. 2013, de Bruijn et al. 2022), levels of certain brain metabolites (Cebeci et al. 2022, Gire et al. 2022) and cerebrospinal fluid proteins (Leifsdottir et al. 2022) as well as an abnormal BAEP (Wang et al. 2020) during the neonatal period have been shown to associate with later neurodevelopmental problems. In our study (II), the prolongation of the conduction time in the auditory pathway in the neonatal period associated with poorer language skills at the corrected age of one year in very preterm infants, and studies have found that this association persists throughout childhood (Cox et al. 1992, Amin et al. 2014, Wang et al. 2020). Of these different methods, BAEP and BA are perhaps the most applicable in clinical practise, but their reliability should be determined in comparison with other methods (brain MRI, brain metabolites, cerebrospinal fluid proteins). A combination of different methods might prove to be beneficial as well. Searching the most accurate and feasible methods for identifying children at risk for adverse neurodevelopmental outcome should continue in order to improve the health of very preterm infants.

7 Summary/Conclusions

1. This thesis work presents reference values for neonatal BAEP and BA recordings to be used in clinical diagnostics. These reference values are also applicable in very preterm infants who are at great risk for abnormal auditory pathway development.

2. The prolongation of conduction velocities and the absence of contralateral responses in the auditory pathway in neonatal period associates with poorer receptive lexicon at the corrected age of one year in preterm infants. This indicates that very preterm children at risk for delayed language development could be identified already at the corrected age of one month in routine neonatal BAEP recording enabling early intervention.

3. Longer duration of CPAP treatment in very preterm infants may be one of the risk factors for delayed auditory maturation seen as prolonged latencies in neonatal BAEP recording. The disruption in auditory pathway function can be caused by the noise generated in the nasal prongs of CPAP device. The hearing and neurodevelopment should be followed in preterm infants treated with CPAP.

4. The reference limit for BA was set at 35 dB nHL. Neonatal BA, evaluated based on this threshold against pure-tone audiometry results five years later, was found to be a reliable method for hearing screening showing perfect sensitivity and negative predictive value.

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