Investigation of fetal brain function and implications for early child development

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

Julia Hartkopf

aus Bergisch Gladbach

Tübingen

2020

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	16.07.2020
Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Dr. Hubert Preissl
2. Berichterstatter:	Prof. Dr. Martin Hautzinger

Contents

Index of abbreviations

AER	auditory evoked response
AGA	appropriate for gestational age
BSID	Bayley Scales of Infant Development
DOHaD	Developmental Origins of Health and Disease
EEG	electroencephalography
ERR	event-related brain response
fMEG	fetal magnetoencephalography
fMRI	functional magnetic resonance imaging
GA	gestational age
GDM	gestational diabetes mellitus
ISI	inter-stimulus interval
ITI	inter-trial interval
IUGR	intrauterine growth restriction
MDI	Mental Development Index
MEG	magnetoencephalography
MMN	mismatch negativity
MRI	magnetic resonance imaging
PDI	Psychomotor Development Index
SGA	small for gestational age
SQUID	superconducting quantum interference device
VER	visually evoked response

Summary

During the last few decades, the “Theory of Developmental Origins of Health and Disease” has increased the interest in early exogenous influences on the life-time prevalence of non-communicable diseases. From conception onwards, sensitive periods in development are discussed. During these phases, the organism is susceptible to exogenous influences that can alter organs and their function. Especially the developing brain, an organ characterized by a high degree of plasticity and mutually affected by genetic preconditions and exogenous factors, is in the focus of interest. It influences numerous physiological processes during the entire life span, determines behavior and is thus closely linked to the preservation of health and to the development of diseases. Fetal magnetoencephalography enables to monitor brain function as early on as *in utero* and provides an unique opportunity to evaluate the course and performance of functional brain development from a very early age.

The first study of this thesis systematically reviewed studies of event-related brain responses and habituation during child development. Neurophysiological measurements of event-related brain responses, their age-related changes and the role of neurophysiological habituation assessment for the evaluation of higher cognitive processes during child development were presented and critically discussed. As a result, age-related changes in event-related brain responses can be postulated. However, both event-related brain response studies and habituation studies had a wide diversity of subjects’ ages and stimulation protocols, and only very few longitudinal studies exist. A general conclusion is thereby limited and future studies should focus on longitudinal designs and methodical standardization.

In line with these recommendations, the second study evaluated event-related brain responses to syllables arranged in a habituation paradigm recorded by fetal magnetoencephalography in fetuses and, later, in infants (0-3 months of age). Early syllable processing and basic learning abilities were investigated in both age groups with a comparable stimulation procedure. Neurophysiological habituation was shown in neither fetuses nor infants. In infants, however, a discriminative neural response to an inserted deviant syllable was observed. These results

may be important for the investigation of early cognitive learning processes and may contribute to a better understanding of language acquisition mechanisms.

In the third study, the impact of intrauterine growth restriction on the development of infantile brain function was investigated. Event-related brain responses in three study groups (fetuses with a pathological intrauterine growth restriction, fetuses that were too small for their gestational age and fetuses with an intrauterine growth appropriate to their gestational age) were recorded by fetal magnetoencephalography. A follow-up assessment of the participants with a psychological developmental test (Bayley Scales of Infant Development, Second Edition) was implemented after two years to examine long-term cognitive and motor development. Latencies of event-related brain responses to visual stimuli were longer in intrauterine growth-restricted fetuses than in the small-for-gestational-age- and appropriate-for-gestational-age group, but this difference did not reach statistical significance. In the follow-up assessment, cognitive capabilities of the intrauterine growth-restricted group were significantly lower than in former small-for-gestational-age and former appropriate-for-gestational-age infants. These findings confirm the hypothesis that intrauterine growth-restriction is a risk factor for cognitive development and future investigations should analyze the underlying neuropathological mechanisms and follow the development of intrauterine growth-restricted children to implement potential intervention strategies.

Zusammenfassung

In den letzten Jahrzehnten hat die „Theory of Developmental Origins of Health and Disease“ das Interesse an frühen exogenen Einflüssen auf die Lebenszeitprävalenz nicht übertragbarer Krankheiten erhöht. Beginnend mit der Empfängnis werden sensitive Phasen während der Entwicklung eines Organismus diskutiert, in denen bestimmte exogene Einflüsse eine Veränderung der Organe und ihrer Funktion einleiten können. Ein Organ im Mittelpunkt des Interesses ist das Gehirn. Es zeichnet sich durch eine hohe Plastizität aus und steht im Wechselspiel zwischen genetischer Disposition und exogenen Einflüssen. Das Gehirn wiederum beeinflusst während der gesamten Lebensdauer zahlreiche physiologische Prozesse, bestimmt das Verhalten und ist somit eng mit dem Erhalt der Gesundheit oder der Entwicklung von Krankheiten verbunden. Die fetale Magnetoenzephalographie ermöglicht die Überwachung der Gehirnfunktion bereits *in utero* und bietet die einzigartige Möglichkeit, den Verlauf und die Leistung der funktionellen Gehirnentwicklung in einem sehr frühen Alter zu untersuchen.

Im ersten Teil dieser Arbeit wurden systematisch Studien über ereignisbezogene Gehirnreaktionen und Habituation während der Kindesentwicklung zusammengestellt und diskutiert. Neurophysiologisch gemessene ereignisbezogene Gehirnreaktionen und deren Veränderungen im Verlauf der Kindesentwicklung sowie die Bedeutung neurophysiologischer Habituation für die Beurteilung höherer kognitiver Prozesse wurden kritisch hinterfragt. In dieser Übersichtsarbeit wird deutlich, dass altersbedingte Veränderungen zwar vorliegen, jedoch die Studien aufgrund der großen Diversität hinsichtlich untersuchter Altersgruppen und verwendeter Stimulationsprotokolle kaum vergleichbar sind. Es liegen zudem wenige Längsschnittuntersuchungen zu ereignisbezogenen Gehirnreaktionen als auch zu Habituation während der Kindesentwicklung vor. Eine allgemeine Aussage über entwicklungsbedingte Veränderungen ist damit nur begrenzt möglich. Zukünftige Studien sollten vermehrt auf Längsschnittdesigns und methodische Standardisierung fokussieren.

In Übereinstimmung mit diesen Empfehlungen wurden in der zweiten Studie mittels fetaler Magnetoenzephalographie ereignisbezogene Gehirnreaktionen von Sprachsilben untersucht, die in einem Habituationsparadigma angeordnet waren. Frühe Silbenverarbeitung und grundlegende Lernfähigkeiten wurden mit einem vergleichbaren Stimulationsverfahren in zwei Altersgruppen, bei Feten und Säuglingen, analysiert. Es wurden drei verschiedene Habituationskriterien untersucht. Weder bei Feten noch bei Säuglingen konnte neurophysiologische Habituation beobachtet werden. Bei Säuglingen zeigte sich jedoch eine neuronale Unterschiedsreaktion auf eine im Paradigma eingefügte abweichende Silbe. Diese Ergebnisse sind für die Untersuchung früher kognitiver Lernprozesse wichtig und tragen möglicherweise zu einem besseren Verständnis der Mechanismen des Spracherwerbs bei.

Die dritte Studie untersuchte Auswirkungen der intrauterinen Wachstumsrestriktion auf die Entwicklung der kindlichen Gehirnfunktion. In drei Studiengruppen (Feten mit intrauteriner Wachstumsrestriktion, Feten, die zu klein für das jeweilige Schwangerschaftsalter waren, und Feten, die hinsichtlich des Schwangerschaftsalters normal entwickelt waren) wurden ereignisbezogene Gehirnreaktionen mittels fetaler Magnetoenzephalographie aufgezeichnet. Im Alter von zwei Jahren erfolgte eine Follow-up-Untersuchung der Kinder mit einem psychologischen Entwicklungstest (Bayley Scales of Infant Development, Second Edition), um die langfristige kognitive und motorische Entwicklung zu dokumentieren. Die Latenzen der ereignisbezogenen Gehirnreaktionen auf visuelle Reize waren bei Feten mit intrauteriner Wachstumsrestriktion im Vergleich zu den anderen Gruppen länger, die Unterschiede erreichten aber keine statistische Signifikanz. In der Follow-up-Untersuchung wies die Gruppe mit intrauteriner Wachstumsrestriktion einen signifikant geringeren Wert bezüglich kognitiver Fähigkeiten auf als Kinder der beiden anderen Gruppen. Diese Ergebnisse bestätigen die Hypothese, dass intrauterine Wachstumsrestriktion ein Risiko für die kognitive Entwicklung darstellt. Zukünftige Untersuchungen sollten zugrundeliegende neuro-pathologische Mechanismen analysieren und die Entwicklung betroffener Kinder verfolgen, um potentielle Interventionsstrategien zu entwickeln.

1 Introduction

1.1 Developmental Origins of Health and Disease – DOHaD

1.1.1 Definition and history

The state of human mental and somatic health is strongly linked to subjective well-being. In particular, the treatment of chronic diseases requires considerable effort on the part of both professionals and patients. Patients with chronic somatic diseases often report a higher degree of mental distress, which increases the incidence for psychological disorders (Moussavi et al., 2007; Snoek et al., 2015; Verhaak et al., 2005). Besides the availability of effective interventions, the expansion of screening procedures to identify individuals at risk and prevention programs to ensure mental and somatic health are becoming increasingly important (Detweiler-Bedell et al., 2008).

During their lifetime, humans are exposed to many risk factors that can increase the incidence of certain diseases. Almost 30 years ago, Barker et al. (1989) emphasized that influences as early as during prenatal development must be taken into consideration to explain the pathogenesis of various diseases that occur in later life. On the basis of a cohort study conducted in the United Kingdom (England and Wales), the authors postulated an association between nutritional conditions during pregnancy and an increased risk for cardiovascular disease and diabetes type 2 in later life (Barker et al., 1993). These observations resulted in the concept of *fetal programming*, which states that influences of the intrauterine environment and of the perinatal period can have an impact on subsequent health status and developmental processes (Barker, 2007; Godfrey & Barker, 2001).

During the years to follow, the findings of Barker and colleagues were evolved into the hypotheses of *Developmental Origins of Health and Disease* (DOHaD), and the DOHaD society was founded in 2003 (Hanson & Gluckman, 2014). Today, DOHaD's field of research takes a broad-based approach, examining the impact of different life experiences on health

and, in particular of non-communicable diseases on the individual and on the population from conception onwards (Penkler et al., 2018; Wadhwa et al., 2009).

1.1.2 Risk factor intrauterine growth restriction (IUGR)

Fetal nutrient deficiency and subsequent restricted fetal growth, resulting in a low birthweight, was one of the first factors believed to cause a higher risk of chronic disease and impaired cognitive development in later life (Barker, 1966, 1991). Generally speaking, fetuses whose estimated birth weight is below the 10th percentile are called small for gestational age (SGA), whereby this term does not necessarily indicate a pathological reason for the low weight and also refers to constitutionally small fetuses. The term intrauterine growth restriction (IUGR), as opposed to the term SGA, should be used for fetuses who do not reach their genetically predisposed growth due to underlying pathological intrauterine processes. In most cases, IUGR is caused by a placental insufficiency that significantly increases the risk of perinatal morbidity and mortality (Figueras & Gardosi, 2011). In the guideline program of the German society for Obstetrics and Gynecology (Deutsche Gesellschaft für Gynäkologie und Geburtshilfe, DGGG), IUGR is defined as an “estimated fetal weight under the 10th percentile and/or a growth course that is not appropriate for the percentile and a pathologic Doppler sonography of the umbilical artery or a pathologic Doppler sonography of the arteria uterinae or the presence of oligohydramnios” (DGGG, 2016). In addition to a reduced birth weight, IUGR can cause neurophysiological alterations which presumably have an impact on the neurocognitive and/or motor development of the children concerned (Murray et al., 2015). With regard to structural brain development, IUGR is associated with alterations in the corpus callosum and a reduction in both intracranial volume and cerebral cortical gray matter (IUGR premature infants compared to premature infants) (Egana-Ugrinovic et al., 2014; Tolsa et al., 2004). Moreover, IUGR might be associated with alterations in connectivity (Fischi-Gomez et al., 2015; Reveillon et al., 2016). As a consequence of neurophysiological alterations, several

authors have proposed that IUGR can even affect mental health in later life (Nilsson et al., 2005; Sizonenko et al., 2006; Thompson et al., 2001).

1.2 Monitoring of fetal brain development

Intact structure, function and connectivity of the brain are important prerequisites for acquiring age-appropriate cognitive, motor, and emotional skills. Several different – preferably non-invasive – methods for tracking structural brain development *in utero* are currently available. Due to technical progress, fetal neurosonography is becoming more and more feasible for accurately monitoring the development of brain structures (Kim et al., 2008). Magnetic resonance imaging (MRI), with its relatively low risk to mother and fetus, provides further information about the state of structural fetal brain development (Clouchoux et al., 2012; Griffiths et al., 2017; Manganaro et al., 2017; Welsh et al., 2011). Also, MRI has recently been supplemented with Diffusion-Tension-Imaging (DTI) to gain information about the (micro-) structure and composition of various kinds of tissue and the development of structural connectivity (Jakab et al., 2017; Kasprian et al., 2008; Mitter et al., 2015). However, neither ultrasound nor MRI/DTI provides information about brain functioning. Non-invasive methods for functional brain examination that are established in adults and children, such as electroencephalography (EEG) and near-infrared spectroscopy (NIRS), are not suitable for measuring fetal brain activity as the maternal tissues weaken and/or distort signals from the fetal brain. A promising approach is functional MRI (fMRI) which enables non-invasive measurement of fetal brain activity by relying on metabolic changes. Interestingly, fMRI enables to study functional connectivity by describing the temporal correlation between different brain regions (Fulford et al., 2004; Moore et al., 2001; Thomason et al., 2013; van den Heuvel & Thomason, 2016; Welsh et al., 2011).

Another method for the assessment of fetal brain function, which has a lower spatial but higher temporal resolution than fMRI, is the completely non-invasive magnetoencephalographic (MEG) approach (Cohen, 1972). The synaptic activity of neurons elicits electrophysiological

currents and induces a magnetic field which can be recorded using special highly sensitive coils. Although these magnetic signals are extremely weak (pico (1 pT = 10^{-12} T) to femto Tesla (1 fT = 10^{-15} T)), the signals measured are not distorted by the skull or by tissue and reach a high temporal resolution in the range of milliseconds (Baillet, 2017).

1.3 Fetal magnetoencephalography (fMEG)

Successful fetal brain activity measurement with MEG was already documented by Blum et al. (1985). Pivotal studies with MEG-devices have shown that magnetically acquired event-related brain responses (ERRs) of auditory stimuli are detectable in the fetus (Schneider et al., 2001; Zappasodi et al., 2001). To improve investigations of fetal brain activity by MEG, a 151-channel *fetal* magnetoencephalograph (fMEG) (known as SARA, Superconducting Quantum Interference Device (SQUID) Array for Reproductive Assessment, VSM MedTech Ltd., Port Coquitlam, Canada) was developed. Installed at the University of Arkansas for Medical Sciences, Little Rock, USA in 2000, this device was especially adapted to the requirements of fetal measurements (Eswaran et al., 2002; Lowery et al., 2009; Preissl et al., 2004).

The fMEG system used in the studies of the current thesis is the follow-up model of SARA. It contains 156 integrated primary and 29 reference sensors (SQUID-coils) and was installed at the fMEG center of the Tuebingen University in 2008 (SARA II: SQUID Array for Reproductive Assessment, VSM MedTech Ltd., Port Coquitlam, Canada). Being a completely non-invasive method, fMEG enables to investigate fetal brain function as well as fetal cardiac activity during the last trimester of pregnancy. It helps to gain insight into the early processes of human neuronal development, on the basis of the measurement of fetal brain activity (Preissl et al., 2004; Sheridan et al., 2010).

During an fMEG measurement, the pregnant woman places her abdomen in a specially constructed sensor array that registers changes in the electromagnetic field (Figure 1). Prior to measurement, the positions of the fetal head and eyes are determined by ultrasound. As displayed in Figure 2A, the pregnant woman leans forward to prevent the occurrence of a vena

cava syndrome (restricted blood backflow to the heart due to a compression of the vena cava caused by the weight of the pregnant uterus, potentially occurring in supine position). Both SARA-devices are also suitable for measuring brain activity of neonates by attaching a cradle on which the neonate can rest its head on the sensor array (Figure 2B). The fMEG also enables to record cardiac activity in the fetus and thus investigate the autonomic nervous system. fMEG measurements take place in a magnetically shielded room (Vakuumschmelze, Hanau, Germany) to avoid environmental artifacts. During the measurements, participants are video-monitored and are able to communicate with the investigator via intercom. For neonatal measurement, one parent remains with the infant in the magnetically shielded room.

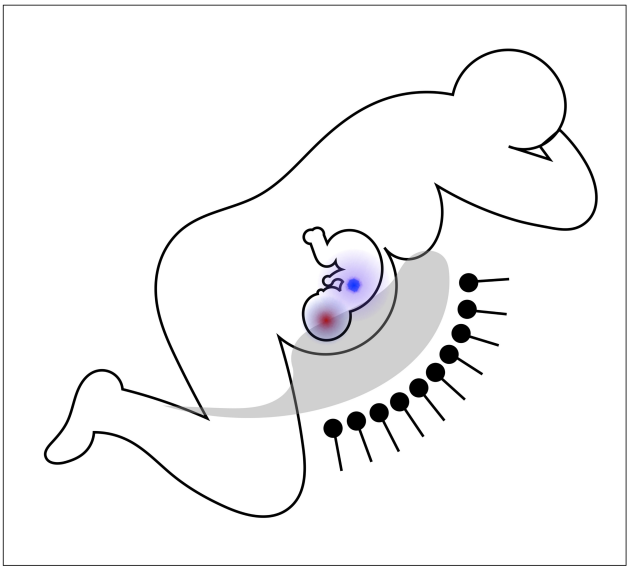

Figure 1: Schematic illustration of the fMEG technique. SQUID-coils (in black) detect changes in fetal heart (blue) and brain (red) activity. Adapted from Sippel et al. (2019).

1.3.1 Stimulation during fMEG measurement

To investigate neuronal responses of fetuses and neonates, auditory and visual stimuli are presented during the fMEG measurements (Sheridan et al., 2010). During visual stimulation, a LED-light pad is placed on the maternal abdomen near the location of the fetal eyes or, in the case of neonates, next to the head near the eyes. Flashes of light are transmitted through fiber optic wires without eliciting electromagnetic interferences (Wilson et al., 2009). Auditory stimuli for fetuses are generated outside the magnetically shielded room and are transmitted via an air-filled tube into an air-filled balloon that is placed between the maternal abdomen and

the SQUID-sensors of the fMEG-device. The stimuli intended for neonates are transmitted via a suitable earphone for small infants (Ear Muffins from Natus, Biologic, San Carlos, USA, Figure 2B). During the fMEG measurements, near-field signals such as muscle activity (uterine, maternal, fetal) or maternal and fetal cardiac activity cause interferences with fetal brain signals and should be avoided or filtered out during data analysis (McCubbin et al., 2006; Preissl et al., 2005; Vrba et al., 2004; Wilson et al., 2008). The fetal position is therefore determined by ultrasound both before and after the fMEG measurement to record changes in fetal position that should be taken into account during data processing.

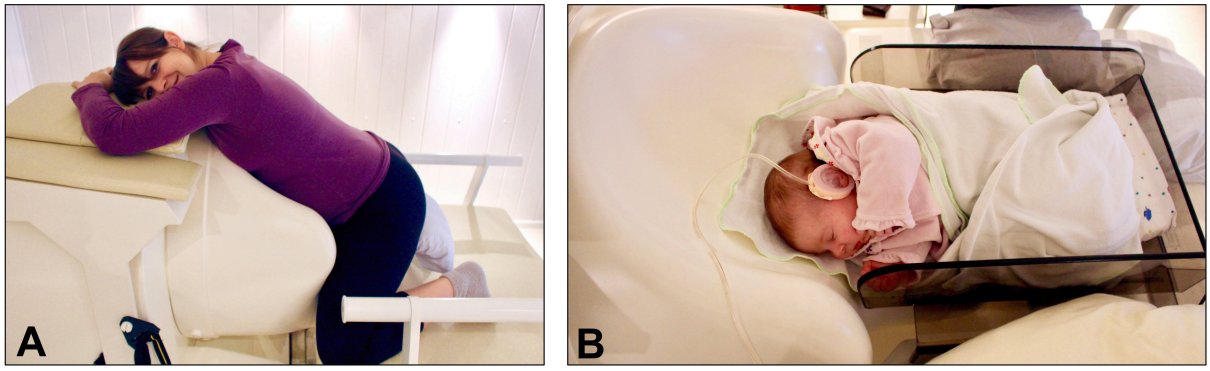

Figure 2: SARA II fMEG system at Tuebingen University. During fetal measurement (A), the maternal abdomen rests in the sensor array. For auditory stimulation, an air-filled balloon for the transmission of acoustic stimuli is placed between the maternal abdomen and the sensors (not visible). During neonatal measurement (B), a cradle is attached to the device and the head rests in the sensor array. Acoustic stimuli are relayed via an earphone.

1.4 Event-related brain responses (ERRs)

During the third trimester of pregnancy, the visual and auditory system of the fetus is already capable of perceiving and processing both visual and auditory stimuli (Anbuhl et al., 2017; Moore & Linthicum, 2007). Both EEG and (f)MEG enable to directly evaluate the neurophysiological activity related to external stimulation with a high temporal resolution. Neurophysiological responses induced by external stimulation were initially recorded as changes in the electrophysiological activation of cortical neurons with EEG and therefore labeled “event-related potentials” (ERPs). Since this thesis includes measurement of changes

in electromagnetic fields, the more comprehensive term *"event-related brain response"* (ERR) is used instead of *ERP* which is established in the EEG field. The analysis of ERRs provides information about stimulus processing, processing speed, and cognitive abilities such as memory and stimulus discrimination. The latter two are realized by analyzing ERRs from sequences of different stimuli. (Draganova et al., 2007; Matuz et al., 2012; Muenssinger et al., 2013b; Schleger et al., 2014). Figure 3 shows the typical time-related (x-axis, in ms) course of the electrical current (y-axis, in μV) induced by the electromagnetic field of an auditory ERR. Its waveform consists of local maxima and minima that reflect the different components of an ERR. Each component can be identified by its amplitude and its latency accordingly. In the nomenclature of ERR components, positive and negative voltage deflections are designated as "P" and "N", followed by a number that indicates either the respective ordinal position or latency.

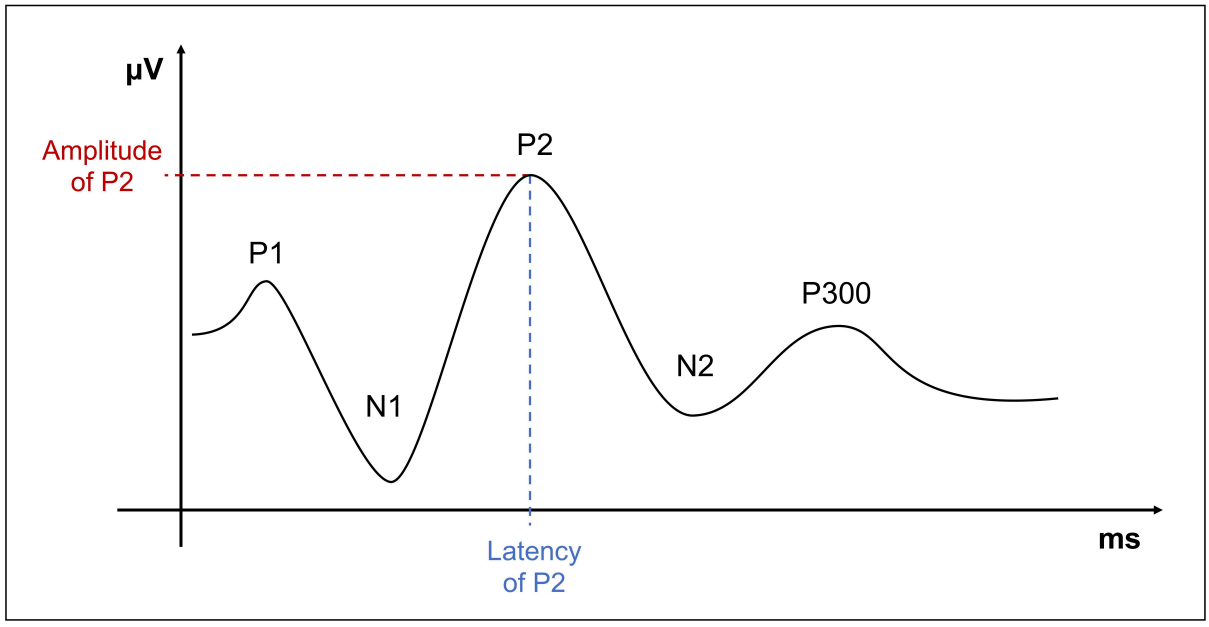

Figure 3: Typical shape of a cortical auditory event-related potential (ERR). The most prominent components are P1, N1, P2, N2 and P300. The amplitude (red) and latency (blue) of P2 are given as examples.

The early components of ERRs (exogenous components) reflect the physical features of the stimulus and occur within the first 150 ms after stimulus presentation. The later components

(endogenous components) reflect task-relevant processes such as attention or consciousness and occur at later time points (150 ms and later) (Kappenman & Luck, 2011). A third category of components, the motor components, represent the readiness for a motor reaction. They are less relevant in the context of fMEG studies, since any deliberate task responses from subjects in this age group are not expected. The component of the ERR to be analyzed depends on the research question, the measuring method, the modality of the stimuli (tactile, auditory, visual) and their specific arrangement. In this context, fMEG is a unique method with which early cognitive skills, and thereby basal learning processes, can be investigated as early as *in utero*. However, not all ERRs can be detected during measurement with fMEG. Various factors can lead to a decreased detection rate, for example fetal position and fetal state, or artifacts caused by fetal or maternal muscle activity (Preissl et al., 2004).

1.5 Neurophysiological habituation in humans

Habituation is generally defined by a successive decrease in response intensity towards a repeatedly presented stimulus (standard) (Thompson, 2009; Thompson & Spencer, 1966). To distinguish this basal form of learning from other possible causes of a response decline, such as neuronal fatigue or sensory adaptation, habituation should be characterized by further criteria (Carandini, 2000; Netser et al., 2011; Rankin et al., 2009). The insertion of a novel stimulus (deviant or dishabituator) into a habituation paradigm (i.e., a series of stimuli to test for habituation) enables to test for stimulus specificity, since the deviant should result in a response that differs from the response to the standard stimulus. Moreover, the presentation of the deviant stimulus should then result in an increase of the decremented response to the again presented standard stimulus (dishabituation). Rankin et al. (2009) compiled a revised overview of criteria that characterize habituation (Table 1) on the basis of the work of Thompson & Spencer (1966).

Table 1: Revised version of Thompson & Spencer's (1966) criteria for habituation (Rankin et al., 2009).

Characteristic	Definition
#1	"Repeated applications of a stimulus result in a progressive decrease in some parameters of response to an asymptotic level. This change may include decreases in frequency and /or magnitude of the response. In many cases, the decrement is exponential, but it may also be linear; in addition, a response may show facilitation prior to decrementing because of (or presumably derived from) a simultaneous process of sensitization."
#2	"If the stimulus is withheld after response decrement, the response recovers at least partially over the observation time ("spontaneous recovery")"
#3	"After multiple series of stimulus repetitions and spontaneous recoveries, the response decrement becomes successively more rapid and/or more pronounced."
#4	"Other things being equal, more frequent stimulation results in more rapid and/or more pronounced response decrement, and more rapid spontaneous recovery (if the decrement has reached asymptotic levels)."
#5	"Within a stimulus modality, the less intense the stimulus, the more rapid and/or more pronounced the behavioral response decrement. Very intense stimuli may yield no significant observable response decrement."
#6	"The effects of repeated stimulation may continue to accumulate even after the response has reached an asymptotic level (which may or may not be zero, or no response). This effect of stimulation beyond asymptotic levels can alter subsequent behavior, for example, by delaying the onset of spontaneous recovery."
#7	"Within the same stimulus modality, the response decrement shows some stimulus specificity. To test for stimulus specificity/stimulus generalization, a second, novel stimulus is presented and a comparison is made between the changes in the response to the habituated stimulus and the novel stimulus."
#8	"Presentation of a different stimulus results in an increase of the decremented response to the original stimulus. This phenomenon is termed "dishabituation"."
#9	"Upon repeated application of the dishabituating stimulus, the amount of dishabituation produced decreases (this phenomenon can be called habituation of dishabituation)."
#10	"Some stimulus repetition protocols may result in properties of the response decrement (e.g. more rapid rehabituation than baseline, smaller initial responses than baseline, smaller mean responses than baseline, less frequent responses than baseline) that last hours, days or weeks. This persistence of aspects of habituation is termed long term habituation."

The term "response" in the concept of habituation was originally based on behavioral changes as an (indirect) correlate of neurophysiological processing. Due to advances in technology, "direct" observation of habituation on a neurophysiological level, i.e., the recording and evaluation of ERR changes, became feasible. Here, the response decrease is reflected by a

decline in the amplitude of a recorded EER component. Other criteria of habituation can be investigated with appropriately designed stimulation paradigms (Matuz et al., 2012; Muenssinger et al., 2013a). An exemplary paradigm for testing response decrement, stimulus specificity, and dishabituation, which was used in study 2 of this thesis, is shown in Figure 4.

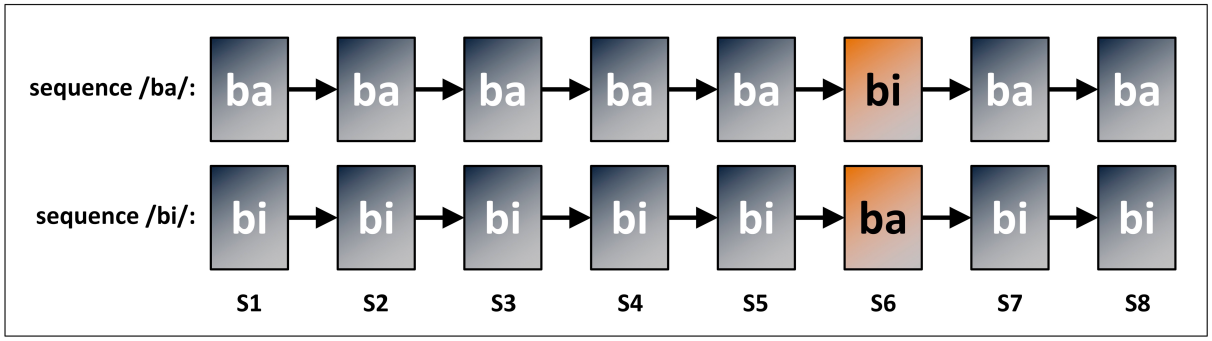

Figure 4: Example of an auditory habituation paradigm. The paradigm enables testing for response decrement, stimulus specificity and dishabituation by presenting the spoken syllables “ba” and “bi” (Hartkopf et al., 2016). The deviant at position S6 is colored in orange. Two sequences (“sequence /ba/” and “sequence /bi/”) are used to control for different responses to the sound of “ba” and “bi”.

1.6 fMEG studies on stimulus processing and higher cognitive functions

The DOHaD theory presumes that influences during pregnancy can already have effects on brain development and function, with profound implications for later life. Earlier studies with fMEG have indicated that fetuses show reliable ERRs to visual and auditory stimulation during the third trimester of pregnancy, thus confirming fetal ability to process acoustic and visual sensory input (Preissl et al., 2004). Fetuses were also able to detect differences between alternating frequencies during tone stimulation and elicited different ERRs towards tones with different rates of amplitude modulation (Draganova et al., 2007; Draganova et al., 2018). To investigate higher cognitive abilities such as habituation, Sheridan et al. (2008) showed response decrement (indicated by a decrease of ERR amplitudes) to a train of visual stimuli in neonates and in fetuses. Likewise, a visual stimulation paradigm devised by Matuz et al. (2012) used two stimulation paradigms consisting of four light flashes followed by a tone with two different inter-train intervals (ITIs). In neonates, response decrement seemed to depend on

the length of the ITI. Moreover, a discriminative response, indicated by higher ERR amplitudes for the inserted tone, was demonstrated in both fetuses and neonates.

Response decrement towards auditory stimulation arranged in a habituation paradigm (eight pure tones, with a deviant tone inserted at place six) was shown by Muenssinger et al. (2013a). In this study, stimulus specificity and dishabituation were also evaluated. While stimulus specificity was present in fetuses and infants, dishabituation could not be confirmed. Schleger et al. (2014) investigated numerosity processing as a further higher cognitive ability in fetuses and babies. By modification of the quantity of presented tones, a change-induced negative component of ERRs (mismatch negativity) was detected.

In addition, fMEG has been used to investigate factors that are expected to impact fetal brain development. Fetuses with an IUGR had delayed ERRs to visual stimulation than SGA and appropriate for gestational age (AGA) fetuses (Morin et al., 2015). Gestational diabetes mellitus (GDM), as a metabolic disorder of the mother, also seems to influence fetal brain development. Fetuses of mothers with GDM showed slower postprandial auditory ERRs than fetuses of healthy mothers (Linder et al., 2015). Moreover, maternal family history of type 2 diabetes impacts the duration of fetal auditory ERRs (Schleger et al., 2018). Schneider et al. (2011), who observed that betamethasone – which is used to induce fetal lung maturation when premature birth is inevitable – had an acute influence on ERRs, concluded that potential long-term effects on brain function should be also examined.

1.7 Developmental assessment with the Bayley Scales of Infant Development

One of the key issues of the DOHaD is the question as to how early events impact health and disease throughout the entire lifespan. It is therefore of utmost importance to determine whether any of the factors found to affect fetal brain function *in utero* also influence developmental processes (Bolin et al., 2019; Linder et al., 2015; Morin et al., 2015). In this context, psychological tests enable standardized follow-up assessments and are valuable tools for detecting and monitoring such potential effects.

The Bayley Scales of Infant Development, Second Edition (BSID-II) is a psychometric assessment tool to measure the developmental status of infants (Figure 5). The first version was developed by Nancy Bayley in 1969. The BSID-II (which was the latest German version of this test at the time of data collection for this thesis) is available in the German language and standardized for infants between one and 42 months of age (Bayley, 1993). Results of the BSID-II are differentiated into a cognitive and a motor domain (represented by the Mental Development Index (MDI) and the Psychomotor Development Index (PDI)) and include tasks involving language skills, attention, memory, visuospatial functioning, sensomotoric skills and motoric abilities.

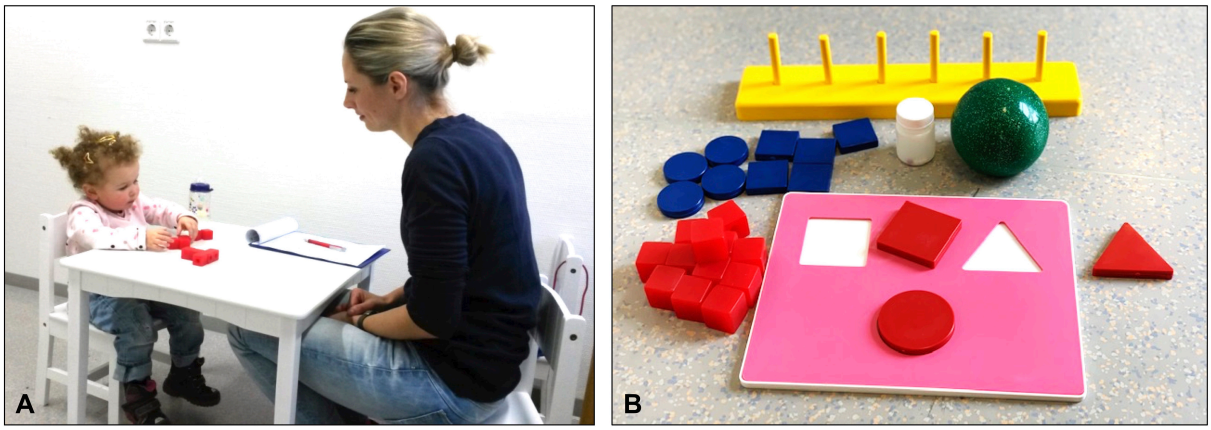

Figure 5: Developmental assessment. (A) Test situation and (B) test material of the BSID-II (exemplary selection).

1.8 Research questions

Measurement of ERRs in fetuses and newborns can indicate whether environmental factors impact brain function and its development, respectively. However, to compare and interpret research results, it is important to understand the physiological changes in ERRs that occur during child development. For this purpose, a systematic review of literature was performed to summarize and evaluate trials about age related changes in ERRs. A second aim was to review literature about habituation assessment during child development with a focus on

neurophysiological measurement methods. Together with an overview of the current state of research, recommendations for future projects were provided (Chapter 2).

The second study aimed to determine whether the investigation of brain function and early learning processes based on complex stimuli is feasible as early as *in utero*. fMEG was used to analyze ERRs towards syllables arranged in a habituation paradigm. Stimulus processing and three criteria of habituation (response decrement, stimulus specificity, dishabituation) were investigated in fetuses and infants (0-3 months of age) (Chapter 3).

The third study investigated whether a pathologic condition (IUGR as a result of restricted fetal nutritional supply) impacts (1) fetal brain function as assessed by fMEG and (2) developmental outcome, determined using psychological follow-up assessments at the age of two. The aim was to evaluate whether the fMEG method might be useful to detect early functional brain impairments that impact later cognitive and motor development. Fetal brain function was assessed by a comparative ERR analysis of IUGR-fetuses, SGA-fetuses and AGA-fetuses. The follow-up developmental assessment examined cognitive and motor development, using the BSID-II to detect long-term effects caused by IUGR (Chapter 4).

The following chapters take the form of manuscripts that can be read separately. This may result in overlaps in content with this introduction and other sections.

2 Changes in event-related brain responses and habituation during child development – a systematic literature review (Study 1)

Habituation allows the examination of both, grade of neuronal maturity and integrity. Being a very basal way of learning, it lends itself particularly well to very early human developmental studies. Habituation can be evaluated by observing behavior as well as by measuring neurophysiological correlates such as ERRs. Since ERRs are assumed to underlie developmental changes, the focus of this systematic review was therefore on (1) the influence of developmental changes of the central nervous system on ERRs with regard to habituation assessment and (2) the investigation of habituation during childhood, beginning at gestation, with a focus on neurophysiological measurements.

Fifty-two studies on habituation during child development were reviewed, and a further eighty-five studies about age-related changes in ERRs were taken into account. Results of both compilations show a very heterogeneous study situation. There was a great diversity with regard to the subjects' ages, stimulation protocols and behavior or components of the ERRs examined. The implementation of methodically matched longitudinal studies as well as the combined measurement of behavioral and neurophysiological habituation could help to draw clearer conclusions about developmental changes in ERRs and neurophysiological habituation assessment.

Author contributions: The material of this chapter was published in Clinical Neurophysiology (Hartkopf et al., 2019). Julia Hartkopf, Jana Keune and Hubert Preissl developed the scientific ideas of this work. Julia Hartkopf and Jana Keune designed the literature search and screened the search results. Julia Hartkopf interpreted the results with the support of Julia Moser, Franziska Schleger and Hubert Preissl. Julia Hartkopf drafted the manuscript. Jana Keune, Hubert Preissl, Julia Moser and Franziska Schleger revised the manuscript.

Acknowledgement: This work was partially supported by the Luminous project (EU- H2020-FETOPEN GA No.686764) and a grant (01GI0925) from the Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.).

Clinical Neurophysiology 130 (2019) 2238–2254

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Changes in event-related brain responses and habituation during child development – A systematic literature review

Julia Hartkopf [a,b,*], Julia Moser [a,b], Franziska Schleger [a,b], Hubert Preissl [a,b], Jana Keune [b,c]

[a] Institute for Diabetes Research and Metabolic Diseases/German Center for Diabetes Research (DZD e.V.) of the Helmholtz Center Munich at the University of Tuebingen, Otfried-Mueller-Strasse 10, 72076 Tuebingen, Germany
[b] fMEG-Center, University of Tuebingen, Otfried-Mueller-Strasse 47, 72076 Tuebingen, Germany
[c] Department of Neurology, Klinikum Bayreuth GmbH, Hohe Warte 8, 95445 Bayreuth, Germany

ARTICLE INFO

Article history:
Accepted 24 August 2019
Available online 14 October 2019

Keywords:
Habituation
Development
Event-related brain responses
Evoked brain responses

HIGHLIGHTS

- With habituation, grade of neuronal maturity/integrity can be investigated from an early age.
- Behavioural studies are being increasingly complemented by neurophysiological measurements.
- Developmental changes in event-related brain responses should be considered.

ABSTRACT

Objective: This systematic review highlights the influence of developmental changes of the central nervous system on habituation assessment during child development. Therefore, studies on age dependant changes in event-related brain responses as well as studies on behavioural and neurophysiological habituation during child development are compiled and discussed.
Methods: Two PubMed searches with terms "(development evoked brain response (fetus OR neonate OR children) (electroencephalography OR magnetoencephalography))" and with terms "(psychology habituation (fetal OR neonate OR children) (human brain))" were performed to identify studies on developmental changes in event-related brain responses as well as habituation studies during child development.
Results: Both search results showed a wide diversity of subjects' ages, stimulation protocols and examined behaviour or components of event-related brain responses as well as a demand for more longitudinal study designs.
Conclusions: A conclusive statement about clear developmental trends in event-related brain responses or in neurophysiological habituation studies is difficult to draw. Future studies should implement longitudinal designs, combination of behavioural and neurophysiological habituation measurement and more complex habituation paradigms to assess several habituation criteria.
Significance: This review emphasizes that event-related brain responses underlie certain changes during child development which should be more considered in the context of neurophysiological habituation studies.

1. Introduction

1.1. Development of the human central nervous system (CNS)

When a human develops up from a fetus over childhood to adolescence, the brain undergoes continuous changes. During the third week after conception, the development of the human nervous system proceeds by expanding the neural plate – the origin of most of the neurons in the CNS. The development of the human CNS is characterized by cell proliferation, cell division, cell migration, cell aggregation, apoptosis and formation of networks (for a detailed

Abbreviations: CNS, central nervous system; EEG, electroencephalography; MEG, magnetoencephalography; fMEG, fetal magnetoencephalography; GA, gestational age; P, positive electroencephalography components; N, negative electroencephalography components; CAEP, cortical auditory evoked potential; CVEP, cortical visual evoked potential; DTI, diffusion tensor imaging; ISI, inter-stimulus interval; SOA, stimulus onset asynchrony; iCNV, early component of contingent negative variation; MMN, mismatch negativity; ITI, inter-train-interval.

* Corresponding author at: University of Tuebingen, fMEG-Center, Otfried-Mueller-Straße 47, 72076 Tuebingen, Germany. Fax: +49 7071 29 5706.

E-mail addresses: julia.hartkopf@med.uni-tuebingen.de (J. Hartkopf), julia.moser@student.uni-tuebingen.de (J. Moser), franziska.schleger@med.uni-tuebingen.de (F. Schleger), hubert.preissl@uni-tuebingen.de (H. Preissl), j.keune@mail.de (J. Keune).

https://doi.org/10.1016/j.clinph.2019.08.029
1388-2457/

overview on intra-uterine anatomical brain development see (Pinel, 2003)). The development of white matter and the related progress of myelination begin before birth and persist, with area-specific differences, until adulthood (Lebel and Beaulieu, 2011; Lebel et al., 2008). The processes of CNS development are therefore not finished at birth but continue over the entire life span.

In earlier days, the development of the human brain, especially before birth, could be investigated only by post mortem studies. The invention of ultrasound observation of fetuses enabled the investigation of brain structure during the fetal period in vivo, as well as fetal inner organs and fetal movements. Moreover, as an indicator for stimulus perception and neuronal processing, fetal behavioural responses to external stimulation were evaluated by ultrasound. In most studies, the stimulation was acoustic or vibro-acoustic and applied outside of the maternal abdomen (Leader et al., 1982b; Bellieni et al., 2005; Shalev et al., 1989). However, despite providing important insight into brain development, behavioural studies could only indirectly explain brain functioning and were unable to directly illuminate functional brain processes. Thanks to technological advances, behavioural studies are now complemented by neurophysiological measurement methods.

Unlike behavioural observation, the neurophysiological measurement methods electroencephalography (EEG) and magnetoencephalography (MEG) meant that neuronal activity could be evaluated directly with a high spatial and temporal resolution. The first EEG recording of a human scalp was performed by Berger in 1911 (Berger, 1929). EEG measurements register changes in electrical neuronal activity on the scalp while MEG measurements register changes in the magnetic fields produced by neurophysiological activity. It should be noted that both methods are completely non-invasive. While in neonates both EEG and MEG are applicable, advances of the fetal magnetoencephalography (fMEG) technique allowed for neurophysiological measurement of event-related responses to auditory and visual stimulation already during the fetal period. In addition to fetal measurements, it is possible to measure event-related brain responses of neonates with fMEG devices. Both EEG and (f)MEG are suitable for measuring event-related responses in infants and children. However, depending on the age of the participants, the kind of stimulation and external conditions, each method has its advantages as well as its specific limitations. MEG devices tend to be more expensive and hence less available. Special MEG systems for children and fMEG devices are rarely accessible. One necessary prerequisite for the evaluation and interpretation of both behavioural and neurophysiological responses related to external stimulation is the functional development of the auditory and visual system. Prenatally, detection and discrimination of sounds is possible, and the development of sound localization, as well as improved sound source acuity and sound discrimination, occurs during the first years of life (Anbuhl et al., 2017). Shortly after the rapid anatomical maturation of the auditory system during the second trimester of pregnancy, the first studies evaluated auditory experiences in fetuses between the 25th and 30th week of gestational age (GA) (for review see (Moore and Linthicum, 2007)). Birnholz et al. detected first responses to vibroacoustic stimulation in fetuses between the 24th and 25th week GA by monitoring blink-startle reflexes ultrasonically (Birnholz and Benacerraf, 1983). Stable behavioural responses across the study group were observed at a GA of 28 weeks. Detection of different pure tone frequencies could be shown over the course of fetal development (Hepper and Shahidullah, 1994). First behavioural responses to 500 Hz tone stimuli in single subjects were detected at 19 weeks GA. At an age of 27 weeks GA, almost the whole sample of fetuses showed reactions to stimulation in the frequency range between 250 Hz and 500 Hz. The authors also ascertained that responses to higher frequencies of around 1000 Hz could be detected at 33 weeks GA, whereas responses to 3000 Hz tones were not detected until 35 weeks GA (Hepper and Shahidullah, 1994). Other groups confirmed similar gestational ages for fetal responses to auditory stimulation (Querleu et al., 1988).

Visual stimulation in the prenatal period has, by comparison, been less well researched than auditory stimulation. It is important to note that not all parts of the visual system are mature before birth, but mature further during the first year of postnatal life. While scotopic vision (vision under low-light condition) becomes functional during the prenatal period between the 24th and 40th week GA, photopic vision (vision under well-lit conditions) requires the influence of direct light and is not functionally mature before birth (Graven and Browne, 2008). First brain responses to light flashes in the prenatal period have been detected as early as at the age of 28 weeks GA (Eswaran et al., 2004).

1.2. Auditory and visual event-related brain responses

Event-related brain responses show characteristic waveforms that consist of different components. The latter can be categorized into three types: exogenous components, endogenous components and motor components. Exogenous components, typically triggered by sensory stimuli, are modulated by characteristics of stimuli and occur in the first 150 ms after stimulus presentation. Unlike exogenous components, endogenous components are influenced by top-down-processes, consciousness and attention. This type of component is modulated by task relevant processes and is not entirely connected to the occurrence and physical aspects of a stimulus. The third category, motor components, emerges during preparation and execution of a motor response (Luck, 2005). Although in some cases the boundaries of these three categories are fluid, it is nevertheless a useful way of differentiating between event-related brain response components. The comparison of specific response components within participants of different age groups can therefore provide information about the development of distinct cognitive skills.

1.3. Habituation

The investigation of event-related responses facilitates the assessment of stimulus perception and processing by the direct examination of underlying neural correlates. The exploration of higher cognitive skills such as recognition or memory, however, requires complex stimulation paradigms. Habituation, as a form of basal learning competence, is one established concept for examining such higher cognitive processes. Habituation is commonly defined as "response decrement to repetitive stimulation" (Thompson and Spencer, 1966). However, alternative explanations for this process could be sensory adaptation or fatigue, defined as a state of decreased neuronal sensitivity as a result of constant stimulation (Carandini, 2000; Netser et al., 2011). To differentiate between these two possible sources of response decrement to repetitively presented stimulation, Thompson et al. developed criteria of habituation, which were later revised to provide ten characteristics of habituation by Rankin et al. (Supplementary Table S1) (Thompson and Spencer, 1966; Rankin et al., 2009).

In habituation studies, many different types of stimulation are applied. In addition to visual, tactile and auditory stimuli, combinations of two different sensory perceptions are used, e.g. vibroacoustic stimulation. The stimulation is selected on the characteristics and age of the participants and on the respective method of measuring habituation. In fetal studies, auditory stimulation is the predominant method. Visual stimulation is less feasible inside the maternal abdomen, even if stimulation with light flashes in fetal studies provides an additional possibility of stimulation. In neonates, visual and tactile stimuli gain importance in

addition to auditory stimulation (Adams and Courage, 1995; Lejeune et al., 2014).

Characteristics of stimuli (loudness, time of repetition, intervals between or modalities of presentation) may affect the processes of habituation as well as the internal condition of the subject under investigation (motivational aspects, state of behaviour, inner evaluation processes and current interests) (Butler, 1968; Barry, 2004; Ponce et al., 2011; Gatchel and Lang, 1974).

The most frequently investigated premises of habituation over the last decades were the characteristics *response decrement*, *stimulus specificity* and *dishabituation*. Many studies reported habituation merely by the occurrence of *response decrement* over repetitively presented stimulation (Clifton et al., 1968; Groome et al., 1995; Hepper et al., 2012). Other studies used two or more of the characteristics to differentiate between habituation and sensory fatigue: *stimulus specificity* and *dishabituation* (Marcus et al., 2012; Lejeune et al., 2014). *Stimulus specificity* is defined by response recovery to a new stimulus (deviant), which is inserted into an array of repetitively presented standard stimuli and which is often wrongly labelled as *dishabituation.* Leader et al. discussed the lack of distinctiveness between stimulus specificity and dishabituation as early as 1982 (Leader et al., 1982a). The term *dishabituation*, however, should be used to describe response recovery to the habituated standard stimulus itself once the deviant stimulus has been inserted (Rankin et al., 2009).

To distinguish habituation clearly from other phenomena that may cause a response decrement (for example sensory fatigue, refractoriness or adaptation), several of the criteria postulated by Rankin et al. should be investigated simultaneously (Berry and Meister, 1998; Perez-Gonzalez and Malmierca, 2014; Rankin et al., 2009). Already Thompson and Spencer suggested that the process of dishabituation should be used as a main indicator for real habituation (Thompson and Spencer, 1966). Rankin et al. propose in addition to test for stimulus specificity and frequency-dependent spontaneous recovery (Rankin et al., 2009). Both, dishabituation and stimulus specificity are therefore particularly suitable criteria to confirm habituation that can easily be integrated into habituation paradigms (Fig. 3).

To investigate habituation in humans, two different types of habituation paradigms are commonly used. In behavioural studies, stimulation is often repeated until response cessation, and habituation is quantified by the number of stimuli required before responses reach an asymptotic level (Kuhlman et al., 1988; Leader et al., 1982b). Alternatively, a predefined number of stimuli is presented and habituation is quantified by the amount of response decrement over this presented array (Sörös et al., 2001; Sheridan et al., 2008; Matuz et al., 2012). This method has the advantage of being less time consuming and is therefore often used in neurophysiological studies of infants and children.

Habituation is an established tool to investigate specific markers of behavioural and neurophysiological measures altered in disease states. Differences in habituation between patients and controls were found in migraine patients, in persons with autism spectrum disorder, with schizophrenia and with neurodegenerative disease (McDiarmid et al., 2017). For the field of neurodevelopmental research, habituation is particular well suitable because it can be applied without any specific behavioural response and is applicable over a wide age range. The recorded neurophysiological or behavioural correlates of habituation can be therefore used as markers for a healthy neurophysiological developmental process.

1.3.1. Behavioural habituation measurement

As early as 1963, Sokolov et al. described a decrement of the orienting reflex (a reaction to a novel or significant stimulus) which is characterized by three criteria: (1) nonspecificity with regard to the quality of the stimulus, (2) nonspecificity with regard to the intensity of the stimulus and (3) selectivity of extinction of various properties of the stimulus with repeated presentation (Sokolov, 1963a). While the term "extinction" is comparable to "habituation", they used the term "disinhibition" similar to response recovery (Sokolov, 1963b). The first behavioural habituation studies were then published in the early 1980s (Schexnider et al., 1981; Leader et al., 1982b). To obtain higher objectivity in these trials, visual examination of a predefined behavioural response to a stimulus paradigm was often combined with videotaped recordings or other technology such as computer assisted recording programs, ultrasound monitoring of fetal behaviour, assessment of eye movements or fixation time (Groome et al., 1993; Groome et al., 1995; Leader et al., 1982b; Sommerville and Woodward, 2005). Moreover, implementation of special observer trainings, categorized documentation systems and calculation of inter-rater reliability are useful tools for minimizing observer effects that might influence data evaluation.

Reflexive action that is not controlled by conscious thoughts, such as the fetal startle response, is a behavioural component to assess habituation that is present in early fetal development. Its decrease is deemed a suitable marker for fetal habituation (Bellieni et al., 2005). The fetal startle response manifests itself not only by body movements but also by vegetative responses such as a change in heart rate (Buss et al., 2009; Sandman et al., 1997). A number of fetal studies therefore combine behavioural assessment and measurement of heart rate changes using ultrasound or transabdominal transducers (Groome et al., 1993; Leader et al., 1984). A further method of assessing habituation is to measure fixation time of participants to visual stimuli. In the past, gaze behaviour was mainly evaluated by observers. However, nowadays, eye-tracking-systems enable us to precisely and objectively record the direction and course of the subject's gaze (Streri and Pecheux, 1986).

Besides the reflexes, participants' conscious actions are an observable dependent variable in behavioural habituation studies. Tactile stimulation is a suitable method for examining habituation in neonates or premature infants and the duration of time that participants explore an object in their hands can be used as a marker for familiarization with its specific features (Lejeune et al., 2012; Sann and Streri, 2008; Lejeune et al., 2014; Streri and Pecheux, 1986).

1.3.2. Neurophysiological habituation measurement

As technology advanced, the behavioural concept of habituation was complemented by studies that analyse neurophysiological activity directly (Weber et al., 2016; Muenssinger et al., 2013c). Habituation, investigated with neurophysiological measurements, enables us to examine the underlying neuronal processes responsible for observable changes in behavioural habituation. In this context, amplitudes of event-related responses are used to detect habituation criteria analogous to behavioural responses (for details, see Section 2).

However, to date, most studies investigating habituation have been based on behavioural observations and only a small number of studies used neurophysiological measurement methods.

1.4. Objective

The aim of this review was to summarize results of habituation studies during child development with a focus on neurophysiological habituation measurements, based on the analysis of event-related brain responses.

Particularly during the first years of life, there is progressive development of the CNS (e.g. myelination, synaptic plasticity, formation of networks). These developmental changes cause changes in event-related responses (especially alterations in amplitude and

latency). As the investigation of event-related responses therefore allows to draw inferences in regard to the development of neural structures and/or neural functions, a general overview of studies on *"age-related changes in event-related brain responses*" is included. Here, the focus is on alterations in amplitude and latency linked to auditory and visual stimulation in the prenatal, postnatal and childhood period as the analysis of event-related brain responses is the most common technique for the investigation of neurophysiological habituation. We then performed a PubMed search on *"habituation studies during child development*" to analyse different methods of stimulation, response evaluation and the criteria used to differentiate between habituation and receptor fatigue. Finally, having drawn conclusions from the insights of both queries, we make recommendations for the evaluation of earlier studies and design of future trials.

2. Methods

This systematic review summarizes research data of studies investigating the development of event-related brain responses and habituation during childhood. For this purpose, relevant literature from the PubMed database was thoroughly reviewed in two independent searches. To summarize studies relating to *"age-related changes in event-related brain responses*", a first search with certain terms "(development evoked brain response (fetus OR neonate OR children) (electroencephalography OR magnetoencephalography))" was performed. The term "evoked" was sought instead of "event-related" since use of the former has become more widespread in the last few decades. A second search with the terms "(psychology habituation (fetal OR neonate OR children) (human brain))" was used to identify *"habituation studies during child development*".

Studies resulting from the search *"age-related changes in event-related brain responses*" were screened for the following inclusion criteria:

- Youngest age group <12 years
- Measurement of brain activity by EEG or (f)MEG
- Developmental processes within research question, including a comparison between at least two different age groups (cross-sectional or longitudinal)
- Healthy subjects or healthy control groups (except preterms)
- Original research articles

To enable a better comparison, it is generally established to label EEG components time-locked to a stimulus by the following definitions: "P" or "N" (P = positivity; N = negativity) combined with an integer number that approximately represents the mean latency of occurrence in milliseconds or the component number (for example "P100" or "P1"). Similarly, responses in (f)MEG studies are typically labelled with "M" followed by latency in ms or by component number (for example "M100", "M1"). Moreover, the respective amplitudes of event-related responses can be analysed. The typically described and frequently analysed components of a "Cortical Auditory Evoked Potential" (CAEP) include P1, followed by N1, P2 and N2 and P300 (Fig. 1). The typical "Cortical Visual Evoked Potential" (CVEP) shows components N1, P1, N2 and P300 (Fig. 2).

In neurophysiological habituation studies, characteristics of habituation can be observed by changes in amplitudes of event-related responses elicited by stimuli arranged in habituation paradigms (Muenssinger et al., 2013b). The observed components depend on the measurement method, the respective stimulation modality (auditory/visual/tactile) and the features of applied stimuli.

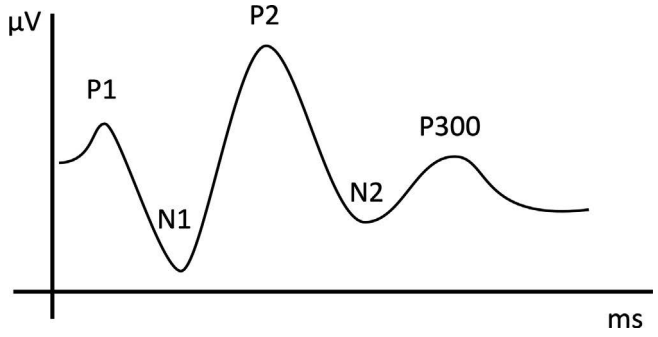

Fig. 1. Typical cortical auditory-evoked potential (CAEP) with the most prominent components P1, N1, P2, N2 and P300.

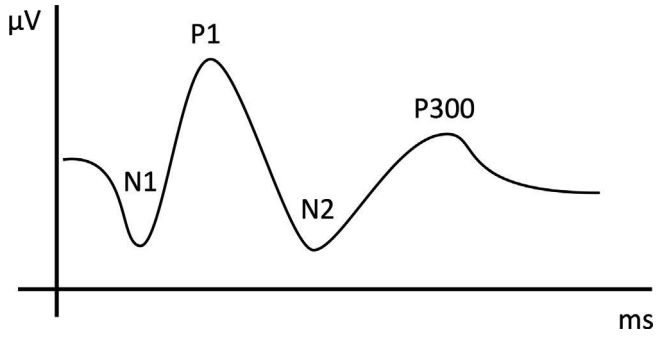

Fig. 2. Typical cortical visual-evoked potential (CVEP) with the most prominent components N1, P1, N2 and P300.

Studies resulting from the second search "*habituation studies during child development*" were screened for the following inclusion criteria:

- Youngest age group <12 years
- Healthy subjects or healthy control groups (except preterms)
- Repeated stimulation with evaluation of response characteristics
- Original research articles

Reference sections of studies that fulfilled inclusion criteria were also screened for further potentially relevant articles. Figs. 4 and 5 show the systematic literature review process. Characteristics, methods and results, as well as limitations and perspectives of exemplary studies, are summarized and discussed in the following sections.

3. Results and discussion

To simplify the overview of studies, age was grouped as depicted in Table 1 (Schneider and Lindenberger, 2012).

3.1. Age-related changes in event-related brain responses

In our first query, we searched for studies investigating event-related brain responses during child development. Table 2 shows a total of 85 studies that fulfilled the inclusion criteria for the first query. The number of participants varied between 10 and 586 with a median of 56. A longitudinal design (identified by "*") was chosen in 13 trials. A total of 72 studies recorded event-related responses by EEG and 12 studies by MEG or fMEG. One study used both EEG and MEG (Fig. 6).

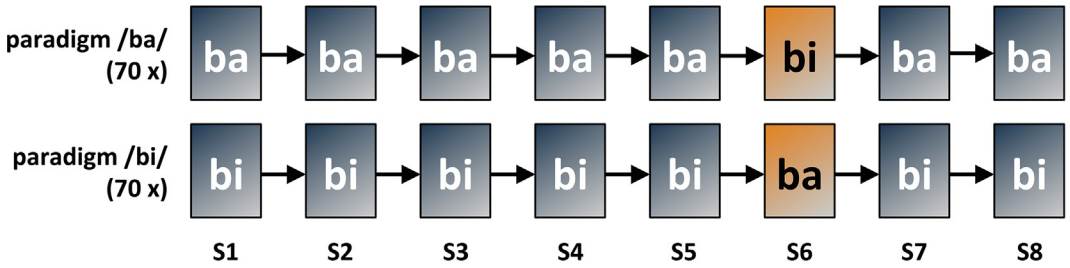

Fig. 3. Example for an auditory habituation paradigm. The paradigm enables testing for response decrement, stimulus specificity and dishabituation by presenting spoken syllables (Hartkopf et al., 2016).

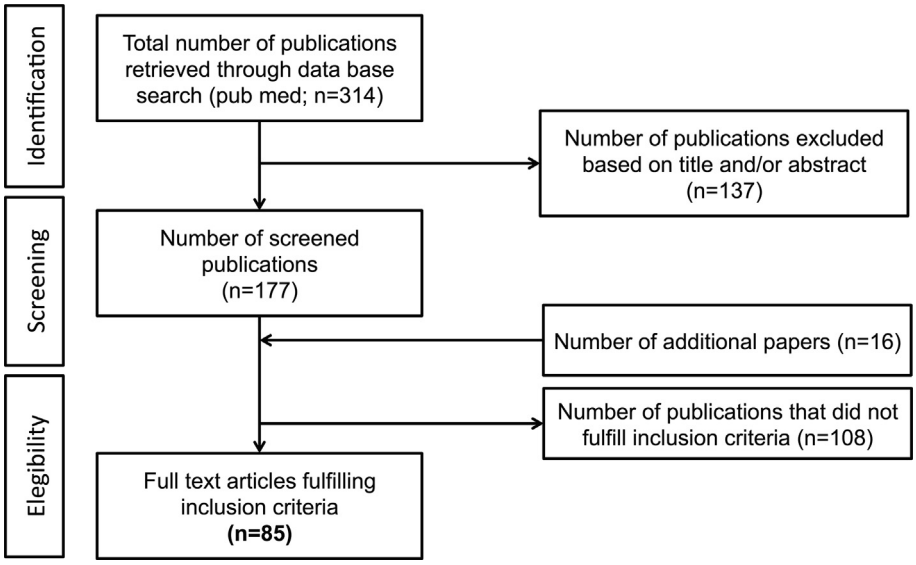

Fig. 4. Flow chart of literature review process and strategy of selection of relevant publications for search *"age-related changes in event-related brain responses"*.

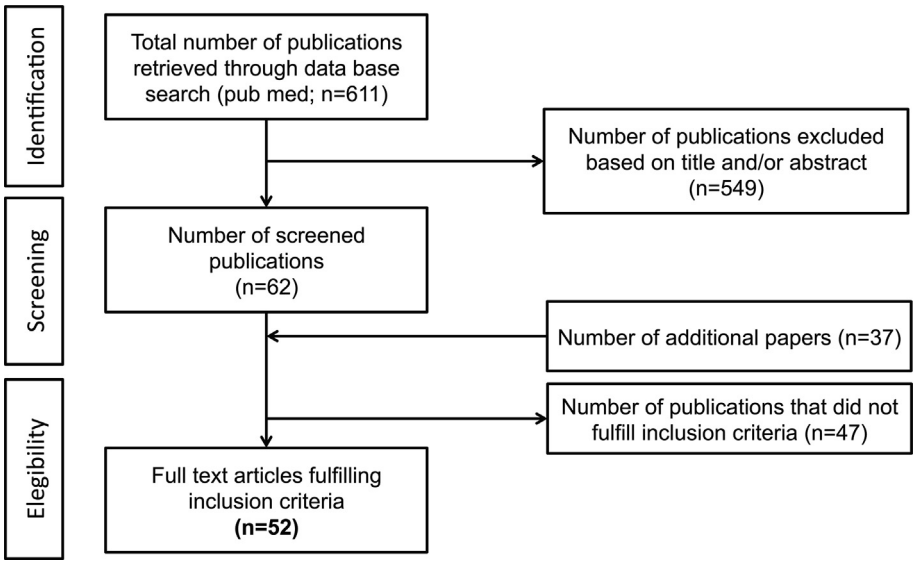

Fig. 5. Flow chart of literature review process and strategy of selection of relevant publications for search *"habituation studies during child development"*.

In one study, MEG was complemented with DTI (Diffusion Tensor Imaging), a non-invasive method for determining the anatomical connectivity of different brain regions by tracking white matter fibres, while another study used MRI alongside MEG to investigate the anatomical brain structure in more detail (Chen et al., 2010).

Changes in event-related brain responses during child development were shown in 73 of 85 studies. These changes may comprise different aspects of event-related brain responses. For example, an MEG-study using visual stimulation that compared children and adults (mean age 11 and 30 years respectively) showed a decrease

Table 1
Age-group classification of study populations.

Age category	Age in years	Developmental stage
1	<0	Fetal
2	0–2	Earliest childhood
3	2–6	Early childhood
4	6–12	Childhood (6–8: middle childhood; 9–12: late childhood)
5	12–18	Adolescence
6	>18	Adulthood

of latency and an increase of response amplitudes with increasing age (Chen et al., 2010). Although a majority of studies investigated age-related changes of event-related brain responses in 0–2 year old children, the changes appeared to persist up until late adolescence (Mahajan and McArthur, 2012). Importantly, fMEG studies demonstrated that developmental changes are already observable during the fetal period (Schneider et al., 2004; Eswaran et al., 2004).

For further investigation, we decided to focus on two component ranges: first, developmental changes in the exogenous components N1 (first negativity) and P1 (first positivity) and second, developmental changes in the later components P2, N2 and P300.

3.2. Age-related changes in primary exogenous event-related brain responses

3.2.1. Auditory P1

Seven studies using auditory stimulation investigated age-related changes in component P1 (Supplementary Table S2). Two studies showed that a typical P1 was not identifiable in very young participants. While one of these studies investigated preterm infants, and ascertained that the P1 component emerged between 37 and 38 weeks of gestational age, a second trial did not detect the component until an age of about 5 years (Weitzman et al., 1967; Lippe et al., 2009). However, different kinds of stimulation were used. The three studies analysing latency reported a decrease of latency with increasing age. Stimulation features such as inter-stimulus interval (ISI) and stimulus onset asynchrony (SOA) also have an influence on the latency and amplitude of the P1 component.

3.2.2. Auditory N1

Four studies investigated changes of the N1 component by comparing latencies and/or amplitudes between different age groups (Supplementary Table S3). However, these studies all differed with respect to the stimulation applied. Two studies showed an increase of the N1 amplitude. In one of these two trials, a go-/no-go task and a stop-signal-task were presented to children between seven and nine years of age, and the increase of amplitude was detectable only for auditory stop-signals in the parietal region (Johnstone et al., 2007). The second study showed an increased amplitude of evoked responses after presenting pure tones and syllables in children and adolescents aged between 10 and 18 years than in adults (Mahajan and McArthur, 2012). A further study, in which clicks were used to stimulate a younger group of children between 23 and 47 weeks of age, reported a decrease in latency (Weitzman et al., 1967). Sussman et al. investigated the influence of different kinds of pure tones with changing stimulation paradigm features (Sussman et al., 2008). Differences in amplitude and latency between age groups were, however, not statistically analysed and the N1 component, which was probably dependent on the stimulus rate, was visible in only a small number of the participants aged between 8 and 11 years.

The latency of the early auditory components P1 and N1 as exogenous components showed a tendency of becoming shorter as age increased. From the 11 studies that investigated either the auditory component N1 or P1, five studies confirmed this tendency by statistical analysis. Due to the fact that the focus was on changes in amplitudes, latency was not analysed in the other six studies. The phenomenon of a decrease of latency with age could be explained by the myelination of nerves that accelerates conduction (Pihko et al., 2009).

Findings were diverse with regard to the amplitudes of event-related responses. Most trials included participants of various age ranges, and almost all of the studies used different kinds of stimuli and stimulation paradigms. Given that age is associated with the degree of maturity of the CNS and that the applied stimulation impacts the elicited event-related responses, the lack of a developmental trend in amplitude heights of the compiled studies seems natural.

3.2.3. Visual N1

In all four studies, an age-dependent decrease in latency of the visual N1 was shown independent of the kind of stimulation and age of the participants (Supplementary Table S4). One of the studies also detected an increase in the amplitude of N1 with age; in the other trials there was no consistent age-related effect on the amplitude of N1. (Hirai et al., 2009; Lippe et al., 2007; Tremblay et al., 2014). In addition to stimulation type and age, the hemisphere under investigation seemed to have an influence on the amplitude and its potential developmental changes, thus contributing to the heterogeneous results.

3.2.4. Visual P1

Four out of eight studies observed a decrease in latency of the visual P1 with increasing age, while one showed only a partial decrease (Supplementary Table S5). An increase in amplitude was shown in only two studies, one of which did not perform a statistical test for significance. Five trials reported a decrease in amplitude as age increased. This observation was independent of stimulation conditions (all five studies applied different stimuli) and observable over various age groups. In three of these five studies, participants were six years of age and older, while very young participants (aged between 0 and 2 years) were investigated in the other two studies.

The type of visual stimulation varied in all five studies and an influence of stimulus complexity on the development of the component P1 can be excepted (Sokol and Jones, 1979). The diversity in age of the participants and the methodical differences of conditions mean that it is difficult to draw a firm conclusion with regard to the developmental changes observed in visual P1.

3.3. Age-related changes in primary endogenous event-related brain responses

3.3.1. Auditory P2

The four studies that investigated auditory P2 did not show consistent results with regard to age-related changes (Supplementary Table S6). P2 component appears to become more prominent during the first months of life and is influenced by the type of stimulation (Lippe et al., 2009). SOA levels of pure tones, as well as different conditions at a go-/no-go-task (response inhibition vs. response execution) affect the P2 (Sussman et al., 2008; Johnstone et al., 2007). Although amplitude of P2 was found to increase in one study stimulating with broadband noise, the P2 did not emerge until about the fifth year of life. Only one study carried out with premature infants registered a decrease in latency of P2 with increasing age within the first weeks after birth (Weitzman et al., 1967).

Table 2
Age-related changes in event-related brain responses. Columns represent number of subjects (N), method of event-related brain response evaluation, the respective age groups and whether or not a developmental trend could be observed. Asterisks (*) indicate studies that used a longitudinal design; EEG = electroencephalography; MEG = magnetoencephalography; DTI = diffusion tensor imaging; MRI = magnetic resonance imaging; PCA = principal component analysis; n.a. = not applicable.

Study	N	Method	Age fetal	Age 0–2	Age 2–6	Age 6–12	Age 12–18	Adults	Development trend	Kind of component investigated
(Agyei et al., 2016)*	20	EEG		x					yes	endogenous
(Anderson et al., 2015)	28	EEG		x					yes	exogenous (brainstem)
(Atchley et al., 2006)	29	EEG				x	x	x	yes	endogenous
(Babkirk et al., 2015)*	32	EEG			x	x			n.a. (prediction)	endogenous
(Brinkman and Stauder, 2007)	112	EEG			x	x		x	yes	exogenous
(Brinkman and Stauder, 2008)	122	EEG			x	x		x	yes	exogenous
(Brusini et al., 2016)	45	EEG			x			x	n.a.	endogenous
(Butler and Trainor, 2013)	60	EEG		x					yes	exogenous
(Byrne et al., 1999)	56	EEG			x	x	x		yes	endogenous
(Chan et al., 2015)	44	EEG				x		x	yes	exogenous/endogenous (NF, P1, N1)
(Chen et al., 2010)	80	MEG/MRI				x	x	x	yes	exogenous
(Chevalier et al., 2015)	64	EEG			x	x			yes	endogenous
(Cho et al., 2015)	188	EEG				x	x	x	yes	not relevant (frequency domain)
(Clery et al., 2012)	24	EEG				x	x	x	yes	exogenous
(Cody and Townsend, 1973)	179	EEG		x	x			x	no	exogenous/endogenous
(Cragg et al., 2009)	56	EEG				x			yes	endogenous
(Cummings et al., 2008)	28	EEG				x	x		yes	endogenous
(Davies et al., 2004a)	124	EEG				x	x	x	yes	endogenous
(Davies et al., 2004b)	153	EEG				x	x	x	yes	endogenous
(Eswaran et al., 2004)	12	fMEG	x						yes	endogenous
(Eswaran et al., 2005)	11	fMEG	x						n.a.	unclear
(Fabrizi et al., 2011)	46	EEG		x					yes	exogenous/endogenous
(Fox et al., 2010)	43	EEG				x	x	x	yes	exogenous
(Friedrich and Friederici, 2005)	113	EEG		x				x	yes	endogenous
(Fuchigami et al., 1993)	175	EEG			x	x	x	x	yes	endogenous
(Fuchigami et al., 2009)	99	EEG			x	x	x	x	yes	endogenous
(Fujioka et al., 2006)*	12	MEG			x	x			yes	exogenous/endogenous
(Gebuis et al., 2009)	51	EEG			x	x		x	yes	endogenous
(Gorga et al., 1989)	535	EEG			x	x			yes	exogenous
(Hadley et al., 2015)*	70	EEG		x	x				n.a. (training and follow up)	exogenous/endogenous
(He et al., 2015)	30	MEG			x			x	yes	exogenous/endogenous (M100, M170)
(Hirai et al., 2009)	60	EEG				x	x		yes	exogenous/endogenous
(Jansen-Osmann and Heil, 2007)	72	EEG				x	x	x	yes	endogenous
(Jeng et al., 2010)	18	EEG		x				x	no	not relevant (frequency domain)
(Johnstone et al., 2007)	24	EEG				x	x		yes	exogenous/endogenous
(Jonkman et al., 2003)	25	EEG					x	x	yes	endogenous
(Kihara et al., 2010)	178	EEG			x	x			yes	exogenous/endogenous
(Kimura et al., 2004)	24	MEG/EEG				x	x	x	yes	exogenous
(Kujawa et al., 2015)*	559	EEG			x	x			n.a.	endogenous
(Kushnerenko et al., 2001)	18	EEG		x				x	n.a.	endogenous
(Lamm and Lewis, 2010)	49	EEG				x	x	x	no	endogenous
(Lasky, 1984)	40	EEG		x				x	yes	exogenous
(Lauronen et al., 2006)	41	MEG		x				x	yes	exogenous
(Lee et al., 2012)	218	EEG		x	x	x		x	yes	exogenous
(Lippe et al., 2007)	79	EEG		x	x			x	yes	exogenous/endogenous
(Lippe et al., 2009)	40	EEG		x	x		x		yes	exogenous/endogenous
(Lo et al., 2013)	50	EEG			x	x			yes	endogenous
(Mahajan and McArthur, 2013)	100	EEG				x	x	x	yes	exogenous
(Marinovic et al., 2014)	66	EEG		x				x	yes	endogenous
(McIntosh et al., 2008)	79	EEG				x	x	x	yes (in PCA)	exogenous
(Mecklinger et al., 2011)	52	EEG				x		x	yes	endogenous
(Missana and Grossmann, 2015)	40	EEG		x					yes	not relevant (frequency domain)
(Missana et al., 2015)	40	EEG		x					yes	endogenous
(Muenssinger et al., 2013a)*	62	fMEG	x	x					n.a.	unclear
(Ojima et al., 2011)*	80	EEG				x	x	x	yes	endogenous
(Paquette et al., 2013)	40	EEG			x	x	x	x	yes	exogenous
(Pihko et al., 2009)	51	MEG		x	x	x		x	yes	exogenous
(Pincham et al., 2015)	60	EEG				x	x		yes	endogenous
(Putkinen et al., 2014a)	117	EEG				x	x		yes	endogenous
(Putkinen et al., 2014b)*	133	EEG				x	x		yes	endogenous
(Richards, 2000)	35	EEG		x					yes	exogenous
(Richardson et al., 2011)	77	EEG				x			no	exogenous/endogenous
(Roberts et al., 2009)	26	MEG, DTI				x	x		yes	exogenous

Table 2 (*continued*)

Study	N	Method	Age fetal	Age 0–2	Age 2–6	Age 6–12	Age 12–18	Adults	Development trend	Kind of component investigated
(Rojas et al., 1998)	22	MEG				x	x		yes	exogenous
(Rojas-Benjumea et al., 2015)	176	EEG				x	x	x	yes	endogenous
(Rueda et al., 2004)	40	EEG			x			x	yes	exogenous/endogenous
(Santesso et al., 2006)	67	EEG				x		x	yes	endogenous
(Schipke et al., 2011)	87	EEG			x	x			yes	endogenous
(Schipke et al., 2012)	109	EEG			x	x		x	yes	endogenous
(Shafer et al., 2000)	78	EEG			x	x		x	yes	endogenous
(Skoe et al., 2015)	586	EEG		x	x	x	x	x	yes	exogenous (brainstem)
(Sussman et al., 2008)	61	EEG				x	x	x	yes	exogenous/endogenous
(Tremblay et al., 2014)	72	EEG		x					yes	exogenous/endogenous (N1)
(Uppal et al., 2016)	53	EEG				x	x		yes	exogenous/endogenous
(van den Boomen et al., 2014)	111	EEG				x	x		yes	exogenous/endogenous
(van der Meer et al., 2012)*	10	EEG		x					yes	endogenous
(Vanvooren et al., 2015)	67	EEG			x			x	yes	not relevant (frequency domain)
(Vuillier et al., 2016)	37	EEG				x		x	yes	endogenous
(Wakai et al., 2007)*	17	MEG		x					yes	endogenous
(Waxer and Morton, 2011a)	80	EEG				x	x	x	yes	exogenous/endogenous
(Weitzman et al., 1967)*	25	EEG		x					yes	exogenous/endogenous
(Yordanova and Kolev, 1996)	80	EEG				x		x	yes	not relevant (frequency domain)
(Yoshimura et al., 2014)*	20	MEG			x	x			yes	exogenous
(Yrttiaho et al., 2014)*	125	EEG		x					n.a.	endogenous
(Zemon et al., 1997)	40	EEG		x	x	x	x	x	yes	not relevant (frequency domain)

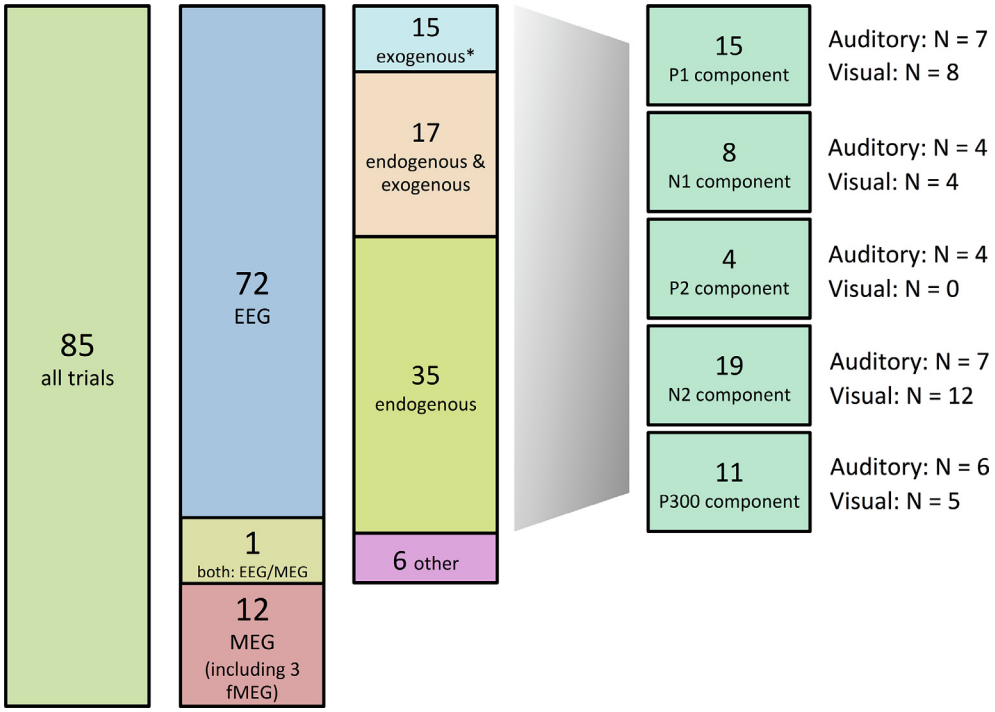

Fig. 6. Overview on studies from the search *"age-related changes in event-related brain responses"*. Applied methods and the investigated components of the event-related responses are displayed. *including brain-stem responses.

3.3.2. Auditory N2

There is no consistency in age-dependent latency changes of N2 (Supplementary Table S7). Four of the seven studies investigating the N2 showed that the development of this component is dependent on the type of stimulus and the location of the electrodes. A decrease in latency was registered in one study only; all other studies found no consistent trend. Changes in amplitude are also heterogeneous. Two studies found no consistent changes, while two others reported that amplitude decreased as age increased.

3.3.3. Auditory P300

Development of auditory P300 was analysed in six trials (Supplementary Table S8). Albeit a decrease in latency with increasing age was found in three trials, two of these showed only a partial decrease, depending on what stimulation was applied. By contrast, one study showed an increase in latency. An increase in amplitude was observed in four studies, even though these changes depended on the kind of stimuli applied. The auditory P300 is supposed to represent higher cognitive processes related to working memory

such as context information, recognition or categorisation (Duncan et al., 2009). The development of this component is assumed to continue until at least adolescence.

3.3.4. Visual N2

The visual N2 was taken into consideration in twelve studies and was therefore the most frequently investigated component (Supplementary Table S9). An age-related decrease in latency was observed in six studies, however, in four of these trials the investigated age groups had a difference of at least six years. A decrease in latency of N2 with increasing age therefore seems to occur relatively slowly (Luck and Kappenman, 2011).

Although an increase of amplitude was established in four studies, one did not report a statistical test for significance (Waxer and Morton, 2011b). A further six studies registered a decline or no change of amplitude. It is, therefore, not possible to draw any conclusions about developmental changes in amplitude of the visual N2 component.

3.3.5. Visual P300

Visual P300 component was analysed in five studies (Supplementary Table S10). The majority of the studies that investigated P300 used go-/no-go or novel-target detection stimulation paradigm. One study, which used a congruency/incongruency task as its stimulus, observed a decrease in latency with age (Rueda et al., 2004). An increase in amplitude was detected in another study that investigated novelty processing by presenting faces and patterns (Kihara et al., 2010). None of the other trials showed consistent results for either a decrease in latency or/and an increase in amplitude for the visual event-related P300 component. The visual P300 is presumably influenced by memory and attention processes (Polich, 2007). The low number of studies and the inconsistent results do not permit us to make any valid conclusion with regard to developmental trends of the P300. Furthermore, the complex and diverse stimulation paradigms complicated the comparison and conclusions from these results.

3.4. Habituation studies during child development

In our second quest, we searched for studies that evaluated habituation during child development. Table 3 summarizes a total of 52 studies and shows the methods applied, type of stimulation and types of habituation criteria tested (Supplementary Table S1).

3.4.1. Participants

A total of 15 of the habituation studies were fetal studies, while 27 studies investigated habituation in infants aged between 0 and 2 years and a further 4 studies examined both fetuses and infants aged between 0 and 2 years. In all, 46 of the 52 trials investigated participants between fetal age and 24 months, thus supporting the particular suitability of habituation for the examination of early learning capability and – directly with measurement of brain activity or indirectly by behavioural analysis – for drawing conclusions about the function of the developing CNS.

3.4.2. Stimulation

Most studies used visual or auditory stimulation (14 visual stimulation, 21 auditory or vibroacoustic stimulation). Tactile/vibrotactile stimulation (passive as well as active) was applied in 11 trials, one of which also combined tactile stimulation with visual stimulation. Six studies applied a combination of visual stimulation and auditory stimulation. In addition to response decrement following repeated stimulation, which was analysed in all of the studies, 26 trials also analysed stimulus specificity. The criterion of response decrement was found to be fulfilled in 35 of the trials, and partially fulfilled in 13 of the studies. Twenty-eight of the studies tested for two criteria (response decrement & stimulus specificity, response decrement & dishabituation or response decrement & spontaneous recovery). Of these, 12 reported that both criteria were fulfilled. Habituation paradigms, which allow the investigation of three or more habituation criteria, were used in only 5 trials. In a tactile habituation study, Marcus et al. ascertained that premature infants already show response decrement, stimulus specificity and dishabituation to a wooden object (Marcus et al., 2012). The authors concluded that premature infants already display developed skills of memorization, discrimination and recognition to tactile explored objects. In a fetal behavioural habituation study, Leader et al. investigated 40 fetuses with vibrotactile stimulation and found evidence of habituation and dishabituation (Leader et al., 1982a). The paradigm design would even have been suitable to test for stimulus specificity, but this step was not performed since the data had not been analysed for this purpose.

3.4.3. Neurophysiological habituation studies

Of all the studies that fulfilled the inclusion criteria, habituation was analysed by direct visual observation of behavioural responses (including videotaped recordings and observation by ultrasound) in 38 studies. Eight studies evaluated changes in heart rate partly in combination with other response types such as respiration or behavioural observation. The neurophysiological activity was directly measured during the habituation procedure to examine habituation criteria in only 8 studies (Table 4).

The first habituation study we identified that used a neuroimaging method was published in the early 1990s and recorded event-related brain responses in children by EEG (McIsaac and Polich, 1992). Beside MEG or fMEG, EEG is still the most widely used neurophysiological approach in habituation studies. Besides its feasibility, the applied method depends on the aims of a study and the respective trial design. EEG, which is feasible in children and adults, is suitable not only for the measurement of temporal dynamics of event-related brain responses but also for examining spatial information by comparing signals at different electrodes. In the search, three EEG studies were found to investigate habituation in children. The study populations of these three studies varied between preterm infants, babies, children and adolescents and investigated habituation in different components of the event-related brain responses, depending on the kind of stimulation and the research question. To compare habituation in 10 infants of approximately 6 months of age with that of a group of 10 young adults, McIsacc et al. chose a paradigm that uses single tones presented in sequences (McIsaac and Polich, 1992). The component P300 was found to have a similar morphology in both groups, but peaked earlier and with lower amplitudes in infants than in adults, indicating a developmental effect. Interindividual differences in P300 were more pronounced in infants. There was no significant response decrement to repeated stimulation in either the group of infants or within young adults. The authors therefore suggested that the kind of stimulation (a passive stimulation paradigm) and the related attention processes inhibited response decline. However, the small sample size limited the generalisability of these results.

Kropp et al. used an auditory two-stimulus reaction time go/no-go task to examine habituation in migraine and healthy participants (Kropp et al., 1999). The early component of contingent negative variation (called iCNV) following the reaction to the go-stimulus was investigated. Response decrement in healthy children was not shown, whereas healthy adults habituated to the stimulation.

The third EEG-study, conducted by Weber et al., examined habituation in 17 healthy preterm infants and 16 term infants with an auditory oddball-paradigm (Weber et al., 2016). The mismatch

Table 3
Habituation studies during child development. Columns represent number of subjects (N), method and objective of response evaluation, method of stimulation, the habituation criteria used and the respective age groups. EEG = electroencephalography; MEG = magnetoencephalography; fMEG = fetal magnetoencephalography; EMG = electromyography; EKG = electrocardiogram. (Y) = habituation criterion was met; (P) = habituation criterion was partially met; (X) = habituation criterion was not met; (n.a.) = not applicable.

Study	N	Method	Objective	Stimulation	Tested habituation criteria	Age fetal	Age 0–2	Age 2–6	Age 6–12	Age 12–18	Adults
(Adams and Courage, 1995)	140	visual observation	behavioural/fixation time	visual	response decrement (Y), stimulus specificity (Y)		x				
(Adams et al., 1991)	40	visual observation	behavioural/fixation time	visual	response decrement (Y), stimulus specificity (P)		x				
(Bellieni et al., 2005)	22	ultrasound	fetal movement	vibro- acoustic	response decrement (Y)	x					
(Clifton et al., 1968)	100	polygraph	heartrate, respiration	auditory	response decrement (P)		x				
(Colombo et al., 2004)	70	EKG	changes in heart rate	visual	response decrement (Y), stimulus specificity (n.a.)		x				
(Fiser and Aslin, 2002)	24	visual observation	behavioural/fixation time	visual/auditory	response decrement (Y), stimulus specificity (P)		x				
(Groome et al., 1993)	90	ultrasound	behavioural	vibro- acoustic	response decrement (Y)	x					
(Groome et al., 1995)	56	ultrasound	behavioural/heartrate	vibro- acoustic	response decrement (Y)	x					
(Hartkopf et al., 2016)*	58	fMEG	brain activity	auditory	response decrement (X), stimulus specificity (P), dishabituation (X)	x	x				
(Hepper et al., 2012)	78	ultra-sound	behavioural	auditory	response decrement (Y)	x					
(Horst et al., 2005)	78	visual observation (videotaped)	behavioural	visual/auditory	response decrement (P), stimulus specificity		x				
(Kirkham et al., 2007)	96	visual observation	behavioural	visual	response decrement (P), stimulus specificity (P)		x				
(Kropp et al., 1999)	320	EEG	brain activity	auditory	response decrement (X)				x	x	x
(Kuhlman et al., 1988)	174	ultrasound	behavioural	vibro- acoustic	response decrement (P)	x					
(Laucht et al., 1994)	226	visual observation	behavioural/fixation time	visual	response decrement (Y), stimulus specificity (Y)			x	x		
(Leader et al., 1982a)	67	ultrasound	fetal movement	vibro- acoustic	response decrement (Y)	x					
(Leader et al., 1982b)	185	ultrasound	behavioural, maternal observation	vibro- acoustic	response decrement (Y)	x					
(Leader et al., 1984)	167	ultrasound	behavioural, heartrate, maternal observation	vibro- tactile	response decrement (Y)	x					
(Lejeune et al., 2010)	24	visual observation	behavioural	tactile	response decrement (Y), stimulus specificity (Y)		x				
(Lejeune et al., 2012)	24	visual observation	behavioural	tactile	response decrement (Y), stimulus specificity (Y)		x				
(Lejeune et al., 2014)	40	visual observation	behavioural	tactile	response decrement (Y), stimulus specificity (Y), dishabituation (P)		x				
(Madison et al., 1986)	39	ultrasound	behavioural, heartrate	vibro- tactile	response decrement (Y)	x					
(Marcus et al., 2012)	48	visual observation (videotaped)	behavioural	tactile	response decrement (Y) stimulus specificity (Y), dishabituation (Y)		x				
(Matuz et al., 2012)*	63	fMEG	brain activity	visual/auditory	response decrement (P), stimulus specificity (P)	x	x				
(McIsaac and Polich, 1992)	20	EEG	brain activity	auditory	response decrement (X)			x			x
(Millar et al., 1991)	42	visual observation	behavioural/fixation time	visual	response decrement (P), stimulus specificity (P)		x	x			
(Milligan et al., 1970)	37	polygraph	behavioural/heart rate	tactile/sensation of falling	response decrement (Y), spontaneous recovery (P)		x				
(Molina et al., 2015)	64	visual observation/measurement of hand pressure	behavioural/grasp time/hand pressure frequency	tactile	response decrement (Y), stimulus specificity (P)		x				
(Morokuma et al., 2004)	26	ultrasound	behavioural	vibro- acoustic	response decrement (P)	x					

(*continued on next page*)

Table 3 (*continued*)

Study	N	Method	Objective	Stimulation	Tested habituation criteria	Age fetal	Age 0–2	Age 2–6	Age 6–12	Age 12–18	Adults
(Morokuma et al., 2008)	21	ultrasound	heartrate characteristics	auditory	response decrement (Y), stimulus specificity (Y)	x					
(Muenssinger et al., 2013b)	62	fMEG	brain activity	auditory	response decrement (P), stimulus specificity (Y), dishabituation (X)	x	x				
(Muenssinger et al., 2013c)	51	MEG	brain activity	auditory	response decrement (X), stimulus specificity (X), dishabituation (X)				x		x
(Oakes et al., 2009)	60	visual observation	behavioural	visual	response decrement (P), stimulus specificity (Y)		x				
(Ornitz et al., 1993)	72	EMG	muscle activity	auditory	response decrement (Y)			x			x
(Perone et al., 2008)	41	visual observation	behavioural	visual/auditory	response decrement (Y), stimulus specificity (Y)		x				
(Pons et al., 2012)	144	visual observation	behavioural	auditory/visual	response decrement (Y), stimulus specificity (P)		x				
(Potter et al., 2000)	50	visual observation	behavioural/head turning	auditory	response decrement (P), stimulus specificity (P)		x				
(Sandman et al., 1997)	84	transabdominal transducers	fetal heart rate	visual	response decrement (Y), dishabituation (Y)	x					
(Sann and Streri, 2008)	24	visual observation	behavioural	tactile	response decrement (Y), stimulus specificity (P)		x				
(Schexnider et al., 1981)	20	visual observation	behavioural/fixation time	visual	response decrement (Y), stimulus specificity (Y)		x				
(Shalev et al., 1989)	103	ultrasound	behavioural	auditory	response decrement (Y)	x					
(Sheridan et al., 2008)*	37	fMEG	brain activity	visual	response decrement (P)	x	x				
(Sinyaya et al., 1992)	72	bipolar electrodes	galvanic skin response	auditory/visual	response decrement (Y)			x			x
(Slater et al., 1984)	52	visual observation	behavioural	visual	response decrement (Y), stimulus specificity (Y)		x				
(Slater et al., 1988)	16	visual observation	behavioural/fixation time	visual	response decrement (Y) stimulus specificity (Y)		x				
(Sommerville and Woodward, 2005)	80	visual observation	behavioural/fixation time	visual	response decrement (P), stimulus specificity (P)		x				
(Sommerville et al., 2005)	30	visual observation	behavioural/fixation time	visual	response decrement (P), stimulus specificity (P)		x				
(Streri and Pecheux, 1986)	64	visual observation	behavioural	Tactile/visual	response decrement (Y), stimulus specificity (Y)		x				
(Streri et al., 2008)	72	visual observation	behavioural	tactile	response decrement (Y), stimulus specificity (P)		x				
(van Heteren et al., 2000)	19	ultrasound	behavioural	vibro- acoustic	response decrement (Y)	x					
(van Heteren et al., 2001)	37	ultrasound	behavioural	vibro- acoustic	response decrement (Y)	x					
(Weber et al., 2016)	33	EEG	brain activity	auditory	response decrement (Y), stimulus specificity (Y)		x				

Table 4
Neurophysiological habituation studies.

Study	Method	Investigated component	Kind of stimulation	Tested habituation criteria and results (fetuses and infants only)	Study population
(Hartkopf et al., 2016)*	fMEG	auditory event-related response	auditory (syllables)	response decrement (X), stimulus specificity (P), dishabituation (X)	30 fetuses (GA: 28–39 weeks), 28 infants (age: 0–3 months)
(Kropp et al., 1999)	EEG	contingent negative variation (CNV)	auditory (two-stimulus reaction time go/no-go task)	response decrement (X)	86 children (age: 8–14 years), 27 youths (age: 15–19 years), 207 adults
(Matuz et al., 2012)*	fMEG	visual event-related response	visual /auditory (light-flashes with tone)	response decrement (P), stimulus specificity (P)	40 fetuses (GA: 30–38 weeks), 26 newborns (age: 6–35 days)
(McIsaac and Polich, 1992)	EEG	P300	auditory (blocks consisting of 10-tone sequences)	response decrement (X)	10 infants (mean age: 6.6 months, SD = 0.49), 10 adults (mean age: 19.8 years, SD = 0.98)
(Muenssinger et al., 2013b)	fMEG	auditory event-related response	auditory (pure tones)	response decrement (P), stimulus specificity (Y), dishabituation (X)	41 fetuses (GA: 30–39 weeks), 22 newborns or babies (age: 6–89 days)
(Muenssinger et al., 2013c)	MEG	M1 component	auditory (tones)	response decrement (P), stimulus specificity (X), dishabituation (X)	29 children (mean age: 9.69 years, SD = 0.47), 14 adults (mean age: 29.29, SD = 3.47)
(Sheridan et al., 2008)*	fMEG	visual event-related response	visual (light flashes)	response decrement (P)	25 fetuses (GA: 29–37 weeks), 12 newborns (age: 6–22 days)
(Weber et al., 2016)	EEG	mismatch negativity (MMN)	auditory (passive oddball paradigm)	response decrement (P), stimulus specificity (P)	17 healthy preterm infants (mean GA: 27.4 weeks, range 25.0–31.3), 16 term infants (mean GA: 40.3 weeks, range 37.9–41.7)

negativity (MMN) component in both groups was investigated at the term-equivalent age of approximately 40.8 weeks of GA. The MMN is assumed to reflect a pre-attentive cognitive discrimination capacity. No significant differences in MMN amplitude and latency between preterm and term infants were found. However, response decrement and stimulus specificity were found in both groups. It is also interesting to note that the authors were able to show a positive correlation between habituation and the scores of a developmental follow-up assessment at approximately 20 months of age in both preterm and term infants.

While the above studies examined endogenous components and MMN, the remaining five developmental related habituation studies analysed exogenous components of event-related brain responses. One research question in the neurophysiological habituation field addressed whether exogenous components, also referred as early components, actually show habituation or if a response decline is potentially caused by adaptation phenomena like refractoriness processes or sensory fatigue (Barry et al., 1992; Verbaten et al., 1986; Budd et al., 1998). However, there is evidence that habituation occurs also in early components, like N1/P1. In adults, for example, habituation of visual N1 was shown. By using a long, varying ISI, refractoriness could be excluded as reason for response decline, since the length of the ISI exceeded the refractory time (Verbaten et al., 1986). It should be mentioned that the variation of the length of the ISI influences the amplitudes of event-related responses and is in general a factor known to influence neurophysiological habituation (Pereira et al., 2014; Miltner et al., 1991). The length of the ISI can modulate how fast habituation occurs and how long the effects persist (Fruhstorfer et al., 1970; Gatchel, 1975; Davis, 1970). Habituation of auditory N1 was moreover shown by Woods and Elmasian, presenting speech sounds and tones (Woods and Elmasian, 1986). Here, the long ISI-condition (1000 ms duration) should be long enough to enable recovery of N1 (Cowper-Smith et al., 2013). The applied paradigm allowed testing for stimulus specificity that was shown additionally to response decrement. The authors examined habituation of N1 by comparing amplitudes of responses and stimulus-specificity across stimulation blocks. This procedure served as a further criterion to exclude adaptation, as the time interval between blocks is usually longer than the ISI.

An MEG-study by Muenssinger et al. examined auditory habituation of component M1 in a sample of 29 children and 14 adults (Muenssinger et al., 2013c). The habituation paradigm consisted of five equal tones, with an inserted different tone followed by two tones as previously, to allow assessment of response decrement, stimulus specificity and dishabituation following the recommendations of Rankin et al. (2009). Response decrement caused by sensory adaption or neuronal fatigue is implausible using this approach, if several habituation criteria are met. In the group of children, none of the three tested habituation criteria within-trains could be confirmed and only response decrement across the stimulation blocks was found. By comparison, the adult group showed response decrement and stimulus specificity within-blocks. Rankin et al. describe stimulus specificity next to frequency-dependent spontaneous recovery as an appropriate marker to prove for habituation (see Supplementary Table S1) (Rankin et al., 2009). The study by Muenssinger et al. also suggested that developmental changes occur in habituation processes across different age groups, as supported by the diverse results in children and adults.

Four neurophysiological habituation studies measured event-related responses with fMEG, where the first detectable peak is examined. This can be interpreted as early component. Sheridan et al. presented repeated trains of four light flashes to 25 fetuses and 12 newborns (Sheridan et al., 2008). In the group of neonates, all infants with detectable event-related responses (9 out of 12) showed a decline in amplitude from the first to the last flash of light. About a third of the fetuses showed detectable event-related response components with a decrement after the first stimulus. The ISI between the stimuli in a block with two seconds should enable recovery of the measured component, however, there is little knowledge about the recovery time of event-related components in fetuses. The second fMEG visual habituation study of Matuz et al. used an advanced version of the light flash paradigm

of the recent study by Matuz et al. (2012) and Sheridan et al. (2008). In the light flash paradigm, a burst tone was also implemented to allow the examination of stimulus specificity. Moreover, two different conditions of inter-train-interval (ITI) duration were implemented to exclude sensory fatigue. In neonates, under the long ITI condition, both response decrement and stimulus specificity could be shown. However, in fetuses, the response rates to the visual stimuli were at a similarly low level as in the study of Sheridan et al. and habituation was not demonstrated consistently (Sheridan et al., 2008). Muenssinger et al. conducted an fMEG study for fetuses and neonates with an auditory habituation paradigm consisting of eight pure tones with one inserted deviant in place of the sixths tone similar to the paradigm as depicted in Fig. 3 (Muenssinger et al., 2013b). The paradigm allowed investigation of response decrement, stimulus specificity and dishabituation. A significant response decrement between the second and the last tone was detected in fetuses, and stimulus specificity was present in both fetuses and neonates. Dishabituation was found neither in fetuses nor in neonates. In a further fMEG study, an auditory habituation paradigm (Fig. 3) consisting of syllables was presented to fetuses and neonates (Hartkopf et al., 2016). Stimulus specificity was demonstrated in neonates only; neither response decrement nor dishabituation could be shown in either age group.

Generally, in future research of neurophysiological habituation studies, the impact of adaptation phenomena on response decrement should be consequently excluded by testing for stimulus specificity and dishabituation. Furthermore, the influence of the ISI should be investigated systematically in this context, with a differentiation of early and late components during child development.

3.4.4. Longitudinal studies

Habituation is widely accepted as a suitable tool for the investigation of the development of early cognitive skills (for example cognitive encoding, information processing skills, discrimination and memory; for a review, see (Kavsek and Bornstein, 2010). Longitudinal designs are particularly suitable for investigating development, for example the timing of specific cognitive changes during childhood. None of the eight neurophysiological habituation studies used the same stimulation paradigm to compare changes in neural correlates from more than two time-points during child development. Further studies should seek to bridge this gap, even if longitudinal research is more time-consuming than cross-sectional studies.

4. Conclusion

In this review, we aimed to collect and describe studies investigating habituation in the context of developmental processes in growing children with an emphasis on neurophysiological habituation measurements, based on the analysis of event-related brain responses.

Age-related changes of event-related brain responses themselves are linked to an increasing maturity of the sensory organs and the central nervous system and, indeed, a large number of the reviewed studies reported age-related changes in components of event-related responses (Birbaumer and Schmidt, 2006). However, the developmental changes observed were not consistent. Although general trends such as a decrease in latency and an increase in amplitude, which can reflect structural or functional maturation processes of the CNS (e.g. increasing myelination or formation of neural networks) are presumed to exist particularly in exogenous components, it is challenging to confirm this on the basis of the studies included in our review. The diversity in methods, selected age groups, chosen stimulation paradigms and investigated components, drastically restricts the comparability of the results.

To facilitate better comparability, the studies were divided according to specific methodical characteristics: a) the kind of investigated component(s) (exogenous versus endogenous components) and b) the stimulus modality (auditory versus visual). In addition, the separate components of the typical CAEP and CVEP were taken into consideration. However, even this differentiated overview did not lead to a generally valid statement about developmental trends in components of event-related brain responses.

Nearly all of the included studies used different stimuli/stimulation paradigms, investigated participants in different age groups and analysed their data on the basis of various methods. Besides the age of participants – and hence the state of maturity of the CNS – changes in event-related brain responses are strongly related to different types of stimulation. Fuchigami et al., for example, showed that response latencies decreased more rapidly with increasing age for complex stimuli than for simple tones (Fuchigami et al., 2009). Moreover, further attributes of the stimuli such as novelty, the emotional context or spatial motion impacted characteristics of event-related brain responses during development (Kihara et al., 2010; Mecklinger et al., 2011; Lamm and Lewis, 2010; van der Meer et al., 2012). This wide range of different study designs and methods limits the interpretation of developmental trends of event-related brain responses or their components and does not endorse a general trend in developmental changes of latency and amplitude during childhood.

Habituation, a basic form of learning, which is manifest very early in development, is a concept to assess cognitive function. It can be measured by two main approaches: the observation of behavioural changes and neurophysiological measurements of event-related brain responses, which enable for a direct evaluation of brain developmental processes. Components of event-related responses measured by EEG and (f)MEG/MEG seem to be suitable for investigating changes in habituation during child development. Studies with fetuses demonstrated that brain responses to auditory and visual stimulation are already measurable during the third trimester of pregnancy, meaning that it is possible to investigate event-related brain responses from an early stage of development. It should be mentioned that fMEG as measuring method has some limitations, for example a low signal-to-noise ratio or the sensitivity to environmental artefacts. The stimuli should meet certain criteria so that they can be perceived by the fetus through tissue layers and amniotic fluid. Therefore, visual light stimuli need to be in a defined range of wavelenghts and auditory stimulation in a certain range of frequency to ensure a satisfactory transferability (Preissl et al., 2004; Sheridan et al., 2010). Most of the habituation studies examining changes in latency and amplitude of event-related responses or their particular components in different age groups found differences that were possibly related to age-dependent stimulus processing. However, it is still difficult to rule out whether other variables, such as the type and the context of the applied stimuli, the examined brain region and the selection of the analysed components impact the results. Unlike behavioural habituation studies, the number of neurophysiological habituation studies remains low.

To rule out sensory fatigue as the driving mechanism for response decrement, habituation studies should use at least three of the postulated habituation criteria: "response decrement", "stimulus specificity" and "dishabituation". Despite the fact that these criteria can be easily implemented in most habituation paradigms, only very few of the included studies used all them. A large number of studies that do not examine these criteria thus lose

quality, as it is not possible to draw clear conclusions about habituation as a driving mechanism for response decrement. This should be also taken into account in future habituation studies.

Despite the large number of studies investigating event-related brain responses and habituation in early development, it is currently not possible to make any conclusive statements about developmental trends. This is mainly due to the fact that stimulation protocols are very diverse. More consistent stimulation paradigms are needed to replicate findings and to better compare different study populations. An interesting issue would also be the combination of behavioural and neurophysiological habituation assessment in order to compare results of both approaches directly with each other. Ultimately, more trials with longitudinal designs are needed to provide clearer information about maturational changes of event-related responses during the process of growing up.

Funding

This word was partially supported by the Luminous project (EU-H2020-FETOPEN GA No.686764) and a grant (01GI0925) from the Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.).

Declaration of Competing Interest

None of the authors have potential conflicts of interest to be disclosed.

Acknowledgements

We are grateful to Shirley Wuerth for help with editing the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2019.08.029.

References

Adams RJ, Courage ML. Development of chromatic discrimination in early infancy. Behav Brain Res 1995;67(1):99–101.
Adams RJ, Courage ML, Mercer ME. Deficiencies in human neonates' color vision: photoreceptoral and neural explanations. Behav Brain Res 1991;43(2):109–14.
Agyei SB, van der Weel FR, van der Meer AL. Longitudinal study of preterm and full-term infants: High-density EEG analyses of cortical activity in response to visual motion. Neuropsychologia 2016;84:89–104.
Anbuhl KL, Uhler KM, Werner LA, Tollin DJ. Early development of the human auditory system. Fetal and neonatal physiology. fifth ed. Elsevier; 2017. p. 1396–411.
Anderson S, Parbery-Clark A, White-Schwoch T, Kraus N. Development of subcortical speech representation in human infants. J Acoust Soc Am 2015;137(6):3346–55.
Atchley RA, Rice ML, Betz SK, Kwasny KM, Sereno JA, Jongman A. A comparison of semantic and syntactic event related potentials generated by children and adults. Brain Lang 2006;99(3):236–46.
Babkirk S, Rios V, Dennis TA. The late positive potential predicts emotion regulation strategy use in school-aged children concurrently and two years later. Dev Sci 2015;18(5):832–41.
Barry RJ. Stimulus significance effects in habituation of the phasic and tonic orienting reflex. Integr Physiol Behav Sci 2004;39(3):166–79.
Barry RJ, Cocker KI, Anderson JW, Gordon E, Rennie C. Does the N100 evoked potential really habituate? Evidence from a paradigm appropriate to a clinical setting. Int J Psychophysiol 1992;13(1):9–16.
Bellieni CV, Severi F, Bocchi C, Caparelli N, Bagnoli F, Buonocore G, et al. Blink-startle reflex habituation in 30–34-week low-risk fetuses. J Perinat Med 2005;33(1):33–7.
Berger H. Über das elektrenkephalogramm des menschen. Arch Psychiatr Nervenkr 1929;87(1):527–70.
Berry 2nd MJ, Meister M. Refractoriness and neural precision. J Neurosci 1998;18(6):2200–11.
Birbaumer N, Schmidt RF. Biologische Psychologie. (6., vollständig überarbeitete und ergänzte Auflage). Heidelberg: Springer; 2006.
Birnholz JC, Benacerraf BR. The development of human fetal hearing. Science 1983;222(4623):516–8.
Brinkman MJ, Stauder JE. Development and gender in the P50 paradigm. Clin Neurophysiol 2007;118(7):1517–24.
Brinkman MJ, Stauder JE. The development of passive auditory novelty processing. Int J Psychophysiol 2008;70(1):33–9.
Brusini P, Dehaene-Lambertz G, Dutat M, Goffinet F, Christophe A. ERP evidence for on-line syntactic computations in 2-year-olds. Dev Cogn Neurosci 2016;19:164–73.
Budd TW, Barry RJ, Gordon E, Rennie C, Michie PT. Decrement of the N1 auditory event-related potential with stimulus repetition: habituation vs. refractoriness. Int J Psychophysiol 1998;31(1):51–68.
Buss C, Davis EP, Class QA, Gierczak M, Pattillo C, Glynn LM, et al. Maturation of the human fetal startle response: evidence for sex-specific maturation of the human fetus. Early Hum Dev 2009;85(10):633–8.
Butler BE, Trainor LJ. Brief pitch-priming facilitates infants' discrimination of pitch-evoking noise: evidence from event-related potentials. Brain Cogn 2013;83(3):271–8.
Butler RA. Effect of changes in stimulus frequency and intensity on habituation of the human vertex potential. J Acoust Soc Am 1968;44(4):945–50.
Byrne JM, Connolly JF, MacLean SE, Dooley JM, Gordon KE, Beattie TL. Brain activity and language assessment using event-related potentials: development of a clinical protocol. Dev Med Child Neurol 1999;41(11):740–7.
Carandini M. Visual cortex: fatigue and adaptation. Curr Biol 2000;10(16):R605–7.
Chan PY, Cheng CH, von Leupoldt A. The effect of development in respiratory sensory gating measured by electrocortical activations. Neural Plast 2015;2015 389142.
Chen Y, Xiang J, Kirtman EG, Wang Y, Kotecha R, Liu Y. Neuromagnetic biomarkers of visuocortical development in healthy children. Clin Neurophysiol 2010;121(9):1555–62.
Chevalier N, Martis SB, Curran T, Munakata Y. Metacognitive processes in executive control development: the case of reactive and proactive control. J Cogn Neurosci 2015;27(6):1125–36.
Cho RY, Walker CP, Polizzotto NR, Wozny TA, Fissell C, Chen CM, et al. Development of sensory gamma oscillations and cross-frequency coupling from childhood to early adulthood. Cereb Cortex 2015;25(6):1509–18.
Clery H, Roux S, Besle J, Giard MH, Bruneau N, Gomot M. Electrophysiological correlates of automatic visual change detection in school-age children. Neuropsychologia 2012;50(5):979–87.
Clifton RK, Graham FK, Hatton HM. Newborn heart-rate response and response habituation as a function of stimulus duration. J Exp Child Psychol 1968;6(2):265–78.
Cody DT, Townsend GL. Some physiologic aspects of the averaged vertex response in humans. Audiology 1973;12(1):1–13.
Colombo J, Kannass KN, Shaddy DJ, Kundurthi S, Maikranz JM, Anderson CJ, et al. Maternal DHA and the development of attention in infancy and toddlerhood. Child Dev 2004;75(4):1254–67.
Cowper-Smith CD, Green J, Maessen H, Bance M, Newman AJ. Refractory effects of the N1 event-related potential in experienced cochlear implant patients. Int J Audiol 2013;52(2):104–12.
Cragg L, Fox A, Nation K, Reid C, Anderson M. Neural correlates of successful and partial inhibitions in children: an ERP study. Dev Psychobiol 2009;51(7):533–43.
Cummings A, Ceponiene R, Dick F, Saygin AP, Townsend J. A developmental ERP study of verbal and non-verbal semantic processing. Brain Res 2008;1208:137–49.
Davies PL, Segalowitz SJ, Gavin WJ. Development of error-monitoring event-related potentials in adolescents. Ann N Y Acad Sci 2004a;1021:324–8.
Davies PL, Segalowitz SJ, Gavin WJ. Development of response-monitoring ERPs in 7- to 25-year-olds. Dev Neuropsychol 2004b;25(3):355–76.
Davis M. Effects of interstimulus interval length and variability on startle-response habituation in the rat. J Comp Physiol Psychol 1970;72(2):177.
Duncan CC, Mirsky AF, Lovelace CT, Theodore WH. Assessment of the attention impairment in absence epilepsy: comparison of visual and auditory P300. Int J Psychophysiol 2009;73(2):118–22.
Eswaran H, Lowery CL, Wilson JD, Murphy P, Preissl H. Functional development of the visual system in human fetus using magnetoencephalography. Exp Neurol 2004;190(S52–S58).
Eswaran H, Lowery CL, Wilson JD, Murphy P, Preissl H. Fetal magnetoencephalography–a multimodal approach. Brain Res Dev Brain Res 2005;154(1):57–62.
Fabrizi L, Slater R, Worley A, Meek J, Boyd S, Olhede S, et al. A shift in sensory processing that enables the developing human brain to discriminate touch from pain. Curr Biol 2011;21(18):1552–8.
Fiser J, Aslin RN. Statistical learning of new visual feature combinations by infants. Proc Natl Acad Sci U S A 2002;99(24):15822–6.
Fox AM, Anderson M, Reid C, Smith T, Bishop DV. Maturation of auditory temporal integration and inhibition assessed with event-related potentials (ERPs). BMC Neurosci 2010;11:49.
Friedrich M, Friederici AD. Phonotactic knowledge and lexical-semantic processing in one-year-olds: brain responses to words and nonsense words in picture contexts. J Cogn Neurosci 2005;17(11):1785–802.
Fruhstorfer H, Soveri P, Järvilehto T. Short-term habituation of the auditory evoked response in man. Electroencephalogr Clin Neurophysiol 1970;28(2):153–61.
Fuchigami T, Okubo O, Fujita Y, Kohira R, Arakawa C, Endo A, et al. Event-related potentials in response to 3-D auditory stimuli. Brain Dev 2009;31(8):577–81.

Fuchigami T, Okubo O, Fujita Y, Okuni M, Noguchi Y, Yamada T. Auditory event-related potentials and reaction time in children: evaluation of cognitive development. Dev Med Child Neurol 1993;35(3):230–7.
Fujioka T, Ross B, Kakigi R, Pantev C, Trainor LJ. One year of musical training affects development of auditory cortical-evoked fields in young children. Brain 2006;129(Pt 10):2593–608.
Gatchel RJ. Effects of interstimulus interval length on short-and long-term habituation of autonomic components of the orienting response. Physiol Psychol 1975;3(2):133–6.
Gatchel RJ, Lang PJ. Effects of interstimulus interval length and variability on habituation of autonomic components of the orienting response. J Exp Psychol 1974;103(4):802–4.
Gebuis T, Herfs IK, Kenemans JL, de Haan EH, van der Smagt MJ. The development of automated access to symbolic and non-symbolic number knowledge in children: an ERP study. Eur J Neurosci 2009;30(10):1999–2008.
Gorga MP, Kaminski JR, Beauchaine KL, Jesteadt W, Neely ST. Auditory brainstem responses from children three months to three years of age: normal patterns of response. II J Speech Hear Res 1989;32(2):281–8.
Graven SN, Browne JV. Visual development in the human fetus, infant and yound child. Newborn Infant Nurs Rev 2008;8(4):194–201.
Groome LJ, Gotlieb SJ, Neely CL, Waters MD. Developmental trends in fetal habituation to vibroacoustic stimulation. Am J Perinatol 1993;10(1):46–9.
Groome LJ, Singh KP, Burgard SL, Neely CL, Deason MA. Motor responsivity during habituation testing of normal human fetuses. J Perinat Med 1995;23(3):159–66.
Hadley H, Pickron CB, Scott LS. The lasting effects of process-specific versus stimulus-specific learning during infancy. Dev Sci 2015;18(5):842–52.
Hartkopf J, Schleger F, Weiss M, Hertrich I, Kiefer-Schmidt I, Preissl H, et al. Neuromagnetic signatures of syllable processing in fetuses and infants provide no evidence for habituation. Early Hum Dev 2016;100:61–6.
He W, Brock J, Johnson BW. Face processing in the brains of pre-school aged children measured with MEG. Neuroimage 2015;106:317–27.
Hepper PG, Dornan JC, Lynch C. Fetal brain function in response to maternal alcohol consumption: early evidence of damage. Alcohol Clin Exp Res 2012;36 (12):2168–75.
Hepper PG, Shahidullah BS. Development of fetal hearing. Arch Dis Child 1994;71 (2):F81–7.
Hirai M, Watanabe S, Honda Y, Kakigi R. Developmental changes in point-light walker processing during childhood and adolescence: an event-related potential study. Neuroscience 2009;161(1):311–25.
Horst JS, Oakes LM, Madole KL. What does it look like and what can it do? Category structure influences how infants categorize. Child Dev 2005;76(3):614–31.
Jansen-Osmann P, Heil M. Developmental aspects of parietal hemispheric asymmetry during mental rotation. NeuroReport 2007;18(2):175–8.
Jeng FC, Schnabel EA, Dickman BM, Hu J, Li X, Lin CD, et al. Early maturation of frequency-following responses to voice pitch in infants with normal hearing. Percept Mot Skills 2010;111(3):765–84.
Johnstone SJ, Dimoska A, Smith JL, Barry RJ, Pleffer CB, Chiswick D, et al. The development of stop-signal and Go/Nogo response inhibition in children aged 7–12 years: performance and event-related potential indices. Int J Psychophysiol 2007;63(1):25–38.
Jonkman LM, Lansbergen M, Stauder JE. Developmental differences in behavioral and event-related brain responses associated with response preparation and inhibition in a go/nogo task. Psychophysiology 2003;40(5):752–61.
Kavsek M, Bornstein MH. Visual habituation and dishabituation in preterm infants: a review and meta-analysis. Res Dev Disabil 2010;31(5):951–75.
Kihara M, Hogan AM, Newton CR, Garrashi HH, Neville BR, de Haan M. Auditory and visual novelty processing in normally-developing Kenyan children. Clin Neurophysiol 2010;121(4):564–76.
Kimura I, Kubota M, Hirose H, Yumoto M, Sakakihara Y. Children are sensitive to averted eyes at the earliest stage of gaze processing. NeuroReport 2004;15 (8):1345–8.
Kirkham NZ, Slemmer JA, Richardson DC, Johnson SP. Location, location, location: development of spatiotemporal sequence learning in infancy. Child Dev 2007;78(5):1559–71.
Kropp P, Siniatchkin M, Stephani U, Gerber WD. Migraine–evidence for a disturbance of cerebral maturation in man? Neurosci Lett 1999;276(3):181–4.
Kuhlman KA, Burns KA, Depp R, Sabbagha RE. Ultrasonic imaging of normal fetal response to external vibratory acoustic stimulation. Am J Obstet Gynecol 1988;158(1):47–51.
Kujawa A, Proudfit GH, Kessel EM, Dyson M, Olino T, Klein DN. Neural reactivity to monetary rewards and losses in childhood: longitudinal and concurrent associations with observed and self-reported positive emotionality. Biol Psychol 2015;104:41–7.
Kushnerenko E, Ceponiene R, Fellman V, Huotilainen M, Winkler I. Event-related potential correlates of sound duration: similar pattern from birth to adulthood. NeuroReport 2001;12(17):3777–81.
Lamm C, Lewis MD. Developmental change in the neurophysiological correlates of self-regulation in high- and low-emotion conditions. Dev Neuropsychol 2010;35(2):156–76.
Lasky RE. A developmental study on the effect of stimulus rate on the auditory evoked brain-stem response. Electroencephalogr Clin Neurophysiol 1984;59 (5):411–9.
Laucht M, Esser G, Schmidt MH. Contrasting infant predictors of later cognitive functioning. J Child Psychol Psychiatry 1994;35(4):649–62.
Lauronen L, Nevalainen P, Wikstrom H, Parkkonen L, Okada Y, Pihko E. Immaturity of somatosensory cortical processing in human newborns. Neuroimage 2006;33 (1):195–203.
Leader LR, Baillie P, Martin B, Molteno C, Wynchank S. Fetal responses to vibrotactile stimulation, a possible predictor of fetal and neonatal outcome. Aust N Z J Obstet Gynaecol 1984;24(4):251–6.
Leader LR, Baillie P, Martin B, Vermeulen E. The assessment and significance of habituation to a repeated stimulus by the human fetus. Early Hum Dev 1982a;7 (3):211–9.
Leader LR, Baillie P, Martin B, Vermeulen E. Fetal habituation in high-risk pregnancies. Br J Obstet Gynaecol 1982b;89(6):441–6.
Lebel C, Beaulieu C. Longitudinal development of human brain wiring continues from childhood into adulthood. J Neurosci 2011;31(30):10937–47.
Lebel C, Walker L, Leemans A, Phillips L, Beaulieu C. Microstructural maturation of the human brain from childhood to adulthood. Neuroimage 2008;40 (3):1044–55.
Lee J, Birtles D, Wattam-Bell J, Atkinson J, Braddick O. Latency measures of pattern-reversal VEP in adults and infants: different information from transient P1 response and steady-state phase. Invest Ophthalmol Vis Sci 2012;53 (3):1306–14.
Lejeune F, Audeoud F, Marcus L, Streri A, Debillon T, Gentaz E. The manual habituation and discrimination of shapes in preterm human infants from 33 to 34+6 post-conceptional age. PLoS ONE 2010;5(2) e9108.
Lejeune F, Berne-Audeoud F, Marcus L, Debillon T, Gentaz E. The effect of postnatal age on the early tactile manual abilities of preterm infants. Early Hum Dev 2014;90(5):259–64.
Lejeune F, Marcus L, Berne-Audeoud F, Streri A, Debillon T, Gentaz E. Intermanual transfer of shapes in preterm human infants from 33 to 34 + 6 weeks postconceptional age. Child Dev 2012;83(3):794–800.
Lippe S, Martinez-Montes E, Arcand C, Lassonde M. Electrophysiological study of auditory development. Neuroscience 2009;164(3):1108–18.
Lippe S, Roy MS, Perchet C, Lassonde M. Electrophysiological markers of visuocortical development. Cereb Cortex 2007;17(1):100–7.
Lo YH, Liang WK, Lee HW, Wang CH, Tzeng OJ, Hung DL, et al. The neural development of response inhibition in 5- and 6-year-old preschoolers: an ERP and EEG study. Dev Neuropsychol 2013;38(5):301–16.
Luck SJ. An introduction to the event-related potential technique. Cambridge, MA: MIT Press; 2005. p. 45–64.
Luck SJ, Kappenman ES. The Oxford handbook of event-related potential components. Oxford University Press; 2011.
Madison LS, Adubato SA, Madison JK, Nelson RM, Anderson JC, Erickson J, et al. Fetal response decrement: true habituation? J Dev Behav Pediatr 1986;7(1):14–20.
Mahajan Y, McArthur G. Maturation of auditory event-related potentials across adolescence. Hear Res 2012;294(1–2):82–94.
Mahajan Y, McArthur G. Maturation of the auditory t-complex brain response across adolescence. Int J Dev Neurosci 2013;31(1):1–10.
Marcus L, Lejeune F, Berne-Audeoud F, Gentaz E, Debillon T. Tactile sensory capacity of the preterm infant: manual perception of shape from 28 gestational weeks. Pediatrics 2012;130(1):e88–94.
Marinovic V, Hoehl S, Pauen S. Neural correlates of human-animal distinction: an ERP-study on early categorical differentiation with 4- and 7-month-old infants and adults. Neuropsychologia 2014;60:60–76.
Matuz T, Govindan RB, Preissl H, Siegel ER, Muenssinger J, Murphy P, et al. Habituation of visual evoked responses in neonates and fetuses: a MEG study. Dev Cogn Neurosci 2012;2(3):303–16.
McDiarmid TA, Bernardos AC, Rankin CH. Habituation is altered in neuropsychiatric disorders-A comprehensive review with recommendations for experimental design and analysis. Neurosci Biobehav Rev 2017;80:286–305.
McIntosh AR, Kovacevic N, Itier RJ. Increased brain signal variability accompanies lower behavioral variability in development. PLoS Comput Biol 2008;4(7) e1000106.
McIsaac H, Polich J. Comparison of infant and adult P300 from auditory stimuli. J Exp Child Psychol 1992;53(2):115–28.
Mecklinger A, Brunnemann N, Kipp K. Two processes for recognition memory in children of early school age: an event-related potential study. J Cogn Neurosci 2011;23(2):435–46.
Millar WS, Weir CG, Supramaniam G. The relationship between encoding, discriminative capacities and perinatal risk status in 4–12-month old infants. J Child Psychol Psychiatry 1991;32(3):473–88.
Milligan JE, Feaster SJ, Hogan GR, Ourth LL. Retention of habituation of the Moro response in the newborn. Dev Med Child Neurol 1970;12(1):6–15.
Miltner W, Johnson R, Braun C. Auditory and somatosensory event-related potentials: II. Effects of inter-stimulus interval. J Psychophysiol 1991;5 (1):27–42.
Missana M, Atkinson AP, Grossmann T. Tuning the developing brain to emotional body expressions. Dev Sci 2015;18(2):243–53.
Missana M, Grossmann T. Infants' emerging sensitivity to emotional body expressions: insights from asymmetrical frontal brain activity. Dev Psychol 2015;51(2):151–60.
Molina M, Sann C, David M, Toure Y, Guillois B, Jouen F. Active touch in late-preterm and early-term neonates. Dev Psychobiol 2015;57(3):322–35.
Moore JK, Linthicum Jr FH. The human auditory system: a timeline of development. Int J Audiol 2007;46(9):460–78.

Morokuma S, Doria V, Ierullo A, Kinukawa N, Fukushima K, Nakano H, et al. Developmental change in fetal response to repeated low-intensity sound. Dev Sci 2008;11(1):47–52.

Morokuma S, Fukushima K, Kawai N, Tomonaga M, Satoh S, Nakano H. Fetal habituation correlates with functional brain development. Behav Brain Res 2004;153(2):459–63.

Muenssinger J, Matuz T, Schleger F, Draganova R, Weiss M, Kiefer-Schmidt I, et al. Sensitivity to auditory spectral width in the fetus and infant - an fMEG study. Front Hum Neurosci 2013a;7:917.

Muenssinger J, Matuz T, Schleger F, Kiefer-Schmidt I, Goelz R, Wacker-Gussmann A, et al. Auditory habituation in the fetus and neonate: an fMEG study. Dev Sci 2013b;16(2):287–95.

Muenssinger J, Stingl KT, Matuz T, Binder G, Ehehalt S, Preissl H. Auditory habituation to simple tones: reduced evidence for habituation in children compared to adults. Front Hum Neurosci 2013c;7:377.

Netser S, Zahar Y, Gutfreund Y. Stimulus-specific adaptation: can it be a neural correlate of behavioral habituation? J Neurosci 2011;31(49):17811–20.

Oakes LM, Kovack-Lesh KA, Horst JS. Two are better than one: comparison influences infants' visual recognition memory. J Exp Child Psychol 2009;104 (1):124–31.

Ojima S, Matsuba-Kurita H, Nakamura N, Hagiwara H. The acceleration of spoken-word processing in children's native-language acquisition: an ERP cohort study. Neuropsychologia 2011;49(5):790–9.

Ornitz EM, Lane SJ, Sugiyama T, de Traversay J. Startle modulation studies in autism. J Autism Dev Disord 1993;23(4):619–37.

Paquette N, Vannasing P, Lefrancois M, Lefebvre F, Roy MS, McKerral M, et al. Neurophysiological correlates of auditory and language development: a mismatch negativity study. Dev Neuropsychol 2013;38(6):386–401.

Pereira DR, Cardoso S, Ferreira-Santos F, Fernandes C, Cunha-Reis C, Paiva TO, et al. Effects of inter-stimulus interval (ISI) duration on the N1 and P2 components of the auditory event-related potential. Int J Psychophysiol 2014;94(3):311–8.

Perez-Gonzalez D, Malmierca MS. Adaptation in the auditory system: an overview. Front Integr Neurosci 2014;8:19.

Perone S, Madole KL, Ross-Sheehy S, Carey M, Oakes LM. The relation between infants' activity with objects and attention to object appearance. Dev Psychol 2008;44(5):1242–8.

Pihko E, Nevalainen P, Stephen J, Okada Y, Lauronen L. Maturation of somatosensory cortical processing from birth to adulthood revealed by magnetoencephalography. Clin Neurophysiol 2009;120(8):1552–61.

Pincham HL, Wu C, Killikelly C, Vuillier L, Fearon RM. Social provocation modulates decision making and feedback processing: examining the trajectory of development in adolescent participants. Dev Cogn Neurosci 2015;15:58–66.

Pinel JP. Development of the nervous system. In: Pinel JP, editor. Biopsychology. Boston: Allyn and Bacon; 2003. p. 221–39.

Polich J. Updating P300: An integrative theory of P3a and P3b. Clin Neurophysiol 2007;118(10):2128–48.

Ponce FP, Quintana GR, Philominraj AS, Vogel EH. Habituation of the eyeblink response in humans with stimuli presented in a sequence of incremental intensity. Biol Res 2011;44(3):295–9.

Pons F, Albareda-Castellot B, Sebastian-Galles N. The interplay between input and initial biases: asymmetries in vowel perception during the first year of life. Child Dev 2012;83(3):965–76.

Potter SM, Zelazo PR, Stack DM, Papageorgiou AN. Adverse effects of fetal cocaine exposure on neonatal auditory information processing. Pediatrics 2000;105(3): E40.

Preissl H, Lowery CL, Eswaran H. Fetal magnetoencephalography: current progress and trends. Exp Neurol 2004;190(Suppl 1):S28–36.

Putkinen V, Tervaniemi M, Saarikivi K, de Vent N, Huotilainen M. Investigating the effects of musical training on functional brain development with a novel Melodic MMN paradigm. Neurobiol Learn Mem 2014a;110:8–15.

Putkinen V, Tervaniemi M, Saarikivi K, Ojala P, Huotilainen M. Enhanced development of auditory change detection in musically trained school-aged children: a longitudinal event-related potential study. Dev Sci 2014b;17 (2):282–97.

Querleu D, Renard X, Versyp F, Paris-Delrue L, Crepin G. Fetal hearing. Eur J Obstet Gynecol Reprod Biol 1988;28(3):191–212.

Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, et al. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem 2009;92(2):135–8.

Richards JE. Localizing the development of covert attention in infants with scalp event-related potentials. Dev Psychol 2000;36(1):91–108.

Richardson C, Anderson M, Reid CL, Fox AM. Neural indicators of error processing and intraindividual variability in reaction time in 7 and 9 year-olds. Dev Psychobiol 2011;53(3):256–65.

Roberts TP, Khan SY, Blaskey L, Dell J, Levy SE, Zarnow DM, et al. Developmental correlation of diffusion anisotropy with auditory-evoked response. NeuroReport 2009;20(18):1586–91.

Rojas DC, Walker JR, Sheeder JL, Teale PD, Reite ML. Developmental changes in refractoriness of the neuromagnetic M100 in children. NeuroReport 1998;9 (7):1543–7.

Rojas-Benjumea MA, Sauque-Poggio AM, Barriga-Paulino CI, Rodriguez-Martinez EI, Gomez CM. Development of behavioral parameters and ERPs in a novel-target visual detection paradigm in children, adolescents and young adults. Behav Brain Funct 2015;11:22.

Rueda MR, Posner MI, Rothbart MK, Davis-Stober CP. Development of the time course for processing conflict: an event-related potentials study with 4 year olds and adults. BMC Neurosci 2004;5:39.

Sandman CA, Wadhwa P, Hetrick W, Porto M, Peeke HV. Human fetal heart rate dishabituation between thirty and thirty-two weeks gestation. Child Dev 1997;68(6):1031–40.

Sann C, Streri A. Inter-manual transfer of object texture and shape in human neonates. Neuropsychologia 2008;46(2):698–703.

Santesso DL, Segalowitz SJ, Schmidt LA. Error-related electrocortical responses in 10-year-old children and young adults. Dev Sci 2006;9(5):473–81.

Schexnider VY, Bell RQ, Shebilske WL, Quinn P. Habituation of visual attention in infants with minor physical anomalies. Child Dev 1981;52(3):812–8.

Schipke CS, Friederici AD, Oberecker R. Brain responses to case-marking violations in German preschool children. NeuroReport 2011;22(16):850–4.

Schipke CS, Knoll LJ, Friederici AD, Oberecker R. Preschool children's interpretation of object-initial sentences: neural correlates of their behavioral performance. Dev Sci 2012;15(6):762–74.

Schneider U, Giessler F, Nowak H, Logemann T, Grimm B, Haueisen J, et al. Fetal MCG and fetal MEG measurements with a 3-channel SQUID system. Neurol Clin Neurophysiol 2004;2004:65.

Schneider W, Lindenberger U. Entwicklungspsychologie. 7th ed. Basel: Beltz; 2012.

Shafer VL, Morr ML, Kreuzer JA, Kurtzberg D. Maturation of mismatch negativity in school-age children. Ear Hear 2000;21(3):242–51.

Shalev E, Benett MJ, Megory E, Wallace RM, Zuckerman H. Fetal habituation to repeated sound stimulation. Isr J Med Sci 1989;25(2):77–80.

Sheridan CJ, Matuz T, Draganova R, Eswaran H, Preissl H. Fetal Magnetoencephalography - achievements and challenges in the study of prenatal and early postnatal brain responses: a review. Infant Child Dev 2010;19(1):80–93.

Sheridan CJ, Preissl H, Siegel ER, Murphy P, Ware M, Lowery CL, et al. Neonatal and fetal response decrement of evoked responses: a MEG study. Clin Neurophysiol 2008;119(4):796–804.

Sinyaya MS, Pinchuk D, Shelyakin AM, Tobias TV, Bogdanov OV. Compensatory plasticity of the brain under conditions of its injury. Neurosci Behav Physiol 1992;22(6):543–9.

Skoe E, Krizman J, Anderson S, Kraus N. Stability and plasticity of auditory brainstem function across the lifespan. Cereb Cortex 2015;25(6):1415–26.

Slater A, Morison V, Rose D. Habituation in the newborn. Infant Behav Dev 1984;7:183–200.

Slater A, Morison V, Somers M. Orientation discrimination and cortical function in the human newborn. Perception 1988;17(5):597–602.

Sokol S, Jones K. Implicit time of pattern evoked potentials in infants: an index of maturation of spatial vision. Vision Res 1979;19(7):747–55.

Sokolov EN. Higher nervous functions: the orienting reflex. Annu Rev Physiol 1963a;25(1):545–80.

Sokolov EN. Perception and the conditioned reflex. Oxford: Pergamon Press; 1963b.

Sommerville JA, Woodward AL. Pulling out the intentional structure of action: the relation between action processing and action production in infancy. Cognition 2005;95(1):1–30.

Sommerville JA, Woodward AL, Needham A. Action experience alters 3-month-old infants' perception of others' actions. Cognition 2005;96(1):B1–B11.

Sörös P, Knecht S, Manemann E, Teismann I, Imai T, Lütkenhöner B, et al. Hemispheric asymmetries for auditory short-term habituation of tones? In: Nenonen J, Ilmoniemi RJ, Katila T, editors. Biomag 2000, Proceedings 12th international conference on biomagnetism. Espoo: Helsinki University of Technology; 2001. p. 47–9.

Streri A, Lemoine C, Devouche E. Development of inter-manual transfer of shape information in infancy. Dev Psychobiol 2008;50(1):70–6.

Streri A, Pecheux MG. Tactual habituation and discrimination of form in infancy: a comparison with vision. Child Dev 1986;57(1):100–4.

Sussman E, Steinschneider M, Gumenyuk V, Grushko J, Lawson K. The maturation of human evoked brain potentials to sounds presented at different stimulus rates. Hear Res 2008;236(1–2):61–79.

Thompson RF, Spencer WA. Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol Rev 1966;73(1):16–43.

Tremblay E, Vannasing P, Roy MS, Lefebvre F, Kombate D, Lassonde M, et al. Delayed early primary visual pathway development in premature infants: high density electrophysiological evidence. PLoS ONE 2014;9(9) e107992.

Uppal N, Foxe JJ, Butler JS, Acluche F, Molholm S. The neural dynamics of somatosensory processing and adaptation across childhood: a high-density electrical mapping study. J Neurophysiol 2016;115(3):1605–19.

van den Boomen C, Lamme VA, Kemner C. Parallel development of ERP and behavioural measurements of visual segmentation. Dev Sci 2014;17(1): 1–10.

van der Meer AL, Svantesson M, van der Weel FR. Longitudinal study of looming in infants with high-density EEG. Dev Neurosci 2012;34(6):488–501.

van Heteren CF, Boekkooi PF, Jongsma HW, Nijhuis JG. Fetal learning and memory. Lancet 2000;356(9236):1169–70.

van Heteren CF, Boekkooi PF, Jongsma HW, Nijhuis JG. Fetal habituation to vibroacoustic stimulation in relation to fetal states and fetal heart rate parameters. Early Hum Dev 2001;61(2):135–45.

Vanvooren S, Hofmann M, Poelmans H, Ghesquiere P, Wouters J. Theta, beta and gamma rate modulations in the developing auditory system. Hear Res 2015;327:153–62.

Verbaten MN, Roelofs JW, Sjouw W, Slangen JL. Habituation of early and late visual ERP components and the orienting reaction: the effect of stimulus information. Int J Psychophysiol 1986;3(4):287–98.

Vuillier L, Bryce D, Szucs D, Whitebread D. The maturation of interference suppression and response inhibition: ERP analysis of a cued go/nogo task. PLoS ONE 2016;11(11) e0165697.

Wakai RT, Lutter WJ, Chen M, Maier MM. On and Off magnetic auditory evoked responses in early infancy: a possible marker of brain immaturity. Clin Neurophysiol 2007;118(7):1480–7.

Waxer M, Morton JB. The development of future-oriented control: an electrophysiological investigation. Neuroimage 2011a;56(3):1648–54.

Waxer M, Morton JB. Multiple processes underlying dimensional change card sort performance: a developmental electrophysiological investigation. J Cogn Neurosci 2011b;23(11):3267–79.

Weber P, Depoorter A, Hetzel P, Lemola S. Habituation as parameter for prediction of mental development in healthy preterm infants: an electrophysiological pilot study. J Child Neurol 2016;31(14):1591–7.

Weitzman L, Graziani L, Duhamel L. Maturation and topography of the auditory evoked response of the prematurely born infant. Electroencephalogr Clin Neurophysiol 1967;23(1):82–3.

Woods DL, Elmasian R. The habituation of event-related potentials to speech sounds and tones. Electroencephalogr Clin Neurophysiol 1986;65(6):447–59.

Yordanova JY, Kolev VN. Developmental changes in the alpha response system. Electroencephalogr Clin Neurophysiol 1996;99(6):527–38.

Yoshimura Y, Kikuchi M, Ueno S, Shitamichi K, Remijn GB, Hiraishi H, et al. A longitudinal study of auditory evoked field and language development in young children. Neuroimage 2014;101:440–7.

Yrttiaho S, Forssman L, Kaatiala J, Leppanen JM. Developmental precursors of social brain networks: the emergence of attentional and cortical sensitivity to facial expressions in 5 to 7 months old infants. PLoS ONE 2014;9(6) e100811.

Zemon V, Hartmann EE, Gordon J, Prunte-Glowazki A. An electrophysiological technique for assessment of the development of spatial vision. Optom Vis Sci 1997;74(9):708–16.

Supplements:

Table S1: Revised version of Thompson & Spencer's (1966) criteria for habituation (Rankin et al., 2009)

Characteristic	Definition
#1	"Repeated applications of a stimulus result in a progressive decrease in some parameters of response to an asymptotic level. This change may include decreases in frequency and /or magnitude of the response. In many cases, the decrement is exponential, but it may also be linear; in addition, a response may show facilitation prior to decrementing because of (or presumably derived from) a simultaneous process of sensitization."
#2	"If the stimulus is withheld after response decrement, the response recovers at least partially over the observation time ("spontaneous recovery")
#3	"After multiple series of stimulus repetitions and spontaneous recoveries, the response decrement becomes successively more rapid and/or more pronounced."
#4	"Other things being equal, more frequent stimulation results in more rapid and/or more pronounced response decrement, and more rapid spontaneous recovery (if the decrement has reached asymptotic levels). "
#5	"Within a stimulus modality, the less intense the stimulus, the more rapid and/or more pronounced the behavioral response decrement. Very intense stimuli may yield no significant observable response decrement."
#6	"The effects of repeated stimulation may continue to accumulate even after the response has reached an asymptotic level (which may or may not be zero, or no response). This effect of stimulation beyond asymptotic levels can alter subsequent behavior, for example, by delaying the onset of spontaneous recovery."
#7	"Within the same stimulus modality, the response decrement shows some stimulus specificity. To test for stimulus specificity/stimulus generalization, a second, novel stimulus is presented and a comparison is made between the changes in the response to the habituated stimulus and the novel stimulus."
#8	"Presentation of a different stimulus results in an increase of the decremented response to the original stimulus. This phenomenon is termed "dishabituation". "
#9	"Upon repeated application of the dishabituating stimulus, the amount of dishabituation produced decreases (this phenomenon can be called habituation of dishabituation)."
#10	"Some stimulus repetition protocols may result in properties of the response decrement (e.g. more rapid rehabituation than baseline, smaller initial responses than baseline, smaller mean responses than baseline, less frequent responses than baseline) that last hours, days or weeks. This persistence of aspects of habituation is termed long term habituation."

Table S2: Developmental trends in latency and amplitude of auditory P1 component
SOA = stimulus onset asynchrony; ISI = inter stimulus interval.

Auditory P1				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/additional information**
(Kihara et al., 2010)	tones	yes	no	decrease in amplitude
(Fox et al., 2010)	tone pairs	not analysed	not analysed	influence of ISI
(Kushnerenko et al., 2001)	tones/different duration	not analysed	not analysed	influence of tone duration and ISI
(Lippe et al., 2009)	broadband noises	not analysed	yes	P1 (0-5.5 years) is not identifiable in children up to 5 years of age
(Mahajan and McArthur, 2012)	pure tones and syllables	yes	no	decrease in amplitude
(Sussman et al., 2008)	two pure tones, 4 SOA-levels	yes	not consistent	latency longer with faster SOA; largest amplitude with 8 years, decrease until 16 years, dependent of SOA
(Weitzman et al., 1967)	clicks	not analysed	not analysed	P1 absent in premature infants, emerges at 37-38 weeks

Table S3: Developmental trends in latency and amplitude of auditory N1 component
SOA = stimulus onset asynchrony.

Auditory N1				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/additional information**
(Johnstone et al., 2007)	go/no-go task, stop-signal-task	not analysed	not consistent	increase in amplitude in parietal region only for stop-signals; tendency to a linear decrease with increasing age for go-trials (frontal region).
(Mahajan and McArthur, 2012)	pure tones and syllables	yes	yes	
(Weitzman et al., 1967)	clicks	yes	not analysed	investigation of preterm infants
(Sussman et al., 2008)	two pure tones, 4 SOA-levels	not analysed	not analysed	N1 in children absent, N1 later dependent of stimulus rate

Table S4: Developmental trends in latency and amplitude of visual N1 component

Visual N1				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/additional information**
(Hirai et al., 2009)	point light walker	yes (linear decrease)	not consistent	amplitude larger in 7-year- olds and adults than in 9-, 10-, & 11-year-olds (left hemisphere). In the right hemisphere significantly larger in the 7-year-olds and adults than in

				the 11-year-olds and in the adults than in the 9-year-olds.
(Lippe et al., 2007)	checkerboard reversals	yes, to 24-66 months, then adult level is reached	not consistent	amplitude appears around 2 months of age and increases from 7 to 12 months. Decrease from 12 to 66 months.
(Rueda et al., 2004)	visual flanker task	yes	no	decrease in amplitude with age
(Tremblay et al., 2014)	sine wave grating, 3 conditions	yes	yes	

Table S5: Developmental trends in latency and amplitude of visual P1 component

Visual P1				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/additional information**
(Hirai et al., 2009)	point light walker	no	no	decrease in amplitude
(Lee et al., 2012)	pattern reversals	yes	not analysed	
(Lippe et al., 2007)	checkerboard reversals	not consistent	yes	
(McIntosh et al., 2008)	face recognition task	yes	yes	not tested for significance
(Richards, 2000)	black and white pattern, changing	no	no	decrease in amplitude (age 14 weeks vs. 20 or 26 weeks)
(Waxer and Morton, 2011a)	dimensional change card sort task (congruency effects)	not analysed	no	decrease in amplitude
(van den Boomen et al., 2014)	texture segmentation task	yes	no	decrease in amplitude 7/8-year-olds vs. 9/10-year- old children
(Tremblay et al., 2014)	sine wave grating	yes	no	decrease in amplitude

Table S6: Developmental trends in latency and amplitude of auditory P2 component
SOA = stimulus onset asynchrony.

Auditory P2				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes**
(Johnstone et al., 2007)	go/no-go task/stop-signal-task	no	No	increase in latency for go-trials
(Lippe et al., 2009)	broadband noise	not analysed	yes	P2 (0-5.5 years) is not identifiable in children up to 5 years of age
(Sussman et al., 2008)	two pure tones, 4 SOA-level	not analysed	not analysed	P2 dependent of SOA-levels (200 ms, 600 ms, 800 ms.)
(Weitzman et al., 1967)	clicks	yes	not analysed	investigation of preterm infants; 35-37 weeks postconceptional age: P2 becomes more prominent

Table S7: Developmental trends in latency and amplitude of auditory N2 component
SOA = stimulus onset asynchrony.

Auditory N2				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/additional information**
(Johnstone et al., 2007)	go/no-go task/stop-signal-task	not consistently	no	amplitude and latency to both no-go and successful stop stimuli decreased linearly with age, but not in the frontal regions
(Lippe et al., 2009)	broadband noises	not consistently	not consistent	no linear changes in amplitude and latency, N2 partly absent
(Kushnerenko et al., 2001)	tones/ different duration	not analysed	not analysed	influence of tone duration on morphology of event-related response was analysed in newborns and adults
(Kihara et al., 2010)	frequent and infrequent pure sinusoidal tones/novel sounds	not consistent	not consistent	no linear trend. Latency of infrequent stimulus was longer for children aged 4–5 years compared to 6–7 years and 8–9 years, but not with age group 10–12 years. Largest amplitude in frequent stimuli in children aged 6–7 years compared to 4/5-year-olds and 10–12-year-olds, but not 8/9- year-olds ($p = 0.175$, ns).
(Sussman et al., 2008)	two pure tones, 4 SOA-level	not consistent	No	latency decrease from 8 to ten years. Amplitude smaller in adolescents than in 8-11-year-olds (interacted with electrode), dependent of SOA condition
(Weitzman et al., 1967)	clicks	not consistently	not analysed	investigation of preterm infants
(Pincham et al., 2015)	feedback processing (reward/punishment (white noise)	yes		

Table S8: Developmental trends in latency and amplitude of auditory P300 component

Auditory P300				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/additional information**
(Brinkman and Stauder, 2008)	harmonic tones and environmental sounds	no	yes	
(Fuchigami et al., 2009)	3D-stimuli	yes (stimuli-dependent)	not analysed	
(Johnstone et al., 2007)	go/no-go task/stop-signal-task	yes (to no-go signal)	yes (for successful stop-trials, go-trials, failed stop trials, dependent of scalp region)	
(Kihara et al., 2010)	frequent and infrequent pure sinusoidal tones/novel sounds	no	yes	increase in latency

(Fuchigami et al., 1993)	auditory oddball paradigm	yes	no	
(Putkinen et al., 2014b)	chords composed of sinusoidal tones	not analysed	yes	Exercise

Table S9: Developmental trends in latency and amplitude of visual N2 component

Visual N2				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/ additional information**
(Hirai et al., 2009)	point light walker	no	no	
(Cragg et al., 2009)	go/no-go paradigm	yes	no	
(Jonkman et al., 2003)	go/no-go paradigm	not analysed	no	
(Kihara et al., 2010)	pictures of faces/patterns (novelty processing)	yes (stimuli dependent)	no	decrease in latency associated with infrequent stimuli
(Lamm and Lewis, 2010)	go/no-go paradigm (emotion inducing)	not analysed	no	amplitude marginally decreased
(Lippe et al., 2007)	pattern reversals	yes	no	decrease in amplitude towards adulthood
(Rueda et al., 2004)	flanker task (congruency)	yes	yes	
(Waxer and Morton, 2011a)	dimensional change card sort task (congruency)	not analysed	yes	study did not test for significance
(Marinovic et al., 2014)	pictures, faces and animals	not analysed	not analysed	N2 absent in children (4 and 7 months of age), only in adults distinguished N1 and N2 were detectable
(Pincham et al., 2015)	feedback processing with reward/punishment (white noise)	yes	yes	
(Agyei et al., 2016)	visual motion, 100 black dots	yes	not analysed	decrease in latency only in full term infants, not in preterm infants. Decreased amplitudes in preterm infants at 4 months compared to full term infants.
(Vuillier et al., 2016)	cued go/no-go-task	yes	yes	

Table S10: Developmental trends in latency and amplitude of visual P300 component

Visual P300				
Study	**Kind of stimulation**	**Decrease in latency**	**Increase in amplitude**	**Other changes/ additional information**
(Cragg et al., 2009)	go/ no-go task	not analysed	not consistent	increase in amplitude only in go trials
(Jonkman et al., 2003)	go/ no-go task	not analysed	not consistent	amplitude differences are dependent of go/no-go condition, location, and age
(Kihara et al., 2010)	faces/ patterns (novelty processing)	no	yes	generally increase of latency with age; increase in amplitude in novel stimuli
(Rueda et al., 2004)	flanker task fish/ congruency	yes	not consistent	
(Rojas-Benjumea et al., 2015)	novel-target visual detection paradigm	not consistent	not consistent	no changes in latency of P3a (fronto-central topography), increase of latency in P3b (parietal).

3 Neuromagnetic signatures of syllable processing in fetuses and infants provide no evidence for habituation (Study 2)

In study 2, neurophysiological processing of spoken syllables (“ba” and “bi"), arranged in a habituation paradigm in healthy fetuses and infants (age 0-3 months), was assessed. The paradigm (illustrated in Figure 4) consisted of eight syllables, whereby the sixth syllable was chosen as a deviant to enable testing for stimulus specificity and dishabituation next to response decrement. In both age groups, EERs were recorded by fMEG and evaluated for the three presumed criteria of habituation (response decrement, stimulus specificity, and dishabituation).

No decrease of ERR amplitudes over continuing stimulation was shown in either age group. In infants, however, the amplitudes of ERRs belonging to the deviant syllable were significantly higher than those of the previous syllable, indicating the presence of a discriminative neural response (stimulus specificity). Further research is required to gain information about early cognitive processing of speech stimuli in relation to later language acquisition.

Author contribution: The material of this chapter was published in Early Human Development (Hartkopf et al., 2016). All authors designed the study together. Julia Hartkopf and Jana Muenssinger acquired the data with the assistance of Franziska Schleger and Magdalene Weiss. Julia Hartkopf was responsible for the data analysis and was supported by Jana Muenssinger and Franziska Schleger. Results were interpreted and discussed by Julia Hartkopf together with Franziska Schleger, Isabelle Kiefer-Schmidt, Hubert Preissl and Jana Muenssinger. Julia Hartkopf drafted the manuscript. Franziska Schleger, Hubert Preissl and Jana Muenssinger revised the manuscript.

Early Human Development 100 (2016) 61–66

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Neuromagnetic signatures of syllable processing in fetuses and infants provide no evidence for habituation

Julia Hartkopf [a,*], Franziska Schleger [a,b,c], Magdalene Weiss [a,d], Ingo Hertrich [e], Isabelle Kiefer-Schmidt [a,d], Hubert Preissl [b,c,f,g], Jana Muenssinger [h]

[a] fMEG Center, University of Tuebingen, Tuebingen, Germany
[b] German Center for Diabetes Research (DZD e.V.), Tuebingen, Germany
[c] Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tuebingen, Tuebingen, Germany
[d] Department of Obstetrics and Gynecology, University Hospital Tuebingen, Tuebingen, Germany
[e] Hertie Center for Neurology, Institute for Clinical Brain Research, University of Tuebingen, Tuebingen, Germany
[f] Department of Internal Medicine, Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, University Hospital Tuebingen, Tuebingen, Germany
[g] Department of Pharmacy and Biochemistry, Faculty of Science, University of Tuebingen, Tuebingen, Germany
[h] Department of Neurology, Klinikum Bayreuth GmbH, Bayreuth, Germany

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 10 April 2016
Accepted 18 April 2016

Keywords:
Fetal magnetoencephalography
Auditory evoked responses
Habituation
Fetuses
Infants

ABSTRACT

Background: Habituation, as a basic form of learning, is characterized by decreasing amplitudes of neuronal reaction following repeated stimuli. Recent studies indicate that habituation to pure tones of different frequencies occurs in fetuses and infants.
Aims: Neural processing of different syllables in fetuses and infants was investigated.
Study design: An auditory habituation paradigm including two different sequences of syllables was presented to each subject. Each sequence consisted of eight syllables (sequence /ba/: 5× /ba/, 1× /bi/ (dishabituator), 2× /ba/; sequence /bi/: 5× /bi/, 1× /ba/ (dishabituator), 2× /bi/). Each subject was stimulated with 140 sequences. Neuromagnetic signatures of auditory-evoked responses (AER) were recorded by fetal magnetoencephalography (fMEG).
Subjects: Magnetic brain signals of N = 30 fetuses (age: 28–39 weeks of gestation) and N = 28 infants (age: 0–3 months) were recorded. Forty-two of the 60 fetal recordings and 29 of the 58 infant recordings were included in the final analysis.
Outcome measures: AERs were recorded and amplitudes were normalized to the amplitude of the first stimulus.
Results: In both fetuses and infants, the amplitudes of AERs were found not to decrease with repeated stimulation. In infants, however, amplitude of syllable 6 (dishabituator) was significantly increased compared to syllable 5 ($p = 0.026$).
Conclusions: Fetuses and infants showed AERs to syllables. Unlike fetuses, infants showed a discriminative neural response to syllables. Habituation was not observed in either fetuses or infants. These findings could be important for the investigation of early cognitive competencies and may help to gain a better understanding of language acquisition during child development.

1. Introduction

Habituation, as a concept for the most basic form of non-associative learning, enables to evaluate the maturation of the fetal central nervous system (CNS) [1,2]. It is represented by a response decrement to repetitively presented stimuli (standard) [3] and makes it possible to investigate the maturation and capacity of cognitive function [4]. Infants with increased risk of cognitive impairment may show delayed performance in habituation tasks [1,5–7].

To differentiate between decrements of reaction due to sensory fatigue, adaptation or habituation, Rankin et al. [8] specified characteristics of habituation on the basis of definitions by Thompson & Spencer [3] and proposed testing for stimulus specificity as well as for dishabituation. To prove stimulus specificity a higher reaction to a new stimulus (dishabituator) must exist, while dishabituation is demonstrated by an increasing reaction to the habituated stimulus that follows the dishabituator as compared to the last standard before the dishabituator.

Abbreviations: AER, auditory evoked response; fMEG, fetal magnetoencephalography; CNS, central nervous system; VER, visual evoked response; GA, gestational age.

* Corresponding author at: fMEG-Center, University of Tuebingen, Otfried-Mueller-Strasse 47, 72076 Tuebingen, Germany.
E-mail address: julia.hartkopf@med.uni-tuebingen.de (J. Hartkopf).

http://dx.doi.org/10.1016/j.earlhumdev.2016.04.002
0378-3782/

Up to now, habituation in fetal research was usually investigated using vibroacoustic stimulation and behavioral observation with ultrasound. Most behavioral studies applied repeated stimulation until response behavior has stopped and reached an asymptotic level or until a previously determined response decrement criteria was reached [9, 10]. Leader et al. used a vibroacoustic habituation procedure that, beside testing for response decrement, also allowed testing for stimulus specificity and dishabituation in fetuses [10]. Hence, they examined three of the characteristics for habituation proposed by Rankin et al. [8] and were therefore able to differentiate between habituation and sensory adaptation. Using acoustic stimulation, Madison et al. demonstrated response recovery after decrement when a novel stimulus was presented. Hence, response decrement during habituation is not based on receptor fatigue but on higher cognitive processes [2]. Morokuma et al. investigated habituation to vibroacoustic stimulation on the basis of body and extremity movement [11]. They noted that habituation was present from at least 32 weeks of gestation and that fetuses with less mature behavior required more stimuli to habituate than fetuses of the same gestational age that were more developed. Other fetal habituation studies used physiological measures like blink-startle reflex or heart-rate parameters or a combination of behavioral and physical assessment to examine characteristics of habituation [1,12–17]. Kisilevsky & Muir could demonstrate a decline of fetal heart rate over repeated stimulation with noise, an increased response to an inserted new stimulus (vibration) and even a response recovery to a re-presented noise stimulus after the new stimulus in fetuses between 37 and 42 weeks of gestation [18].

A noninvasive method for the investigation of brain activity and neuronal reaction in fetuses and infants is fetal magnetoencephalography (fMEG) [19]. This technique makes it possible to directly visualize the presence of habituation by monitoring evoked brain responses. Using fMEG, Sheridan et al. already reported fetal and infant habituation to visual evoked responses (VERs) [20]. Although the number of fetuses with detectable VERs was rather small (29%), decreasing amplitudes of VERs to repeated stimuli were evident in all of them. However, since the habituation paradigm consisted of four identical light-flashes but no dishabituator, the decrease of amplitudes could also have been due to sensory fatigue or adaptation.

Stimulus specificity measured with fMEG was shown by Matuz et al. [21], who used a paradigm consisting of visual standards and an auditory dishabituator. Moreover, we recently evaluated an auditory habituation paradigm consisting of sequences of five identical standard tones, one deviant tone (dishabituator) and two further standard tones [22]. Surprisingly, there was an increase of amplitudes between tone one and tone two, but a decrease of amplitudes in fetuses between tone 2 and tone 5 (response decrement). Higher amplitudes were observed for the dishabituator (tone 6) than for tone 5 or tone 7 (stimulus-specificity). Although there was an increase of amplitudes for tone 7 over tone 5 (dishabituation) in a majority of fetuses and newborns, the results did not reach significance. One of the reasons for these findings could be the small discrepancies between the stimuli, in which case the dishabituator may not have been strong enough to elicit dishabituation.

The ability to discriminate between pairs of syllables /babi/ and /biba/ by fetuses from 35 to 38 weeks of gestation was already shown by Lecanuet et al. [23]. Differences between syllables might be more pronounced and complex than differences between the pure tone frequencies that have already been investigated [22]. The current fMEG study therefore aimed to investigate the presence of habituation in fetuses and infants by using a paradigm consisting of the syllables /ba/ and /bi/.

2. Methods

2.1. Participants

Thirty healthy women, with singleton pregnancy and normal course of gravidity between 28–39 weeks of gestational age (GA) participated in fetal measurement. All participants were native German speakers, ≥18 years of age. Criteria for exclusion were smoking (influence of evoked responses caused by nicotine [24]) or other substance abuse, and high-risk pregnancies. All women were invited to participate with their infants (0–3 months of age) in a secondary measurement. For the secondary measurement, infants had to be healthy and pass a newborn hearing screening. The study was approved by the local Ethical Committee of the Medical Faculty of the University of Tuebingen.

2.2. Stimuli

The /ba/ and /bi/ stimuli were generated by a formant synthesizer using the synthesis program “tuesyn”, as documented in [25,26]. The syllable /ba/ was characterized by a 30 ms transition phase (F1 = 500–750; F2 = 700–1300; F3 = 2700; F4 = 3700; F5 = 4500 Hz) followed by a stationary formant structure. Fundamental frequency declined from 130 to 120 Hz during the transition and ended at 110 Hz at stimulus offset. The syllable /bi/ was similarly generated, but with different formant values of the lower three formants (F1 = 200–280, F2 = 1200–2400, F3 = 2800 Hz).

2.3. Stimulation procedure

An auditory habituation paradigm including two different sequences of syllables was presented to each subject. As illustrated in Fig. 1, each sequence consisted of eight syllables (S1–S8). In one sequence (sequence /ba/), the syllables were: 5× /ba/, 1× /bi/ (dishabituator) and 2× /ba/. The other sequence (sequence /bi/) consisted of 5× /bi/, 1× /ba/ (dishabituator) and 2× /bi/. Two stimulation blocks, each consisting of 70 repeatedly presented equal sequences, were consecutively presented to each subject: block /ba/ consisted of 70 sequences /ba/ and block /bi/ consisted of 70 sequences /bi/. All subjects were randomly assigned to begin with either block /bi/ or block /ba/. The length of syllables and inter-stimulus interval was 500 ms. The interval between sequences was set at random between 4000 and 5000 ms.

The total recording time for both consecutively presented blocks was about 30 min. Stimulation intensity was 95 dB for fetal measurements (approximately attenuated to 65 dB through maternal tissue and amniotic fluid [27]) and 65 dB for infant measurements. The selected intensity of 95 dB for the fetal stimulation is based on previous fMEG studies which showed reliable brain responses in fetuses even younger than the present sample [28,29].

Syllables were generated by a speaker outside the shielded room and transmitted to the fetuses or infants via an air-filled tube. Fetuses received stimuli by way of an air-filled balloon which was placed between the maternal abdomen and the sensor array, while infants had an earphone (bio-logic, USA) placed at their left ear.

2.4. Data acquisition

Magnetic brain signals were recorded with a magnetoencephalographic system designed for fetal and infant studies (SARA II: SQUID Array for Reproductive Assessment, VSM MedTech Ltd., Port Coquitlam, Canada). The 156 primary sensors and 29 reference sensors are placed in an ergonomically shaped array that fits the pregnant abdomen. For localizing the fetal head position in relation to the sensors, one localization coil was placed on the mother's abdomen and three localization coils were placed on her left and right side and on her spine.

Ultrasound imaging (Ultrasound Logiq 500MD, GE, UK) was performed immediately before and after measurement to localize the fetal head position. In case of fetal movement from breech position to cephalic presentation or reverse, the measurement was excluded from further analysis.

By attaching a cradle, the fMEG device can also be used to record infant brain activity. Infants were measured sleeping or awake but at rest. One parent remained inside the shielded room with the infant for the

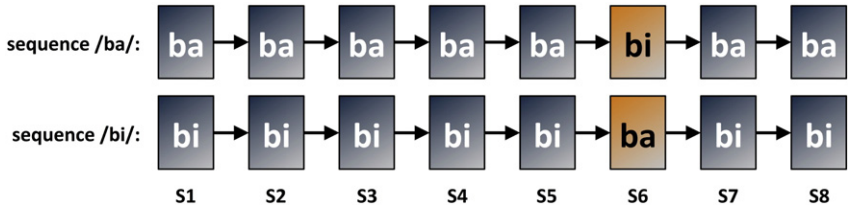

Fig. 1. Auditory habituation paradigm. Each sequence consisted of seven uniform syllables (S1–S5, S7, S8) and one different syllable (S6, dishabituator). Each sequence is consecutively repeated 70 times (=block). Two blocks (block /ba/ = 70× sequence /ba/ and block /bi/ = 70× sequence /bi/) are consecutively presented to each fetus and each infant. Each subject is randomly assigned to either start with block /ba/ or with block /bi/.

duration of the infant measurements. Magnetic signals were recorded in continuous mode with a sampling rate of 610.352 Hz.

2.5. Data analysis

Preprocessing was performed by attenuation of maternal and fetal heart signals using standard algorithms [30–32]. Data were filtered offline with a low-pass filter of 10 Hz and a high-pass filter of 1 Hz for fetuses and a low-pass filter of 15 Hz and a high-pass filter of 1 Hz for infants. Data were divided into segments ranging from 100 ms before to 500 ms after the onset of each syllable. After excluding segments with artifacts higher than 2 pT, an event-related average was calculated. The five channels with the highest amplitudes were determined by visual inspection and their root-mean-square represented the auditory evoked responses (AERs) for further statistical analysis (see Muenssinger et al. [22]).

For each subject, four datasets were analyzed: the fetal and infant data of block /ba/ and /bi/, respectively. A dataset consisted of the AERs of S1–S8 and was included in the analysis if at least five of the eight AERs were detectable. Missing data was replaced by mean imputation.

A repeated measures ANOVA was conducted to determine whether there were statistically significant differences between AERs of different syllables. Two within-subject factors were used: syllable (S1; S5; S6; S7) and block (/ba/; /bi/). Moreover, for analysis of fetal datasets, age (GA < 34 weeks; GA ≥ 34 weeks) was used as between-subjects factor. Data was normally distributed, as assessed by Shapiro-Wilk test ($p > 0.05$). Mauchly's test of sphericity was performed and Epsilon (ε) was calculated according to Huynh-Feldt and used to correct the ANOVA, if the assumption of sphericity was violated. Post hoc analyses were performed with a Bonferroni adjustment. All reported data were normalized to the first syllable of each dataset (AERs of syllable S1–S8 divided by the AER of syllable S1 of the respective data set). All statistical tests were carried out with PASW Statistics 22 (SPSS Inc., Chicago, IL, USA) with significance levels set to $p < 0.05$.

3. Results

Forty-two of 60 fetal and 29 of 58 infant datasets were included in the analysis. Table 1 displays the normalized mean amplitudes of the AERs from syllable S1–S8 for block /bi/ and block /ba/ in fetuses and infants, respectively. To analyze these for the predefined criteria of habituation (Table 2) we compared the amplitudes of S1 and S5 (response decrement), S5 and S6 (stimulus specificity) and S5 and S7 (dishabituation).

3.1. Fetal data

From a total of 60 fetal datasets, 13 datasets had to be excluded because the criterion of five detectable AERs could not be met. The dataset of one fetus was excluded because of fetal movement. Two datasets showed metal artifacts which could not be eliminated and two others were discarded due to technical problems. Of the remaining 42 datasets, 20 were performed with block /ba/ and 22 with block /bi/. The mean gestational age of fetuses was 32.86 (SD: 3.12) weeks. We analyzed both habituation patterns in a combined approach as presented in Fig. 2A by using a two-way repeated measure ANOVA with two within-subject factors (syllable and block) and one between-subjects factor (age). There was no statistically significant interaction between block and syllable ($p = 0.122$) and no statistically significant interaction between age and syllable ($p = 0.540$). Although AERs significantly differed for the different syllables (S1, S5, S6, S7; $p = 0.013$), post hoc analyses revealed that the criteria for habituation were not met (Table 2).

3.2. Infant data

Twenty-nine mothers returned with their infants for the secondary measurement. However, 24 of the 58 datasets had to be excluded because of restlessness or crying of the infant. Five datasets were excluded because the criterion of five detectable AERs was not met. Of the remaining 29 datasets, 15 were performed with block /ba/ and 14 with block /bi/. The mean age of infants was 41.77 (SD: 16.87) days. Normalized mean amplitudes are provided in Fig. 2B. Two-way repeated measure ANOVA with two within-subject factors (syllable and block) revealed that AERs of syllable S1, S5, S6 and S7 were significantly different ($p < 0.001$). Although, the criteria for habituation were not met (Table 2), there was a statistically significant increase from syllable S5 to S6 (stimulus specificity, $p < 0.001$). A statistically significant interaction between block and syllable was not found ($p = 0.324$).

4. Discussion

The present study aimed to investigate the presence of habituation in fetuses and infants by directly observing changes of AERs using fMEG. For this purpose, we presented a habituation paradigm consisting of the two syllables /ba/ and /bi/. We hypothesized a response decrement to a repeated stimulus (see Matuz et al. [21]) and stimulus specificity as shown by a response increase to a new stimulus (dishabituator) (see Muenssinger et al. [22]). In addition, we expected dishabituation, a response increase between the last stimulus before and the first stimulus after the dishabituator.

Table 1
Normalized mean amplitudes of the AERs from syllable S1–S8 for block /bi/ and block /ba/ in fetuses and infants.

Syllable	Fetus		Infants	
	/bi/ Mean (SD)	/ba/ Mean (SD)	/bi/ Mean (SD)	/ba/ Mean (SD)
S1	1 (0)	1 (0)	1 (0)	1 (0)
S2	1.08 (0.38)	0.91 (0.24)	0.94 (0.48)	0.72 (0.30)
S3	1.03 (0.41)	1.10 (0.26)	1.02 (0.51)	0.64 (0.31)
S4	0.98 (0.34)	0.97 (0.26)	1.47 (0.43)	0.77 (0.41)
S5	1.16 (0.45)	0.93 (0.18)	1.0 (0.44)	0.84 (0.39)
S6 (dishabituator)	1.06 (0.54)	1.15 (0.52)	1.40 (0.61)	0.99 (0.60)
S7	1.28 (0.70)	1.04 (0.19)	1.03 (0.58)	0.92 (0.49)
S8	1.08 (0.68)	0.93 (0.25)	0.95 (0.40)	0.77 (0.49)

Table 2
Pairwise comparison according to the criteria of habituation using post hoc analysis with Bonferroni adjustment.

Criteria for habituation	Fetus		Infants	
	Difference of normalized mean amplitudes (95% CI[a])	p-Value	Difference of normalized mean amplitudes (95% CI[a])	p-Value
S1 > S5 (response decrement)	−0.042 (−0.150–0.066)	1.000	0.081 (−0.013–0.174)	0.127
S5 < S6 (stimulus specificity)	−0.067 (−0.212–0.077)	1.000	−0.262 (−0.356 to −0.167)	<0.001
S5 < S7 (dishabituation)	−0.108 (−0.238–0.016)	0.118	−0.064 (−0.190–0.061)	0.946

[a] Confidence interval.

AERs to the presented stimuli were detected in both fetuses and infants. The latency for the AER did not differ between the syllables /ba/ and /bi/, hence an ability of discrimination between /ba/ and /bi/ based on latency analysis was neither shown in fetuses nor in infants (data not shown). With respect to the analysis of amplitudes, however, infants but not fetuses fulfilled the criteria of stimulus specificity and were able to discriminate between the two syllables /ba/ and /bi/. Habituation, however, which is expressed in a response decrement to repeated stimulation, was shown in neither fetuses nor infants. Accordingly, dishabituation (increased response to the standard stimulus after presentation of a dishabituator) was not found either.

Results of an earlier fMEG-study by our group show indications of habituation to pure tones in fetuses [22]. Likewise, in other studies carried out in late trimester fetuses, habituation was indirectly demonstrated by measuring stimulus-associated behavioral changes [12–14,33,34].

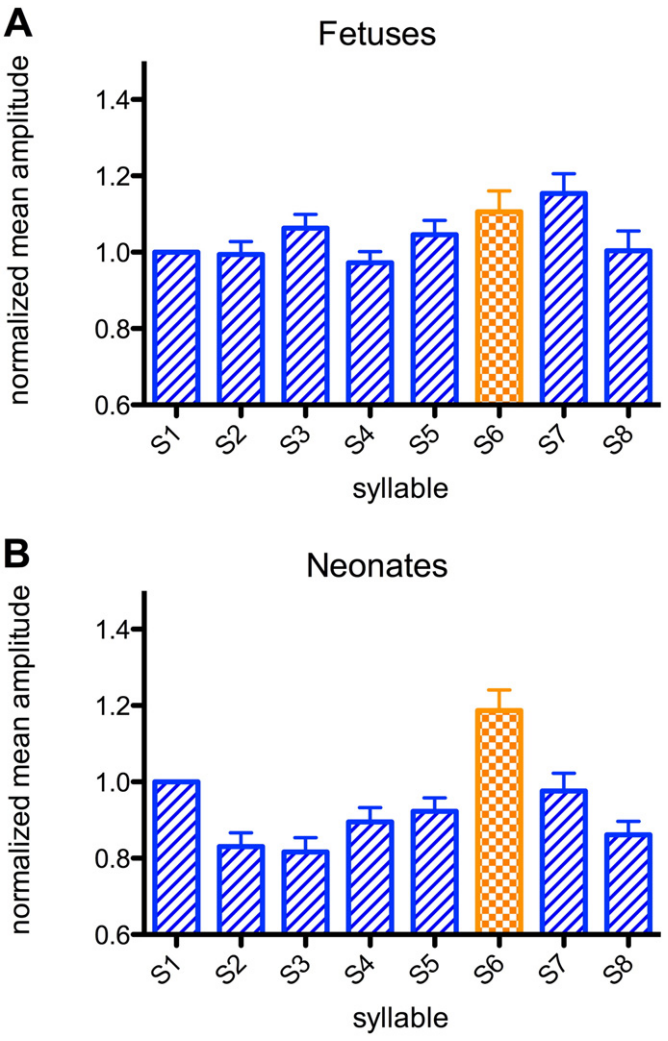

Fig. 2. Normalized mean amplitudes of S1–S8 in fetuses (A) and infants (B).

The lack of habituation in the present study could be due to several factors. While it has not been conclusively established how acoustical stimuli are transmitted to the fetal auditory system, several studies suggest that fetuses are able to detect the applied stimuli (syllables /ba/ and /bi/) through maternal tissues and the amniotic fluid. By using stimuli similar to those in our present study, Lecanuet et al. demonstrated changes in the fetal heart rate variability [23]. Animal studies with hydrophones implanted into ewe's uteri indicate that our chosen stimuli are still discernible for fetuses in utero [35,36]. However, the level of sound attenuation was associated with fetal position and status of development [37].

On the basis of the findings of AERs to the different syllables, we can safely assume that acoustic features of the syllables were transmitted to the fetus and stimulated the fetal auditory system. A behavioral fetal reaction to auditory stimulation with syllables /ba/ and /bi/ seems to be already well developed in the last trimester of pregnancy [23]. However, we cannot rule out the possibility that discrimination between /bi/ and /ba/ is biased due to the quality of transmission. Differences between pure tones, such as were used in our previous study, might be more pronounced. Nevertheless, syllables contain a broad and discontinuous spectrum of frequencies in comparison to pure tones [22]. Complex auditory stimuli require higher and more differentiated neural processing for recognition and discrimination, and so fetal brain function has to be more mature to show stimulus specificity to syllables. A separate analysis of younger (<34 weeks of GA) versus more mature fetuses (≥34 weeks of GA) showed no evidence of an age effect. Provided that more mature brain functions and higher cognitive processes are required to distinguish between different syllables, this would also explain why the neonatal sample, despite being able to discriminate, failed to reach the criteria for habituation. In contrast to our findings, earlier studies using behavioral measures showed habituation to auditory stimuli in neonates and fetal discrimination of syllable changes [23,38]. Combined studies using both, neuronal and behavioral measures, are warranted to explain these contrary findings.

The definition of habituation used in the current study referred to the ten criteria postulated by Rankin et al. [8]. These criteria are based on adult studies. Some studies suggest that characteristics of habituation change during development [11,39–41] and that the differences between habituation in infants and adults are not only associated with physical features of stimuli, but also to the relevance that the subject assigns to the stimuli presented [42–44]. A glance at the adult data of a number of habituation studies (e.g. [42,45,46]) shows a decrease in amplitude from the first to the second tone in a sequence, but no further decrease thereafter, as opposed to the classic criterion of an asymptotic decrease. It has been proposed that these results in adults are better explained by a refractoriness than by a habituation account. An adult MEG-study by Okamoto et al. [42] compared habituation by using pure tones in a silent background and repetitive silences in a pure tone background as indicating stimuli. Surprisingly, silences in a continuing pure tone caused a successive/gradual decrement, while pure tones in a silence showed a decrement from the first to the second stimulus and then an almost constant response pattern. The authors concluded that the novelty of a stimulus is a critical feature for the occurrence of “classic” gradual decrement of responses as postulated

for habituation, whereas more familiar stimuli might result in other neural processes. In accordance with these results on repeated auditory stimulation, we also found a significant decrement from S1 to S2 ($p = 0.003$, data not shown) in infants, albeit no asymptotic decrement. According to the hypothesis proposed by Okamoto et al. [42], one explanation could be that infants are frequently exposed to auditory stimulation similar to the syllables used in our trial.

The capacity and speed of the working memory increases during childhood development. Given that syllables are more complex stimuli than pure tones, it might take longer to habituate to them. A sequence of six repeated syllables before presentation of the dishabituator would then be too short for habituation and more repetitions of the standard syllable before introduction of the dishabituator might induce a decrement of AERs. In earlier studies, a response decrement criteria was set before initiating a stimulus change [2,47]. However, duration of fMEG measurement with pregnant women and young infants is limited due to practical and ethical reasons (special sitting position, avoidance of physical movements, restricted interaction between parent and child) and real-time observation of brain data is not feasible. Nevertheless, we suggested to increase the number of habituation stimuli before the dishabituator in future trials. Moreover, it should also be borne in mind that the duration of ISI between syllables was 500 ms. Compared to other fetal and infant habituation studies, this interval may have been too long for the fetal brain to process it as a continuously performed sequence. In the study by Muenssinger et al. [22], for example, the ISI was 300 ms. Additionally, lack of habituation might be due to the intensity of the auditory stimulus.

Unlike the fetal sample, infants lacked response decrement or dishabituation, but showed a significant stimulus-specificity. AERs to the dishabituator were significantly increased, indicating that infants are able to differentiate between the spoken syllables /bi/ and /ba/. This result is supported by other studies in which infants were shown to be able to process phonological input and their neural correlates reflected changes in auditory stimulation (for review see [48]).

In the present study, we did not analyze the habituation responses in relation to fetal or neonatal behavioral states [49]. The limited number of recorded segments did not permit the extraction of AERs according to the behavioral state. In addition, there is evidence that habituation is independent of behavioral state [14].

Since language impairment in children is often associated with early impairment in auditory perception, the investigation into the age at which the ability to discriminate between different syllables occurs in human development is an important topic for future research. Early disorders like fetal growth restriction can affect auditory perception as well as processing and indicate that it may be possible to identify individual fetuses and newborns at risk for language deficits [38]. In this context, our findings are important for a better understanding of the early development of language acquisition. However further research is warranted to assess whether fMEG might be useful to identify early impairments of auditory-system functioning that may eventually lead to early interventions in specific language impairments.

Acknowledgements

We gratefully thank all families for their participation.

References

[1] L.R. Leader, P. Baillie, B. Martin, C. Molteno, S. Wynchank, Fetal responses to vibrotactile stimulation, a possible predictor of fetal and neonatal outcome, Aust. N. Z. J. Obstet. Gynaecol. 24 (4) (1984) 251–256.
[2] L.S. Madison, S.A. Adubato, J.K. Madison, R.M. Nelson, J.C. Anderson, J. Erickson, et al., Fetal response decrement: true habituation? J. Dev. Behav. Pediatr. 7 (1) (1986) 14–20.
[3] R.F. Thompson, W.A. Spencer, Habituation: a model phenomenon for the study of neuronal substrates of behavior, Psychol. Rev. 73 (1) (1966) 16–43.
[4] S. Schmid, D.A. Wilson, C.H. Rankin, Habituation mechanisms and their importance for cognitive function, Front. Integr. Neurosci. 8 (2014) 97.
[5] P.G. Hepper, J.C. Dornan, C. Lynch, Fetal brain function in response to maternal alcohol consumption: early evidence of damage, Alcohol. Clin. Exp. Res. 36 (12) (2012) 2168–2175.
[6] J.R. Swartz, J.L. Wiggins, M. Carrasco, C. Lord, C.S. Monk, Amygdala habituation and prefrontal functional connectivity in youth with autism spectrum disorders, J. Am. Acad. Child Adolesc. Psychiatry 52 (1) (2013) 84–93.
[7] D. Sontheimer, Visual information processing in infancy, Dev. Med. Child Neurol. 31 (6) (1989) 787–796.
[8] C.H. Rankin, T. Abrams, R.J. Barry, S. Bhatnagar, D.F. Clayton, J. Colombo, et al., Habituation revisited: an updated and revised description of the behavioral characteristics of habituation, Neurobiol. Learn. Mem. 92 (2) (2009) 135–138.
[9] K.A. Kuhlman, K.A. Burns, R. Depp, R.E. Sabbagha, Ultrasonic imaging of normal fetal response to external vibratory acoustic stimulation, Am. J. Obstet. Gynecol. 158 (1) (1988) 47–51.
[10] L.R. Leader, P. Baillie, B. Martin, E. Vermeulen, Fetal habituation in high-risk pregnancies, Br. J. Obstet. Gynaecol. 89 (6) (1982) 441–446.
[11] S. Morokuma, K. Fukushima, N. Kawai, M. Tomonaga, S. Satoh, H. Nakano, Fetal habituation correlates with functional brain development, Behav. Brain Res. 153 (2) (2004) 459–463.
[12] C.F. van Heteren, P.F. Boekkooi, R.H. Schiphorst, H.W. Jongsma, J.G. Nijhuis, Fetal habituation to vibroacoustic stimulation in uncomplicated postterm pregnancies, Eur. J. Obstet. Gynecol. Reprod. Biol. 97 (2) (2001) 178–182.
[13] C.V. Bellieni, F. Severi, C. Bocchi, N. Caparelli, F. Bagnoli, G. Buonocore, et al., Blink-startle reflex habituation in 30–34-week low-risk fetuses, J. Perinat. Med. 33 (1) (2005) 33–37.
[14] C.F. van Heteren, P.F. Boekkooi, H.W. Jongsma, J.G. Nijhuis, Fetal habituation to vibroacoustic stimulation in relation to fetal states and fetal heart rate parameters, Early Hum. Dev. 61 (2) (2001) 135–145.
[15] J.P. Lecanuet, C. Granier-Deferre, H. Cohen, R. Le Houezec, M.C. Busnel, Fetal responses to acoustic stimulation depend on heart rate variability pattern, stimulus intensity and repetition, Early Hum. Dev. 13 (3) (1986) 269–283.
[16] L.J. Groome, S.J. Gotlieb, C.L. Neely, M.D. Waters, Developmental trends in fetal habituation to vibroacoustic stimulation, Am. J. Perinatol. 10 (1) (1993) 46–49.
[17] C.A. Sandman, P. Wadhwa, W. Hetrick, M. Porto, H.V. Peeke, Human fetal heart rate dishabituation between thirty and thirty-two weeks gestation, Child Dev. 68 (6) (1997) 1031–1040.
[18] B.S. Kisilevsky, D.W. Muir, Human fetal and subsequent newborn responses to sound and vibration, Infant Behav. Dev. 14 (1) (1991) 1–26.
[19] H. Preissl, C.L. Lowery, H. Eswaran, Fetal magnetoencephalography: current progress and trends, Exp. Neurol. 190 (Suppl. 1) (2004) S28–S36.
[20] C.J. Sheridan, H. Preissl, E.R. Siegel, P. Murphy, M. Ware, C.L. Lowery, et al., Neonatal and fetal response decrement of evoked responses: a MEG study, Clin. Neurophysiol. 119 (4) (2008) 796–804.
[21] T. Matuz, R.B. Govindan, H. Preissl, E.R. Siegel, J. Muenssinger, P. Murphy, et al., Habituation of visual evoked responses in neonates and fetuses: a MEG study, Dev. Cogn. Neurosci. 2 (3) (2012) 303–316.
[22] J. Muenssinger, T. Matuz, F. Schleger, I. Kiefer-Schmidt, R. Goelz, A. Wacker-Gussmann, et al., Auditory habituation in the fetus and neonate: an fMEG study, Dev. Sci. 16 (2) (2013) 287–295.
[23] J.P. Lecanuet, C. Granier-Deferre, A.J. DeCasper, R. Maugeais, A.J. Andrieu, M.C. Busnel, Fetal perception and discrimination of speech stimuli; demonstration by cardiac reactivity; preliminary results, C. R. Acad. Sci. III 305 (5) (1987) 161–164.
[24] A.P. Key, M. Ferguson, D.L. Molfese, K. Peach, C. Lehman, V.J. Molfese, Smoking during pregnancy affects speech-processing ability in newborn infants, Environ. Health Perspect. 115 (4) (2007) 623–629.
[25] I. Hertrich, H. Ackermann, A vowel synthesizer based on formant sinusoids modulated by fundamental frequency, J. Acoust. Soc. Am. 106 (1999) 2988–2990.
[26] I. Hertrich, H. Ackermann, Modelling voiceless speech segments by means of an additive procedure based on the computation of formant sinusoids, in: P. Wagner, J. Abresch, S. Breuer, W. Hess (Eds.), Proceedings of the 6th ISCA Workshop on Speech Synthesis, University of Bonn, Bonn 2007, pp. 178–181.
[27] D. Querleu, X. Renard, F. Versyp, L. Paris-Delrue, G. Crepin, Fetal hearing, Eur. J. Obstet. Gynecol. Reprod. Biol. 28 (3) (1988) 191–212.
[28] K. Linder, F. Schleger, I. Kiefer-Schmidt, L. Fritsche, S. Kummel, M. Heni, et al., Gestational diabetes impairs human fetal postprandial brain activity, J. Clin. Endocrinol. Metab. 100 (11) (2015) 4029–4036.
[29] K. Linder, F. Schleger, C. Ketterer, L. Fritsche, I. Kiefer-Schmidt, A. Hennige, et al., Maternal insulin sensitivity is associated with oral glucose-induced changes in fetal brain activity, Diabetologia 57 (6) (2014) 1192–1198.
[30] J. McCubbin, S.E. Robinson, R. Cropp, A. Moiseev, J. Vrba, P. Murphy, et al., Optimal reduction of MCG in fetal MEG recordings, IEEE Trans. Biomed. Eng. 53 (8) (2006) 1720–1724.
[31] J. Vrba, S.E. Robinson, J. McCubbin, C.L. Lowery, H. Eswaran, J.D. Wilson, et al., Fetal MEG redistribution by projection operators, IEEE Trans. Biomed. Eng. 51 (7) (2004) 1207–1218.
[32] J.D. Wilson, R.B. Govindan, J.O. Hatton, C.L. Lowery, H. Preissl, Integrated approach for fetal QRS detection, IEEE Trans. Biomed. Eng. 55 (9) (2008) 2190–2197.
[33] E. Shalev, M.J. Benett, E. Megory, R.M. Wallace, H. Zuckerman, Fetal habituation to repeated sound stimulation, Isr. J. Med. Sci. 25 (2) (1989) 77–80.
[34] L.J. Groome, K.P. Singh, S.L. Burgard, C.L. Neely, M.A. Deason, Motor responsivity during habituation testing of normal human fetuses, J. Perinat. Med. 23 (3) (1995) 159–166.
[35] K.J. Gerhardt, R.M. Abrams, X. Huang, S.K. Griffiths, A.J. Peters, Intra-abdominal sound pressure levels during impulse noise exposure in sheep, Mil. Med. 165 (2) (2000) 153–156.
[36] K.J. Gerhardt, R.M. Abrams, Fetal exposures to sound and vibroacoustic stimulation, J. Perinatol. 20 (8 Pt 2) (2000) S21–S30.

[37] K.J. Gerhardt, R. Otto, R.M. Abrams, J.J. Colle, D.J. Burchfield, A.J. Peters, Cochlear microphonics recorded from fetal and newborn sheep, Am. J. Otolaryngol. 13 (4) (1992) 226–233.
[38] B.S. Kisilevsky, B. Chambers, K.C.H. Parker, G.A.L. Davies, Auditory processing in growth-restricted fetuses and newborns and later language development, Clin. Psychol. Sci. 2 (4) (2014) 495–513.
[39] J. Muenssinger, K.T. Stingl, T. Matuz, G. Binder, S. Ehehalt, H. Preissl, Auditory habituation to simple tones: reduced evidence for habituation in children compared to adults, Front. Hum. Neurosci. 7 (2013) 377.
[40] S. Morokuma, V. Doria, A. Ierullo, N. Kinukawa, K. Fukushima, H. Nakano, et al., Developmental change in fetal response to repeated low-intensity sound, Dev. Sci. 11 (1) (2008) 47–52.
[41] H. McIsaac, J. Polich, Comparison of infant and adult P300 from auditory stimuli, J. Exp. Child Psychol. 53 (2) (1992) 115–128.
[42] H. Okamoto, R. Kakigi, Neural adaptation to silence in the human auditory cortex: a magnetoencephalographic study, Brain Behav. 4 (6) (2014) 858–866.
[43] P.S. Kaplan, J.S. Werner, Habituation, response to novelty, and dishabituation in human infants: tests of a dual-process theory of visual attention, J. Exp. Child Psychol. 42 (2) (1986) 199–217.
[44] M.A. Hunter, E.W. Ames, R. Koopman, Effects of stimulus complexity and familiarization time on infant preferences for novel and familiar stimuli, Dev. Psychol. 19 (3) (1983) 338–352.
[45] T. Rosburg, P. Soros, The response decrease of auditory evoked potentials by repeated stimulation - is there evidence for an interplay between habituation and sensitization? Clin. Neurophysiol. 127 (1) (2016) 397–408.
[46] T.W. Budd, R.J. Barry, E. Gordon, C. Rennie, P.T. Michie, Decrement of the N1 auditory event-related potential with stimulus repetition: habituation vs. refractoriness, Int. J. Psychophysiol. 31 (1) (1998) 51–68.
[47] L.R. Leader, P. Baillie, B. Martin, E. Vermeulen, The assessment and significance of habituation to a repeated stimulus by the human fetus, Early Hum. Dev. 7 (3) (1982) 211–219.
[48] E.V. Kushnerenko, B.R. Van den Bergh, I. Winkler, Separating acoustic deviance from novelty during the first year of life: a review of event-related potential evidence, Front. Psychol. 4 (2013) 595.
[49] J.G. Nijhuis, Behavioural states: concomitants, clinical implications and the assessment of the condition of the nervous system, Eur. J. Obstet. Gynecol. Reprod. Biol. 21 (5–6) (1986) 301–308.

4 Impact of intrauterine growth restriction on cognitive and motor development at 2 years of age (Study 3)

In study 3, the impact of IUGR as a risk factor for pathological intrauterine development on fetal brain development and later child development was investigated. Three fetal groups were compared: IUGR, SGA and AGA. During the fetal period, visual and auditory ERRs were recorded by fMEG. When the children reached the age of about 24 months, BSID-II was conducted to assess cognitive (represented by MDI) and motor developmental status (represented by PDI) of the infants.

In the IUGR group, the latencies of the visual ERRs were longer than those of the SGA and AGA groups (however, the difference was not statistically significant). The MDI was significantly lower in former IUGR-infants than in former SGA- and AGA-infants, whereas there were no differences in the PDI. Results of this study endorse the assumption that IUGR affects brain development *in utero*, as reflected by the lower MDI scores at 24 months, and supported by longer latencies of fetal EERs during visual stimulus processing. Such differences may therefore have long-term-effects on future neurodevelopment. The underlying causes of these changes and potentially persisting long-term effects should be further explored.

Author contribution: The material of this chapter was published in Frontiers in Physiology (Hartkopf et al., 2018). Julia Hartkopf, Hubert Preissl and Isabelle Kiefer-Schmidt conceived the study. Magdalene Weiss, Jan Pauluschke-Froehlich, Sara Brucker and Isabelle Kiefer-Schmidt recruited participants and performed fetal ultrasounds and Doppler measurements. Julia Hartkopf, Jana Keune and Magdalene Weiss acquired fMEG-data. Cornelia Wiechers conducted physical examinations of neonates. Julia Hartkopf and Jana Keune performed the cognitive and motor development assessments. Data were statistically analyzed and interpreted by Julia Hartkopf, who was assisted by Annette Conzelmann, Franziska Schleger and Hubert Preissl. Julia Hartkopf drafted the manuscript with the support of Franziska Schleger, Hubert Preissl and Isabelle Kiefer-Schmidt. All authors revised the final manuscript.

frontiers in Physiology

ORIGINAL RESEARCH
published: 19 September 2018
doi: 10.3389/fphys.2018.01278

Impact of Intrauterine Growth Restriction on Cognitive and Motor Development at 2 Years of Age

Julia Hartkopf[1,2,3], Franziska Schleger[1,2,3], Jana Keune[4], Cornelia Wiechers[5], Jan Pauluschke-Froehlich[6], Magdalene Weiss[3,6], Annette Conzelmann[7], Sara Brucker[6], Hubert Preissl[1,2,3,8] and Isabelle Kiefer-Schmidt[3,6]*

[1] Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tuebingen, Tuebingen, Germany, [2] German Center for Diabetes Research (DZD e.V.), Tuebingen, Germany, [3] fMEG Center, University of Tuebingen, Tuebingen, Germany, [4] Department of Neurology, Klinikum Bayreuth GmbH, Bayreuth, Germany, [5] Department of Neonatology, University of Tuebingen, Tuebingen, Germany, [6] Department of Women's Health, University of Tuebingen, Tuebingen, Germany, [7] Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University of Tuebingen, Tuebingen, Germany, [8] Department of Internal Medicine, Division of Endocrinology, Diabetology, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Tuebingen, Germany

OPEN ACCESS

Edited by:
Suzanne Lee Miller, Monash University, Australia

Reviewed by:
Michelle Welsh, University of Glasgow, United Kingdom
Atul Malhotra, Monash Health, Australia

****Correspondence:***
Julia Hartkopf
Julia.hartkopf@med.uni-tuebingen.de

Specialty section:
This article was submitted to Reproduction, a section of the journal Frontiers in Physiology

Received: *17 May 2018*
Accepted: *22 August 2018*
Published: *19 September 2018*

Citation:
Hartkopf J, Schleger F, Keune J, Wiechers C, Pauluschke-Froehlich J, Weiss M, Conzelmann A, Brucker S, Preissl H and Kiefer-Schmidt I (2018) Impact of Intrauterine Growth Restriction on Cognitive and Motor Development at 2 Years of Age. Front. Physiol. 9:1278. doi: 10.3389/fphys.2018.01278

Intrauterine growth restriction (IUGR), which is already known to be a risk factor for pathological intrauterine development, perinatal mortality, and morbidity, is now also assumed to cause both physical and cognitive alterations in later child development. In the current study, effects of IUGR on infantile brain function were investigated during the fetal period and in a follow-up developmental assessment during early childhood. During the fetal period, visual and auditory event-related responses (VER and AER) were recorded using fetal magnetoencephalography (fMEG). VER latencies were analyzed in 73 fetuses (14 IUGR fetuses) while AER latencies were analyzed in 66 fetuses (11 IUGR fetuses). Bayley Scales of Infant Development, Second Edition (BSID-II) were used to assess the developmental status of the infants at the age of 24 months. The Mental Development Index (MDI) was available from 66 children (8 IUGR fetuses) and the Psychomotor Development Index (PDI) from 63 children (7 IUGR fetuses). Latencies to visual stimulation were more delayed in IUGR than in small for gestational age (SGA) or appropriate for gestational age (AGA) fetuses, albeit not to any significant extent (p = 0.282). The MDI in former IUGR infants was significantly lower (p = 0.044) than in former SGA and AGA infants. However, IUGR had no impact on PDI (p = 0.213). These findings support the hypothesis that IUGR may constitute a risk factor for neurodevelopmental delay. Further investigation of the possible underlying mechanisms, as well as continued long-term developmental research, is therefore necessary.

Keywords: intrauterine growth restriction, child development, fetal magnetoencephalography, visual event-related responses (VER), auditory event-related responses (AER)

Abbreviations: AER, auditory event-related responses; AGA, appropriate for gestational age; BSID-II, Bayley Scales of Infant Development, Second Edition; BSID-III, Bayley Scales of Infant and Toddler Development, Third Edition; fMEG, fetal magnetoencephalography; fT, femto Tesla; GA, gestational age; Hz, Hertz; ISI, inter-stimulus interval; IUGR, intrauterine growth restriction; MDI, Mental Development Index; ms, millisecond; PDI, Psychomotor Development Index; SGA, small for gestational age; VER, visual event-related responses.

INTRODUCTION

Over the last few decades, it has become evident that events during early development in humans – even during the prenatal phase – can have long-term effects on health and disease. This concept is commonly known as Developmental Origins of Health and Disease (Barker, 2007; Wadhwa et al., 2009).

One trademark of anomalous prenatal development is intrauterine growth restriction (IUGR). Intrauterine growth restriction is characterized by a pathological restriction of fetal weight, as is presumed to be the case when a fetus is "small for gestational age" (SGA), i.e., when its estimated fetal weight and birth weight are below the 10th percentile for gestational age (GA). The literature and practice often does not distinguish clearly between IUGR and SGA. Consistent criteria are therefore required to establish general valid guidelines in diagnosis and treatment (Barker et al., 2013; Unterscheider et al., 2014; Levine et al., 2015). SGA, which is a more general term for those fetuses and infants whose estimated and actual birth weights are below the 10th percentile, is not necessarily connected with a pathological finding. The term also includes cases of below-average weight caused by genetic preconditions. By contrast, IUGR is associated with pathological intrauterine changes that cause restricted fetal growth. It is also linked to a higher risk of perinatal mortality and morbidity and requires appropriate medical support (Craigo, 1994; Bamberg and Kalache, 2004). It is important to distinguish between pathologically growth-restricted fetuses and constitutionally small fetuses. Placental insufficiency, the most frequently observed pathological cause for restricted fetal growth, should be diagnosed by the umbilical artery Doppler velocity (Figueras and Gardosi, 2011). Placental insufficiency is associated with metabolic and hormonal influences on the fetuses and manifests itself by reduced fetal growth and weight gain during pregnancy. These processes can lead to specific alterations in later physical and cognitive development known as "fetal programming" (Godfrey and Barker, 2001; Martin-Gronert and Ozanne, 2012). Since this influence begins during pregnancy, an early investigational approach is advisable.

Recent follow-up studies with former IUGR infants often used only reduced body size or abnormal Doppler for diagnosis of IUGR. A review by Murray et al. (2015) showed that only a small number of studies on the neurodevelopmental outcome in children with IUGR born at 35 weeks of gestation or later used both abnormal Doppler and small size as diagnostic criteria. The authors reported that IUGR is associated with an increased risk for neurodevelopmental delay. Children with fetal circulatory redistribution (i.e., a pathological Doppler) were reported to be more severely affected.

Neurodevelopmental impairments in IUGR infants are reflected by morphological and structural brain alterations and impaired brain function even in utero (D'Hooghe and Odendaal, 1991; Vindla et al., 1997; Nijhuis et al., 2000; Tolsa et al., 2004; Dubois et al., 2008; Lodygensky et al., 2008). In earlier trials, changes in body movements and heart rate were the two main indicators for stimulus processing for investigating the influence of IUGR on functional brain development in utero. Following acoustic or vibroacoustic stimulation, heart rate responses in IUGR fetuses were delayed and their body movement patterns lower than in controls (Gagnon et al., 1988, 1989; Kisilevsky et al., 2014).

Fetal magnetoencephalography (fMEG) is a non-invasive method for measuring fetal brain activity. From the GA of 28 weeks onward, fetal auditory event-related brain responses (AER) and visual event-related brain responses (VER) can be recorded and a decrease of latency can be assumed to be a marker of the maturation and integrity of functional fetal brain development (Schleussner et al., 2001; Eswaran et al., 2002; Schleussner and Schneider, 2004; Holst et al., 2005; Kiefer et al., 2008). Against this background, by demonstrating that IUGR fetuses have slower VER than their appropriate for gestational age (AGA) control counterparts, we recently ascertained that VER latency is associated with fetal outcome (Morin et al., 2015). A follow-up study to determine the impact of VER latencies on early childhood development, i.e., from birth to 24 months of age, is currently under way.

In the present study, we aimed to determine whether fetal outcome affects early childhood development. This entailed a developmental assessment using BSID-II that was performed at the age of 24 months in former IUGR, SGA, and AGA children. Furthermore, we investigated whether VER and AER latencies, as assessed by fMEG, differed between the fetal outcome groups.

MATERIALS AND METHODS

Participants

One hundred and seven women with singleton pregnancies were recruited by the Department of Obstetrics and Gynecology at the University Hospital, Tuebingen. They gave written informed consent of their and their infant's participation prior to the study, which was approved by the local Ethical Committee of the Medical Faculty of the University of Tuebingen (No. 476/2008MPG1). The study was performed in accordance with the relevant guidelines and regulations.

Fifteen of the infants had birth weights below the 10th percentile, and an increased umbilical artery pulsatility index above the 90th percentile for the respective GA was observed during pregnancy. These 15 fetuses were classified as IUGR due to an insufficient placental blood supply. Although 32 of the infants were born with weights below the 10th percentile, they had a normal umbilical artery Doppler during pregnancy and no placental insufficiency was found. These 32 fetuses were classified as constitutionally SGA. Sixty healthy children with an AGA birth weight were included as controls.

fMEG Measurement

To investigate potential differences in brain development already during pregnancy, all participants underwent an fMEG measurement with visual and auditory stimulation to record event-related brain responses of the fetuses from 28 weeks of GA. The fMEG measurement was performed with a magnetoencephalographic system for fetal and neonatal studies (SARA II: SQUID Array for Reproductive Assessment,

VSM MedTech Ltd., Port Coquitlam, BC, Canada). During the measurement, the woman placed her abdomen in an ergonomically shaped array containing 156 primary and 29 reference sensors. Visual stimuli were presented during 10 min of the measurement and consisted of light flashes delivered by fiber optic wire to an LED-light pad that was placed on the maternal abdomen near the location of a fetal eye, as determined via ultrasound. The light flashes had a wavelength of 625 nm and an intensity of 8000 lux; stimulus duration was 500 ms and the ISI was set at random between 1.5 and 2.5 s (Morin et al., 2015).

Auditory stimulation consisted of an oddball-paradigm with pure tones and was presented for a further 10 min of the measurement. Stimulus duration was 500 ms and the ISI was randomly selected between 1900 and 2100 ms. Standard tones, presented with a frequency of 500 Hz, were interspersed with deviant tones presented at 750 Hz to avoid habituation to the standard tone. Stimuli were delivered into a balloon via an air-filled tube placed on the maternal abdomen. The sequence of visual and auditory stimulation was randomized over subjects. Fetal data were recorded with a sampling rate of 610.352 Hz (Muenssinger et al., 2013).

fMEG Data Analysis

Recorded fetal auditory and visual datasets were filtered offline with a high-pass filter of 0.5 Hz and were transformed by a first-order gradiometer to eliminate any external interference. Maternal and fetal heart signals were attenuated by signal space projection (McCubbin et al., 2006). The data was cut into segments ranging from 200 ms before to 1000 ms after the stimulus. A 10 Hz low-pass filter was applied and the average of the segments was calculated. VER and AER were analyzed by visual examination and defined as a peak in a time window of 80–500 ms after the stimulus, with a minimal amplitude of 4 fT in at least four sensors around the fetal head coil. The latency between stimulus onset and peak was documented for further statistical analysis.

Developmental Test

Two years after fMEG measurement, all families were invited to participate in an assessment of their child's development with BSID-II. Of a total of 107 participants, 66 returned for the follow-up assessment. The 41 participants who discontinued were distributed as follows: IUGR group: 7 of 15 children (46.7%), SGA group: 14 of 32 children (43.8%) and AGA group: 20 of 40 children (50%). The most common reasons for non-participation are summarized in **Figure 1**.

The BSID-II was developed for the measurement of the current developmental state of infants and children between 1 and 42 months of age (Nellis and Gridley, 1994). An experienced and trained psychologist, who was unaware of the medical history of the infant, conducted the test with the child in the presence of a parent. The BSID-II is divided into two scales: the MDI and PDI. The cognitive and psychomotor development of a child can therefore be assessed separately. MDI and PDI both have a mean of 100 and a standard deviation (SD) of 15.

Statistics

Data was described as mean ± SD. A preliminary assumption check revealed that data was normally distributed, as assessed by Shapiro–Wilk test ($p > 0.05$), and that there were no univariate or multivariate outliers, as assessed by boxplot and Mahalanobis distance ($p > 0.001$), respectively. MDI, PDI, VER latency, and AER latency were analyzed for differences between fetal outcome groups (IUGR, SGA, and AGA) using one-way ANOVA and Welch's test of unequal variances, respectively. *Post hoc* analyses were performed using the Games-Howell correction method. PASW Statistics 21 (SPSS Inc., Chicago, IL, United States) was used for statistical analysis and the significance level was set to $p < 0.05$.

RESULTS

Auditory event-related responses and VER latencies were measured using fMEG in 107 fetuses. Of these, 15 were IUGR fetuses, 32 were SGA fetuses and 60 were AGA fetuses. **Table 1** shows mean and SD for GA at birth and birth weight. VER latencies could be analyzed in a total of 73 fetuses (14 IUGR, 22 SGA, and 37 AGA) at a mean GA of 34.1 weeks. AER latencies were detectable in a total of 66 fetuses (11 IUGR, 22 SGA, and 33 AGA) at a mean GA of 34.0 weeks.

Mental Development Index and PDI were assessed using BSID-II at a mean (± SD) age of 24.10 (± 0.79) months. MDI was assessed in 66 children (8 IUGR, 18 SGA, and 40 AGA) and was 96 (± 6), 100 (± 16), and 103 (± 13), respectively. PDI was assessed in 63 children (7 IUGR, 16 SGA, and 40 AGA) and was 94 (± 7), 96 (± 11), and 100 (± 10), respectively. Results are presented as box plots in **Figure 2**.

Table 2 shows the results of the univariate one-way ANOVA in a comparison of MDI, PDI, VER latency, and AER latency in the IUGR, SGA, and AGA groups. There were no statistically significant differences between PDI ($p = 0.213$), VER latency ($p = 0.282$), and AER latency ($p = 0.206$). However, the MDI differed significantly between groups ($p = 0.044$) and increased from the IUGR (96 ± 6) to the SGA (100 ± 16) as well as to the AGA group (103 ± 13). Games-Howell *post hoc* analysis (**Table 3**) revealed that the difference between IUGR and AGA was statistically significant ($p = 0.035$).

DISCUSSION

In the current study, we aimed to investigate the impact of IUGR on early child development. At the age of two, children's developmental status was assessed using BSID-II. The MDI was significantly lower in the IUGR than in the AGA group. Although scores for the PDI decreased from AGA to SGA, and IUGR, these differences were not statistically significant. In addition, fetal brain responses to visual and sound stimulation were assessed via fMEG before birth. We observed an increase in VER latencies from AGA over SGA to IUGR fetuses. These latency differences were, however, not statistically significant. Our results suggest that functional brain development maybe

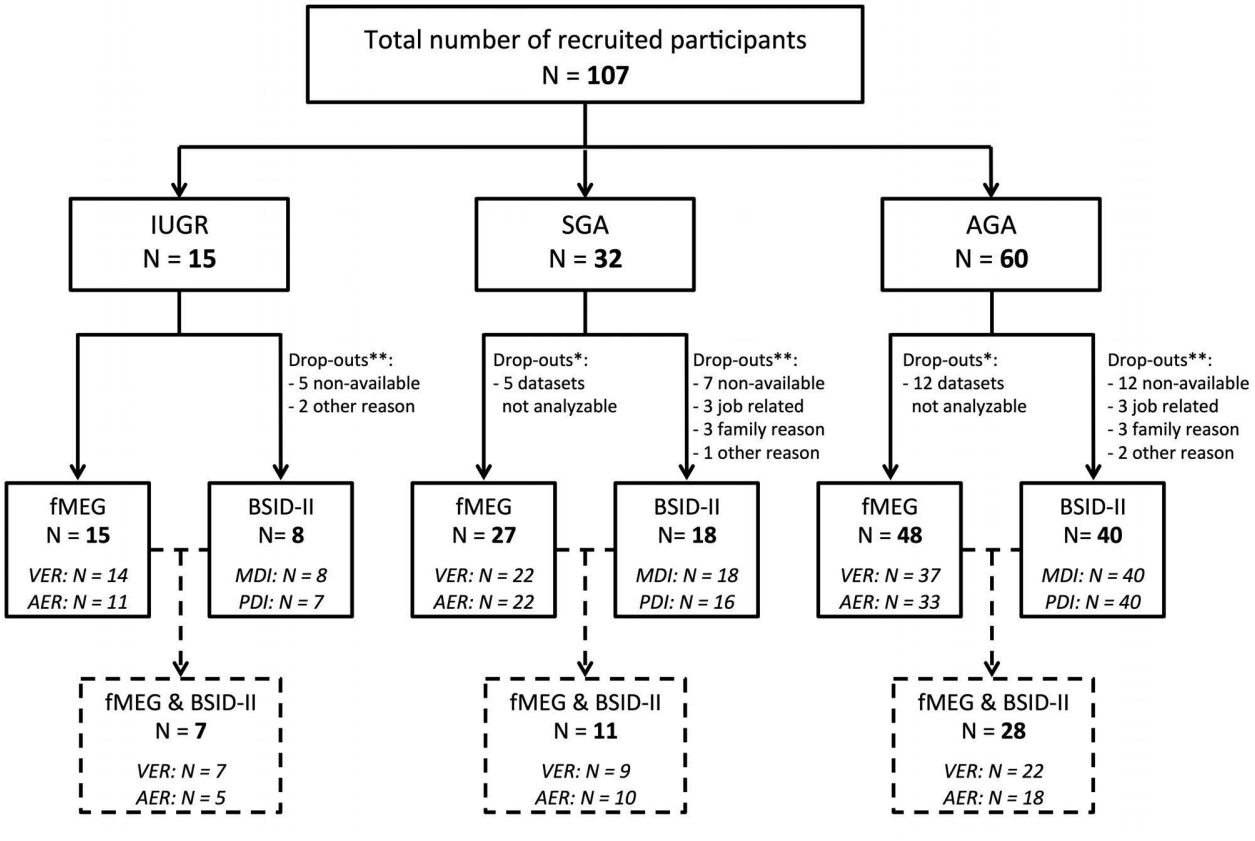

FIGURE 1 | Flow chart showing enrollment and follow-up. All 107 infants were evaluated using fMEG. At the age of 2 years, developmental assessment was performed using BSID-II. *Indicates the number of fMEG data sets that were not analyzable. **Indicates the number of children that were lost to follow-up. At the bottom of the figure, the number of children for whom BSID-II and fMEG was available is shown.

TABLE 1 | Mean weeks (wks) of gestational age (GA) at the time of VER measurement, AER measurement and birth as well as birth weight in grams (g) of IUGR versus SGA versus AGA fetus.

Fetal group		GA at VER (wks)	GA at AER (wks)	GA at birth (wks)	Birth weight (g)
IUGR	Mean	33.6	33.8	35.5	1720
	N	14	11	15	15
	SD	2.7	2,6	2.8	405
SGA	Mean	34.7	34.1	38.6	2446
	N	22	23	32	32
	SD	3.0	3.4	2.1	401
AGA	Mean	33.9	33.9	40.0	3454
	N	37	34	60	60
	SD	3.0	3.1	1,6	412

already altered during gestation and might cause an alteration in the neurological developmental trajectory in later life. However, it must be emphasized that these findings are based on a relatively small group of children.

Intrauterine growth restriction, a pathologic growth restriction of fetuses, is associated with significant neonatal morbidity and mortality (Nardozza et al., 2017). It is also believed to impact morphological and structural brain development (D'Hooghe and Odendaal, 1991; Vindla et al., 1997; Nijhuis et al., 2000; Tolsa et al., 2004; Dubois et al., 2008; Lodygensky et al., 2008). We recently reported that latencies of fetal AER and VER assessed by fMEG are delayed in fetuses with IUGR (Morin et al., 2015). In the present study, however, the differences in VER and AER latencies between IUGR, SGA, and AGA fetuses were not statistically different. A possible explanation for these seemingly contradictory findings may be due to the fact that we had used a case control approach in the previous study to match subjects for GA and fetal behavioral state. Since our primary focus in the present study was on the effect of IUGR on neurodevelopmental changes at 2 years of age, we decided to increase sample size by including not only matched pairs of SGA-AGA and IUGR-AGA subjects but also of all other subjects. For proof of the possible predictive value of fMEG, further studies with larger population sizes and longitudinal assessment of functional brain development are necessary.

In the current study, we used simple tone stimulation only. However, since several cognitive capabilities such as discrimination and habituation are already established in the last trimester of gestation, it would be worthwhile to apply these stimulation paradigms to determine whether they are

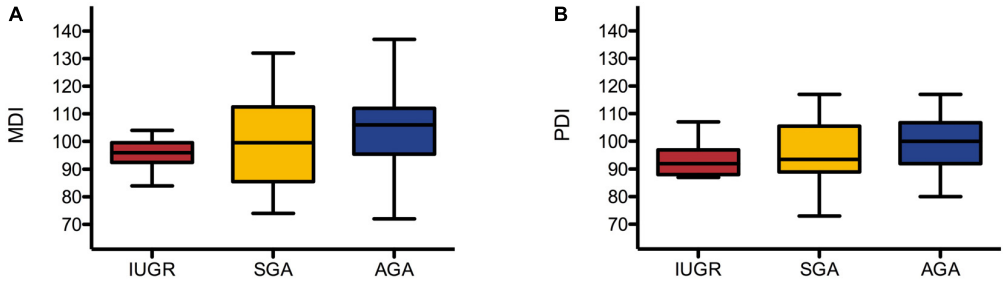

FIGURE 2 | Box plots of MDI **(A)** and PDI **(B)** in IUGR, SGA, and AGA group. Box plots represent median, interquartiles, and ranges.

TABLE 2 | MDI, PDI, VER latency and AER latency in IUGR, SGA, and AGA groups as calculated using one-way ANOVA.

		N	Mean	*SD*	*p*-value
MDI	IUGR	8	96	6	
	SGA	18	100	16	
	AGA	40	103	13	
	Total	66	101	13	0.044*
PDI	IUGR	7	94	7	
	SGA	16	96	11	
	AGA	40	100	10	
	Total	63	98	10	0.213
VER latency	IUGR	14	233	59	
	SGA	22	217	64	
	AGA	37	204	57	
	Total	73	213	60	0.282
AER latency	IUGR	11	204	65	
	SGA	22	220	61	
	AGA	33	188	69	
	Total	66	201	67	0.206

*Welch's t-test was used on account of unequal variances and sample-sizes and *indicates a significant group difference.*

TABLE 3 | Comparisons of the MDI between the IUGR, SGA, and AGA groups.

	IUGR	SGA	AGA
IUGR	–	−4.44 (± 4.33) *p* = 0.568	−7.83 (± 2.91) *p* = 0.035
SGA	4.44 (± 4.33) *p* = 0.568	–	−3.38 (± 4.26) *p* = 0.710
AGA	7.83 (± 2.91) *p* = 0.035	3.38 (± 4.26) *p* = 0.710	–

The differences of mean MDI values (± standard error) are shown and the respective p-values were calculated by Games-Howell post hoc analysis.

more specific for alterations of early fetal brain development (Draganova et al., 2005; Matuz et al., 2012; Muenssinger et al., 2013; Hartkopf et al., 2016). Interestingly, intrauterine auditory stimulation with the maternal voice in growth-restricted fetuses has been proposed as a potential tool to compensate brain alterations that might be responsible for later language impairment (Kisilevsky et al., 2014).

When it came to childhood development, we observed lower cognitive and psychomotor abilities in IUGR than in AGA children, although only the differences in cognitive (mental) scores were of statistical significance. In line with our results, earlier trials showed that former IUGR infants are more liable to achieve lower scores in neurocognitive and/or motor developmental assessment tests than control children without IUGR (for a review, see Murray et al., 2015). The comparability of studies on neurocognitive development of IUGR children, is, however, limited due to the selection criteria for growth restriction. Unlike reduced growth in SGA fetuses, which is usually constitutional, the growth delay in IUGR has a pathological cause. We therefore identified IUGR fetuses by using ultrasound to estimate fetal weight as well as to measure the umbilical artery pulsatility index. The latter is a marker of placental blood supply and a clinical standard to monitor intrauterine malnutrition (Murray et al., 2015). In a follow-up sample of 83 very-low-birth-weight infants, Leppanen et al. used the mental scale of BSID-II to show that only the subgroup with a pathological Doppler was affected by an altered cognitive outcome at the age of 2 years, whereas motor development remained unaffected (Leppanen et al., 2010). This is akin to the present study: PDI of BSID-II did not reveal any differences in psychomotor development between IUGR, SGA, and AGA children at 2 years of age. Several other studies investigating motor outcomes in IUGR children also reported that no differences were observed (Wienerroither et al., 2001; Eixarch et al., 2008; Padilla et al., 2010). Some study results indicate an influence of prematurity and severity of IUGR on motor development (Gazzolo et al., 1995; Padilla et al., 2011).

The MDI of the BSID-II includes measures for different cognitive skills, i.e., active and passive speech development, problem solving, or memory performance. The updated version "Bayley Scales of Infant and Toddler Development, Third Edition" (BSID-III) provides more specific subscores: a cognitive scale, a receptive language and an expressive language scale. To establish specific approaches to support affected infants and their families with early interventions, the assessment should be performed with the updated version in future investigations. However, the German version of the third edition was not available at the time of this study, nor is a behavioral scale, as provided by the original versions of BSID-II and BSID-III, available in the German language to date. Results of studies investigating behavioral

changes in former IUGR children indicate that attention, social-interactive skills or mood might also be affected (Roza et al., 2008; Beukers et al., 2017).

The major limitation of the current study is the low sample size, particularly for the IUGR group. Future studies with larger sample sizes should consider co-factors such as onset, duration and severity of IUGR to gain more detailed information about the impact of different types of IUGR (Miller et al., 2016). Moreover, loss to follow-up might be influenced by socioeconomic or demographic factors and might therefore bias our results (see **Figure 1** for drop-out at the different stages).

CONCLUSION

The results of this study support the hypothesis that IUGR might be a risk factor for a delay in neurocognitive development (MDI) in two-year old children. However, the differences were only modest, and not significant with respect to the PDI, and the three study groups did not differ significantly in fetal event-related brain activity. The investigation of underlying physiological processes and their impact on human brain development should be the focus of further research. Moreover, larger trials with a standardized definition of IUGR and well-defined outcome measures are required to identify factors that impact the role of IUGR on child development. These findings would be instrumental in developing specific treatment and support for the affected infants and their families.

AUTHOR CONTRIBUTIONS

JH, HP and IK-S conceived and designed the study. MW, JP-F, SB, and IK-S recruited the participants. JP-F, SB, and IK-S performed fetal ultrasound and Doppler measurements. JH, JK, and MW carried out fMEG measurements. CW conducted the physical examination of neonates. JK and JH performed Bayley testing. JH, AC, FS and HP analyzed the data and were responsible for statistics. JH, FS, HP and IK-S prepared the draft manuscript. All authors made substantial corrections for the final manuscript.

FUNDING

This work was partly supported by the German Research Foundation (DFG BI 195/50 and KI 1306/3-1), the University of Tuebingen (E.05.00303 and E.05.0259.1), Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.), and the Foundation of Baden-Wuerttemberg. We acknowledge support by the German Research Foundation and Open Access Publishing Fund of University of Tuebingen.

ACKNOWLEDGMENTS

We are very grateful to all the families who participated in our study.

REFERENCES

Bamberg, C., and Kalache, K. D. (2004). Prenatal diagnosis of fetal growth restriction. *Semin. Fetal Neonatal. Med.* 9, 387–394. doi: 10.1016/j.siny.2004.03.007

Barker, D. J. (2007). The origins of the developmental origins theory. *J. Intern. Med.* 261, 412–417. doi: 10.1111/j.1365-2796.2007.01809.x

Barker, E. D., McAuliffe, F. M., Alderdice, F., Unterscheider, J., Daly, S., Geary, M. P., et al. (2013). The role of growth trajectories in classifying fetal growth restriction. *Obstet. Gynecol.* 122(2 Pt 1), 248–254. doi: 10.1097/AOG.0b013e31829ca9a7

Beukers, F., Aarnoudse-Moens, C. S. H., van Weissenbruch, M. M., Ganzevoort, W., van Goudoever, J. B., and van Wassenaer-Leemhuis, A. G. (2017). Fetal growth restriction with brain sparing: neurocognitive and behavioral outcomes at 12 years of age. *J. Pediatr.* 188:e102. doi: 10.1016/j.jpeds.2017.06.003

Craigo, S. D. (1994). The role of ultrasound in the diagnosis and management of intrauterine growth retardation. *Semin. Perinatol.* 18, 292–304.

D'Hooghe, T. M., and Odendaal, H. J. (1991). Fewer accelerations and decreased long term variability in the heart rate of small for gestational age fetuses. *Int. J. Gynaecol. Obstet.* 35, 133–138. doi: 10.1016/0020-7292(91)90816-N

Draganova, R., Eswaran, H., Murphy, P., Huotilainen, M., Lowery, C., and Preissl, H. (2005). Sound frequency change detection in fetuses and newborns, a magnetoencephalographic study. *Neuroimage* 28, 354–361. doi: 10.1016/j.neuroimage.2005.06.011

Dubois, J., Benders, M., Borradori-Tolsa, C., Cachia, A., Lazeyras, F., Ha-Vinh Leuchter, R., et al. (2008). Primary cortical folding in the human newborn: an early marker of later functional development. *Brain* 131(Pt 8), 2028–2041. doi: 10.1093/brain/awn137

Eixarch, E., Meler, E., Iraola, A., Illa, M., Crispi, F., Hernandez-Andrade, E., et al. (2008). Neurodevelopmental outcome in 2-year-old infants who were small-for-gestational age term fetuses with cerebral blood flow redistribution. *Ultrasound Obstet. Gynecol.* 32, 894–899. doi: 10.1002/uog.6249

Eswaran, H., Wilson, J., Preissl, H., Robinson, S., Vrba, J., Murphy, P., et al. (2002). Magnetoencephalographic recordings of visual evoked brain activity in the human fetus. *Lancet* 360, 779–780. doi: 10.1016/S0140-6736(02)09905-1

Figueras, F., and Gardosi, J. (2011). Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. *Am. J. Obstet. Gynecol.* 204, 288–300. doi: 10.1016/j.ajog.2010.08.055

Gagnon, R., Hunse, C., Carmichael, L., and Patrick, J. (1989). Vibratory acoustic stimulation in 26- to 32-week, small-for-gestational-age fetus. *Am. J. Obstet. Gynecol.* 160, 160–165. doi: 10.1016/0002-9378(89)90111-7

Gagnon, R., Hunse, C., Fellows, F., Carmichael, L., and Patrick, J. (1988). Fetal heart rate and activity patterns in growth-retarded fetuses: changes after vibratory acoustic stimulation. *Am. J. Obstet. Gynecol.* 158, 265–271. doi: 10.1016/0002-9378(88)90135-4

Gazzolo, D., Visser, G. H., Santi, F., Magliano, C. P., Scopesi, F., Russo, A., et al. (1995). Behavioural development and Doppler velocimetry in relation to perinatal outcome in small for dates fetuses. *Early. Hum. Dev* 43, 185–195. doi: 10.1016/0378-3782(95)01676-7

Godfrey, K. M., and Barker, D. J. (2001). Fetal programming and adult health. *Public Health Nutr.* 4, 611–624. doi: 10.1079/PHN2001145

Hartkopf, J., Schleger, F., Weiss, M., Hertrich, I., Kiefer-Schmidt, I., Preissl, H., et al. (2016). Neuromagnetic signatures of syllable processing in fetuses and infants provide no evidence for habituation. *Early. Hum. Dev.* 100, 61–66. doi: 10.1016/j.earlhumdev.2016.04.002

Holst, M., Eswaran, H., Lowery, C., Murphy, P., Norton, J., and Preissl, H. (2005). Development of auditory evoked fields in human fetuses and newborns: a longitudinal MEG study. *Clin. Neurophysiol.* 116, 1949–1955. doi: 10.1016/j.clinph.2005.04.008

Kiefer, I., Siegel, E., Preissl, H., Ware, M., Schauf, B., Lowery, C., et al. (2008). Delayed maturation of auditory-evoked responses in growth-restricted fetuses revealed by magnetoencephalographic recordings. *Am. J. Obstet. Gynecol.* 199, 503.e1–503.e7. doi: 10.1016/j.ajog.2008.04.014

Kisilevsky, B. S., Chambers, B., Parker, K. C. H., and Davies, G. A. L. (2014). Auditory processing in growth-restricted fetuses and newborns and

later language development. *Clin. Psychol. Sci.* 2, 495–513. doi: 10.1177/2167702613509371

Leppanen, M., Ekholm, E., Palo, P., Maunu, J., Munck, P., Parkkola, R., et al. (2010). Abnormal antenatal Doppler velocimetry and cognitive outcome in very-low-birth-weight infants at 2 years of age. *Ultrasound Obstet Gynecol.* 36, 178–185. doi: 10.1002/uog.7694

Levine, T. A., Grunau, R. E., McAuliffe, F. M., Pinnamaneni, R., Foran, A., and Alderdice, F. A. (2015). Early childhood neurodevelopment after intrauterine growth restriction: a systematic review. *Pediatrics* 135, 126–141. doi: 10.1542/peds.2014-1143

Lodygensky, G. A., Seghier, M. L., Warfield, S. K., Tolsa, C. B., Sizonenko, S., Lazeyras, F., et al. (2008). Intrauterine growth restriction affects the preterm infant's hippocampus. *Pediatr. Res.* 63, 438–443. doi: 10.1203/PDR.0b013e318165c005

Martin-Gronert, M. S., and Ozanne, S. E. (2012). Mechanisms underlying the developmental origins of disease. *Rev. Endocr. Metab. Disord.* 13, 85–92. doi: 10.1007/s11154-012-9210-z

Matuz, T., Govindan, R. B., Preissl, H., Siegel, E. R., Muenssinger, J., Murphy, P., et al. (2012). Habituation of visual evoked responses in neonates and fetuses: a MEG study. *Dev. Cogn. Neurosci.* 2, 303–316. doi: 10.1016/j.dcn.2012.03.001

McCubbin, J., Robinson, S. E., Cropp, R., Moiseev, A., Vrba, J., Murphy, P., et al. (2006). Optimal reduction of MCG in fetal MEG recordings. *IEEE Trans. Biomed. Eng.* 53, 1720–1724. doi: 10.1109/TBME.2006.876619

Miller, S. L., Huppi, P. S., and Mallard, C. (2016). The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. *J. Physiol.* 594, 807–823. doi: 10.1113/JP271402

Morin, E. C., Schleger, F., Preissl, H., Braendle, J., Eswaran, H., Abele, H., et al. (2015). Functional brain development in growth-restricted and constitutionally small fetuses: a fetal magnetoencephalography case-control study. *BJOG* 122, 1184–1190. doi: 10.1111/1471-0528.13347

Muenssinger, J., Matuz, T., Schleger, F., Kiefer-Schmidt, I., Goelz, R., Wacker-Gussmann, A., et al. (2013). Auditory habituation in the fetus and neonate: an fMEG study. *Dev. Sci.* 16, 287–295. doi: 10.1111/desc.12025

Murray, E., Fernandes, M., Fazel, M., Kennedy, S. H., Villar, J., and Stein, A. (2015). Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review. *BJOG* 122, 1062–1072. doi: 10.1111/1471-0528.13435

Nardozza, L. M., Caetano, A. C., Zamarian, A. C., Mazzola, J. B., Silva, C. P., Marcal, V. M., et al. (2017). Fetal growth restriction: current knowledge. *Arch. Gynecol. Obstet.* 295, 1061–1077. doi: 10.1007/s00404-017-4341-9

Nellis, L., and Gridley, B. E. (1994). Review of the bayley scales of infant development—second edition. *J. Sch. Psychol.* 32, 201–209. doi: 10.1016/0022-4405(94)90011-6

Nijhuis, I. J., ten Hof, J., Mulder, E. J., Nijhuis, J. G., Narayan, H., Taylor, D. J., et al. (2000). Fetal heart rate in relation to its variation in normal and growth retarded fetuses. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 89, 27–33. doi: 10.1016/S0301-2115(99)00162-1

Padilla, N., Falcon, C., Sanz-Cortes, M., Figueras, F., Bargallo, N., Crispi, F., et al. (2011). Differential effects of intrauterine growth restriction on brain structure and development in preterm infants: a magnetic resonance imaging study. *Brain Res.* 1382, 98–108. doi: 10.1016/j.brainres.2011.01.032

Padilla, N., Perapoch, J., Carrascosa, A., Acosta-Rojas, R., Botet, F., and Gratacos, E. (2010). Twelve-month neurodevelopmental outcome in preterm infants with and without intrauterine growth restriction. *Acta Paediatr.* 99, 1498–1503. doi: 10.1111/j.1651-2227.2010.01848.x

Roza, S. J., Steegers, E. A., Verburg, B. O., Jaddoe, V. W., Moll, H. A., Hofman, A., et al. (2008). What is spared by fetal brain-sparing? Fetal circulatory redistribution and behavioral problems in the general population. *Am. J. Epidemiol.* 168, 1145–1152. doi: 10.1093/aje/kwn233

Schleussner, E., and Schneider, U. (2004). Developmental changes of auditory-evoked fields in fetuses. *Exp. Neurol.* 190(Suppl. 1), S59–S64. doi: 10.1016/j.expneurol.2004.04.008

Schleussner, E., Schneider, U., Kausch, S., Kahler, C., Haueisen, J., and Seewald, H. J. (2001). Fetal magnetoencephalography: a non-invasive method for the assessment of fetal neuronal maturation. *BJOG* 108, 1291–1294.

Tolsa, C. B., Zimine, S., Warfield, S. K., Freschi, M., Sancho Rossignol, A., Lazeyras, F., et al. (2004). Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. *Pediatr. Res.* 56, 132–138. doi: 10.1203/01.PDR.0000128983.54614.7E

Unterscheider, J., Daly, S., Geary, M. P., Kennelly, M. M., McAuliffe, F. M., O'Donoghue, K., et al. (2014). Definition and management of fetal growth restriction: a survey of contemporary attitudes. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 174, 41–45. doi: 10.1016/j.ejogrb.2013.11.022

Vindla, S., James, D. K., Sahota, D. S., and Coppens, M. (1997). Computerised analysis of behaviour in normal and growth-retarded fetuses. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 75, 169–175. doi: 10.1016/S0301-2115(97)00131-0

Wadhwa, P. D., Buss, C., Entringer, S., and Swanson, J. M. (2009). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Semin. Reprod. Med.* 27, 358–368. doi: 10.1055/s-0029-1237424

Wienerroither, H., Steiner, H., Tomaselli, J., Lobendanz, M., and Thun-Hohenstein, L. (2001). Intrauterine blood flow and long-term intellectual, neurologic, and social development. *Obstet. Gynecol.* 97, 449–453.

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.

The reviewer AM and handling Editor declared their shared affiliation at the time of the review.

5 General Discussion

As hypothesized by the theory of DOHaD, early exogenous influences may impact development and the subsequent state of health and disease. In this thesis, it was demonstrated that fMEG is applicable in examining brain function of fetuses and neonates by recording ERRs and in evaluating habituation as a basal form of learning. It was also ascertained that an early deprived exogenous condition (IUGR, due to an impaired placental blood flow) impacts fetal brain function which, in turn, may lead to an impaired cognitive development during early childhood.

The analysis of electromagnetic signatures to an external stimulation facilitates the evaluation of stimulus processing by recording its neural correlates. The use of different stimulation paradigms enables to draw conclusions about the state of sensory processing, basal learning competencies, and thus about functional brain development. For example, patients with, or persons with an enhanced risk of developing psychiatric disorders, habituate differently from healthy controls (Ludewig et al., 2003; Taiminen et al., 2000). ERR measurement provides the possibility of investigating habituation from a very early developmental state. For this purpose, however, it is necessary to compare the results of studies that assess ERRs and habituation in different age groups.

In a critical review of scientific articles, developmental changes of ERRs in different age groups were investigated (Study 1). Although changes were detected during infancy and/or childhood in 73 of the 85 studies, it was difficult to draw any general conclusions. Due to increasing myelination and formation of neural networks, it was anticipated that most of these changes would be present in the exogenous components of ERRs. However, even after a classification of studies into the respective sensory modality examined, and after splitting ERRs into separate components (P/N 100, P/N 200, P/N 300), there was a wide heterogeneity in results. Several variables, such as age of the participants, features of the stimulation paradigm selected, and the respective components investigated, seem to influence the results. In conclusion, studies that analyze ERRs in different age groups should take developmental

changes into account. Due to the diversity of the current study situation, however, this is rather challenging. Trials with comparable stimulation paradigms that allow replication as well as longitudinal studies are therefore required to generate more general and valid statements about developmental trends in ERRs.

In addition to studies concerning developmental changes of ERRs, developmental habituation studies were reviewed. Habituation enables to investigate basal learning abilities by observing behavior or by measuring neural responses to distinct stimulation paradigms. A total of 52 habituation studies were reviewed, the majority of which investigated relatively young subjects between the fetal stage and aged about 24 months (46 of 52 trials). Like in the review of developmental ERR changes, one focus was on studies with a neurophysiological approach. Neurophysiological measurements make it possible to directly evaluate the underlying neurophysiological correlates of habituation and do not rely on the observation of behavioral changes. In the eight neurophysiological habituation studies included here, only five tested for response decrement and/or stimulus specificity. However, response decrement to a repeated stimulus can also be explained by other factors such as sensory fatigue, or adaptation phenomena like refractoriness (Budd et al., 1998). It is therefore advisable to analyze several criteria of habituation, as stated by Rankin et al. (2009). Nevertheless, only three trials tested for response decrement, stimulus specificity and dishabituation. Another impact on ERRs analyzed in habituation paradigms is the length of the inter-stimulus interval (ISI) (Miltner et al., 1991). Like the studies about age-dependent changes in ERRs included in the review, the eight neurophysiological habituation studies showed a wide variety depending on the age groups, sensory modalities, stimulation paradigms, ISIs and the habituation criteria investigated. None of the eight studies investigated habituation with the same paradigm in more than two different age groups and, consequently, general statements about developmental changes on neurophysiological habituation are not possible. Longitudinal studies with standardized methods constitute an urgent prerequisite to deploy these techniques for the identification of groups at risk for impaired brain development.

In accordance with the recommendations of the literature review, an adapted habituation paradigm consisting of the syllables “ba” and “bi” in a neurophysiological measurement setting using fMEG in healthy fetuses and neonates was tested (Study 2). The aim was to show that the fMEG method can be used to monitor early processing of language stimuli and early basal learning competencies. Using a similar habituation paradigm consisting of pure tones, our group has already shown that habituation is present as early as in the fetus and assessable *in utero* via fMEG (Muenssinger et al., 2013a). ERRs of the syllables were recorded and three criteria of habituation (response decrement, stimulus specificity and dishabituation) were hypothesized to be found after data analysis. Since amplitudes of ERRs showed no decrement with repeated stimulation in either age group, habituation to the presented stimuli could not be verified. With significantly higher ERRs towards the dishabituator than to the subsequent stimulus, however, a discriminative neural response (stimulus specificity) was shown in infants but not in fetuses. This is at variance with earlier studies by our group as well as with behavioral habituation studies during the last trimester of pregnancy, indicating fetal and neonatal habituation to less complex stimuli (Bellieni et al., 2005; Hepper et al., 2012; Muenssinger et al., 2013a). Although it was possible to analyze ERRs to syllables, several reasons as to why habituation was not shown must be taken into consideration.

Firstly, the quality of the stimuli transferred to the fetuses cannot be determined with certainty. The ERRs elicited to the syllables presented indicate that they are transmitted to the auditory sensory system. However, evidence of discriminatory effects on both syllables was found in the infant group only. One reason could be that the acoustic transmission through maternal tissues and amniotic fluid does not adequately reflect the differences between the two syllables. Another possible reason for these results could be the complexity of the stimuli applied. Syllables, which have a broader and more discontinuous spectrum of frequencies than pure tones, might be more challenging in both transmission and perceptional processing (Muenssinger et al., 2013a).

Secondly, pregnant women and newborns cannot be expected to undergo an excessively long examination. To avoid artifacts caused by movements, restlessness or muscle tension, which may have negative influences on the analysis of ERRs, a reasonably short measurement period was used. Additionally, the fetal and neonatal behavioral state may impact stimulus processing and therefore influence characteristics of the ERRs (Nijhuis, 1986). Our group previously ascertained that the fetal state influences latency of ERRs towards auditory stimulation (Kiefer-Schmidt et al., 2013). An EEG study with auditory stimuli presented to neonates also suggested that the fetal sleep state may influence the amplitude of ERRs (Weitzman et al., 1965). Other studies indicated that habituation (measured by the decrease in fetal movement) occurs independently of the behavioral state (van Heteren et al., 2001). In the present study, the number of segments included in the analysis of ERRs was too low to reliably evaluate fetal state.

Thirdly, the relevance that participants attribute to a stimulus can influence habituation (Kenemans et al., 1988; Schupp et al., 2006). Speech stimuli may be more common to fetuses than artificial sounds such as pure tones, since the former occur frequently in a natural fetal environment. The extent to which habituation differs from more or less familiar auditory stimuli at this early age should be the subject of subsequent studies.

In future trials, stimuli with more pronounced differences should be selected, and more repetitions of the standard before the dishabituator occurs should be included. Differentiated knowledge about early auditory language perception and processing may help to identify children at risk for later language deficits.

In the third study, to examine whether exogenous factors impact fetal brain development, visual and auditory ERRs using fMEG in fetus and neonates with or without IUGR were investigated. In addition, children were reassessed at the age of two years using a standardized developmental test (BSID II). The long-term objective of such follow-up assessments would be to determine whether fMEG in fetuses at risk can be used to predict future development.

Previous results of our group indicate that, unlike AGA and SGA fetuses, IUGR fetuses have delayed visual ERRs, as measured by fMEG (Morin et al., 2015). In the present study, longer latencies for visual ERRs (albeit not to a statistically significant extent) and a significantly decreased MDI of the BSID II indicate that IUGR has a negative influence on early functional brain development, beginning as early as during gestation and possibly persisting until childhood. One explanation for the non-significant differences between EERs of IUGR, SGA and AGA fetuses might be the small sample sizes, rendering it inappropriate to statistically control for GA at the time of fMEG measurement. At the age of about two years, the MDI was significantly lower in former IUGR infants than in former SGA or AGA infants. Although several other studies have reported similar results, inconsistent definitions of IUGR in the present literature challenge their comparisons. IUGR fetuses should be clearly distinguished from constitutionally small fetuses by including markers that indicate a pathologic growth delay. The umbilical artery pulsatility index is a clinical standard for the observation of intrauterine nutritional and oxygen insufficiency that leads to pathologic metabolic conditions. In line with the present results, a study by Leppanen et al. (2010) reported lower scores in the mental, but not in motoric abilities only in those fetuses with a pathological umbilical doppler. Other studies investigating the cognitive development of children with a former IUGR also found deficits (Eixarch et al., 2008; Wienerroither et al., 2001). It should be noted that the MDI and PDI scores of BSID-II include several cognitive and motor domains which should be further differentiated. For this purpose, the 3rd version of the BSID, which is now available in German, should be used in future trials (Bayley, 2006).

Due to the theory of DOHaD, early exogenous influences may induce changes in developmental processes, and subsequently in health status. Measurement of ERRs gives the chance to gain information about functional brain development from fetal age onwards. To compare results of different trials, standardized methods should be used in well-defined age groups. The assessment of fetal habituation processes using ERR detection by fMEG is feasible, and complex stimuli like syllables are recognized by the fetus. Nevertheless, it remains unclear as to whether different syllables are distinguished from each other and a

response decrement was not shown. Since ERRs are influenced by pathological factors like IUGR, the fMEG method might be useful to detect impairments in early functional brain development. This is essential not only to comprehend the underlying neuropathological mechanisms, but also for clinical purposes, as early detection might help to implement interventions to compensate the subsequent developmental constraints that were observed in children with former IUGR.

Acknowledgements

I would like to express my gratitude to Professor Dr. Hubert Preissl and to Professor Dr. Martin Hautzinger for their supervision and advice. They provided me with professional guidance throughout my doctoral thesis and supported me with their time and scientific knowledge.

I would also like to thank my colleagues of the fMEG research group, without whose support my scientific work would not have been possible. Their constructive feedback and continuous encouragement have contributed greatly to the success of my doctoral thesis.

I would express my grateful thanks to all our participants and their infants who took the time to take part in our studies.

I would also like to thank Shirley Würth for proofreading and linguistic revision.

Finally, but most importantly, I would like to thank my family for their continued emotional support and their infinite love. Thank you for always believing in me.

References

Anbuhl, K., Uhler, K., Werner, L., & Tollin, D. (2017). Early Development of the Human Auditory System. In R. Polin, S. Abman, D. Rowitch, W. Benitz, & W. Fox (Eds.), *Fetal and Neonatal Physiology* (5 ed., pp. 1396-1411). Elsevier.

Baillet, S. (2017). Magnetoencephalography for brain electrophysiology and imaging. *Nat Neurosci, 20*(3), 327-339. https://doi.org/10.1038/nn.4504

Barker, D. J. (1966). Low intelligence. Its relation to length of gestation and rate of foetal growth. *Br J Prev Soc Med, 20*(2), 58-66. https://doi.org/10.1136/jech.20.2.58

Barker, D. J. (1991). The intrauterine origins of cardiovascular and obstructive lung disease in adult life. The Marc Daniels Lecture 1990. *J R Coll Physicians Lond, 25*(2), 129-133. https://www.ncbi.nlm.nih.gov/pubmed/2066923

Barker, D. J. (2007). The origins of the developmental origins theory. *J Intern Med, 261*(5), 412-417. https://doi.org/10.1111/j.1365-2796.2007.01809.x

Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., & Robinson, J. S. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet, 341*(8850), 938-941. https://www.ncbi.nlm.nih.gov/pubmed/8096277

Barker, D. J., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ, 298*(6673), 564-567. https://doi.org/10.1136/bmj.298.6673.564

Bayley, N. (1969). Manual for the Bayley Scales of infant development. *New York, Psychological Corporation*.

Bayley, N. (1993). Bayley scales of infant development (BSID-II). *San Antonio, Psychological Corporation*.

Bayley, N. (2006). Bayley scales of infant development (BSID-III). *San Antonio, Psychological Corporation*.

Bellieni, C. V., Severi, F., Bocchi, C., Caparelli, N., Bagnoli, F., Buonocore, G., & Petraglia, F. (2005). Blink-startle reflex habituation in 30-34-week low-risk fetuses. *J Perinat Med, 33*(1), 33-37. https://doi.org/10.1515/JPM.2005.005

Blum, T., Saling, E., & Bauer, R. (1985). First magnetoencephalographic recordings of the brain activity of a human fetus. *Br J Obstet Gynaecol, 92*(12), 1224-1229. https://doi.org/10.1111/j.1471-0528.1985.tb04866.x

Bolin, E. H., Escalona-Vargas, D., Daily, J. A., Siegel, E. R., Lowery, C. L., Coker, J., Stowe, Z. N., & Eswaran, H. (2019). Magnetocardiographic identification of prolonged fetal corrected QT interval in women receiving treatment for opioid use disorder. *J Obstet Gynaecol Res*. https://doi.org/10.1111/jog.14055

Budd, T. W., Barry, R. J., Gordon, E., Rennie, C., & Michie, P. T. (1998). Decrement of the N1 auditory event-related potential with stimulus repetition: habituation vs. refractoriness. *Int J Psychophysiol, 31*(1), 51-68. https://doi.org/10.1016/s0167-8760(98)00040-3

Carandini, M. (2000). Visual cortex: Fatigue and adaptation. *Curr Biol, 10*(16), R605-607. https://doi.org/10.1016/s0960-9822(00)00637-0

Clouchoux, C., Kudelski, D., Gholipour, A., Warfield, S. K., Viseur, S., Bouyssi-Kobar, M., Mari, J. L., Evans, A. C., du Plessis, A. J., & Limperopoulos, C. (2012). Quantitative in vivo MRI measurement of cortical development in the fetus. *Brain Struct Funct, 217*(1), 127-139. https://doi.org/10.1007/s00429-011-0325-x

Cohen, D. (1972). Magnetoencephalography: detection of the brain's electrical activity with a superconducting magnetometer. *Science, 175*(4022), 664-666. https://doi.org/10.1126/science.175.4022.664

Detweiler-Bedell, J. B., Friedman, M. A., Leventhal, H., Miller, I. W., & Leventhal, E. A. (2008). Integrating co-morbid depression and chronic physical disease management: identifying and resolving failures in self-regulation. *Clin Psychol Rev, 28*(8), 1426-1446. https://doi.org/10.1016/j.cpr.2008.09.002

DGGG. (2016). Leitlinie - Intrauterine Wacxhstumsrestriktion. *AWMF-Registernummer 015/080*. https://www.awmf.org/leitlinien/detail/ll/015-080.html

Draganova, R., Eswaran, H., Murphy, P., Lowery, C., & Preissl, H. (2007). Serial magnetoencephalographic study of fetal and newborn auditory discriminative evoked responses. *Early Hum Dev, 83*(3), 199-207. https://doi.org/10.1016/j.earlhumdev.2006.05.018

Draganova, R., Schollbach, A., Schleger, F., Braendle, J., Brucker, S., Abele, H., Kagan, K. O., Wallwiener, D., Fritsche, A., Eswaran, H., & Preissl, H. (2018). Fetal auditory evoked responses to onset of amplitude modulated sounds. A fetal magnetoencephalography (fMEG) study. *Hear Res, 363*, 70-77. https://doi.org/10.1016/j.heares.2018.03.005

Egana-Ugrinovic, G., Sanz-Cortes, M., Couve-Perez, C., Figueras, F., & Gratacos, E. (2014). Corpus callosum differences assessed by fetal MRI in late-onset intrauterine growth restriction and its association with neurobehavior. *Prenat Diagn, 34*(9), 843-849. https://doi.org/10.1002/pd.4381

Eixarch, E., Meler, E., Iraola, A., Illa, M., Crispi, F., Hernandez-Andrade, E., Gratacos, E., & Figueras, F. (2008). Neurodevelopmental outcome in 2-year-old infants who were small-for-gestational age term fetuses with cerebral blood flow redistribution. *Ultrasound Obstet Gynecol, 32*(7), 894-899. https://doi.org/10.1002/uog.6249

Eswaran, H., Preissl, H., Wilson, J. D., Murphy, P., Robinson, S. E., Rose, D., Vrba, J., & Lowery, C. L. (2002). Short-term serial magnetoencephalography recordings offetal auditory evoked responses. *Neurosci Lett, 331*(2), 128-132. https://doi.org/10.1016/s0304-3940(02)00859-5

Figueras, F., & Gardosi, J. (2011). Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. *Am J Obstet Gynecol, 204*(4), 288-300. https://doi.org/10.1016/j.ajog.2010.08.055

Fischi-Gomez, E., Vasung, L., Meskaldji, D. E., Lazeyras, F., Borradori-Tolsa, C., Hagmann, P., Barisnikov, K., Thiran, J. P., & Huppi, P. S. (2015). Structural Brain Connectivity in School-Age Preterm Infants Provides Evidence for Impaired Networks Relevant for Higher Order Cognitive Skills and Social Cognition. *Cereb Cortex, 25*(9), 2793-2805. https://doi.org/10.1093/cercor/bhu073

Fulford, J., Vadeyar, S. H., Dodampahala, S. H., Ong, S., Moore, R. J., Baker, P. N., James, D. K., & Gowland, P. (2004). Fetal brain activity and hemodynamic response to a vibroacoustic stimulus. *Hum Brain Mapp, 22*(2), 116-121. https://doi.org/10.1002/hbm.20019

Godfrey, K. M., & Barker, D. J. (2001). Fetal programming and adult health. *Public Health Nutr, 4*(2B), 611-624. https://www.ncbi.nlm.nih.gov/pubmed/11683554

Griffiths, P. D., Bradburn, M., Campbell, M. J., Cooper, C. L., Graham, R., Jarvis, D., Kilby, M. D., Mason, G., Mooney, C., Robson, S. C., Wailoo, A., & group, M. c. (2017). Use of MRI in the diagnosis of fetal brain abnormalities in utero (MERIDIAN): a multicentre, prospective cohort study. *Lancet, 389*(10068), 538-546. https://doi.org/10.1016/S0140-6736(16)31723-8

Hanson, M. A., & Gluckman, P. D. (2014). Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev, 94*(4), 1027-1076. https://doi.org/10.1152/physrev.00029.2013

Hartkopf, J., Moser, J., Schleger, F., Preissl, H., & Keune, J. (2019). Changes in event-related brain responses and habituation during child development - A systematic literature review. *Clin Neurophysiol, 130*(12), 2238-2254. https://doi.org/10.1016/j.clinph.2019.08.029

Hartkopf, J., Schleger, F., Keune, J., Wiechers, C., Pauluschke-Froehlich, J., Weiss, M., Conzelmann, A., Brucker, S., Preissl, H., & Kiefer-Schmidt, I. (2018). Impact of Intrauterine Growth Restriction on Cognitive and Motor Development at 2 Years of Age. *Front Physiol, 9*, 1278. https://doi.org/10.3389/fphys.2018.01278

Hartkopf, J., Schleger, F., Weiss, M., Hertrich, I., Kiefer-Schmidt, I., Preissl, H., & Muenssinger, J. (2016). Neuromagnetic signatures of syllable processing in fetuses and infants provide no evidence for habituation. *Early Hum Dev, 100*, 61-66. https://doi.org/10.1016/j.earlhumdev.2016.04.002

Hepper, P. G., Dornan, J. C., & Lynch, C. (2012). Sex differences in fetal habituation. *Dev Sci, 15*(3), 373-383. https://doi.org/10.1111/j.1467-7687.2011.01132.x

Jakab, A., Tuura, R., Kellenberger, C., & Scheer, I. (2017). In utero diffusion tensor imaging of the fetal brain: A reproducibility study. *Neuroimage Clin, 15*, 601-612. https://doi.org/10.1016/j.nicl.2017.06.013

Kappenman, E., & Luck, S. (2011). *The Oxford handbook of event-related potential components*. Oxford university press.

Kasprian, G., Brugger, P. C., Weber, M., Krssak, M., Krampl, E., Herold, C., & Prayer, D. (2008). In utero tractography of fetal white matter development. *Neuroimage, 43*(2), 213-224. https://doi.org/10.1016/j.neuroimage.2008.07.026

Kenemans, J. L., Verbaten, M. N., Sjouw, W., & Slangen, J. L. (1988). Effects of task relevance on habituation of visual single-trial ERPs and the skin conductance orienting response. *Int J Psychophysiol, 6*(1), 51-63. https://doi.org/10.1016/0167-8760(88)90034-7

Kiefer-Schmidt, I., Raufer, J., Brandle, J., Munssinger, J., Abele, H., Wallwiener, D., Eswaran, H., & Preissl, H. (2013). Is there a relationship between fetal brain function and the fetal behavioral state? A fetal MEG-study. *J Perinat Med, 41*(5), 605-612. https://doi.org/10.1515/jpm-2013-0022

Kim, M. S., Jeanty, P., Turner, C., & Benoit, B. (2008). Three-dimensional sonographic evaluations of embryonic brain development. *J Ultrasound Med, 27*(1), 119-124. https://doi.org/10.7863/jum.2008.27.1.119

Leppanen, M., Ekholm, E., Palo, P., Maunu, J., Munck, P., Parkkola, R., Matomaki, J., Lapinleimu, H., Haataja, L., Lehtonen, L., Rautava, P., & Group, P. S. (2010). Abnormal antenatal Doppler velocimetry and cognitive outcome in very-low-birth-weight infants at 2 years of age. *Ultrasound Obstet Gynecol, 36*(2), 178-185. https://doi.org/10.1002/uog.7694

Linder, K., Schleger, F., Kiefer-Schmidt, I., Fritsche, L., Kummel, S., Bocker, M., Heni, M., Weiss, M., Haring, H. U., Preissl, H., & Fritsche, A. (2015). Gestational Diabetes Impairs Human Fetal Postprandial Brain Activity. *J Clin Endocrinol Metab, 100*(11), 4029-4036. https://doi.org/10.1210/jc.2015-2692

Lowery, C. L., Govindan, R. B., Preissl, H., Murphy, P., & Eswaran, H. (2009). Fetal neurological assessment using noninvasive magnetoencephalography. *Clin Perinatol, 36*(3), 701-709. https://doi.org/10.1016/j.clp.2009.07.003

Ludewig, K., Geyer, M. A., & Vollenweider, F. X. (2003). Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. *Biol Psychiatry, 54*(2), 121-128. https://doi.org/10.1016/s0006-3223(02)01925-x

Manganaro, L., Bernardo, S., Antonelli, A., Vinci, V., Saldari, M., & Catalano, C. (2017). Fetal MRI of the central nervous system: State-of-the-art. *Eur J Radiol, 93*, 273-283. https://doi.org/10.1016/j.ejrad.2017.06.004

Matuz, T., Govindan, R. B., Preissl, H., Siegel, E. R., Muenssinger, J., Murphy, P., Ware, M., Lowery, C. L., & Eswaran, H. (2012). Habituation of visual evoked responses in neonates and fetuses: a MEG study. *Dev Cogn Neurosci, 2*(3), 303-316. https://doi.org/10.1016/j.dcn.2012.03.001

McCubbin, J., Robinson, S. E., Cropp, R., Moiseev, A., Vrba, J., Murphy, P., Preissl, H., & Eswaran, H. (2006). Optimal reduction of MCG in fetal MEG recordings. *IEEE Trans Biomed Eng, 53*(8), 1720-1724. https://doi.org/10.1109/TBME.2006.876619

Miltner, W., Johnson, R., & Braun, C. (1991). Auditory and somatosensory event-related potentials: II. Effects of inter-stimulus interval. *Journal of Psychophysiology*.

Mitter, C., Jakab, A., Brugger, P. C., Ricken, G., Gruber, G. M., Bettelheim, D., Scharrer, A., Langs, G., Hainfellner, J. A., Prayer, D., & Kasprian, G. (2015). Validation of In utero Tractography of Human Fetal Commissural and Internal Capsule Fibers with Histological Structure Tensor Analysis. *Front Neuroanat, 9*, 164. https://doi.org/10.3389/fnana.2015.00164

Moore, J. K., & Linthicum, F. H., Jr. (2007). The human auditory system: a timeline of development. *Int J Audiol, 46*(9), 460-478. https://doi.org/10.1080/14992020701383019

Moore, R. J., Vadeyar, S., Fulford, J., Tyler, D. J., Gribben, C., Baker, P. N., James, D., & Gowland, P. A. (2001). Antenatal determination of fetal brain activity in response to an acoustic stimulus using functional magnetic resonance imaging. *Hum Brain Mapp, 12*(2), 94-99. https://doi.org/10.1002/1097-0193(200102)12:2<94::aid-hbm1006>3.0.co;2-e

Morin, E. C., Schleger, F., Preissl, H., Braendle, J., Eswaran, H., Abele, H., Brucker, S., & Kiefer-Schmidt, I. (2015). Functional brain development in growth-restricted and constitutionally small fetuses: a fetal magnetoencephalography case-control study. *BJOG, 122*(9), 1184-1190. https://doi.org/10.1111/1471-0528.13347

Moussavi, S., Chatterji, S., Verdes, E., Tandon, A., Patel, V., & Ustun, B. (2007). Depression, chronic diseases, and decrements in health: results from the World Health Surveys. *Lancet, 370*(9590), 851-858. https://doi.org/10.1016/S0140-6736(07)61415-9

Muenssinger, J., Matuz, T., Schleger, F., Kiefer-Schmidt, I., Goelz, R., Wacker-Gussmann, A., Birbaumer, N., & Preissl, H. (2013a). Auditory habituation in the fetus and neonate: an fMEG study. *Dev Sci, 16*(2), 287-295. https://doi.org/10.1111/desc.12025

Muenssinger, J., Stingl, K. T., Matuz, T., Binder, G., Ehehalt, S., & Preissl, H. (2013b). Auditory habituation to simple tones: reduced evidence for habituation in children compared to adults. *Front Hum Neurosci, 7*, 377. https://doi.org/10.3389/fnhum.2013.00377

Murray, E., Fernandes, M., Fazel, M., Kennedy, S. H., Villar, J., & Stein, A. (2015). Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review. *BJOG, 122*(8), 1062-1072. https://doi.org/10.1111/1471-0528.13435

Netser, S., Zahar, Y., & Gutfreund, Y. (2011). Stimulus-specific adaptation: can it be a neural correlate of behavioral habituation? *J Neurosci, 31*(49), 17811-17820. https://doi.org/10.1523/JNEUROSCI.4790-11.2011

Nijhuis, J. G. (1986). Behavioural states: concomitants, clinical implications and the assessment of the condition of the nervous system. *Eur J Obstet Gynecol Reprod Biol, 21*(5-6), 301-308. https://doi.org/10.1016/0028-2243(86)90008-0

Nilsson, E., Stalberg, G., Lichtenstein, P., Cnattingius, S., Olausson, P. O., & Hultman, C. M. (2005). Fetal growth restriction and schizophrenia: a Swedish twin study. *Twin Res Hum Genet, 8*(4), 402-408. https://doi.org/10.1375/1832427054936727

Penkler, M., Hanson, M., Biesma, R., & Muller, R. (2018). DOHaD in science and society: emergent opportunities and novel responsibilities. *J Dev Orig Health Dis*, 1-6. https://doi.org/10.1017/S2040174418000892

Preissl, H., Lowery, C. L., & Eswaran, H. (2004). Fetal magnetoencephalography: current progress and trends. *Exp Neurol, 190 Suppl 1*, S28-36. https://doi.org/10.1016/j.expneurol.2004.06.016

Preissl, H., Lowery, C. L., & Eswaran, H. (2005). Fetal magnetoencephalography: viewing the developing brain in utero. *Int Rev Neurobiol, 68*, 1-23. https://doi.org/10.1016/S0074-7742(05)68001-4

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C. F., & Thompson, R. F. (2009). Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol Learn Mem, 92*(2), 135-138. https://doi.org/10.1016/j.nlm.2008.09.012

Reveillon, M., Borradori Tolsa, C., Monnier, M., Huppi, P. S., & Barisnikov, K. (2016). Response inhibition difficulties in preterm children aged 9-12 years: Relations with emotion and behavior. *Child Neuropsychol, 22*(4), 420-442. https://doi.org/10.1080/09297049.2014.994486

Schleger, F., Landerl, K., Muenssinger, J., Draganova, R., Reinl, M., Kiefer-Schmidt, I., Weiss, M., Wacker-Gussmann, A., Huotilainen, M., & Preissl, H. (2014). Magnetoencephalographic signatures of numerosity discrimination in fetuses and neonates. *Dev Neuropsychol, 39*(4), 316-329. https://doi.org/10.1080/87565641.2014.914212

Schleger, F., Linder, K., Walter, L., Heni, M., Brandle, J., Brucker, S., Pauluschke-Frohlich, J., Weiss, M., Haring, H. U., Preissl, H., & Fritsche, A. (2018). Family History of Diabetes Is Associated With Delayed Fetal Postprandial Brain Activity. *Front Endocrinol (Lausanne), 9*, 673. https://doi.org/10.3389/fendo.2018.00673

Schneider, U., Arnscheidt, C., Schwab, M., Haueisen, J., Seewald, H. J., & Schleussner, E. (2011). Steroids that induce lung maturation acutely affect higher cortical function: a fetal magnetoencephalography study. *Reprod Sci, 18*(1), 99-106. https://doi.org/10.1177/1933719110381140

Schneider, U., Schleussner, E., Haueisen, J., Nowak, H., & Seewald, H. J. (2001). Signal analysis of auditory evoked cortical fields in fetal magnetoencephalography. *Brain Topogr, 14*(1), 69-80. https://www.ncbi.nlm.nih.gov/pubmed/11599534

Schupp, H. T., Stockburger, J., Codispoti, M., Junghofer, M., Weike, A. I., & Hamm, A. O. (2006). Stimulus novelty and emotion perception: the near absence of habituation in the visual cortex. *Neuroreport, 17*(4), 365-369. https://doi.org/10.1097/01.wnr.0000203355.88061.c6

Sheridan, C. J., Matuz, T., Draganova, R., Eswaran, H., & Preissl, H. (2010). Fetal Magnetoencephalography - Achievements and Challenges in the Study of Prenatal and Early Postnatal Brain Responses: A Review. *Infant Child Dev, 19*(1), 80-93. https://doi.org/10.1002/icd.657

Sheridan, C. J., Preissl, H., Siegel, E. R., Murphy, P., Ware, M., Lowery, C. L., & Eswaran, H. (2008). Neonatal and fetal response decrement of evoked responses: a MEG study. *Clin Neurophysiol, 119*(4), 796-804. https://doi.org/10.1016/j.clinph.2007.11.174

Sippel, K., Moser, J., Schleger, F., Preissl, H., Rosenstiel, W., & Spuler, M. (2019). Fully Automated R-peak Detection Algorithm (FLORA) for fetal magnetoencephalographic data. *Comput Methods Programs Biomed, 173*, 35-41. https://doi.org/10.1016/j.cmpb.2019.02.016

Sizonenko, S. V., Borradori-Tolsa, C., Bauthay, D. M., Lodygensky, G., Lazeyras, F., & Huppi, P. (2006). Impact of intrauterine growth restriction and glucocorticoids on brain development: insights using advanced magnetic resonance imaging. *Mol Cell Endocrinol, 254-255*, 163-171. https://doi.org/10.1016/j.mce.2006.04.035

Snoek, F. J., Bremmer, M. A., & Hermanns, N. (2015). Constructs of depression and distress in diabetes: time for an appraisal. *Lancet Diabetes Endocrinol, 3*(6), 450-460. https://doi.org/10.1016/S2213-8587(15)00135-7

Taiminen, T., Jaaskelainen, S., Ilonen, T., Meyer, H., Karlsson, H., Lauerma, H., Leinonen, K. M., Wallenius, E., Kaljonen, A., & Salokangas, R. K. (2000). Habituation of the blink reflex in first-episode schizophrenia, psychotic depression and non-psychotic depression. *Schizophr Res, 44*(1), 69-79. https://doi.org/10.1016/s0920-9964(99)00140-1

Thomason, M. E., Dassanayake, M. T., Shen, S., Katkuri, Y., Alexis, M., Anderson, A. L., Yeo, L., Mody, S., Hernandez-Andrade, E., Hassan, S. S., Studholme, C., Jeong, J. W., & Romero, R. (2013). Cross-hemispheric functional connectivity in the human fetal brain. *Sci Transl Med, 5*(173), 173ra124. https://doi.org/10.1126/scitranslmed.3004978

Thompson, C., Syddall, H., Rodin, I., Osmond, C., & Barker, D. J. (2001). Birth weight and the risk of depressive disorder in late life. *Br J Psychiatry, 179*, 450-455. https://doi.org/10.1192/bjp.179.5.450

Thompson, R. F. (2009). Habituation: a history. *Neurobiol Learn Mem, 92*(2), 127-134. https://doi.org/10.1016/j.nlm.2008.07.011

Thompson, R. F., & Spencer, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol Rev, 73*(1), 16-43. https://doi.org/10.1037/h0022681

Tolsa, C. B., Zimine, S., Warfield, S. K., Freschi, M., Sancho Rossignol, A., Lazeyras, F., Hanquinet, S., Pfizenmaier, M., & Huppi, P. S. (2004). Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. *Pediatr Res, 56*(1), 132-138. https://doi.org/10.1203/01.PDR.0000128983.54614.7E

van den Heuvel, M. I., & Thomason, M. E. (2016). Functional Connectivity of the Human Brain in Utero. *Trends Cogn Sci, 20*(12), 931-939. https://doi.org/10.1016/j.tics.2016.10.001

van Heteren, C. F., Boekkooi, P. F., Jongsma, H. W., & Nijhuis, J. G. (2001). Fetal habituation to vibroacoustic stimulation in relation to fetal states and fetal heart rate parameters. *Early Hum Dev, 61*(2), 135-145. https://doi.org/10.1016/s0378-3782(00)00130-4

Verhaak, P. F., Heijmans, M. J., Peters, L., & Rijken, M. (2005). Chronic disease and mental disorder. *Soc Sci Med, 60*(4), 789-797. https://doi.org/10.1016/j.socscimed.2004.06.012

Vrba, J., Robinson, S. E., McCubbin, J., Lowery, C. L., Eswaran, H., Wilson, J. D., Murphy, P., & Preissl, H. (2004). Fetal MEG redistribution by projection operators. *IEEE Trans Biomed Eng, 51*(7), 1207-1218. https://doi.org/10.1109/TBME.2004.827265

Wadhwa, P. D., Buss, C., Entringer, S., & Swanson, J. M. (2009). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Semin Reprod Med, 27*(5), 358-368. https://doi.org/10.1055/s-0029-1237424

Weitzman, E. D., Fishbein, W., & Graziani, L. (1965). Auditory Evoked Responses Obtained from the Scalp Electroencephalogram of the Full-Term Human Neonate during Sleep. *Pediatrics, 35*, 458-462. https://www.ncbi.nlm.nih.gov/pubmed/14260305

Welsh, R. C., Nemec, U., & Thomason, M. E. (2011). Fetal magnetic resonance imaging at 3.0 T. *Top Magn Reson Imaging, 22*(3), 119-131. https://doi.org/10.1097/RMR.0b013e318267f932

Wienerroither, H., Steiner, H., Tomaselli, J., Lobendanz, M., & Thun-Hohenstein, L. (2001). Intrauterine blood flow and long-term intellectual, neurologic, and social development. *Obstet Gynecol, 97*(3), 449-453. https://doi.org/10.1016/s0029-7844(00)01158-3

Wilson, J. D., Adams, A. J., Murphy, P., Eswaran, H., & Preissl, H. (2009). Design of a light stimulator for fetal and neonatal magnetoencephalography. *Physiol Meas, 30*(1), N1-10. https://doi.org/10.1088/0967-3334/30/1/N01

Wilson, J. D., Govindan, R. B., Hatton, J. O., Lowery, C. L., & Preissl, H. (2008). Integrated approach for fetal QRS detection. *IEEE Trans Biomed Eng, 55*(9), 2190-2197. https://doi.org/10.1109/TBME.2008.923916

Zappasodi, F., Tecchio, F., Pizzella, V., Cassetta, E., Romano, G. V., Filligoi, G., & Rossini, P. M. (2001). Detection of fetal auditory evoked responses by means of magnetoencephalography. *Brain Res, 917*(2), 167-173. https://doi.org/10.1016/s0006-8993(01)02901-8