Neuronal Biomarkers of Cognitive Development in Preterm and Term Born Infants: a Multidimensional Approach Combining Electrophysiology and Peripheral Blood Biomarkers

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Table of Contents

ABSTRACT

Prematurity is a global health problem, affecting about 11% of infants born worldwide. Due to the recent advancements in neonatal medicine the rates of preterm births are increasing and especially the survival of very and extremely preterm. Preterm born infants are at high risk for neurodevelopmental deficits, which have a lifelong impact. Therefore it is of utmost clinical importance to find a screening tool to detect infants at high risk that can possibly benefit from early intervention programs.

In the present work we aim at investigating neuronal biomarkers in preterm and term born infants in order to examine their ability to predict neurodevelopmental outcome. Behavioral tests alone are inadequate to assess cognition in early infancy and therefore neuronal biomarkers are considered. Two methods are explored: an electrophysiological approach, using auditory event related potentials (AERPs) and a protein of neuroaxonal injury found in the blood named neurofilament (Nf). Three publications are included. First a systematic review of the literature, examining the association between AERPs and cognitive outcome in preterm born infants. This review is followed by a pilot study, where neonatal AERPs are investigated and correlated with neurodevelopmental outcome at 2 years in healthy very preterm and term born infants. In particular discrimination and habituation are examined as early forms of attention and learning respectively. Finally, we investigated a promising biomarker of neuroaxonal injury Nf light chain (NfL) for the very first time in preterm and term born infants during the first week of life.

I. INTRODUCTION

1.1. Prematurity and neurodevelopmental outcome

Preterm birth is defined as delivery before 37 completed weeks of gestation (WHO, 2018). The prevalence of preterm birth is about 11% worldwide, with higher incidence in Africa and South Asia (Blencowe et al., 2012). Prematurity is the main cause of infant death (Liu et al., 2015) and surviving preterm born infants are at higher risk for neurodevelopmental deficits as compared to infants born at term (Bhutta, Cleves, Casey, Cradock, & Anand, 2002). Based on the gestational age (GA), preterm infants are classified as follows: moderate to late preterm (32-36 weeks GA), very preterm (29-31 weeks GA) and extremely preterm (below 28 weeks GA) (WHO, 2018). The risk of cognitive impairments due to preterm birth increases exponentially with the degree of prematurity at birth (Larroque et al., 2008). It is estimated that 52% of extremely preterm infants, 24% of very preterm infants and 5% of moderate to late preterm infants suffer from neurodevelopmental impairments (Blencowe et al., 2012). Cognitive impairments include a decrement in IQ points, attention and language problems as well as deficits in executive functions (i.e. inhibition, planning, cognitive flexibility, working memory and verbal fluency) (Aarnoudse-Moens, Weisglas-Kuperus, van Goudoever, & Oosterlaan, 2009; Johnson & Marlow, 2017; Ribeiro et al., 2011). Socio-behavioral sequelae are also common and preterm infants are at higher risk for autism and hyperactivity disorders (Johnson & Marlow, 2017; Moreira, Magalhaes, & Alves, 2014). These deficits may persist into adulthood and therefore influence later academic and professional achievements of former preterm children (Johnson & Marlow, 2017). Besides cognitive and behavioral deficits associated with prematurity, motor impairments are also common (Moreira et al., 2014), but exceed the scope of the current work.

With increasing medical knowledge and expertise, the survival rate of preterm and especially very and extremely preterm born infants rises dramatically (Blencowe et al., 2013). With a global total of 15 million preterm infants born in 2010 (Blencowe et al., 2012), the worldwide burden of this incidence and accompanying complications should not be underestimated. It is unclear whether increasing neonatal medicine improvements lead to a decrease in later neurodevelopmental deficits (Allotey et al., 2018; Johnson & Marlow, 2017). Hence there is a need for cognitive assessment tools to identify those preterm born infants at higher risk for later deficits. A reliable method to assess neonates at risk would allow allocation to early intervention programs. Intervention programs focusing on developmental care, parenting, environmental and behavioral factors are found to be effective (Spittle, Orton, Anderson, Boyd, & Doyle, 2015; Van Hus et al., 2013; Verkerk et al., 2012). Given the neuroplasticity in infants, it is important to offer such programs as early as possible during and after discharge from the neonatal intensive care unit (NICU). However due to the economic costs of these programs, it is not feasible to provide this preventive intervention to all premature infants, especially in low-income countries.

Objective methods to detect neonates at higher risk are currently lacking (Streri, de Hevia, Izard, & Coubart, 2013; Thierry, 2005; Ullman et al., 2015) and behavioral tests are not reliable to assess cognitive functioning in neonates (Picton & Taylor, 2007; Wickremasinghe et al., 2012). In the next sections we investigate neuroimaging tools as well as blood biomarkers in order to evaluate its efficacy to detect preterm infants with a high-risk profile for developmental disorders.

1.2. Neuroimaging as screening and prediction tool

Neuroimaging techniques such as cranial ultrasound (CUS) and structural magnetic resonance imaging (MRI) are routinely used in clinical settings to detect brain abnormalities as well as predict neurodevelopmental outcome (Rademaker et al., 2005). CUS is a non-invasive, simply feasible and highly effective bedside tool to serially evaluate the preterm brain. Using this tool common brain conditions associated with prematurity, such as hemorrhages or white matter damage, can be detected (Plaisier et al., 2015). Most common complications are periventricular leukomalacia (PVL) and intraventricular hemorrhage (IVH) (Ortinau & Neil, 2015; Volpe, 2009a). CUS is also found to be an effective prediction tool for cognitive outcome. In particular ventricomegaly at term equivalent age was associated with a worse cognitive outcome at 2 years corrected age in very preterm infants (Brouwer et al., 2014), as well as severe IVH or intraparenchymal hemorrhages shown on an early CUS (Franckx, Hasaerts, Huysentruyt, & Cools, 2018).

For a more detailed screening of preterm brain injury, such as cerebellar malformations or more subtle white and grey matter abnormalities, a CUS is unable to provide adequate information (Plaisier et al., 2015). MRI has a higher spatial resolution and is therefore more precise to detect brain lesions. This comes at a cost of being more invasive, not applicable in bedside testing and less affordable. However prospective studies in preterm infants have found several indications of unfavorable outcome either using structural or functional MRI. Larger ventricular volumes are associated with an adverse neurodevelopmental outcome until early school age (Keunen et al., 2016). Also moderate to severe white matter abnormality and the presence of cerebellar lesions as detected by MRI are related to lower cognitive outcomes scores (Anderson, Cheong, & Thompson, 2015; Hintz et al., 2015). Even though brain damage has serious implications on outcome, preterm

infants without CUS/MRI documented lesions still present neurodevelopmental impairments (Lemola et al., 2017). The underlying neurologic injury due to preterm birth has been described as “encephalopathy of prematurity” (Ortinau & Neil, 2015; Volpe, 2009b). From functional MRI (fMRI) studies, different patterns of brain activity are observed between preterm and term born infants during the neonatal period (Baldoli et al., 2015) and persisting in adulthood (White et al., 2014). Moreover associations between fMRI paradigms and cognitive outcome tests are found (He et al., 2018; Ullman et al., 2015).

In contrast to MRI, electroencephalography (EEG) has a high temporal resolution and allows to investigate brain functioning in a non-invasive manner (Mantini, Marzetti, Corbetta, Romani, & Del Gratta, 2010). EEG can be passively administered in neonates in a bedside setting. To monitor neonatal brain activity at the NICU amplitude-integrated EEG (aEEG) is frequently used. aEEG has also proven to be effective as predictor of neurodevelopmental outcome (Wikstrom et al., 2012). In order to evaluate cognitive functioning more precisely and in a time-efficient manner, event-related potentials (ERPs) are considered suitable (Picton & Taylor, 2007). ERPs are characterized by positive and negative amplitude peaks following stimulation and give very precise information about the timing of cognitive processes (Key, Dove, & Maguire, 2005). In particular oddball paradigms are used as stimulation paradigms, since they are a simple and effective cognitive discrimination task to test early cognitive functions (Ceponiene et al., 2002; Zhang, Li, Zheng, Dong, & Tu, 2017). The stimuli used in an oddball paradigm exist in several modalities (i.e. visual, tactile, somatosensory, etc.), but in language studies the auditory modality is used. The auditory oddball paradigm consists of presenting repetitive “standard” stimuli (i.e. tones, phonemes or syllables) sporadically interrupted by “deviant” stimuli. Several auditory ERP (AERP) components have

been investigated as possible predictors of cognitive outcome. Common AERPs are P1, N2, P3, MMN, N400 and reflect different cognitive processes: auditory inhibition, stimulus discrimination, memory updating and response preparation, preattentive sensory memory and sematic processing respectively (Key et al., 2005). Some AERP components in children are found to be predictive of neurodevelopmental outcome (Hovel et al., 2015; Korpilahti, Valkama, & Jansson-Verkasalo, 2016).

A combination of structural and functional neuroimaging tools, in particular MRI and ERPs, seem to provide the best neuronal biomarkers for detection of brain injuries and for prediction of neurodevelopmental outcome. Using both techniques one avoids the trade-off between spatial and temporal information (Jorge, van der Zwaag, & Figueiredo, 2014; Mantini et al., 2010). Although very precise, it can be time consuming and costly to perform in each preterm born infant at the NICU and normative standards are challenging to create. These parameters need to be taken into account when selecting biomarkers used to discriminate between patients at high and low risk and select those eligible for intervention programs.

1.3. Blood biomarkers as screening and prediction tool

Whereas neuroimaging tools provide us with an objective assessment of brain injuries and cognitive functioning, it can be challenging to perform in all preterm born infants. Moreover neuroimaging techniques are still indirect measurements and not every NICU owns these facilities. Blood biomarkers on the other hand offer a rapid indication of injury as seen in increased levels (Disanto et al., 2017; Michetti et al., 2012). Blood punctures are routinely taken at the NICU, which makes it less complicated and less invasive to analyze an additional neuronal biomarker.

Especially in neurodegenerative diseases blood biomarkers have been investigated in order to improve early diagnostics, monitor disease progression and assess efficacy of new drugs. A promising biomarker of neuraxonal injury, neurofilament (Nf), has been found very valuable in acute and chronic neuronal damage in adults. Nf consists of several subunits and is released into the cerebrospinal fluid and eventually the peripheral blood in case of neuronal injury (Petzold, 2005). Cerebrovascular accident, traumatic brain injury, dementia and multiple sclerosis are examples where Nf levels are dramatically increased as compared to healthy controls (Barro et al., 2018; De Marchis et al., 2018; Mattsson, Andreasson, Zetterberg, Blennow, & Alzheimer's Disease Neuroimaging, 2017; Rohrer et al., 2016; Shahim, Zetterberg, Tegner, & Blennow, 2017). Moreover Nf is found to be associated with MRI markers of disease severity and can be administered as indicator of disease progression (Kuhle et al., 2016). Other studies confirmed the correlation between Nf and imaging measurements of the injury (Barro et al., 2018; Gattringer et al., 2017). Furthermore Nf was found to be predictive of neurological outcome (Rana et al., 2013; Shahim et al., 2016).

Data on Nf are sparse in neonates, but some studies point out the promising use in infants. As shown by a study in children with febrile seizures, prolonged febrile seizures led to raised serum Nf heavy chain (NfH) (Matsushige et al., 2012). Similarly serum NfH was increased in neonates with hypoxic-ischemic encephalopathy (HIE) compared to healthy neonates (Douglas-Escobar et al., 2010; Toorell, Zetterberg, Blennow, Savman, & Hagberg, 2018). A more recent study (Shah et al., 2018) found NF light chain (NfL) predictable of MRI outcome, in particular higher NfL levels were observed in case of unfavorable MRI outcome. Taken together, the potential use of this biomarker might also be extended to the preterm population.

1.4. Research objectives

The present PhD thesis aims at investigating neuronal biomarkers in preterm born infants in order to discriminate infants at higher risk for neurodevelopmental deficits. Given the advances in neonatal medicine, there is an increasing worldwide incidence of preterm births and a rising survival rate of extremely preterm born infants. Therefore it is of utmost clinical importance to find a screening tool to detect infants that might benefit from early intervention programs. In this thesis two neuronal biomarker methods are explored: an electrophysiology approach using AERPs and a neuronal scaffolding protein, Nf.

First a systematic review of the literature was performed about the predictive capacity of AERPs in preterm born infants, in other words investigating the association between AERPs and cognitive outcome. This review is followed by a pilot study, where neonatal AERPs are investigated and correlated with neurodevelopmental outcome at 2 years in healthy very preterm and term born infants. In particular discrimination and habituation are examined as early forms of attention and learning respectively. Finally, we investigated a promising biomarker of neuroaxonal injury NfL in preterm and term infants during the first week of life.

II. PUBLICATIONS

2.1. Manuscript 1

Predicting Neurodevelopmental Outcome in Preterm Born Infants Using Auditory Event-Related Potentials: a Systematic Review.

Journal: Neuroscience and Biobehavioral Reviews - published

Authors: Depoorter A, Früh J, Herrmann K, Zanchi D, Weber P.

Abstract: Prematurity is a known risk factor for later cognitive deficits. At present there are neither behavioral nor neurological tests available to detect those preterm infants who would benefit most from early interventions. Neurophysiologic methods, and more specifically, auditory event-related potentials (AERPs) are convenient tools to investigate early cognitive functioning. However, the capability of AERPs as a prognostic factor for mental development in preterm infants remains unclear. The present systematic search of the literature yielded 1016 articles, out of which 13 were included. Both prospective and cross-sectional studies reported a relationship between AERPs and cognitive outcome. Our results show that larger amplitudes and shorter latencies of late AERPs are related to better cognitive outcomes. Additional studies are needed to corroborate our findings regarding this potential use of AERPs in the individual evaluation of preterm born infants.

Authorship statement: AD and JF have a shared first authorship. AD and JF equally divided the work of reviewing the literature, collecting the data, writing and correcting the manuscript. As corresponding author AD took care of the submitting and reviewing process.

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Review article

Predicting neurodevelopmental outcome in preterm born infants using auditory event-related potentials: A systematic review

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ABSTRACT

Prematurity is a known risk factor for later cognitive deficits. At present there are neither behavioral nor neurological tests available to detect those preterm infants who would benefit most from early interventions. Neurophysiologic methods, and more specifically, auditory event-related potentials (AERPs) are convenient tools to investigate early cognitive functioning. However, the capability of AERPs as a prognostic factor for mental development in preterm infants remains unclear. The present systematic search of the literature yielded 1016 articles, out of which 13 were included. Both prospective and cross-sectional studies reported a relationship between AERPs and cognitive outcome. Our results show that larger amplitudes and shorter latencies of late AERPs are related to better cognitive outcomes. Additional studies are needed to corroborate our findings regarding this potential use of AERPs in the individual evaluation of preterm born infants.

1. Introduction

Preterm birth is defined by the WHO as birth before 37 completed weeks of gestation (WHO, 2016). Children born preterm are at significant risk for impaired cognitive development (Bhutta et al., 2002; Luu et al., 2011). It is estimated that 52% of children born before 28 weeks of gestation, 24% of children born at 28–31 weeks, and 5% of children born at 32–36 weeks suffer from neurodevelopmental impairments (Blencowe et al., 2012), including cognitive and behavioral issues, which may persist into adulthood (Johnson and Marlow, 2017). With a global total of 15 million babies born preterm in 2010, the burden of these impairments should not be underestimated (Blencowe et al., 2012).

While there is evidence that early intervention can significantly improve cognitive development in preterm born children and adolescents (Spittle et al., 2015), there is no reliable cognitive assessment tool to identify newborns in need of early intervention (Lobo and Galloway, 2013). Bearing in mind the importance of brain plasticity for early development, a timely start of targeted interventions is of utmost importance (Wass, 2015).

Over the past few years, an increasing amount of research has focused on the detection of differences in neonatal brain activity between infants born preterm and those born at term using various neuroimaging techniques (Mento and Bisiacchi, 2012). The apparent preference of functional neuroimaging techniques over behavioral assessments in infants born preterm is the possibility to objectively administer these tests as early as the neonatal period, when early forms of cognitive skills are already present (Streri et al., 2013). In particular, the use of electroencepahlography (EEG) allows for a non-invasive, time-efficient bedside evaluation of neonatal brain activity with a high temporal resolution (Kamel and Malik, 2015). A few research groups investigated whether neurophysiologic techniques such as EEG, amplitude-integrated EEG and auditory event-related potentials (AERPs) could be used to detect or predict cognitive impairments in preterm born children (deRegnier, 2005, 2008; Fogtmann et al., 2017).

The objective of this systematic review is to investigate whether defined AERP peaks can be used as a marker for cognitive and language functioning in children born preterm. Both cohort studies and cross-sectional studies will be considered. Implications for clinical practice and future research are discussed. This is, to our knowledge, the first systematic review to summarize the evidence of AERPs used to assess and predict cognitive functioning in children born preterm.

Abbreviations: EEG, electroencephalography; AERP, auditory event-related potential; GA, gestational age; MMN, mismatch negativity; BSID, Bayley Scales of Infant Development; WPPSI, Wechsler Preschool and Primary Scale of Intelligence; BNT, Boston Naming Test; NEPSY, Developmental NEuroPSYchological Assessment; CDI, MacArthur Communicative Development Inventory; DAYC, Developmental Assessment of Young Children

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Table 1
Quality assessment of included studies using an adapted QUIPS checklist.

Study	Participation	Attrition	Prognostic Factor Measurement	Outcome Measurement	Confounding	Statistical Analysis and reporting	Overall Score	Quality Rating
Weber et al. (2016)	3 + + +	2 + + -	3 + + +	3 + + +	1 – +	2 + - +	14	II
Korpilahti et al. (2016)	2 - + +	N/A	3 + + +	3 + + +	2 + + -	3 + + +	13	IV
Hovel et al. (2015)	3 + + +	N/A	3 + + +	3 + + +	3 + + +	2 + + -	14	IV
Paquette et al. (2015)	3 + + +	N/A	3 + + +	2 + - +	3 + + +	3 + + +	14	IV
Maitre et al. (2014)	3 + + +	0 —	2 - + +	2 - + +	2 + - +	2 - + +	11	II
Maitre et al. (2013)	3 + + +	1 – +	2 - + +	2 - + +	1 – +	3 + + +	12	II
Leipala et al. (2011)	2 + + -	1 - + -	2 - + +	1 - + -	0 —	2 + + -	8	II
Jansson-Verkasalo et al. (2010)	0 —	1 + –	3 + + +	3 + + +	0 —	3 + + +	8	II
Mikkola et al. (2010)	2 + + -	N/A	2 + + -	3 + + +	0 —	1 – +	8	IV
Mikkola et al. (2007)	2 + + -	N/A	3 + + +	3 + + +	0 —	3 + + +	11	IV
Fellman et al. (2004)	3 + + +	2 + + -	2 + + -	1 – +	0 —	2 - + +	10	II
Jansson-Verkasalo et al. (2004)	2 - + +	1 + –	3 + + +	3 + + +	1 – +	0 —	10	II
Jansson-Verkasalo et al. (2003)	2 - + +	1 + –	3 + + +	3 + + +	0 —	3 + + +	11	IV

+ = criteria fulfilled, - = criteria not fulfilled, N/A = not applicable, II = prospective cohort study, IV = cross-sectional study.

2. Methods

2.1. Search strategy

This review is written in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). We did not use a review protocol. A.D. and J.F. carried out a systematic search of the databases Pubmed, Embase, and PsycINFO between May 1st and May 31st, 2017. Records published between 01.01.1990 and 31.05.2017 were screened for studies whose title or abstract indicated relevance to our research questions. In addition, we searched reference lists of published reviews for eligible studies.

A detailed description of the search strategy is given in the Appendix A.

2.2. Eligibility criteria

Articles were selected according to the following inclusion criteria: 1. Cross-sectional, prospective, or retrospective original research papers investigating the relation between AERPs and cognitive outcome in children born preterm (< 37 weeks of gestation); 2. The study investigated long latency event-related potentials including N1, P2, P300, Mismatch Negativity (MMN), and Contingent Negative Variation; 3. Subjects were no older than 18 years at the time of study participation; 4. Studies were published in one of the following languages: English, French, German, Italian, or Dutch. We excluded studies if only an abstract or conference paper was published at the time of our search, in the absence of a full-text scientific article. In case of uncertainty, A.D. and J.F. consulted with P.W. to discuss the studies in question until agreement was reached.

2.3. Data extraction

From the included studies, we extracted data on study design, number of subjects, subject characteristics, EEG methodology, AERP peaks investigated, type of neuropsychological testing, as well as statistical correlations between AERPs and outcome. Only significant ($p < .05$) correlations between AERPs and cognitive outcome are presented. A number of studies investigated not only the correlation between AERPs and cognitive outcome, but also the differences in AERPs between infants born preterm and at term, as well as technical questions regarding the administration of AERPs. Since these data do not directly pertain our research question, we did not report them in this review. Similarly, if a study comprised of a group of preterm children as well as a term-born control group, information is given only on characteristics of participants born preterm. No standardized data extraction tool was used.

2.4. Quality assessment

The included studies were assessed for their methodology by A.D. and J.F. using an adapted version of the Quality in Prognostic Studies (QUIPS) Checklist (Hayden et al., 2013), which grades studies regarding study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. We divided each of these six categories into three items. Studies received a score from 0 to 3 depending on how many items they fulfilled, with a score of 0 reflecting the poorest and a score of 3 reflecting the highest methodological quality. Studies which do not contain a 0 in any category are considered of good overall quality. The attrition category was scored as “N/A” (not applicable) in cross-sectional studies. The studies also received a grade from I to V (strongest to weakest) for their study design according to the The American Society of Plastic Surgeons Evidence Rating Scale (The ASPS Evidence Rating Scales, n.d.) adapted from the 2011 Oxford CEBM Levels of Evidence (OCEBM Levels of Evidence Working Group).

Our adapted version of the QUIPS Checklist can be found in the Appendix A and the study-by-study quality assessment in Table 1.

3. Results

3.1. Identified studies

The electronic literature search led to the identification of 1016 articles in total, of which 167 full-text articles were screened for eligibility after excluding 849 non-relevant studies based on the title and abstract. Out of the full-text articles, 146 studies were eliminated: We excluded 79 duplicates, 67 studies which did not investigate the relation between AERPs and cognition in preterm infants, two studies which used outdated AERP nomenclature, and six conference papers which did not provide enough data to compare them to full-text articles. 13 studies met the inclusion criteria for the qualitative synthesis. No additional articles were identified through hand search of reference lists. A flowchart of the search and selection process is shown in Fig. 1.

3.2. Study characteristics

The 13 included studies investigating the relationship between AERPs and cognitive outcome were published between 2003 and 2016.

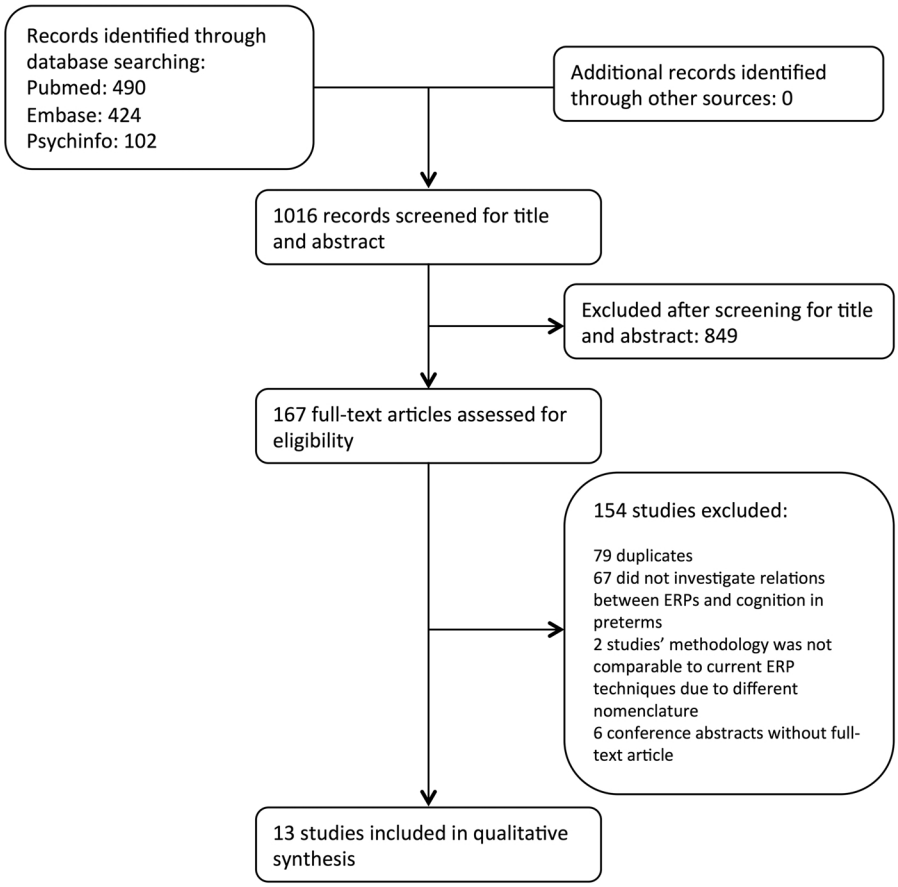

Fig. 1. Search flowchart.

Out of those 13 studies, seven were prospective cohort studies (Fellman et al., 2004; Jansson-Verkasalo et al., 2003, 2004, 2010; Leipala et al., 2011; Maitre et al., 2013, 2014; Paquette et al., 2015; Weber et al., 2016) and six were cross-sectional studies (Hovel et al., 2015; Jansson-Verkasalo et al., 2003; Korpilahti et al., 2016; Mikkola et al., 2007, 2010; Paquette et al., 2015). One of these studies (Jansson-Verkasalo et al., 2003) was part of a prospective follow-up, however the findings in which we are interested were taken at one point in time and therefore it was treated as a cross-sectional study. The studies were conducted by only six research groups. Specifically, we included several papers published by three research groups from the following study sites: Oulu University Hospital, Helsinki University Central Hospital, and Vanderbilt University Medical Center.

Looking at the study participants, 12 studies included both preterm and term born infants, with 10 studies dividing participants into a preterm group and a term born control group (Fellman et al., 2004; Jansson-Verkasalo et al., 2003, 2004, 2010; Korpilahti et al., 2016; Leipala et al., 2011; Mikkola et al., 2007, 2010; Paquette et al., 2015; Weber et al., 2016) and two studies including one large cohort consisting of both preterm and term born infants (Maitre et al., 2013, 2014). One study (Hovel et al., 2015) exclusively evaluated preterm born infants. Overall, the gestational age (GA) of the prematurely born participants was 22–37 weeks. The age at assessment ranged from < 38 weeks to 9 years and at follow-up from 6 months to 6 years. Two studies (Maitre et al., 2013, 2014) conducted AERP measurements before the corrected term age of 40 weeks, if participants were clinically stable. Five out of the seven prospective studies (Fellman et al., 2004; Leipala et al., 2011; Maitre et al., 2013, 2014; Weber et al., 2016) assessed AERPs at neonatal age, while one study group which performed several prospective studies measured AERPs at a later age. Eight studies (Hovel et al., 2015; Jansson-Verkasalo et al., 2003, 2004; Korpilahti et al., 2016; Leipala et al., 2011; Maitre et al., 2013, 2014; Mikkola et al., 2007) included subjects with severe brain damage, such as intracranial hemorrhage grade III or IV or cerebral palsy, while three studies (Fellman et al., 2004; Paquette et al., 2015; Weber et al., 2016) only included individuals with no major brain abnormalities. From two studies (Jansson-Verkasalo et al., 2010; Mikkola et al., 2010) it was not possible to extract information on participants' clinical diagnoses. The two studies (Maitre et al., 2013, 2014) which followed the mixed cohort of preterm and term born children recruited participants who were hospitalized at the NICU, without mentioning the reason for their NICU admission. Also, no age range or number of children born preterm was given.

The included studies used different auditory stimulation paradigms to investigate the AERPs. Five studies (Fellman et al., 2004; Hovel et al., 2015; Leipala et al., 2011; Mikkola et al., 2007; Weber et al., 2016) presented tones in an oddball paradigm. Four out of these five studies used syllables (Jansson-Verkasalo et al., 2003, 2004, 2010; Paquette et al., 2015) and one study (Mikkola et al., 2010) used animal sounds. Two studies randomly presented syllables (Maitre et al., 2013, 2014) and one study (Korpilahti et al., 2016) used words and pseudowords as stimuli in a pseudo-random order. The authors also used different AERP nomenclatures to describe the investigated peaks, but all were long latency AERPs and therefore endogenous (Sur and Sinha, 2009).

Different standardized tests were used to assess the neurodevelopmental outcome across the studies: The Bayley Scales of Infant Development (BSID), Boston Naming Test (BNT), Developmental NEuroPSYchological Assessment (NEPSY), Wechsler Preschool and Primary Scale of Intelligence (WPPSI), MacArthur Communicative Development Inventory (CDI) and Developmental Assessment of Young Children (DAYC).

The study characteristics are visualized in Table 2.

Table 2
Characteristics of included studies.

Authors, Year of publication	Study Design	Subjects (male)	Preterm GA (mean, weeks)	Subject Characteristics	Age at Assessment	Methods: Stimuli, auditory paradigm, AERPs investigated	Neuropsychological Testing
Weber et al. (2016)	PC	P: n = 17 (9)	27.4	Preterms < 32 w GA	ERP: P: 40.8 w	Tones (85% standard tones of 1000 Hz and 15% deviant tones of 2000 Hz) presented in a passive auditory oddball paradigm of tones.	BSID-I
		T: n = 16 (10)		Normal hearing Exclusion: severe brain lesions, genetic syndromes or congenital infection	T: 40.8 w NDA: 2 y	3 × 500 stimuli MMN block 1 and MMN block 3	
Korpilahti et al. (2016)	CS	P: n = 14 (4)	< 34	Very low birth weight < 1500 g	9 y	95 naturally occurring words and 95 pseudowords were presented in pseudo-random order; participants indicated by clicking on icons on a computer screen whether it was a real or a pseudoword.	WISC-III,
		T: n = 14		Normal hearing and vision n = 3 with learning problems as reported by school, n = 1 w/ cerebral palsy		N200 and N400	BNT, NEPSY-II[a]
Hovel et al. (2015)	CS	P: n = 70 (35)	27.4	Preterms < 32 w GA	5y	Tones (70% standard tones of 1000 Hz and 10% frequency, direction and duration deviants) presented in an auditory oddball paradigm while watching silent movie.	WPPSI-R, NEPSY[a], SDQ
				Normal hearing Exclusion: major congenital malformations		3 × 610 stimuli P1, N2, MMN and P3a	
Paquette et al. (2015)	CS	P: n = 32 (16), divided into 3 age groups	29.4, range 22–34	No CUS abnormalities, no major brain damage	3 m, 1 y, 3 y	Speech stimuli /da/ and /ba/ and non-speech sounds were presented in passive auditory oddball paradigm.	BSID-III[a]
		T: n = 14		Normal hearing		P150, N250, and MMN	
Maitre et al. (2014)	PC	One cohort of both term and preterm infants hospitalized at the NICU (n = 5, 60%)	Median 28, range 24–40	Measured at minimum of 32 w GA and 31cm head circumference	ERP: 2 m	Syllables ba/, da/, ga/, bu/, du/, gu/ randomly presented over 15 min at random intervals.	DAYC[a],
				Clinically stable	NDA: 6 m, 1 y	Mean amplitude calculated 250–400 ms after stimulus onset.	parent questionnaires
				n = 4 had grade III or IV IVH, n = 1 had Turner syndrome, n = 2 had unilateral auditory neuropathy		Investigation of hemisphere differences in ERP peaks.	
Maitre et al. (2013)	PC	One cohort of both term and preterm infants hospitalized at the NICU (n = 5, 60%)	Median 28, range 24–40	Measured at minimum of 32 w GA and 31 cm head circumference	ERP: median 37 w	Syllables ba/, da/, ga/, bu/, du/, gu/ randomly presented over 15 min in random intervals.	12 m: DAYC[a], parent questionnaires
				Clinically stable	NDA: 1 y, 2 y	Mean amplitude calculated 250–400 ms after stimulus onset	24 m: BSID III[a]
				n = 3 w/ severe abnormalities on CUS n = 3 had abnormal ABR on one side			
Leipala et al. (2011)	PC	P ICH: n = 9 (5)	P: 27.9 (median)	Preterms with and without ICH	ERP: At term, 6m and 1y	Tones (85% three-partial harmonic standard tones of 500 Hz and 15% harmonic deviant tones of 750 Hz) presented in an auditory oddball paradigm.	Normal (score 0), moderately abnormal (score 1) or severely abnormal (score 2)
		P control: n = 16 (10) T with ICH: n = 5 or T with asphyxia: n = 4	P: 26.5 (median)	Preterms with and without ICH	NDA: 2 y	P150, N150, P350 and MMR	

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Table 2 (*continued*)

Authors, Year of publication	Study Design	Subjects (male)	Preterm GA (mean, weeks)	Subject Characteristics	Age at Assessment	Methods: Stimuli, auditory paradigm, AERPs investigated	Neuropsychological Testing
		T control: n = 22					
Jansson-Verkasalo et al. (2010)	PC	P: n = 11	29, all < 32	Normal hearing	ERP: 6 m, 1 y	Native Finnish vowels /e/ (standard) and /ö/ (deviant) and non-native vowel /õ/ (deviant) presented in a passive auditory oddball paradigm.	CDI
		T: n = 13			NDA: 1 y, 2 y	MMN	
Mikkola et al., 2010	CS	P: n = 24 (14)	28.2	Very low birth weight	5 y	3 × 18 blocks, each block consists of 36 stimuli. 2 different animal sounds, 89% standards and 11% direction deviants.	WPPSI-R, NEPSY[a]
		T: n = 11 (7)		Normal hearing		P1, MMN, P3a	
Mikkola et al., 2007	CS	P SGA: n = 15 (9)	P SGA: 27.8	Normal hearing	5y	Easy paradigm: 800 stimuli consisting of 80% three-partial harmonic standard tones of 500 Hz and 10% harmonic frequency deviant tones of 750 Hz and 10% novel sounds.	WPPSI-R, NEPSY[a]
		P AGA: n = 13 (7)	P AGA: 27.1			Challenging paradigm: 1800 stimuli consisting of 80% harmonic standard tones of 1000 Hz and 10% duration and frequency deviant tones.	
		T: n = 13 (8)				P1, N2, MMN and P3a	
Fellman et al., 2004	PC	P SGA: n = 15 (8)	P SGA: 29.0	Preterms < 1500 g	ERP: At term, 6 m and 1 y	Tones (85% three-partial harmonic standard tones of 500 Hz and 15% harmonic deviant tones of 750 Hz) presented in a frequency oddball paradigm.	BSID
		P AGA: n = 20 (10)	P AGA: 26.9	Exclusion: acute birth asphyxia, chromosomal abnormalities and major congenital heart disease	NDA: 2 y	P150, N150, P350, P3a, Nc and MMN	
		T: n = 22 (14)					
Jansson-Verkasalo et al. (2004)	PC	P: n = 12 (6)	29, range 27–33	Very low birth weight < 1500 g	ERP: 4 y	Syllables taa/, ta/, kaa/ presented using passive auditory oddball paradigm while child watched silenced cartoon.	BNT
		10 of 12 subjects from Jansson-Verkasalo et al. (2003)		Normal hearing	NPT: 6 y	MMN	
		T: n = 12 (6)		n = 11 had MRI abnormalities			
Jansson-Verkasalo et al. (2003)	CS	P: n = 12, (7)	29, range 24–33	Very low birth weight < 1500 g	4 y	Syllables taa/, ta/, kaa/ presented using passive auditory oddball paradigm while child watched silenced cartoon.	BNT
		T: n = 12 healthy term-born children (7)		Normal hearing		P1, N2, N4, and MMN	
				n = 11 had MRI abnormalities			

PC = prospective cohort, CS= cross-sectional; P = preterm group, T = term group (healthy controls), w = weeks, m = months, y = years.
ABR = Auditory Brainstem Reaction, CUS = Cranial Ultrasonography, ICH = Intracerebral Hemorrhage, IVH = Intraventricular Hemorrhage.
BSID = Bayley Scales of Infant Development, NEPSY = A Developmental NEuroPSYchological Assessment, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, BNT = Boston Naming Test, DAYC = Developmental Assessment of Young Children, CDI = MacArthur Communicative Development Inventory, SDQ = Strengths and Difficulties Questionnaire.
ERP = Event-related Potential, AERP = auditory ERP, NDA = Neurodevelopmental Assessment, NPT = Neuropsychological Testing; MDI = Mental Developmental Index, PDI = Performance Developmental Index.
[a] = Partial test administration using select subscales.

Table 3
Relation between AERPs and neurodevelopmental outcome.

Authors, year of publication	Statistical analysis	Statistical results	Main findings
Weber et al. (2016)	Pearson correlation	dMMN at Cz and correlated positively with MDI ($r = 0.65^{**}$) and PDI ($r = 0.48^{*}$). Words: - Auditory attention correlated negatively with N200 A at right ($r = -0.41^{*}$), left ($r = -0.47^{**}$), midline ($r = -0.47^{**}$), frontal ($r = -0.46^{*}$), central ($r = -0.54^{**}$), parietal ($r = -0.45^{*}$) electrodes. - Pseudoword repetition correlated negatively with N200 A at frontal ($r = -0.42^{*}$) electrodes. Pseudowords: - Auditory attention correlated negatively with N200 A at parietal ($r = -0.38^{*}$) electrodes. - Pseudoword repetition correlated negatively with N200 A at right ($r = -0.39^{*}$), left ($r = -0.39^{*}$), frontal ($r = -0.43^{*}$) electrodes.	Larger difference in MMN between the first and third stimulation block correlated with better MDI and PDI scores.
Korpilahti et al. (2016)	Pearson correlation		Larger N200 amplitude correlated with better auditory attention and pseudoword repetition (term and preterm subjects combined).
Hovel et al. (2015)	Pearson correlation and linear or binary logistic regression analysis	P1 ($r = 0.25^{*}$) and N2 ($r = 0.32^{**}$) L correlated positively with VAt. Duration deviant: - 150–200 ms MA: positive correlation with PP ($r = 0.27^{*}$) - 200–250 ms MA: positive correlation with PP ($\beta = 0.26^{*}$) - 300–350 ms MA: positive correlation with IVQ ($r = 0.23^{***}$), PIQ ($r = 0.20^{***}$), PSQ ($r = 0.36^{**}$), FSIQ ($r = 0.26^{*}$), VAco ($r = 0.22^{***}$), PP ($r = 0.21^{***}$) and SR ($r = 0.25^{*}$) - 350–400 ms MA: positive correlation with PIQ ($\beta = 0.21^{***}$), PSQ ($\beta = 0.35^{**}$) and VAco ($\beta = 0.34^{**}$) - 400–450 ms MA: positive correlation with PSQ ($\beta = 0.27^{*}$), VAco ($\beta = 0.24^{***}$) and negative correlation with VAt ($\beta = -0.25^{***}$) Direction deviant: - 150–200 ms MA: positive correlation with VAt ($r = 0.25^{*}$) - 250–300 ms MA: positive correlation with VAt ($r = 0.29^{*}$)	Shorter P1 and N2 latencies correlated with faster visual attention. More positive mean amplitudes 150-450ms post stimulus onset correlated with better cognitive test results.
Paquette et al. (2015)	Pearson correlation	Speech stimuli: - MMN L correlated negatively with exp. language ($r = -0.39^{***}$) and rec. language ($r = -0.26^{*}$) - P150 L correlated negatively with exp. language ($r = -0.34^{**}$) and rec. language ($r = -0.25^{*}$) - P150 A correlated positively with exp. language ($r = 0.23^{*}$) - N250 L correlated negatively with exp. language ($r = -0.34^{**}$) and rec. language ($r = -0.34^{**}$) Non-speech stimuli: N250 L correlated negatively with cognition ($r = -0.26^{*}$)	Shorter latency of MMN, P150, and N250 correlated with better expressive and receptive language. Larger P150 amplitude correlated with better expressive language. Shorter N250 latency correlated with better cognition.
Maitre et al. (2014)	Linear regression	At 6 months: - Temporal hemispheric differences in MA correlated with DAYC communication scores (coefficient: 1.9^{*}) (adjusted model) - Frontal hemispheric differences in MA correlated with DAYC cognitive scores (coefficient: -1.9^{*}) (adjusted model) At 1 year: Temporal hemispheric differences in MA correlated with DAYC communication scores (coefficient: 2.4^{*}) and cognitive scores (coefficient: 2.0^{*}) (non-adjusted model.)	Larger hemisphere differences in ERPs predicted better communication and cognitive scores at 6 months and to a smaller extent at 1 year.
Maitre et al. (2013)	Ordinary least squares linear regression model Pearson correlation Linear regression	At 1 year: ERP responses contributed 34% to the model predicting communication and 21% to the model predicting cognitive scores. At 2 year: ERP responses contributed 14% for prediction of cognition and 9% for prediction of rec. language At 1 year: F3 (/ba/-/ga/ contrast) and communication ($r = 0.41^{*}$) At 2 year: F3 ($r = 0.44^{*}$), T6 ($r = 0.45^{*}$) for /du/-/gu/ sound contrast and cognition F4 (/ba/-/ga/ contrast)and rec. language ($r = -0.51^{**}$)	Larger ERP amplitude significantly predicted better communication and cognitive scores at 1 year as well as receptive language and cognition at 2 year.

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Table 3 (*continued*)

Authors, year of publication	Statistical analysis	Statistical results	Main findings
Leipala et al. (2011)	Pearson correlation	No numeric data available	3 out of 72 AERP variables correlated significantly with the 2 year outcome.
Jansson-Verkasalo et al. (2010)	Spearman correlation	MMN A correlated positively with the number of produced words ($r^2 = 0.20^{*}$), developed word morphology ($r^2 = 0.27^{*}$) and mean sentence length ($r^2 = 0.38^{**}$)	Larger MMN amplitude correlated with fewer produced words, less developed word morphology, and shorter mean sentence length.
Mikkola et al. (2010)	Correlation analysis not specified	/	No significant correlations were found.
Mikkola et al. (2007)	Pearson correlation	Easy paradigm: - Standard P1 correlated with sentence repetition ($r = 0.43^{*}$) - Frequency MMN correlated with verbal IQ ($r = 0.43^{*}$) and verbal fluency ($r = 0.50^{*}$) - Novel P1 correlated with verbal fluency ($r = 0.57^{*}$) Challenging paradigm: - Frequency N2 correlated with verbal fluency ($r = 0.54^{*}$) - Duration P1 correlated with language domain ($r = 0.44^{*}$) and phonological processing ($r = 0.52^{*}$) All AERP peaks were recorded at Fz and all correlations were positive.	Larger P1, N2 and MMN amplitudes correlated with better verbal IQ and language performance (sentence repetition, verbal fluency, language domain and phonological processing).
Fellman et al. (2004)	Correlation analysis not specified	At term: MDI correlated with parietal P150 A in the 150–250 ms time window ($r = 0.60^{*}$) and P350 A in the 250–350 ms time window ($r = 0.64^{*}$) for standard stimuli At 6 months: - MDI correlated with parietal P150 A in the 50–150 ms time window for deviant ($r = 0.87^{**}$) and standard ($r = 0.63^{*}$) stimuli - MDI correlated with frontal N250 A in the 250–350 ms time window ($r = -0.63^{*}$) for standard stimuli At 1 year: - MDI correlated with frontal N250 A in the 250 350 ms time window ($r = -0.59^{*}$) for standard stimuli - MDI correlated with frontal P3a A in the 250–350 ms time window ($r = 0.61^{*}$) and central P3a A ($r = 0.64^{*}$) for the difference waveform All correlations were positive.	At term: Larger P150 and P350 amplitudes correlated with better MDI scores. At 6 months: Larger P150 and N250 amplitudes correlated with better MDI scores. At 1 year: Larger P3a and N250 amplitudes correlated with better MDI scores.
Jansson-Verkasalo et al. (2004)	Spearman correlation	No numeric data available	Individual inspection of ERPs: If MMN absent in max. 5 out of 9 electrodes, naming ability was normal (n = 3). If MMN was absent or questionable in 8 or 9 electrodes, naming ability was decreased (n = 5).
Jansson-Verkasalo et al. (2003)	Repeated measures analysis of variances	MMN amplitude for vowel change significantly smaller in preterms w/ naming difficulties than in preterms w/out naming difficulties ($F(1,10) = 5.30^{*}$) and controls ($F(1,19) = 6.56^{*}$). MMN amplitude for consonant change significantly smaller in preterms w/ naming difficulties than in controls ($F(1,19) = 7.79^{*}$)	MMN amplitude significantly smaller in preterms w/ naming difficulties than in preterms w/out naming difficulties and controls.

A = amplitude, L = latency, MA = mean amplitude.
MDI = Mental Developmental Index, PDI = Performance Developmental Index, DAYC = Developmental Assessment of Young Children.
VIQ = Verbal IQ, PIQ = Performance IQ, PSQ = Processing Speed Quotient, FSIQ= Full-Scale IQ, VAco = Visual Attention; number of correct markings, Vat = Visual Attention; time to complete test, PP = Phonological Processing, SR = Sentence Repetition.
* $= p < .05$.
** $= p < .01$.
*** $= p < .001$.

3.3. Methodological quality of included studies

Based on the adapted QUIPS checklist 5 out of the 13 articles were considered to be of good methodological quality, two of which were prospective cohort studies (Table 1). Across all studies, the methodological strengths lay in the prospective factor (e.g. EEG administration) and outcome measurement (e.g. cognitive testing). The main shortcomings of the included studies were seen in the "attrition" and "confounding" categories, with a majority of studies not mentioning or controlling for important possible confounders.

3.4. Correlations between defined AERPs and neurodevelopmental outcome

The results of the 13 included studies are summarized in the following paragraphs and presented in Table 3. One study (Leipala et al., 2011) was not considered in the results section, since it did not report explicitly which correlations the authors performed and what the results were.

The correlations between the AERPs and outcome are described according to the peak latencies and the results are presented in ascending order of age of the study participants.

3.4.1. Long latency auditory event-related potentials

3.4.1.1. First positive peak. This peak was defined as follows by the five investigating studies: P150 between 150–250 ms (Fellman et al., 2004), P150 between 50–250 ms (Paquette et al., 2015), P1 between 80–180 ms (Hovel et al., 2015), P1 between 50–150 ms (Mikkola et al., 2007) and P1 between 90–120 ms (Mikkola et al., 2010). Four of these five studies found significant correlations between either the amplitude or the latency of the first positive peak and neuropsychological test scores. Fellman et al. (2004) found that the P150 amplitude at term equivalent age and 6 months correlated positively with the mental developmental index (MDI) of the BSID at 2 years. Paquette et al. (2015) conducted AERP measurements in children of three age groups: 3 months, 1 year, and 3 years. For all ages, larger P150 amplitude and shorter latency correlated with better BSID language, but not with cognitive scores. In the study by Hovel et al. (2015), a larger positive amplitude between 150 and 200ms after stimulus onset correlated with better phonological processing, while shorter P1 latency correlated with better visual attention in 5 year-old children. Mikkola et al. (2007), who examined the cohort from the study by Fellman et al. (2004), found significant positive correlations between the amplitude of P1 and language scores on the NEPSY in 5 year-old children. However, Mikkola et al. (2010) reported that there were no significant correlations between P1 and any of the outcome measures.

In summary, one prospective study found P150 to be predictive of later neurodevelopmental outcome, while three cross-sectional studies reported significant correlations between the first positive peak and neurodevelopmental test scores in various age groups. One cross-sectional study with 5 year-old participants yielded no significant correlations.

3.4.1.2. First negative peak. This peak was defined as follows by the five investigating studies: N250 between 250–350 ms (Fellman et al., 2004), N250 between 150–350 ms (Paquette et al., 2015), N2 between 170–330 ms (Hovel et al., 2015), N2 between 200–300 ms (Mikkola et al., 2007) and N200 between 200–350 ms (Korpilahti et al., 2016). All studies reported significant correlations between this AERP-peak and outcome tests. Fellman et al. (2004) found that the N250 amplitude at 6 and 12 months correlated negatively with the MDI of the BSID at 2 years. Paquette et al. (2015) found that the N250 latency correlated negatively with the BSID language and cognitive scores in children aged between 3 months and 3 years. Hovel et al. (2015) reported that shorter N2 latencies are related to faster visual attention in 5 year-old children. In the study of Mikkola et al. (2007) the N2 amplitude correlated positively with verbal fluency on the NEPSY in 5 year-old children. Whereas Korpilahti et al. (2016) found that the larger N200 amplitude, the better the language and attention subtest scores of the NEPSY in 9 year-old children.

In summary, one prospective study found that a decreased N250 peak is related to a later increased risk for cognitive dysfunction, so did two cross-sectional studies. Regarding N2/N250 latency two cross-sectional studies observed that the shorter the latency, the better the test results.

3.4.1.3. Second positive peak. This peak was defined as follows by the two investigating studies: P350, P3a between 250–350 ms (Fellman et al., 2004) and P3a between 330–360 ms (Mikkola et al., 2010). Fellman et al. (2004) reported that P350 at term equivalent age as well as the P3a at 1 year of age correlated positively with the MDI of the BSID at 2 years. However, Mikkola et al. (2010) did not find any significant correlations between P3a and neurodevelopmental outcome in 5 year-olds.

In summary, P3 was reported to predict cognitive development by one prospective study, while one cross-sectional study did not report any significant correlations.

3.4.1.4. MMN. The MMN is a negative component elicited in an auditory oddball paradigm. It peaks around 160–220 ms post stimulus onset and is related to pre-attention and auditory discrimination (Beres, 2017).

Seven studies investigated MMN in relation to the cognitive outcome. Weber et al. (2016) investigated the MMN by looking at the difference in amplitude between the first and last stimulation block at term equivalent age and found that it was predictive of the MDI of the BSID at 2 years. Jansson-Verkasalo et al. (2010) reported that the MMN amplitude at 1 year was related to the number of produced words assessed by the CDI at 2 years. One study (Paquette et al., 2015) reported a negative correlation between the MMN latency and language scores, but not cognition, on the BSID in children aged between 3 months and 3 years. Two prospective studies of the same cohort (Jansson-Verkasalo et al., 2003, 2004) found that lower MMN amplitude or the absence of MMN at 4 years was associated with naming difficulties assessed by the BNT at the age of 4 and 6 years, although they were not statistically supported. Mikkola et al. (2007) found positive correlations between the MMN amplitude and verbal intelligence quotient (IQ) and verbal fluency in 5-year old children. In their other study (Mikkola et al., 2010) they did not find any significant correlations between MMN and attention or executive functioning.

In summary, MMN was the most commonly investigated AERP component in the included studies, and six studies found it to be predictive of cognitive outcome. One study did not find any relation.

3.4.1.5. N400. Korpilahti et al. (2016) was the only study that investigated the N400 component, defined as the most negative peak in the 400–800 ms time window.They did not find any significant correlations between this peak and behavioral tests in 9-year old children.

3.4.2. Non-defined peaks

Three studies reported correlations for specific latency time windows without evaluating defined AERP peaks. The group of Maitre et al. (2013, 2014) used the same study cohort for two different analyses: In their 2013 study, they calculated the mean amplitude measured 250 until 400 ms post stimulus onset. This value at term equivalent age correlated positively with the communication subscale of the DAYC at 1 year and with cognitive subscales of the BSID at 2 years, but negatively with BSID receptive language scores at 2 years. In a predictive model the amplitude of AERP differentiation responses predicted the communication scores and the cognitive ability at 1 year. At 2 years the AERP responses predicted cognition and receptive language, while expressive language contributed less to the predictive model. In their 2014 study, the same cohort was evaluated, but instead of AERP amplitudes the hemispheric differences in AERPs from 250 until 400 ms after stimulus onset were evaluated. The authors found that larger differences in AERP amplitudes between the left and right hemisphere correlated with better DAYC communication and cognitive scores at 6 and 12 months. Even though Hovel et al. (2015) presented correlations for P1 and N2, they reported correlations for later AERPs in 50ms time windows. AERP amplitudes between 300 and 450ms after stimulus onset correlated positively with IQ as measured by the WPPSI as well as attention and language on the NEPSY in 5 year-olds.

In summary, across three studies, late AERP amplitudes were found to correlate with cognitive development.

4. Discussion

4.1. Summary of main findings and strength of evidence

To our knowledge, this is the first review to systematically assess the predictive power of AERPs on neurodevelopmental outcome in the population of preterm born infants.

Our results show that the majority of studies included in this review reported significant correlations between AERPs and neuropsychological test scores, both in prospective and cross-sectional designs. A wide range of auditory stimulation paradigms was used according to the studies' research questions and the age of their participants. While in very young children passive paradigms were used, some studies with older participants included attentional tasks during auditory stimulation. Significant correlations between AERPs and neurodevelopmental outcome were found regardless of stimulation paradigm and participant age. Only one study (Mikkola et al., 2010) did not find any significant correlations between AERPs and outcome. However, the authors of this study state that not all participants were able to complete the attentional task which was linked to the AERP measurements; therefore the used paradigm might have been too challenging for their study population.

Two studies are of particular interest to this review, since they both used a prospective design and were of sufficient methodological quality according to our scoring system. Both studies recorded AERPs in the neonatal period and administered the BSID at the age of 2 years. Weber et al. (2016) found that habituation, as reflected by the decrease in MMN over a 30-minute auditory stimulation period, significantly predicted cognitive outcome in very preterm infants. Similarly, in the study by Maitre et al. (2013), AERP amplitudes between 250–400 ms after stimulus onset predicted cognitive development on the BSID.

In general, shorter latencies and larger amplitudes of late AERP peaks were related to a better cognitive outcome. This also applies for the MMN in a simple oddball paradigm using tones or syllables. However, in more complicated paradigms the interpretation of MMN is different: Jansson-Verkasalo et al. (2010) described that the presence of MMN in a paradigm using native and non-native phoneme contrasts indicates a deficiency of perceptual narrowing. Therefore in this paradigm larger MMN amplitude was related to poorer vocabulary.

As many as eight prospective cohort studies investigated and supported the predictive capacities of AERPs for later mental development. We therefore consider AERPs to be, at least to some extent, a viable, simple, bedside applicable, cognitive assessment tool in neonates born preterm. While our main research question was whether AERPS in neonates could predict neurodevelopmental development at an older age, cross-sectional studies of somewhat older children provided valuable information as well. Firstly, the inclusion of cross-sectional studies increased the number of correlations between AERPs and neuropsychological test scores and thereby allowed us to make a more powerful statement regarding individual AERP components. Secondly, a significant correlation at a single point in time might strengthen the assumption that neonatal AERPs also correlate with later neuropsychological outcomes. Thirdly, including cross-sectional studies increases the amount of information on AERPs in preterm born children. Both the analysis of AERPs and the administration of neuropsychological test batteries become easier as children grow older.

Overall, the studies included a range of preterm born infants. This implies that moderate to late, very, but also extremely preterm infants were included. This created a heterogeneous group of infants with different expected outcomes based on their gestational age (Boyle et al., 2012; Glass et al., 2015). The included studies made intragroup comparisons based on birth weight (Fellman et al., 2004; Mikkola et al., 2007), neonatal brain damage (Hovel et al., 2015; Leipala et al., 2011) or cognitive performance (Jansson-Verkasalo et al., 2003, 2004), however not based on gestational age. Therefore we were not able to investigate the effect of the degree of prematurity on the correlations between AERPs and outcome.

4.2. General interpretation and context with other evidence

While the studies included in our review utilized a wide range of auditory stimulation paradigms and heterogeneous cohorts, our findings corroborate previous reports of AERPs emerging as screening tools for cognitive functioning (deRegnier, 2005, 2008). AERPs have been successfully used to assess cognition in a range of other populations, such as children at risk for dyslexia (Lyytinen et al., 2015; Molfese, 2000) or adults (Naatanen et al., 1997, 1993; Rugg and Coles, 1995). Another use of AERPs is predicting the survival of neonates with brain injuries. Studies emerging from this background also included data on risk stratification for neurodevelopmental outcome in preterm infants (Pasman et al., 1997). The given outcomes, however, only provide limited information regarding the predictive value of AERPs for cognitive development.

The administration and interpretation of AERPs in neonates poses several additional challenges when compared to adult populations: In neonates, the typical AERP potentials may not be reliably detected, as these positive and negative peaks do not appear until 1 year of age (Kushnerenko et al., 2002). This fact is reflected by the variety of AERP peaks investigated across the included studies. Mean amplitudes from longer time windows post stimulus onset have been used in a neonatal population (Maitre et al., 2013, 2014), yielding similar results as the analysis of defined AERP peaks. However, the definition of latency windows for individual AERP peaks varied across studies. Especially in the preterm population, longer latencies are found which reflect the immaturity of the auditory processing in this group. With increasing age, the latency is shown to decrease (Silva et al., 2017). Given the variability in AERP responses in very young children (Kushnerenko et al., 2002; Leppanen et al., 2004), we consider this heterogeneity to be justified. Korpilahti et al. (2016) state that AERPs are more broadly distributed in preterm born children than in those born at term. For this reason, measuring AERPs at only a few positions might not discover all relevant activity. However, more research is needed to establish a consensus regarding the AERP characteristics that are most indicative of cognition in newborns.

4.3. Strengths and limitations

The most apparent strength of this review is its systematic methodology. A thorough search of relevant databases was conducted, and no additional records were identified through hand-searching of reference lists, which is an indicator that all eligible studies were identified through our online search. While reviews have been published on this topic, none of them were systematic. We based our critical appraisal checklist on a standardized tool. By including both cross-sectional and prospective study designs, we made sure to adequately depict today's state of knowledge regarding AERPs and cognitive development in children born premature. Another strength of this review is the fact that the majority of included studies used reliable tools for the measurement of AERPs as well as neuropsychological outcome. Also, the testing conditions for both prognostic factor and outcome measurement were comparable across studies. This speaks to the fact that AERPs are a feasible bedside test regardless of environmental circumstances, even in neonates and infants. Finally, the reported outcomes were comparable across all included studies, which supports the overall validity of our findings.

However several limitations have to be acknowledged to our review and the studies we included. We did not contact research teams directly, but relied solely on our search of online databases and reference lists. As for the studies we included, it was striking to see that numerous papers did not clearly explain their methodology and the factors which were considered for the analyses. This made it difficult for us to tell whether non-significant results for a certain outcome were not reported

or whether there was no analysis conducted. Likewise they often did not provide an interpretation of the correlations they found between the AERPs and outcome tests.

For their correlations, one study (Jansson-Verkasalo et al., 2010) used the MacArthur Communicative Development Inventories, which is a parent questionnaire. While it is a standardized tool, the risk of bias might be higher than in an assessment conducted by trained professionals. In another study (Leipala et al., 2011), neurodevelopmental outcome was simply scored as normal, moderately abnormal, or severely abnormal by a pediatric neurologist. The same study reported the number of AERP variables correlating with neurodevelopmental outcome without mentioning which variables those were. While clinically relevant, these results do not allow for an easy reproduction of the study findings. Another two studies reported their results in a narrative fashion (Jansson-Verkasalo et al., 2004) and calculated the probability of having abnormal AERPs in the presence of naming difficulties (Jansson-Verkasalo et al., 2003). Further, the preterm born cohorts were heterogeneous regarding age and clinical diagnoses. Across the 13 published papers we included, the number of preterm born children examined was just short of 300 - which is a relatively small number. Some studies had such small sample sizes that it was not possible to calculate adequate statistical correlations. In addition the number of preterm born infants who underwent an AERP-assessment around term equivalent age was very limited. Only few research groups are investigating the prediction of cognition in prematurely born children, which means that the bulk of evidence comes from only a handful of clinical centers with some overlap between study cohorts. Another limitation is the fact that, due to the rapid advancement in AERP methodology, some older studies pertaining to this topic could not be compared to studies using the newest techniques. A more general limitation to our review is the overall poor quality of prognostic studies compared to other study designs (Altman, 2001).

4.4. Implications for clinical practice and future research

Prematurity is a known risk factor for the occurrence of cognitive deficits (Bhutta et al., 2002). Neonatologists, pediatricians and developmental psychologists are in daily contact with parents of preterm born infants who are worried about their child's cognitive development (Howe et al., 2014). Therefore a tool that allows early identification is needed in clinical practice. The earlier patients at risk are identified, the earlier an intervention can start and possibly the better the outcomes will be (Spittle et al., 2015). The most advantageous features of EEG are that it is a non-invasive and bedside functional neuroimaging tool. Due to the long hospitalization duration, it is feasible to perform an AERP-assessment with premature infants. However few studies have focused on AERPs recorded in the neonatal period and the ones who did are quite diverse. In general based on the five prospective studies containing neonatal AERPs, larger peaks and shorter latencies were related to a better cognitive outcome. None of those studies provided cut-off levels based on sensitivity or specificity analyses. Recommendations for different levels of prematurity cannot be made due to the heterogeneity of the study participants.

Most of the studies utilized an auditory oddball paradigm with tones or syllables to investigate different AERP peaks and components. One study (Jansson-Verkasalo et al., 2010) reported that not all preschool-aged participants were able to complete a rather complex attentional task. This might imply that across all age groups, simple auditory tasks such as an oddball paradigm should be used in order to avoid confounding by individual performance levels.

All included studies except for one reported significant correlations between AERP measurements and cognitive development. This finding should encourage more in-depth research in this field in order to establish the clinical use of AERPs for the purpose of cognitive assessment in premature infants.

This systematic review does not report enough data to be translated into normative data or cut-off values. More studies with similar cohorts and methods are needed as well as sensitivity and specificity analyses in order to establish individual routine screening. Larger sample sizes also improve the power; therefore performing multicenter studies can be helpful. Additionally, more prospective cohort studies, starting at neonatal age, with longer follow-up periods are required. As well as the elaboration of guidelines (Duncan et al., 2009; Picton et al., 2000) for the predictive use of AERPs in the preterm population. Such guidelines can serve as the base for the development of a clinical test consisting of well-defined parameters.

5. Conclusion

The present systematic review investigates the predictive power of AERPs in preterm born infants. Our findings highlight the potential use of this method in an at risk population. Overall it was found that larger amplitudes and shorter latencies of late AERPs are related to better cognitive outcomes. In order to clinically apply this method, more evidence based on high quality studies is needed.

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Appendix A

Search strategy

Pubmed

Keyword and Medical Subject Headings Search

((((((preterm*[Text Word] OR premature*[Text Word] OR very low birth weight*[Text Word]))) AND ((neonat*[Text Word] OR newborn*[Text Word] OR infant*[Text Word] OR toddler*[Text Word] OR child*[Text Word] OR adolescent*[Text Word] OR preschool*[Text Word] OR school-age*[Text Word]))) AND ((((event-related potential*[Text Word] OR auditory evoked potential*[Text Word] OR mismatch respons*[Text Word] OR mismatch negativit*[Text Word] OR contingent negative variation*[Text Word] OR evoked cortical respons*[Text Word]))) OR (((N1[Text Word] OR N100[Text Word] OR P2[Text Word] OR P200[Text Word] OR P300[Text Word] OR ERP*[Text Word] OR MMN*[Text Word] OR CNV*[Text Word])) AND (EEG*[Text Word] OR electroencephalogr*[Text Word])))))) OR ((((((("Premature Birth"[Mesh]) OR "Infant, Premature"[Mesh]) OR "Infant, Very Low Birth Weight"[Mesh])) AND (((((("Infant, Newborn"[Mesh]) OR "Infant"[Mesh]) OR "Child"[Mesh]) OR "Child, Preschool"[Mesh]) OR "Adolescent"[Mesh])) AND ((("Event-Related Potentials, P300"[Mesh]) OR "Evoked Potentials, Auditory"[Mesh]) OR "Contingent Negative Variation"[Mesh])).

Embase

Keyword and Emtree Search

1 'preterm*':ab,ti OR 'prematur*':ab,ti OR 'very low birth weight*':ab,ti
2 'neonat*':ab,ti OR 'newborn*':ab,ti OR 'infant*':ab,ti OR 'toddler*':ab,ti OR 'child*':ab,ti OR 'adolescent*':ab,ti OR 'preschool*':ab,ti OR 'school-age*':ab,ti
3 'event-related potential*':ab,ti OR 'auditory evoked potential*':ab,ti OR 'mismatch respons*':ab,ti OR 'mismatch negativit*':ab,ti OR 'contingent negative variation*':ab,ti OR 'evoked cortical respons*':ab,ti
4 'n1':ab,ti OR 'n100':ab,ti OR 'p2':ab,ti OR 'p200':ab,ti OR 'p300':ab,ti OR 'erp*':ab,ti OR 'mmn*':ab,ti OR 'cnv*':ab,ti AND ('eeg*':ab,ti OR 'electroencephalogr*':ab,ti)
5 #3 OR #4
6 #1 AND #2 AND #5
7 'newborn'/exp OR 'infant'/exp OR 'child'/exp OR 'adolescent'/exp
8 'immature and premature labor'/exp OR 'very low birth weight'/exp
9 'event related potential'/exp OR 'auditory evoked potential'/exp OR 'evoked cortical response'/exp
10 #7 AND #8 AND #9
11 #6 OR #10

PsycINFO
Keyword and Thesaurus Search

1 (preterm* or premature* or very low birth weight*).mp. [mp = title, abstract, heading word, table of contents, key concepts, original title, tests & measures]
2 (neonat* or newborn* or infant* or toddler* or child* or adolescent* or preschool* or school-age*).mp. [mp = title, abstract, heading word, table of contents, key concepts, original title, tests & measures]
3 (event-related potential* or auditory evoked potential* or mismatch respons* or mismatch negativit* or contingent negative variation* or evoked cortical respons*).mp. [mp = title, abstract, heading word, table of contents, key concepts, original title, tests & measures]
4 (N1 or N100 or P2 or P200 or P300 or ERP* or MMN* or CNV*).mp. [mp = title, abstract, heading word, table of contents, key concepts, original title, tests & measures]
5 (EEG* or electroencephalogr*).mp. [mp = title, abstract, heading word, table of contents, key concepts, original title, tests & measures]
6 4 and 5
7 3 or 6
8 1 and 2 and 7
9 evoked potentials/ or auditory evoked potentials/ or contingent negative variation/ or mismatch negativity/ or p300/
10 birth weight/ or premature birth/
11 9 and 10
12 8 or 11

2. QUIPS-adapted appraisal checklist

Item	Criteria	Score
Participation	a Adequate description of recruitment and participation (min. n = 20)	a.
	a Adequate description of study sample	b.
	a Adequate description of inclusion and exclusion criteria	c.
		total:
Attrition	a Adequate percentage returning for follow-up (> 50%)	a.
	a Adequate description of participants lost to follow-up	b.
	a Participants lost to follow-up did not differ significantly from participants who completed the follow-up	c.
		total:
Prognostic Factor Measurement*	a The prognostic test / prognostic factor was adequately described	a.
	a The prognostic test conditions were equal for all participants	b.
	a Adequate proportion of the study sample has complete data	c.
		total:
Outcome Measurement	a The outcome is clearly defined	a.
	a Adequate administration of outcome measurement across participants	b.
	a Outcome measure is a standardized validated test	c.
		total:
Study Confounding	a Adequate description and measurement of relevant confounders	a.
	a Important potential confounders are accounted for	b.
	a Authors report possible study limitations	c.
		total:
	a Sufficient data presented to assess analytic strategy	a.

Statistical Analysis and Reporting	a Adequate selection of statistical model	b.
	a No selective reporting of results	c.
		total:

Scoring: 1 point for each criterion fulfilled.
*ERPs.

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2.2. Manuscript 2

Habituation as Parameter for Prediction of Mental Development in Healthy Preterm Infants: an Electrophysiological Pilot Study.

Journal: Journal of Child Neurology - published

Authors: Weber P, Depoorter A, Hetzel P, Lemola S.

Abstract: The aim of this prospective pilot study was to evaluate the predictive value of discrimination and habituation, which was measured by mismatch negativity in 17 healthy very preterm (mean gestational age 27.4 weeks; range 25.0-31.3) and 16 term (mean gestational age 40.3 weeks; range 37.9-41.7) born infants at term equivalent age. Developmental outcome was measured by Bayley Scales of Infant Development-I in 13 preterm and 13 term-born children at a mean age of 21.7 months (±2.18) and 18.5 months (±1.9), respectively. No differences in amplitude and latency of the mismatch negativity were found between both groups at term equivalent age. Within the preterm group habituation capacity was positively correlated with the Mental Developmental Index ($r = .654$, $P = .008$) and Performance Developmental Index ($r = .482$, $P = .048$) at 21 months. Early learning capability, as measured by habituation, may be associated with a better prognosis for early mental development in healthy preterm infants.

Authorship statement: AD and PW have a shared first authorship. PW designed the study, collected and analyzed the data. AD and PW interpreted the data. AD wrote the manuscript and made figures and tables. PW and AD corrected and improved the manuscript. As corresponding author AD took care of the submitting and reviewing process.

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Habituation as Parameter for Prediction of Mental Development in Healthy Preterm Infants: An Electrophysiological Pilot Study

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Abstract

The aim of this prospective pilot study was to evaluate the predictive value of discrimination and habituation, which was measured by mismatch negativity in 17 healthy very preterm (mean gestational age 27.4 weeks; range 25.0-31.3) and 16 term (mean gestational age 40.3 weeks; range 37.9-41.7) born infants at term equivalent age. Developmental outcome was measured by Bayley Scales of Infant Development–I in 13 preterm and 13 term-born children at a mean age of 21.7 months ($\pm$2.18) and 18.5 months ($\pm$1.9), respectively. No differences in amplitude and latency of the mismatch negativity were found between both groups at term equivalent age. Within the preterm group habituation capacity was positively correlated with the Mental Developmental Index ($r = .654$, $P = .008$) and Performance Developmental Index ($r = .482$, $P = .048$) at 21 months. Early learning capability, as measured by habituation, may be associated with a better prognosis for early mental development in healthy preterm infants.

Keywords

extreme prematurity, mental development, auditory event-related potentials, mismatch negativity, habituation

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Preterm birth is the main cause of infant mortality and a very important risk factor for the development of intellectual disability.[1] Whereas individual prediction of cerebral palsy—the severe form of a movement disorder following prematurity—by clinical bedside procedures is already possible with sufficient validity within the first 3 to 6 months of life,[2,3] accurate identification of individual children with increased risk for cognitive impairments is still a challenge because of the difficulties in finding any valid bedside tests of estimating cognitive abilities in infancy.[4] Closing this diagnostic gap is therefore of utmost clinical importance in long-term follow-up of preterm children.[5] Early identification of preterm infants at risk for neuropsychological sequelae is particularly relevant because early intervention programs are found to be effective up to preschool age.[6] Even if the concept of brain plasticity recommends an early start of intervention, ''high rate of attrition in routine clinical follow-up and consequent difficulty in accurately determining rates of delay highlight challenges for centers providing ongoing care.''[7] Offering these programs to all very preterm children is not feasible in many countries due to limited resources. In addition in some countries, such as Switzerland, a routine evaluation of the development of preterm infants is offered, but not an early intervention for all children. Thus, there is potential need to develop diagnostic tools which allow risk-stratification by early identification of preterm infants at increased risk for mental impairment, who might possibly benefit the most from such intervention programs.

Numerous studies show that several event-related components in an auditory oddball paradigm are easily measurable and indicative of mental functioning in neonates.[8-11] Moreover sound discrimination tasks, used in the neonatal intensive care unit, appear to be predictive of cognitive outcome in preterm infants at 2 years of age.[12] Such an auditory oddball paradigm consists of presenting sequences of repetitive ''standard'' tones, sporadically interrupted by ''deviant'' tones, resulting in an auditory event-related potential component called mismatch negativity. This event-related potential component is calculated by subtracting the average event-related potential of the standard tones from the average event-related potential

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Table 1. Cohort Characteristics: Group Differences Tested by Unpaired *t*-Test and Chi-Quadrat Test.

	Preterm infants (n = 17)	Term-born infants (n = 16)	*P* value
GA at birth, mean (range)	27.4 weeks (25.0-31.3)	40.3 weeks (37.9-41.7)	<.001*
GA at examination, mean (range)	40.8 weeks (38.4-43.0)	40.8 weeks (38.1-42.0)	.943
Sex (m: f)	9: 8	10: 6	.257
Birth weight, mean (range)	893 g (470-1530)	3603 g (2880-4300)	<.001*

Abbreviations: f, female; GA, gestational age; m, male.
*$P < .05$.

of the deviant tones. The by subtraction resulting difference waveform is known to have a negative amplitude peaking between 100 and 250 ms post deviant stimulus onset in adults.[13] It is interpreted as a preattentive cognitive discrimination ability and can already be observed from 30 weeks of gestational age onward.[14]

Preterm infants may show a diminished capability for habituation and dishabituation as early signs of delay in mental processing such as encoding, attention, discrimination, and memory.[15] Nevertheless until now habituation and its predictive value for later mental development of very preterm children has not yet been studied in an electrophysiological setting. Therefore the authors aimed to assess discrimination, as an early preattentive parameter, and habituation, as an early parameter of learning, measured by auditory event-related potentials in very preterm compared to healthy term-born infants in a clinical routine setting. To test the predictive value of habituation in preterm infants at term-equivalent age for the mental development, authors correlated the habituation capability at this age, measured by the difference in mismatch negativity, with the developmental level at corrected age of 21 months.

Methods

Participants and Procedure

In this prospective pilot study preterm infants were recruited at the Neonatal Intensive Care Unit of the University Children's Hospital Basel and healthy term-born infants were recruited at the child-bed ward of the Division of Gynaecology and Obstetrics of Bruderholzspital in the canton Basel-Landschaft. The authors examined 17 very preterm infants and 16 healthy term-born infants at a mean gestational age of 40.8 weeks (range 38.1-43.0). Preterm infants with severe brain lesions (ie, > grade II intracerebral hemorrhages, neonatal seizures), genetic syndromes, or confirmed congenital infection were excluded from the study to avoid a heterogeneous cohort. The cohort characteristics are documented in Table 1. Routinely in all infants and neonates transient-evoked otoacoustic emissions were recorded and analyzed, before discharge from the hospital (36-42 weeks of gestational age). All participants fulfilled the "pass" criterion. Even if this procedure doesn't allow to quantify the hearing level, it is accepted to be demonstrating a normal cochlear function.[16]

Follow-up was conducted and 13 preterm and 13 term-born children were tested with regard to developmental outcome at a corrected mean age of 21.7 months ($\pm$2.18) and 18.5 months ($\pm$1.9), respectively. Thus there was a drop-out of 4 preterm and 3 term-born children due to moving of the families, including 1 family with preterm triplets.

The Ethics Committee of Basel approved the study protocol, parents gave written informed consent, and the study has been carried out in accordance with the Declaration of Helsinki.

Auditory Event-Related Potentials

The auditory event-related potentials were conducted with electrodes located according to the International 10-20 system at midline electrode sites (Fz, Pz, and Oz) and referenced to the left-sided mastoid electrode A1. To control for artifacts by eye movements, 1 electrode was located at the lower outer side of the left eye. The passive auditory oddball paradigm was used in the study to elicit auditory event-related potentials. It consists of presenting 2 tones with different frequencies (Hz). The standard (ie, frequently occurring) tone was 1000 Hz and the deviant (ie, rarely occurring) tone 2000 Hz, both presented in a pseudo-randomized order. The standard tone occurred with a probability of 85%, while the deviant tone occurred with a probability of 15%. The stimulus frequency was 0.9 Hz and the intensity 80 dB normal hearing level. In the experimental setting, 3 blocks of 500 tones were presented via headphones to the silent awake or sleeping infants. A break of 2 minutes was taken between each block. The event-related potentials were recorded with the Viking Select 4-channel in a bedside setting. Even if the use of this simple equipment with only 4 active electrodes implicated some limitations, such as renouncement of information about laterality or automatically grand averaging, it was used since it is an equipment which is available in the clinical setting of nearly all Neonatal Intensive Care Units, underlining the aspect of the feasibility of this data collection in daily routine on the ward. The data were acquired offline with a band-pass filter of 0.5-40 Hz and a sampling rate of 20 kHz.

Data Analysis

The artifact rejection was performed manually per stimulation block. Samples with an amplitude difference between rare and frequent tones of >0.5 μV at the electrode under the eye were excluded, as indication of a relevant influence by eye blinks. The electrophysiological response was then averaged over the standard and the deviant tones independently for each of the 3 stimulus blocks.

To compute the mismatch negativity, at first the authors manually identified the peak of the event-related potential in the time window between 150 and 300 ms after stimulus presentation for every proband. Thereafter the differences of the amplitude between rare and frequent tones were calculated, defining the mismatch negativity (Figure 1). The resulting mismatch negativity is investigated regarding amplitude (in μV), latency (in ms) and polarity (negative or positive) and corresponds to the discrimination ability of the subjects. The habituation capability is then calculated by the difference in amplitude of mismatch negativity between the first and third stimulus block.

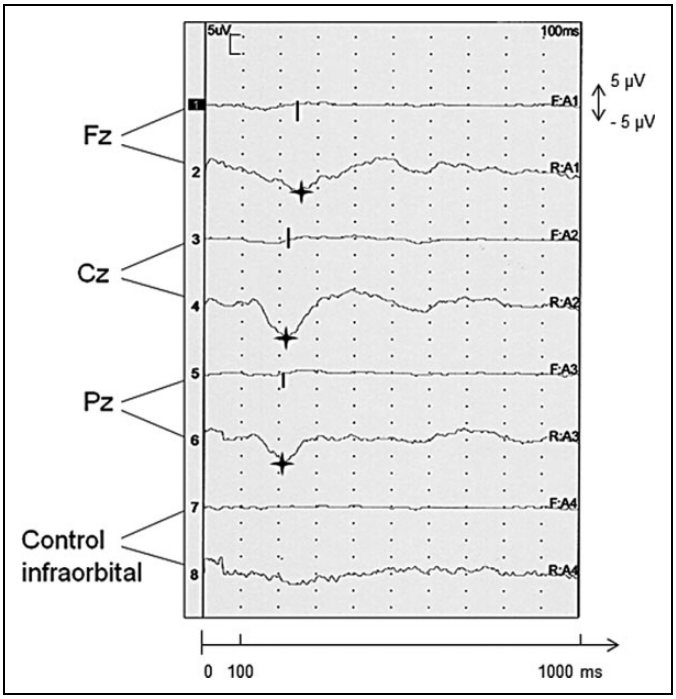

Figure 1. Example of an averaged auditory event-related potential (AERP) of the first stimulus block of 1 participant. The maximum amplitude peaks between 150-300 ms are indicated at the midline electrodes (Fz, Cz, Pz) for rare (R, $n = 75$) tones and the correspondent amplitude value for frequent (F, $n = 425$) tones. By subtracting these amplitude values respectively, the mismatch negativity (MMN) component is calculated.

Developmental Outcome

The developmental outcome was measured by the Bayley Scales of Infant Development–I[17] and the Mental Developmental Index and Performance Developmental Index were computed. The examiners were blinded with regard to the electrophysiological data.

Statistical Analysis

Statistical computations were performed using SPSS version 22.0 (IBM) for Windows. Pearson's chi-square test, calculation of Pearson's correlation coefficient, or *t*-test for independent cohorts were used accordingly. The significance level was set at $P < .05$ (2-tailed).

In respect to the proof-of-concept initiation of the study and the small sample size, no Bonferroni corrections were applied.

Results

Mismatch Negativity

No significant group differences in latency or mean amplitude of the mismatch negativity were found at the midline electrodes between preterm and term-born infants. However, significantly more preterm infants showed a lower arbitrary chosen mismatch negativity amplitude of < 2 µV ($\chi^2 = 4.29$, $P = .038$) at Fz compared to term infants. In addition no significant difference was found in the habituation effect calculated by the difference in mismatch negativity between first and third stimulus blocks, just as no difference was observed in the frequency of a habituation or dishabituation effect between the groups (Table 2).

Outcome

The developmental outcome measured by Bayley Scales of Infant Development–I showed significant differences between the preterm and control group. In particular, both groups differed significantly on the Mental Developmental Index (74 ±18.28 in the preterm group vs 95 ±10.78 in the control group), $t(19.5) = 3.49$, $P = .002$, and the Performance Developmental Index (89 ±13.01 in the preterm group vs 109 ±8.9 in the control group), $t(24) = 4.57$, $P < .001$. No differences were found in the behavioral scales: orientation, $t(23) = –0.30$, $P = .766$, emotion, $t(23) = 2.02$, $P = .568$, or motor quality, $t(23) = 2.14$, $P = .43$.

Correlations

Within the group of preterm infants no significant correlation was found between gestational age and amplitude or latency of mismatch negativity at any of the 3 electrode positions. However gestational age significantly correlated with difference in mismatch negativity at electrode position Fz ($r = .551$, $P = .013$), Cz ($r = .531$, $P = .017$), and Pz ($r = .478$, $P = .031$) (Table 3; Figure 2).

With regard to the developmental outcome test, the Mental Developmental Index was significantly correlated with difference in mismatch negativity at the electrode position Cz ($r = .654$, $P = .008$), but not at position Fz ($r = .313$, $P = .149$) or Pz ($r = .145$, $P = .319$). The Performance Developmental Index was also significantly correlated with difference in mismatch negativity at position Cz ($r = .482$, $P = .048$), but no correlation was observed between the Performance Developmental Index and difference in mismatch negativity at position Fz ($r = .157$, $P = .304$) and Pz ($r = –.047$, $P = .439$) (Figure 3).

Discussion

The main finding of this study is the association between the habituation capability (difference in mismatch negativity) and the mental and performance outcome subtest at the age of 21 months within the group of preterm infants. This positive relation suggests that the lower the habituation effect, the lower the scores on the mental and performance scale. The relationship between event-related potential components and the Bayley Scales of Infant Development–I highlights the power of auditory event related measures in prediction of cognitive abilities,[8,9,18] although the authors have taken into account the low statistical power of this study including the fact that no Bonferroni correction considering the number of electrodes was done, which would decrease the statistical significance. Other studies have found similar relationships between auditory event-related potentials and cognitive outcomes even at later ages.[10,12] The authors examined habituation, a variable

Table 2. Latency, Amplitude, Frequency of Low Mismatch Negativity (MMN) Amplitudes (<2 μV), Difference of the Amplitude (Habituation Effect) of MMN Between the First and Second (dMMN 1-2) and Between the First and the Third Stimulus Block (dMMN 1-3), and Frequency of Dishabituation in Both Groups at the 3 Electrode Positions Fz, Cz, and Pz after Unpaired *t*-Tests.

		Preterm infants (n = 17)	Term-born infants (n = 16)	*P* value
Fz	MMN latency (ms) (mean ± SD)	220.9 ± 42.8	234.9 ± 27.3	.275
	MMN amplitude (μV) (mean ± SD)	3.27 ± 1.88	4.08 ± 1.71	.205
	Frequency MMN amplitude < 2 μV	4/17	0/16	.038*
	dMMN 1-2 amplitude (μV) (mean ± SD)	–0.01 ± 1.53	0.75 ± 1.93	.236
	Frequency of dishabituation	8/17	6/16	.740
	dMMN 1-3 amplitude (μV) (mean ± SD)	0.42 ± 3.36	1.33 ± 1.56	.330
	Frequency of dishabituation	7/17	3/16	.127
Cz	MMN latency ms (mean ± SD)	204.3 ± 21.7	213.3 ± 20.5	.230
	MMN amplitude (μV) (mean ± SD)	4.22 ± 2.79	4.65 ± 2.35	.644
	Frequency MMN amplitude < 2 μV	4/17	1/16	.144
	dMMN 1-2 amplitude (μV) (mean ± SD)	0.43 ± 2.50	1.17 ± 2.38	.406
	Frequency of dishabituation	7/17	5/16	.849
	dMMN 1-3 amplitude (μV) (mean ± SD)	0.63 ± 3.32	0.65 ± 2.77	.986
	Frequency of dishabituation	9/17	6/16	.288
Pz	MMN latency ms (mean ± SD)	213.9 ± 36.9	205.0 ± 27.2	.438
	MMN amplitude (μV) (mean ± SD)	2.90 ± 1.41	3.08 ± 1.70	.743
	Frequency MMN amplitude < 2 μV	5/17	4/16	.694
	dMMN 1-2 amplitude (μV) (mean ± SD)	0.20 ± 1.44	1.0 ± 1.78	.179
	Frequency of dishabituation	9/17	7/16	.777
	dMMN amplitude (μV) (mean ± SD)	0.23 ± 1.68	0.30 ± 2.02	.915
	Frequency of dishabituation	7/17	8/16	.723

*$P < .05$.

Table 3. Pearson Correlations Between Gestational Age at Birth (GA), Mental Developmental Index (MDI), and Performance Developmental Index (PDI) and the Difference Between MMN During the First and the Third Stimulus Block (dMMN = Habituation Effect).

	GA	MDI	PDI
dMMN (μV) at Fz	.551*	.313	.157
dMMN (μV) at Cz	.531*	.654*	.482*
dMMN (μV) at Pz	.478*	.154	.047

*$P < .05$.

that has not been adequately assessed in previous studies using auditory event-related potentials in preterm children. In an older behavioral study[19] fast movement habituation was found to be related to higher scores on the Mental Developmental Index. Habituation is regarded as the most elementary form of learning and can be observed in neonates in behavioral studies[15] as well as in neuroimaging studies.[18,20] Since the competence of learning is an important constituent of cognitive development, it is a potentially useful predictor of neurodevelopmental outcomes in infants at greatest risk for cognitive impairment.[20]

This pilot study points to the possible early predictive value of habituation measured by mismatch negativity for later individualized developmental outcomes in very preterm infants. Moreover, it implies that this procedure might be useful as a simply applicable risk stratification tool in a bedside setting. The fact that the closest correlation between the developmental level at age of 21 months and the habituation effect was documented at the Cz lead, could evoke some suggestions about the cortical function involved in the tasks. In respect of the small head circumference of the infants as well as the insufficient spatial resolution of electroencephalography from scalp electrodes, the authors disclaim this speculation at this point.

In contrast to most studies with larger samples sizes,[21] in this study the authors found no differences regarding amplitude or latency of mismatch negativity in a passive auditory oddball paradigm between very preterm and term-born infants at neonatal age. Even though no significant group differences were found in mean values, using an arbitrary cut off amplitude value of < 2 μV more control infants exceeded this mismatch negativity limit than preterm infants. Often it is reported that the lower the gestational age, the lower the event-related potential amplitudes.[21] Lower amplitudes might indicate lower capacities in detection of sound differences, meaning a less preattentive discrimination ability, and therefore immaturity of the auditory system.[22] Consequently the current results suggest that only a subgroup of very preterm infants show reduced mental processing during discrimination tasks. In this way, measuring habituation as a possible predictor of cognitive impairment in early infancy might open the window for more targeted follow up neurodevelopmental therapies in certain newborns.

Contrary to what might have been expected the habituation capacity—defined by the reduction of the mismatch negativity amplitude between the first and third session—did not differ among the preterm as compared to the control children, neither between the first and second stimulus block, nor between the first and third stimulus block. As a limitation of the authors' technical methods, the authors could not analyze a possible

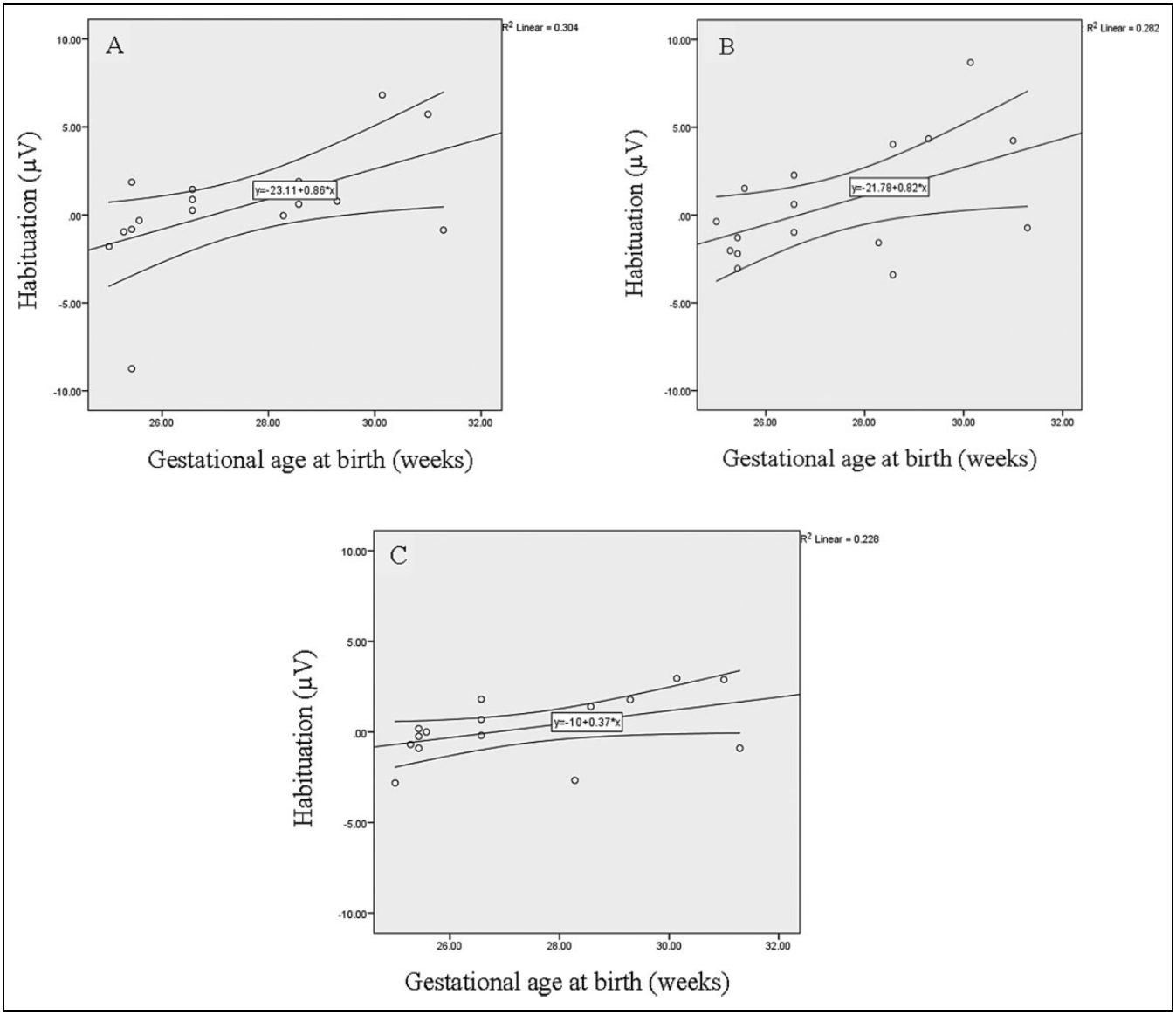

Figure 2. Simple linear regression of the amplitude difference between mismatch negativity (MMN) in the first stimulus block minus MMN in the third stimulus block (= habituation effect, dMMN) and gestational age at electrode position Fz (A), Cz (B), and Pz (C).

habituation effect within 1 single (ie, the first) stimulus block. It is possible that habituation is a fast learning process, which could be detected only in a short time window. In addition, the failure to replicate this result might be due to limited statistical power in the present study and the fact that only a subgroup of preterm infants at higher risk might show this deficiency.

As expected, very preterm children showed lower scores in mental development compared to children born at term which is in line with existing evidence.[23,24]

Furthermore, difference in mismatch negativity was also related to the gestational age, meaning the earlier the infant is born, the lower the habituation effect and vice versa. Gestational age is an important risk factor in general and known to correlate with cognitive outcomes.[12] Therefore in a larger sample size difference in mismatch negativity has to be confirmed as independent predictive variable. In addition infants with prematurity under 25 gestational weeks and higher risk of neurodevelopmental problems should be included for further testing the discussed hypothesis.

Beside the small sample size, the main constraint of this pilot study is that only 3 electrode positions were used and therefore does not provide us with many strong correlations between the event-related potential components and the mental outcome test. Moreover the low number of participants and the drop-out for the outcome measurement at the age of 21 months leaves us with less data to support the prediction hypothesis. The outcome was only investigated in early childhood, so data from the subjects at a later age in their development would be interesting.

In conclusion, this proof-of-concept pilot study suggests the auditory event-related potential approach as a potentially valuable tool to assess early cognitive abilities, such as habituation, in neonates in a bedside setting.

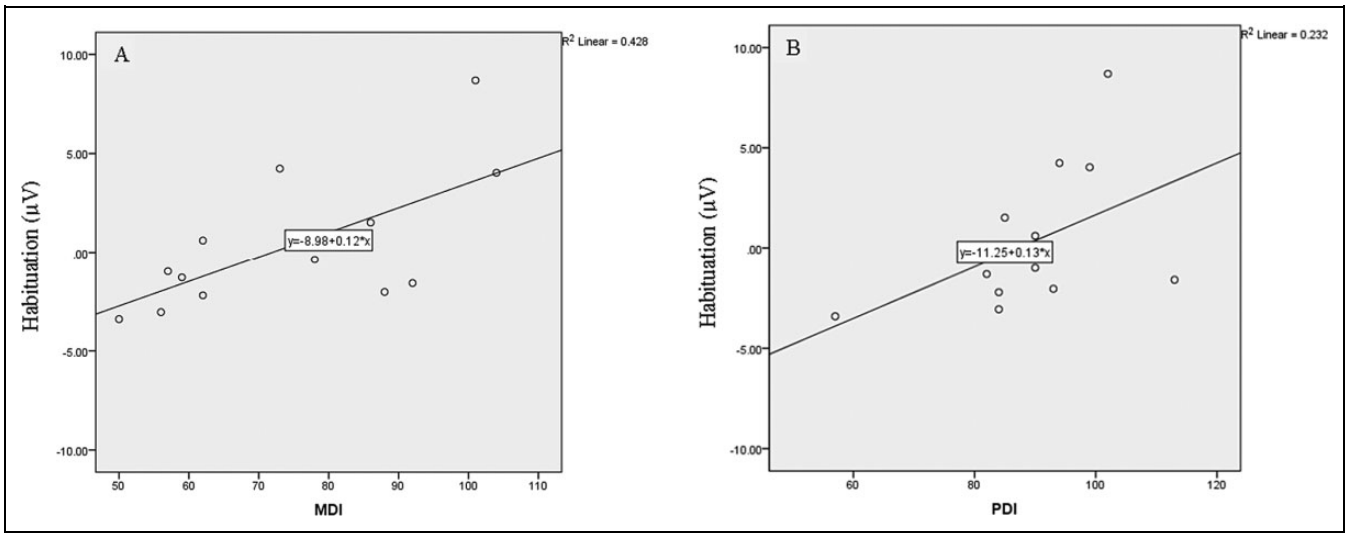

Figure 3. Simple linear regression of the amplitude difference between mismatch negativity (MMN) in the first stimulus block minus MMN in the third stimulus block (= habituation effect) and Mental Developmental Index (MDI) (A) and Performance Developmental Index (PDI) (B) at the electrode position Cz.

Author Contributions

The study design and acquisition of the data were done by PW and PH. PW and AD analyzed and interpreted the data. Drafting and revising the manuscript wad done by PW, AD, and SL.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The Ethics Committee of Basel approved the study protocol, parents gave written informed consent, and the study has been carried out in accordance with the Declaration of Helsinki.

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2.3. Manuscript 3

Neurofilament Light Chain: Blood Biomarker of Neonatal Neuronal Injury.

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Authors: Depoorter A, Roland Neumann R, Barro C, Fisch U, Weber P, Kuhle J, Wellmann S.

Abstract: Neurofilament light chain (NfL) is a highly promising biomarker of neuroaxonal injury that has mainly been studied in adult neurodegenerative disease. Its involvement in neonatal disease remains largely unknown. Our aim was to establish NfL plasma concentrations in preterm and term infants in the first week of life. Plasma NfL was measured by single molecule array immunoassay in two neonatal cohorts: cohort 1 contained 203 term and preterm infants, median gestational age (GA) 37.9 weeks (interquartile range [IQR] 31.9-39.4), in whom venous and arterial umbilical cord blood was sampled at birth and venous blood at day of life (DOL) 3; cohort 2 contained 98 preterm infants, median GA 29.3 weeks (IQR 26.9-30.6), in whom venous blood was sampled at DOL 7. Median NfL concentrations in venous blood increased significantly from birth (18.2 pg/mL [IQR 12.8-30.8, cohort 1]) to DOL 3 (50.9 pg/mL [41.3-100, cohort 1]) and DOL 7 (126 pg/mL [78.8-225, cohort 2]) ($p<.001$). In both cohorts NfL correlated inversely with birth weight (BW, Spearman's rho -.403, $p<.001$, cohort 1; R -.525, $p<.001$, cohort 2) and GA (R -.271, $p<.001$, cohort 1; R -.487, $p<.001$, cohort 2). Additional significant correlations were found for maternal age at delivery, preeclampsia, delivery mode, 5-min Apgar, duration of oxygen supplementation, sepsis, and brain damage (intraventricular hemorrhage or periventricular leukomalacia). Multivariable logistic regression analysis identified the independent predictors of NfL in cohort 1 as BW

(beta=-.297, p=.003), delivery mode (beta=.237, p=.001) and preeclampsia (beta=.183, p=.022) and in cohort 2 as BW (beta=-.385, p=.001) and brain damage (beta=.222, p=.015). Neonatal NfL levels correlate inversely with maturity and BW, increase during the first days of life, and relate to brain injury factors such as intraventricular hemorrhage and periventricular leukomalacia, and also to vaginal delivery.

Authorship statement: AD and RN have a shared first authorship. RN collected the data and AD analyzed the data and wrote the manuscript. Together the manuscript was corrected and reviewed. As corresponding author AD took care of the submitting and reviewing process.

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Neurofilament Light Chain: Blood Biomarker of Neonatal Neuronal Injury

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Background: Neurofilament light chain (NfL) is a highly promising biomarker of neuroaxonal injury that has mainly been studied in adult neurodegenerative disease. Its involvement in neonatal disease remains largely unknown. Our aim was to establish NfL plasma concentrations in preterm and term infants in the first week of life.

Methods: Plasma NfL was measured by single molecule array immunoassay in two neonatal cohorts: cohort 1 contained 203 term and preterm infants, median gestational age (GA) 37.9 weeks (interquartile range [IQR] 31.9–39.4), in whom venous and arterial umbilical cord blood was sampled at birth and venous blood at day of life (DOL) 3; cohort 2 contained 98 preterm infants, median GA 29.3 weeks (IQR 26.9–30.6), in whom venous blood was sampled at DOL 7.

Results: Median NfL concentrations in venous blood increased significantly from birth (18.2 pg/mL [IQR 12.8–30.8, cohort 1]) to DOL 3 (50.9 pg/mL [41.3–100, cohort 1]) and DOL 7 (126 pg/mL [78.8–225, cohort 2]) ($p < 0.001$). In both cohorts NfL correlated inversely with birth weight (BW, Spearman's rho −0.403, $p < 0.001$, cohort 1; R −0.525, $p < 0.001$, cohort 2) and GA (R −0.271, $p < 0.001$, cohort 1; R −0.487, $p < 0.001$, cohort 2). Additional significant correlations were found for maternal age at delivery, preeclampsia, delivery mode, 5-min Apgar, duration of oxygen supplementation, sepsis, and brain damage (intraventricular hemorrhage or periventricular leukomalacia). Multivariable logistic regression analysis identified the independent predictors of NfL in cohort 1 as BW (beta = −0.297, $p = 0.003$), delivery mode (beta = 0.237, $p = 0.001$) and preeclampsia (beta = 0.183, $p = 0.022$) and in cohort 2 as BW (beta = −0.385, $p = 0.001$) and brain damage (beta = 0.222, $p = 0.015$).

Conclusion: Neonatal NfL levels correlate inversely with maturity and BW, increase during the first days of life, and relate to brain injury factors such as intraventricular hemorrhage and periventricular leukomalacia, and also to vaginal delivery.

Keywords: cerebral injury, neuropathology, biomarker, infant, parturition, prematurity

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INTRODUCTION

As direct access to the central nervous system (CNS) is almost impossible, neuronal biomarkers have been investigated for decades in order to improve early diagnostics, monitor disease progression and optimize care. Neurofilaments (Nf) are highly specific major neuronal scaffolding proteins comprising 4 subunits: the triplet of Nf light chain (NfL), Nf medium chain, and Nf heavy chain (NfH), and α-internexin in the CNS, or peripherin in the peripheral nervous system (1). Acute or chronic neuronal damage, including traumatic brain injury, stroke, dementia and multiple sclerosis, releases Nf fragments into the cerebrospinal fluid and eventually the blood compartment (2–6). Recent advances using highly sensitive single molecule array (Simoa) immunoassay have improved NfL detection, particularly in peripheral blood, making it a promising and readily accessible biomarker for neuroaxonal injury (7).

Whereas, circulating Nf has been extensively characterized in adults and older children with neurologic disease, data in infants and particularly newborns are sparse. One study reported raised serum NfH in children older than 6 months with febrile seizures lasting >30 min, suggesting that prolonged seizures cause some degree of neuronal damage (8). Plasma NfH in newborns with hypoxic-ischemic encephalopathy (HIE) was also higher than in healthy neonates (9, 10). Moreover, NfL levels in infants undergoing therapeutic hypothermia for HIE were significantly higher in those with unfavorable vs. favorable brain magnetic resonance imaging (MRI) outcome (11). As for mode of delivery, serum NfH levels at day of life (DOL) 2 in a small cohort of newborns did not differ between those born vaginally and those born by cesarean section (12).

Given the potential of Nf in adults with acute or chronic CNS damage and promising results in infants with HIE, we aimed to measure NfL levels by Simoa in two cohorts of preterm and term neonates in umbilical cord blood at birth and in venous blood a few days after birth.

MATERIALS AND METHODS

Study Participants

The study was based on data and blood samples prospectively collected from two neonatal cohorts. Cohort 1 comprised data and blood samples from 203 preterm and term neonates, median gestational age (GA) 37.9 weeks (interquartile range [IQR] 31.9–39.4), born and cared for at the University Hospitals of Zurich and Basel, Switzerland. More specifically, it comprised 89 preterm infants (GA < 37 weeks), including 52 with GA < 32 weeks, and 114 term infants (GA ≥ 37 weeks). The study was approved by the institutional review boards of both university hospitals (Ethikkommission beider Basel, EKBB07/09, Kantonale Ethikkommission Zurich, KEK08/09). Cohort 2 comprised data and blood samples from 98 very preterm neonates (GA < 32 weeks), median GA 29.3 weeks (IQR 26.9–30.6), born and cared for at the University Hospital of Basel, Switzerland. The study was approved by the institutional review board (Ethikkommission beider Basel, EK233/13) and was carried out in accordance with the declaration of Helsinki. Written informed consent was obtained from the parents prior to enrollment.

Abbreviations: Nf, Neurofilament; NfL, Neurofilament Light Chain; GA, Gestational Age; BW, Birth Weight; DOL, Day of Life; MPT, Moderate Preterm and Term.

Clinical Characteristics (Table 1)

Details of pregnancy (presence/absence of preeclampsia, amniotic infection, preterm labor, maternal age, premature rupture of membranes), delivery (umbilical artery pH, delivery modality), birth (GA, BW, sex, 5- and 10-min Apgar scores), and postnatal course to discharge home (presence/absence of sepsis and/or necrotizing enterocolitis, ultrasound brain damage with periventricular intraventricular hemorrhage [PIVH] or periventricular leukomalacia [PVL], duration of oxygen) were collected from the charts. Definitions of clinical characteristics, including preeclampsia, clinical chorioamnionitis, PIVH, and PVL, have been described previously (13), based on standardized definitions of the Swiss Neonatal Network.

Sample Preparation and Assessment of NfL

In cohort 1, venous blood (0.5 mL) was collected from the umbilical cord at birth ($n = 185$) and simultaneously with mandatory neonatal metabolic screening at DOL 3 ($n = 39$); 68 paired umbilical arterial samples were also collected at birth. In cohort 2, venous blood was collected with diagnostic blood samples at DOL 7 ($n = 98$). All samples were handled according to standard operating procedures for blood sampling in EDTA tubes, subsequent sample transfer to the central laboratory service, centrifugation, preparation of aliquots, and storage at −80°C until batch-wise analysis as described previously (14). Assay technicians were blinded to clinical information and pregnancy outcome.

NfL levels were measured by Simoa immunoassay using capture monoclonal antibody (mAB) 47:3 and biotinylated detector mAB 2:1 (UmanDiagnostics, Umea, Sweden), as previously described (15). Calibrators (neat) and serum samples (1:4 dilution) were measured in duplicate. Bovine lyophilized NfL was obtained from UmanDiagnostics. Calibrators ranged from 0 to 2,000 pg/mL. Batch-prepared calibrators were stored at −80°C. Intra- and interassay variabilities were < 10%; the few samples with intra-assay coefficients of variation >20% were remeasured.

Data Analysis

Statistical analyses were performed using SPSS for Windows version 24 (IBM) and included descriptive statistics, Spearman's rank-order correlation analyses and multiple linear regressions (MLR) using NfL as dependent variable. NfL variables were log10 transformed for the correlations and MLR. The independent variables included for MLR were based on significant correlations and significant non-parametric univariate analyses such as the Mann-Whitney U (2 levels) and Kruskal-Wallis tests (>2 levels). For cohort 1 these variables were: BW, 5-min Apgar, delivery mode (3 levels), preeclampsia, sepsis, and oxygen duration. For cohort 2 they were: BW, 5-min Apgar, sex, brain damage, sepsis,

amniotic infection, and oxygen duration. Due to collinearity between BW and GA, we used only BW in MLR, where it showed stronger correlation with NfL than GA.

RESULTS

Baseline NfL Levels

In cohort 1 overall median venous NfL concentrations were 18.2 pg/mL (IQR 12.8–30.8) at birth and 50.9 pg/mL (41.3–100.1) at DOL 3; in cohort 2 they were 128.5 pg/mL (78.8–224.8) at DOL 7.

We split cohort 1 into a very preterm group (GA < 32 weeks; $n = 52$) and a moderate preterm and term (MPT) group (GA ≥32 weeks; $n = 151$) with fewer prematurity complications ($n = 1$ in our sample). This also enabled us to compare the first group with cohort 2. NfL levels were significantly higher in very preterm infants than in the MPT group at birth (median 32.5 pg/mL, $n = 47$ vs. 15.3 pg/mL, $n = 138$; $p < 0.001$), but not at DOL 3 (median 48.5 pg/mL, $n = 16$ vs. 51.4 pg/mL, $n = 23$; $p = 0.668$). Moreover, levels increased significantly from birth to DOL 3 in both the very preterm and MPT groups (median 32.5 vs. 48.5 pg/mL, $p = 0.002$; and median 15.3 vs. 51.4 pg/mL, $p < 0.001$), and from DOL 3 to DOL 7 in the very preterm group (median 48.5 vs. 128.5 pg/mL, $p = 0.001$) (**Table 2**). This increase was confirmed in cohort 1 when comparing paired samples from same infants (MPT group $n = 16$, very preterm group $n = 11$) at birth and DOL 3 (median 18.2 pg/mL vs. 49.4 pg/mL). Out of these, only in 2 very preterm infants NfL levels remained unchanged, in all other infants they increased from birth until DOL 3. Paired umbilical cord arterial and venous plasma were closely related ($R = 0.875$, $p < 0.001$). Given this close correlation and the greater number of subjects ($n = 185$), we performed all further analyses using the venous blood samples collected at birth.

TABLE 1 | Descriptive statistics.

	Cohort 1 $n = 203$		Cohort 2 $n = 98$
	Moderate Preterm and Term (≥32 weeks GA) $n = 151$	Very preterm (< 32 weeks GA) $n = 52$	Very preterm (< 32 weeks GA) $n = 98$
NEONATAL CHARACTERISTICS			
GA (weeks)	38.3 (37.0–40.0)	30.1 (28.3–31.3)	29.3 (26.9–30.6)
BW (g)	3270 (2710–3630)	1360 (1063–1463)	1145 (788–1413)
Sex (male, %)	87 (57.6)	25 (48.1)	52 (53.1)
Brain damage (%)	1 (0.7)	10 (19.2)	12 (12.2)
O_2 duration (days)	0	4 (1–15.8)	2.38 (0.05–22.8)
pH umbilical artery	7.30 (7.26–7.33)	7.32 (7.29–7.37)	7.32 (7.28–7.36)
NEC (%)	0	0	3 (3.1)
Sepsis (%)	0	11 (21.2)	13 (13.3)
5-min Apgar	9 (9–9)	7 (5.25–8)	7 (6–8)
Death (%)	0	6 (11.5)	2 (2.0)
MATERNAL CHARACTERISTICS			
Age (years)	32 (29–36)	33 (28.3–36.0)	33 (29–36)
Amniotic infection (%)	5 (3.3)	13(25)	20 (20.4)
Preeclampsia (%)	16 (10.6)	20 (38.5)	16 (16.3)
PROM (%)	14 (9.3)	14 (26.9)	28 (28.6)
DM (%):			
Primary CS	76 (50.3)	26 (50)	27 (27.6)
Secondary CS	29 (19.2)	21 (40.4)	59 (60.2)
VD	46 (30.5)	5 (9.6)	12 (12.2)

GA, gestational age; BW, birth weight; BD, brain damage (PIVH and/or PVL); NEC, necrotizing enterocolitis; PROM, premature rupture of membranes; DM, delivery mode; CS, cesarean section; VD, vaginal delivery. GA, BW, O_2 duration, Apgar, pH and maternal age are presented as median and interquartile range.

NfL and Perinatal Characteristics in Cohort 1

Venous cord blood at birth correlated negatively with BW ($R = -0.403$, $p < 0.001$, **Figure 1**), GA ($R = -0.271$, $p < 0.001$), 5-min Apgar ($R = -0.295$, $p < 0.001$), and 10-min Apgar ($R = -0.363$, $p < 0.001$). In contrast, levels correlated positively with oxygen duration ($R = 0.333$, $p < 0.001$) and delivery mode ($R = 0.156$, p = 0.034).

Presence of preeclampsia (31.0 pg/mL vs. 16.2, $p < 0.001$) and sepsis (32.6 pg/mL vs. 17.85, $p = 0.033$) were associated with higher NfL levels.

In the MPT group NfL levels at birth were significantly higher in infants delivered vaginally than by primary or secondary cesarean section (21.8 vs. 13.9 and 14.4 pg/mL; $p = 0.002$) (**Figure 2**). This was not the case in the very preterm group, presumably due to the few vaginal deliveries ($n = 5$ vs. $n = 47$ cesarean sections). At DOL 3 there was no significant difference ($p = 0.07$) in NfL levels between birth modalities except for vaginal delivery vs. cesarean section (110 pg/mL, $n = 8$ vs. 48.7 pg/mL, $n = 31$; $p = 0.031$).

MLR testing for the best independent predictors of NfL levels at birth used BW, 5-min Apgar, delivery mode, preeclampsia, sepsis and oxygen duration as explanatory variables. The model was significant ($F_{(6, 176)} = 8.655$, $p < 0.001$), explaining around 23% of NfL variance ($R^2 = 0.228$). The predictors were BW (beta = −0.297, $p = 0.003$), delivery mode (beta = 0.237, $p = 0.001$), and preeclampsia (beta = 0.183, $p = 0.022$).

NfL and Perinatal Characteristics in Cohort 2

NfL at DOL 7 correlated negatively with the main neonatal characteristics such as BW ($R = -0.525$, $p < 0.001$, **Figure 1**), GA ($R = -0.487$, $p < 0.001$), and 5- and 10-min Apgar ($R = -0.247$, $p = 0.014$; $R = -0.228$, $p = 0.024$). Correlation was positive with oxygen duration ($R = 0.358$, $p < 0.001$) and maternal age ($R = 0.353$, $p < 0.001$).

Brain damage (211.5 pg/mL vs. 123, $p = 0.002$) and sepsis (184 pg/mL vs. 124.5, $p = 0.020$) were associated with higher NfL levels. Delivery mode had no significant impact ($p = 0.624$).

MLR analysis of cohort 2 used BW, 5-min Apgar, sex, brain damage, sepsis, amniotic infection, and oxygen duration as explanatory variables. The regression model explained around 37% of NfL variance ($R^2 = 0.366$, $F_{(7, 89)} = 7.331$, $p < 0.001$). Only BW (beta = −0.385, $p = 0.001$) and brain damage (beta = 0.222, $p = 0.015$) contributed significantly to predicting NfL (**Figure 2**).

DISCUSSION

Neuronal injury marker NfL has proved a sensitive and specific biomarker in adult peripheral blood, serving as a promising adjunct to monitoring and decision-making in acute and chronic neurologic disease (16, 17). Our study provides a first insight into neonatal NfL levels in term and preterm infants. The major findings are that NfL levels increase over the first few days of life, relate inversely to prematurity and BW, and identify BW, delivery mode, preeclampsia and brain damage as independent predictors.

NfL levels at birth in MPT infants resemble those in healthy adults (15). By DOL 3 they rise to the levels seen in adults with neurodegenerative disease such as multiple sclerosis (15). At DOL 7 in very preterm infants NfL levels are in the range of asphyxiated neonates at DOL 4 (11).

The main influencers of NfL in both cohorts were BW and maturity: birth and neonatal levels were both higher in low BW infants (**Figure 1**), perhaps because brain vulnerability to neuronal injury increases with prematurity. Alternatively, high NfL levels in preterm infants might be due to high neuronal turnover in general, with the much higher postnatal levels at DOL 3 and DOL 7 (**Figure 2**) simply reflecting a neuronal stress reaction to birth, as in healthy term neonates.

Preterm infants are at risk for perinatal brain damage, in particular PIVH and PVL (18). In our sample those with evidence of brain damage had significantly higher NfL levels than those without (**Figure 2**). Brain damage leads directly to neuronal injury, to a degree objectifiable by NfL: levels are higher in asphyxiated neonates with unfavorable brain MRI outcome (11). As in adults, cerebrovascular accident results in immediately higher NfL levels (19), compared to the more gradual neuronal damage seen in neurodegenerative disease (20).

In addition to a direct effect of brain damage, we identified two other stressors that increase NfL, namely delivery mode and preeclampsia. Levels were higher in infants delivered vaginally than by cesarean section (**Figure 2**), suggesting greater neuronal injury and confirming vaginal delivery as one of life's strongest stressors, causing incommensurable release of various fetal stress hormones (21). Preeclampsia, a pregnancy-specific syndrome defined by high blood pressure and other morbidities (22), was the additional stressor, raising NfL levels at birth even after adjustment for BW and GA. Our finding is consistent with the recent report of raised NfL levels in women with preeclampsia (23). Maternal hypertension is closely linked to placental insufficiency which compromises fetal perfusion and may cause cardiovascular disease later in life (24). Our data indicate that preeclampsia involves a risk of neuronal damage in the unborn child.

While the main source of NfL is considered to be the central nervous system, peripheral damage may contribute to increased NfL values as well, as recently revealed by studies on peripheral neuropathies (25, 26). Increased blood levels of the muscle enzyme creatine kinase in newborn infants after vaginal deliveries compared to cesarean sections have been reported (27). They support the notion that increased NfL in these babies may result, at least in part, from peripheral neuronal damage. However, data on the central nervous system biomarker S100 B measured in the maternal serum and cord blood show clearly increased S100B values after vaginal delivery compared to cesarean section (28). It has been shown previously that

TABLE 2 | Cohort neurofilament light chain concentrations at birth and at days of life (DOL) 3 and 7.

Cohort	Neurofilament light chain concentrations (pg/mL)			
	Birth (arterial)	Birth (venous)	DOL 3 (venous)	DOL 7 (venous)
1: Very preterm group (GA < 32 weeks) $n = 52$		32.5 (17.6–52.5) $n = 47$	48.5 (37.6–138) $n = 16$	
1: Moderate Preterm and Term group (GA ≥32 weeks) $n = 151$	17.7 (12.4–25.4) $n = 68$	15.3 (12.2–23.9) $n = 138$	51.4 (41.4–86.4) $n = 23$	
2: Very preterm group (GA < 32 weeks) $n = 98$				126 (78.8–225) $n = 98$

Median and interquartile range. GA, gestational age.

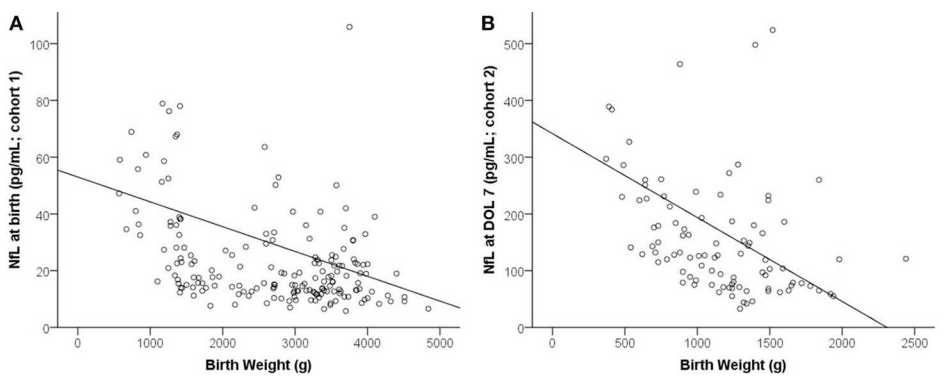

FIGURE 1 | Correlation between birth weight and NfL. **(A)** Birth (cohort 1). **(B)** DOL 7 (cohort 2).

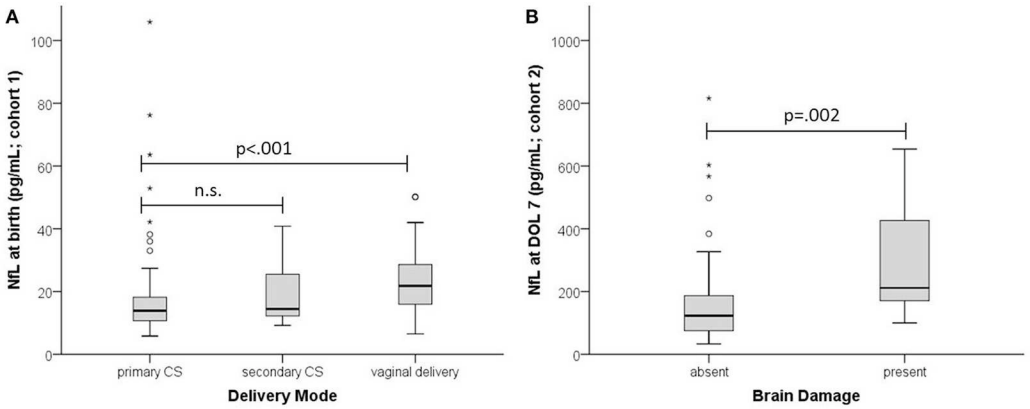

FIGURE 2 | Effect of delivery mode and brain damage on NfL. **(A)** NfL at birth in infants with GA ≥ 32 weeks (cohort 1). **(B)** NfL at DOL 7 (cohort 2). Absent, no brain damage; present, PIVH and/or PVL. Boxplots are presented with median and IQR. The * are extreme outliers.

extracranial sources of S100B do not affect serum levels (29). Taken together, the findings of Schulpis KH et al. corroborate our data that increased levels of the neuronal injury markers S100B and NfL might be caused by the compression on the fetus' brain during delivery.

Further, S100B levels in neonates with HIE exceeded those in healthy controls, proportionately to disease severity and worse outcome (30). Although S100B levels decreased overall from DOL 1 through DOL 9 (31), levels in preterm and term neonatal saliva followed a pattern similar to NfL, being higher in preterm than in term infants and correlating negatively with GA (32). Nerve growth factor (NGF) is a neurotrophic factor involved in brain development and neuroplasticity following brain damage. Unlike NfL, NGF levels in maternal and cord plasma are lower in preterm than in term deliveries (33).

To date the metabolism of NfL in cerebrospinal fluid (CSF) and blood is largely unknown, ways of elimination or protein degradation have not been described. One study examined the influence of blood brain barrier permeability and blood NfL levels. In this study there was no correlation between serum NfL concentration and CSF/serum albumin ratio (34).

Study limitations include the relatively few subjects sampled at DOL 3, which may account for the non-significant difference between very preterm and MPT infants at DOL 3. In the first week of life there is an apparent increase in NfL levels, but in the absence of data points post-DOL 7, the subsequent profile of NfL requires elucidation in further studies. Nor can we exclude other confounders that might influence and explain NfL. Cognitive outcome studies will need to confirm the use of NfL as a predictive biomarker of brain damage and eventual neurodevelopmental deficit. Such early biomarkers are sorely needed to complement ultrasound or MRI in conditions such as PVL (18). In addition, future studies may explore NfL together with other potentially promising biomarkers of brain damage (35). More generally, research is required to explore and disentangle the causes of the high degree of neuronal injury in the preterm brain.

CONCLUSION

This study provides an initial insight into neuronal injury marker NfL in term and preterm infants. Levels increase through the first week of life. They relate inversely to GA and BW and are higher in brain injury. Obstetric parameters such as delivery mode and preeclampsia also raise NfL levels. Our study supports the use of NfL in neonates to help us understand the factors leading to neuroaxonal injury and how we might monitor and prevent them.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

SW and UF designed the study. SW and RN collected the data. JK and CB assayed the serum samples. AD analyzed the data and wrote the manuscript together with SW and PW. All authors provided critical feedback and helped to improve the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

III. DISCUSSION

The aim of the present PhD thesis is to explore neuronal biomarkers as tool for detection of preterm infants at higher risk for neurodevelopmental delays. First, through a systematic review of the literature we examined previous studies that assessed the association between AERPs and cognitive outcome in preterm infants. Second, we performed a prospective pilot study to assess discrimination and habituation in preterm and term infants using AERPs and to correlate the habituation capability with the developmental outcome. Third, a blood biomarker of neuronal injury was investigated in a cohort of term and preterm infants during the first week of life. Given the potential of this biomarker in adults, we want to see whether it is possible to measure it and utilize in a preterm population.

These three studies provide a brief overview of the current state of AERP research in the field of neuropediatrics together with an investigation of two neuronal biomarkers.

3.1. Predicting neurodevelopmental outcome in preterm infants

The first two publications focus on EEG methodology, in particular AERPs, used in preterm infants to investigate whether defined AERP peaks can be used as a marker for cognitive functioning.

After systematically reviewing the literature, it appeared that only few studies (Fellman et al., 2004; Hovel et al., 2015; Jansson-Verkasalo et al., 2003; Jansson-Verkasalo et al., 2004; Jansson-Verkasalo et al., 2010; Korpilahti et al., 2016; Leipala, Partanen, Kushnerenko, Huotilainen, & Fellman, 2011; Maitre, Lambert, Aschner, & Key, 2013; Maitre, Slaughter, Aschner, & Key, 2014; Mikkola et al., 2007; Mikkola et al., 2010; Paquette et al., 2015; Weber, Depoorter, Hetzel, & Lemola, 2016) have examined the association between AERPs and cognitive outcome. In

particular 13 studies, of which seven prospective and six cross-sectional, have been found and included in the systematic review. Five long latency AERPs, P1, N2, P3, MMN and N400 were examined in the studies and mismatch negativity (MMN) was the most commonly investigated component. According to six studies MMN was predictive of cognitive outcome. The majority of the included studies found significant correlations between the AERPs and outcome tests. In summary, shorter latencies and larger amplitudes of long latency AERPs were related to better cognitive outcome. However it is challenging to create guidelines (Tome, Barbosa, Nowak, & Marques-Teixeira, 2015) based on this review due to the heterogeneity of participants (regarding gestational age and brain injury), variety of stimulation paradigms and investigated peaks. Two studies (Maitre et al., 2013; Maitre et al., 2014) did not define specific late AERP peaks, but specified time windows of interest. Given the challenges associated with detecting typical potentials in neonates, in contrast to older children and adults, this is reasonable. The variability in AERPs in very young children can be explained by the immaturity of auditory processing (Ceponiene et al., 2002; Kushnerenko et al., 2002; Leppanen et al., 2004), hence GA has a big influence on the apparition of peaks (Bisiacchi, Mento, & Suppiej, 2009).

This is to our knowledge the first review to systematically study the potential of AERPs to predict neurodevelopmental outcome. This review demonstrates that more research is needed on neonatal AERPs, in order to apply this method in a clinical setting. It is important that studies use a similar setting, paradigm and time of assessment to increase comparability among study sites.

In the second manuscript, a closer look is taken at the predictive capacities of the MMN at neonatal age in healthy preterm and term born infants. MMN is an AERP component commonly investigated in ERP research in the auditory modality

(Naatanen, Paavilainen, Rinne, & Alho, 2007). MMN is elicited in an oddball paradigm and calculated by subtracting the average waveform of the standard stimuli from the average waveform of the deviant stimuli. In adults it displays a negative amplitude peaking between 100 and 250 ms post stimulus onset (Naatanen et al., 2007). MMN reflects a preattentive cognitive discrimination ability and can be observed in neonates (Ceponiene et al., 2002; Haden, Nemeth, Torok, & Winkler, 2016). From previous studies its potential role as predictor of early language development has been shown (Mikkola et al., 2007; Paquette et al., 2015). In the second manuscript we took a closer look at the properties of MMN, not yet assessed by other AERP studies. In particular habituation, an elementary form of learning, can provide an indication of cognitive development in young infants (Kavsek & Bornstein, 2010). We found that a diminished habituation capability is associated with a lower mental and psychomotor outcome at the corrected age of 21 months in very preterm infants. This is in line with the findings of Kavsek et al. from behavioral studies (Kavsek & Bornstein, 2010). Moreover the habituation capability strongly correlated with GA, as previously confirmed by Morokuma's fMRI study (Morokuma et al., 2004). In our study we did not find significant differences in MMN amplitude or latency between preterm and term infants at 40 weeks GA, although preterm infants showed considerably lower amplitudes (<2 μV). The absence of group differences in MMN is in accordance with other studies using speech (Rago, Honbolygo, Rona, Beke, & Csepe, 2014) and non-speech stimuli (Paquette et al., 2015). The finding that proportionally more preterm infants showed lower amplitudes than their peers, is understood as diminished preattentive sound discrimination and consequently a sign of immaturity of the auditory system (Ceponiene et al., 2002). It is important to note that the very preterm infants included in this study did not have any severe brain

malformations or damage, therefore the results are supposed to be solely concerning the “preterm brain”.

Taken together, AERP studies provide evidence to use this particular EEG task for investigating early cognitive functioning in neonates. It is a bedside tool that has the potential to individually stratify preterm infants at risk during hospitalization at the NICU. Additionally it is non-invasive, inexpensive and can be passively administered.

3.2. Blood biomarker of neonatal neuronal injury

Similar to neuroimaging studies, blood biomarkers have been looked into and explored in order to develop techniques to screen for brain injuries and functioning. Neuronal biomarkers have been comprehensively studied in adults, but to a lesser extent in neonates. Nevertheless, several neuronal biomarkers have been discovered useful. Blood biomarkers such as S100B and Activin were found suitable to predict occurrence of IVH (Douglas-Escobar & Weiss, 2012; Michetti et al., 2012). Data on prediction of outcome in infants using neuronal biomarkers are currently missing (Daoud et al., 2014). In the third manuscript we took a closer look at a novel biomarker with high potential that was investigated for the very first time in preterm and term born infants.

The main findings were that NfL at birth was increased in very preterm compared to moderate preterm-term infants and increased over the first days of life. Clinical factors that influenced NfL in both groups were GA, BW, Apgar scores, duration of oxygen supply and presence of sepsis. So in general the “sicker” the infant, the higher the NfL levels and therefore potentially higher neuroaxonal injury. Exclusively in very preterm infants brain damage was an independent predictor of NfL, besides BW or GA. Whereas in the cohort of moderate preterm and term infant obstetric parameters, such as delivery modality and preeclampsia in the mother, as well as

BW or GA had the highest impact on NfL. In particular infants delivered vaginally presented increased levels compared to those delivered by cesarean section. This finding supports vaginal delivery as a stressor for infants and is in accordance with the S100B biomarker (Schulpis et al., 2006). Other neuronal biomarkers such as brain-derived neurotrophic factor (Flock et al., 2016) and nerve growth factor (Dhobale, Mehendale, Pisal, Nimbargi, & Joshi, 2012), linked to immaturity of the central nervous system, are reduced in the cord blood of preterm infants and corroborate our findings that GA/BW is the main influencer of increased neuronal injury. In this study it is important to underline that in the preterm cohort there are some infants that have brain injury, but only few with IVH Grade 3-4.

Based on what we know from the adult literature and the few promising studies in infants (Shah et al., 2018; Toorell et al., 2018), we expect that NfL can also act as a predictor of later cognitive functioning.

3.3. Limitations and outlook

After providing a brief overview and discussing the three PhD publications, some limitations of the studies need to be addressed as well as suggestions for future studies investigating neuronal biomarkers in the neonatal population. First of all the small sample size in the EEG studies is a big weakness in order to generalize findings to the preterm population, as well as the accompanying low statistical power and the few electrode positions in most cases. High dropout rates in longitudinal studies are also a concern for outcome predictions. Due to the ongoing brain development and immaturity it is also difficult to validate AERPs in neonates. In EEG research there is a high variety in paradigms, electrodes and outcome tests which also makes it difficult to compare studies. To improve this, multicenter studies using identical study protocols should be encouraged. The same holds for research on

body fluid biomarkers where many different immunoassay methods exist. Consequently there is a need for standard protocols and guidelines in order to translate findings from research to clinical practice (Berger et al., 2012). One of the benefits of studies investigating blood biomarkers in neonates is that it is less complicated to obtain big cohorts and to create norms as compared to neuroimaging. In order to validate a biomarker it is important to have distinct groups, such as “healthy” preterm (as included in the second publication) versus “sick” preterm (with morbidities) and healthy term infants as well as groups based on GA (extreme, very, moderate preterm). In case of documented brain injuries (i.e. IVH or PVL) it is important to control for, in order to clearly investigate the “premature brain”. The next important steps for Nf as a marker of neuronal injury in preterm infants are to correlate it with CUS, MRI and EEG findings plus to link it to neurodevelopmental outcome tests.

3.4. Conclusion

Preterm birth is associated with a range of morbidities and neurodevelopmental deficits. This poses a serious concern for clinicians worldwide, especially given the increasing rate of preterm births. While not all preterm infants develop later cognitive deficits, a screening tool to detect those at higher risk is required. Based on the present work we can propose a complementary use of the investigated neuronal biomarkers, AERPs and Nf, to assess cognitive development. For instance in case of elevated Nf levels, an AERP test can be performed in order to investigate early cognitive functioning. However more high quality studies with similar, large cohorts are needed before guidelines can be elaborated.

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