Early Development of Cortical Brain Responses in Newborns and Fetuses – Cognitive Studies with Fetal Magnetoencephalography

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ZUSAMMENFASSUNG

Entwicklungsdefizite entstehen häufig schon während der pränatalen Kortexgenese, wohingegen ihre Diagnose zumeist viel später erfolgt, wenn sich z.B. das Verhalten des Kindes nicht Altersgemäß entwickelt.

In den pränatalen und beginnenden neonatalen Entwicklungsstadien verfügt der Kortex über eine hohe Plastizität, die mit zunehmendem Alter abnimmt. Somit ist der Einsatz von Interventionsmethoden möglichst früh im Entwicklungsverlauf wünschenswert, um irreversiblen Defiziten und deren physiologischen sowie psychosozialen Folgeschäden vorzubeugen. Voraussetzung hierfür ist allerdings eine frühzeitige Diagnose von Entwicklungsdefiziten. Mit Hilfe neuer Technologien – wie der fetalen Magnetoenzephalographie (fMEG) – ist die Erfassung auditorisch oder visuell evozierter Gehirnantworten schon vor der Geburt möglich. Das fMEG ist nicht invasiv und misst fetale Magnetfelder, die bei neuronaler Aktivität über der Hirnrinde entstehen. Dabei leiten hochsensible Sensoren – die sogenannten SQUID (Superconducting Quantum Interference Device) – die fetalen Magnetfelder über der mütterlichen Bauchoberfläche ab. Bei Kindern und Erwachsenen können evozierte Felder dazu dienen, Probanden mit Defiziten von nicht beeinträchtigten Personen zu unterscheiden. Somit könnte das fMEG zu einer frühen Erkennung von Entwicklungsdefiziten beitragen. Interventionsmethoden in

diesen frühen Stadien könnten die Plastizität des sich entwickelnden Gehirns dazu nutzen, Defiziten entgegen zu wirken und weitere Entwicklungsrückstände zu verhindern.

In den Anfangsstadien der Entwicklung spielen Wahrnehmung und Gedächtnis eine zentrale Rolle, da sie erst den Erwerb vieler weiterer Funktionen ermöglichen.

Das Ziel dieser Arbeit ist es, kognitive Paradigmen in frühen Entwicklungsstadien anzuwenden, um einen besseren Einblick in die fetalen und neonatalen Grundlagen komplexer kortikaler Prozesse zu bekommen. Mit dem fMEG wurden 2 Studien zur fetalen und neonatalen Entwicklung evozierter Potenziale durchgeführt. In der ersten Untersuchung geht es um die Erfassung integrativer Prozesse, auch Habituation genannt. Die zweite Studie untersucht die Entwicklung der Lautwahrnehmung als Basis für Spracherwerb.

In Studie 1 nahmen 25 Föten zwischen der 29. und 37. Schwangerschaftswoche, sowie 12 Neugeborene im Alter von 6 bis 22 Tagen teil. Vier aufeinander folgende Lichtblitze wurden präsentiert um kortikale Antworten zu evozieren. Die Reaktionsintensität der vier Stimuli wurde zueinander in Beziehung gesetzt, wobei Habituation durch eine Abnahme der Intensität von der ersten Gehirnantwort zu den darauf folgenden Reaktionen definiert ist.

In Studie 2 wurden auditorisch evozierte Felder zu Tonpaaren verschiedener Länge präsentiert. Sie unterscheiden sich durch den Abstand zwischen den beiden Tönen – in einer Version war

dieser kürzer als in der anderen. Die Eigenschaften der evozierten Felder auf beide Tonpaare wurden bei 22 Feten zwischen der 29. und der 38. Schwangerschaftswoche, sowie bei 15 Säuglingen im Alter zwischen 2 und 38 Tagen untersucht.

In beiden Untersuchungen wurden die Neugeborenen aus der Stichprobe der jeweils vorausgehenden fetalen Studie rekrutiert.

Studie 1 ergab eine Abnahme der Reaktionsintensität von der ersten bis zur letzten Stimuluspräsentation bei 9 der 12 Säuglinge. Dies weist auf Habituation hin, was als einer der wichtigsten Indikatoren für kognitive Prozesse gilt. Die drei übrigen Erhebungen wurden vorzeitig abgebrochen. In nur 29% der fetalen Daten waren evozierte Felder erkennbar. Allerdings zeigten diese Feten ebenfalls eine Abnahme der Reaktionsintensität.

Studie 2 ergab einen Trend zu zwei Gehirnantworten mit zunehmendem Alter der Neugeborenen, zumindest in der längeren Version des Tonpaares. Die jüngeren Säuglinge reagierten dagegen zumeist mit nur einer Gehirnantwort. In der pränatalen Studie konnte kein Trend verzeichnet werden – die meisten Feten zeigten eine Gehirnantwort auf das Tonpaar. Allerdings war die Anzahl der erfassten fetalen Felder mit 76% bis 86% größer als in vergleichbaren Studien.

Die Anwendung beider Paradigmen an Feten und Neugeborenen war erfolgreich, und die entsprechenden Gehirnantworten konnten mit dem fMEG erfasst werden. Die fetalen Daten ergaben trotz der

Herausforderungen in der Datenanalyse Hinweise für die Gestaltung zukünftiger Studienprotokolle.

Beide Studien könnten einen ersten Schritt zu einem besseren Einblick in die funktionelle Kortexgenese in Utero darstellen, und damit zu einer frühzeitigen Erkennung von Entwicklungsverzögerungen beitragen.

ABSTRACT

During early developmental stages the brain is more vulnerable to physiological insult than the more mature brain. However, an early diagnosis of deficits might enhance the chances for successful interventions due to the high cortical plasticity of the immature brain. This thesis contains two studies on early cognitive development, conducted with a device called fetal magnetoencephalography (fMEG). It allows non-invasive recordings of cortical responses in utero.

Objective: Two study paradigms that assess cognitive functioning, such as integrative processes and the development of speech perception, were applied to neonates and fetuses.

Methods: In study 1, brain responses to visual stimuli (VER) in a habituation paradigm were recorded in 25 fetuses (aged between 29 and 37 weeks gestational age (GA)) and 12 newborns (aged between 6 and 22 days).

In study 2, auditory evoked responses (AER) to tone pairs were presented to 22 fetuses (aged between 29 and 38 weeks GA) and 15 neonates (aged between 2 and 38 days). This paradigm was applied in two difficulties – with short and long gaps between the tones. In both studies, the newborns already participated in the corresponding antecedent fetal recordings.

Results: In study 1, nine of the 12 newborns showed a response decrement for consecutive visual stimuli, indicating habituation. The remaining three recordings were discontinued

early. The prenatal VER rate was only 29%. But these fetuses exhibited a response decrement as well.

In study 2, the newborns showed a trend to 2 responses with increasing age in the long gap tone pair. The prenatal study revealed single responses in most cases and no trend was observed. However, the fetal response rate between 76% and 86% was higher than in previous studies.

Conclusions: Both paradigms have been successfully applied on neonates and fetuses, and cortical responses were recorded with fMEG technology. Despite the challenges in fetal data analysis, these investigations might help to improve future study paradigms and technological advances.

Significance: These studies might contribute to a more accurate detection of early developmental delays and the development of subsequent impairments.

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Abbreviations

AER	auditory evoked response
dB	decibel
DF	degress of freedom
EEG	electroencephalogram
ERP	event-related potential
FD	frequency discrimination
fMEG	fetal magnetoencephalography
fMRI	functional magnetic resonance imaging
fT	femto-tesla
GA	gestational age
ISI	interstimulus interval
LED	light emitting diode
MEG	magnetoencephalography
MMN	mismatch negativity
MMR	mismatch response
MSR	magnetically shielded room

pT	pico-tesla
RMS	root mean square
RTP	rapid temporal processing
SARA	squid array for reproductive assessment
SLI	specific language impairment
SOA	stimulus onset asynchrony
Squid	superconducting quantum interference device
VER	visual evoked response

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Early Development of Cortical Brain Responses in Newborns and Fetuses – Cognitive Studies with Fetal Magnetoencephalography

1. Introduction

The acquisition of complex behavior in humans is a long-lasting process, which can continue well into adulthood. However, many important precursors for later cortical functioning are established long before the anatomical and functional development is observable (deRegnier, 2005). Most of these basic functions can be acquired during limited time periods only. Lack of certain inputs or harmful events in these early periods can negatively affect the maturation of the brain and lead to irreversible consequences for subsequent development. If motor or sensory modalities are impaired on a very basic level, the subsequent development of higher cognitive functioning will most likely be disturbed as well. Therefore, the brain is more vulnerable to physiological insult during early developmental stages, than the more mature brain of older participants. The detection of impairments at very early stages could contribute to the implementation of intervention methods and the prevention of subsequent developmental delays.

As yet, developmental delays are typically diagnosed by the observation of functional deficits, long after the presumed pathogenic process has occurred (deRegnier, 2005). Dyslexia, for example, is believed to develop as early as in the prenatal stages (Galaburda, Sherman, Rosen, Aboitiz, & Geschwind, 1985; Galaburda, & Kemper, 1979; Humphreys, Kaufmann, & Galaburda, 1990) but is frequently not diagnosed until the child enters school and the adverse ability to read and spell becomes evident (Snowling, Bishop, & Stothard, 2000; Vellutino, Fletcher, Snowling, & Scanlon, 2004). Therefore, these deficits often remain undetected for years and become irreversibly established in the brain.

In prenatal and early postnatal developmental stages, functionality of cortical structures concerns mainly perception and memory, because both constitute the foundation for many other cortical functions. The ability to perceive and remember sounds, for example, is essential for the acquisition of language, and in turn, facilitates social interactions and the acquisition of complex social skills.

A better insight into early functional brain development, preferably as early as in the prenatal stages, might be crucial for the prevention of maturational delays and deficits. Technological advances in brain imaging, such as fetal magnetoencephalography (fMEG), enable the direct investigation of the human brain in uterus. This thesis presents two novel neurophysiological studies on pre- and

neonatal brain development. Both investigations address the detection of early precursors for higher cognitive functioning with an fMEG device called SARA – Squid (Superconducting Quantum Interference Device) Array for Reproductive Assessment. This system is designed specifically for the direct and non-invasive recording of fetal and neonatal brain signals and other biomagnetic fields generated by the mother or the fetus(Preissl, Lowery, & Eswaran, 2004, 2005).

1.1 Human Brain Development

The prenatal development of brain anatomy is well documented (for a recent review see Kintner et al., 1999), whereas the functional development in utero is not yet well understood. This thesis focuses on the functional brain development, since it is crucial for the diagnosis of cortical impairments. While in postnatal development the functional meaning of developing brain structures is more easily observable, this has been a difficult attempt in the prenatal stages of life, due to the limited accessibility of the fetal brain in utero. Previous studies on early functional brain development in infants have been conducted with brain imaging technology such as Electroencephalogram (EEG) (e.g. Benasich, Thomas, Choudhury, & Leppanen, 2002; Benasich et al., 2006). Some authors started at even earlier developmental stages by investigating premature babies (e.g. Cheour-Luhtanen et al.,

1996; Tokioka, Pearce, & Crowell, 1995). But these results from premature neonates are not necessarily representative for the normal development in utero. These babies often experienced dramatic environmental alterations; and the causal conditions for premature delivery usually indicate a poor state of health. Other studies have used non-human primates to assess early brain development (e.g. Kuboshima-Amemori, & Sawaguchi, 2007; Schneider, & Coe, 1993; Schneider et al., 1998) since the anatomical similarities of human and primate cortices suggest parallels in their functional development. Nevertheless, drawing conclusions from primates to humans has to be regarded as hypothetical.

With advanced technology, such as fMEG (e.g. Eswaran, Lowery, Wilson, Murphy, & Preissl, 2005) and functional magnetic resonance imaging (fMRI) (e.g. Fulford, Vadeyar, Dodampahala, Ong, Moore, et al., 2004; Fulford, Vadeyar, Dodampahala, Moore, Young, et al., 2003; Moore, Vadeyar, Fulford, Tyler, Gribben, et al., 2001; Stiles, Moses, Passarotti, Dick, & Buxton, 2003), the assessment of early functional brain development has been improved. Among these, fMEG technology is the only method that allows completely non-invasive recordings on human fetuses.

The following chapter explains the importance of early brain development on the basis of physiological mechanisms during cortical maturation.

1.1.1 Cortical Plasticity and Critical Periods

Many cell characteristics, their future location and functions, are determined from the beginning of cell segmentation, which occurs within the first days of embryonic development (for a more detailed review see Kintner et al., 1999). But most cortical functions develop over time and are shaped by experience. The acquisition of complex behavior and the brain’s capability to adjust to new challenges is enabled by the potential to change size, organization and capacity of its structures and networks. This ability to constantly adapt to new requirements is called cortical plasticity. The experience-based changes in the structure of the brain are believed to be caused by different underlying neuronal mechanisms (Knudsen, 2004): (1) Axons establish new projection fields if more axonal connections are required for the acquisition of certain skills (axonal elaboration). (2) In order to specialize in certain fields, unused synapses are eliminated(synapse elimination) and the remaining ones are strengthened. (3) Repeated exposure to stimuli or events leads to activation of the same synapses and postsynapses, which stabilizes the structure of these synaptic connections (synapse consolidation).

Despite this stabilization, the efficacy of a consolidated synapse can still be adjusted according to its use. Experience

can lead to a modification in the number of presynaptic vesicles and/or alter the quantity of postsynaptic receptors.

Axon elaboration enables the acquisition of new skills. Synapse elimination and consolidation strengthen neuronal circuits, but at the same time, limit the plasticity of the brain. The time periods during which the architecture of neuronal circuits can be shaped are called sensitive periods. Specific experiences have to be made in these developmental stages in order for certain skills to develop. The ability to acquire these skills or functions ceases after a certain age or level of maturation. This decline in plasticity occurs in stages rather than a constant time course. If sensitive periods lead to irreversible changes in brain functioning, they are called critical periods. After a critical period has ended, neuronal regeneration is irreversibly lost (Knudsen, 2004). Some studies have shown the brain to be plastic even in high ages (e.g. Erickson et al., 2007; Mahncke et al., 2006). However, this is true for select functions only (e.g. Jones, et al., 2006). Language, for example, can reach the level of the native tongue only if one was exposed to it at a very early age (e.g. Kuhl, 1994). Also, social and coping skills have been shown to require early positive interactions with the parents (e.g. Liu et al., 1997; Thompson, 1999).

Thus, cortical plasticity is most potent during the immature stages of brain development and becomes more and more

limited with inclining age and specialization of cortical functions.

The next chapter addresses the physiological mechanisms involved in higher cortical functioning.

1.1.2 Cognitive Functioning

The human cortex is divided into different functional areas. The most basic discrimination differentiates between the areas that process basic sensory input (primary areas) from higher cortical areas, which are involved in more complex tasks. The latter mainly involve the frontal cortex. Cognitive functions usually require the integration of information from different cortical areas. One of the most commonly used definitions for the term cognition was published by Neisser (1967): “. . . Cognition refers to all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered, and used . . . . cognition is involved in everything a human being might possibly do . . .” (p. 4). Thus, cognition stands for higher-level functions of the brain. The complexity of different behaviors and skills is also reflected by the cortical area that is responsible for their processing. In order to control behavior and actions, the frontal cortex communicates with and integrates information from all brain areas. This communication between brain regions is conducted through highly interactive networks or circuits. A network consists of different neurons or groups

of neurons that communicate with each other. There are multiple signaling possibilities for neurons, but the major signal transmission is conducted via electrochemical signaling. Electric currents depolarize the cell, which in turn releases messenger molecules called neurotransmitters from the so-called presynapse into the synaptic cleft. The neurotransmitters diffuse across the cleft and bind to receptors that are located on the postsynapse. This elicits a chain of excitatory and/or inhibitory mechanisms in the postsynaptic neuron, which in turn, can evoke further signal transmission to other neurons in the network. If the interconnected neurons or groups of neurons are involved in functional entities, they are called neuronal circuits. Such circuits facilitate the integration of information from different parts of the brain that is required for higher-level cognitive functions.

The majority of complex functions, such as language, decision making, reasoning etc. are first observable during the postnatal years of life.

Even though different brain regions follow different developmental courses, a general progression of maturation is preset. The lower, sensorimotor brain areas develop first and the higher, frontal lobe regions last (Stiles et al., 2003). Since the frontal lobe integrates information from the lower-level areas, these brain regions need to be fully developed first, in order to transmit reliable information (Knudsen,

2004). A part of the frontal cortex, the prefrontal cortex, is very important for different cognitive functions, such as working memory and speech development. This is also reflected by the fact that the postnatal development of many cognitive functions correlates with the morphological development (myelinisation) of the prefrontal cortex (Fuster, 2002).

As yet, the early development of cognitive functions is not well understood, mainly because they are not established until cortical maturation is in a more advanced stage. However, there have been attempts to study cognitive development on a basic level. The following section provides an overview over study paradigms that have been useful in the assessment of precursors for cognitive processes, such as speech and memory.

1.2 Assessment of Cognitive Development

One common technique to investigate the maturation of cortical functioning, is to test the participants' reactions to different stimuli by observing their behavior. These methods are called behavioral tests. In prenatal studies, fetal body movements in response to a presented stimulus can be observed with ultrasound technology.

Another, more direct way to study cortical functionality is the recording of evoked brain responses. With this method, a participant's brain activity in response to a repeatedly

presented stimulus is detected with EEG electrodes or magnetoencephalography (MEG) sensors. The raw recordings contain random or spontaneous brain activity as well as stimulus-related brain responses. In order to obtain the evoked brain responses devoid of the random activity, the raw dataset is averaged over all the presented stimuli. The resulting evoked field usually contains several different components, depending on the age of the participant and the stimulus modality. These components are defined by their peak amplitude and the latency (usually in ms after stimulus onset) at which the peak occurred. For a more detailed description of evoked responses, see deRegnier (2005).

The recording of evoked fields from the fetal cortex has been enabled with the development of fMEG technology. The most basic way to assess prenatal cortical maturation is the presentation of simple auditory or visual stimuli, such as non-speech tones or light flashes. This tests the functionality of the primary sensorimotor areas.

Different study paradigms have been used to investigate more complex cortical functions that require higher-level processing on infants and fetuses: Classical conditioning, the “Oddball” paradigm, the assessment of rapid temporal processing (RTP) skills and habituation studies. These paradigms can be conducted with the help of behavioral tests or with brain imaging methods, such as fMEG or fMRI technology.

1.2.1 Classical Conditioning

Classical Conditioning was one of the first methods used to assess cognitive functioning. It relates to a learning process called “associative learning”. In this approach, two different stimuli are presented simultaneously or in quick succession of one another: a neutral stimulus, that does not normally evoke a response (conditioned stimulus) and a stimulus that necessarily evokes a response (unconditioned stimulus). After several repetitions, the participant starts to associate the two stimuli with one another and the previously neutral stimulus now evokes a response as well. The participant has “learned” the association between the two stimuli. In early approaches, Ray (1932) and Spelt (1948) studied classical conditioning in fetuses. More recently, Hepper (1997) reported that 50% of the participants in a prenatal ultrasound study showed conditioned reactions.

1.2.2 Oddball Paradigm

In the oddball paradigm, sequences of frequent standard stimuli, intermixed with rare deviant stimuli are presented. It is being used to elicit evoked responses, such as the P300 component or the N200, that occur 300 ms or 200 ms after the presentation of a stimulus. Moreover, it assesses the ability to differentiate between the prevalent standard and the infrequent deviant stimulus. In order to differentiate two stimuli, the participant has to be able to compare the memory

traces of the standard stimulus with the attributes of the deviant one. The physiological correlate of the auditory discriminative ability in adults is called mismatch negativity (MMN) – a negative peak latency between 100 ms and 250 ms after stimulus onset. This difference wave is obtained by subtracting the response to the standard from the one to the deviant stimulus (Näätänen, 2001; Näätänen, Gaillard, & Mäntysalo, 1978; Sams, Paavilainen, Alho, & Näätänen, 1985). The MMN is independent of attention and therefore even suitable for the assessment of an infant’s ability to discriminate auditory stimuli. Several studies have shown this brain component to be evident in preterm and full-term neonates (Alho, Sainio, Sajaniemi, Reinikainen, & Näätänen, 1990; Cheour, Leppänen, & Kraus, 2000; Cheour et al., 2002; Cheour-Luhtanen et al., 1996; Dehaene-Lambertz, & Baillet, 1998; Dehaene-Lambertz, & Dehaene, 1994; Huotilainen, et al., 2003; Kushnerenko et al., 2001; Tanaka, Okubo, Fuchigami, & Harada, 2001). However, some authors found this change-detection response to be of positive polarity in newborns and young children (Duclaux, Challamel, Collet, Roullet-Solignac, & Revol, 1991; Friederici, Friedrich, & Weber, 2002; Pihko et al., 1999; Ruusuvirta, Huotilainen, Fellman, & Näätänen, 2003; Tokioka et al., 1995). The reasons for this difference in polarity are not yet resolved. Some researchers argue that maturational changes, such as myelination or changes in the architecture and anatomy of the brain, lead to an adverse

polarity (Leppänen et al., 2004). Other explanations are the high-pass filters used for data analysis (Trainor et al., 2003; Weber, Hahne, Friedrich, & Friederici, 2004) or the length of the interstimulus interval (ISI) between the presented triggers (Cheour et al., 2002). Friederici et al. (2002) found the neonatal alertness state (asleep or awake) to be the causal factor for the different polarities. Due to this controversy, the according component is called mismatch response (MMR) when referred to in this thesis. By means of fetal MEG, the MMR has been shown in fetuses as early as 28 weeks GA (Draganova, Eswaran, Murphy, Lowery, & Preissl, 2007).

1.2.3 Rapid Temporal Processing Task

Yet another paradigm has been used to study speech development. In this thesis it is called RTP task. It is similar to the “Oddball” paradigm and has been shown to be predictive of later speech performance (Benasich, & Tallal, 2002). RTP is believed to facilitate the sufficient perception of sounds and might therefore constitute a precursor for the development of speech perception. This phenomenon will be described in more detail in chapter 5 of the Introduction (page 35 ff.).

1.2.4 Habituation Paradigm

Habituation is defined as the decrease of an elicited response as a result of repeated stimulation (Thompson, & Spencer, 1966). It is one of the primary phenomena used to assess neurological integrity of the central nervous system (Leader, Baillie, Martin, Molteno, & Wynchank, 1984; Madison et al., 1986a). Chapter 4 of the Introduction (page 31 ff.) will give a more detailed explanation of this paradigm.

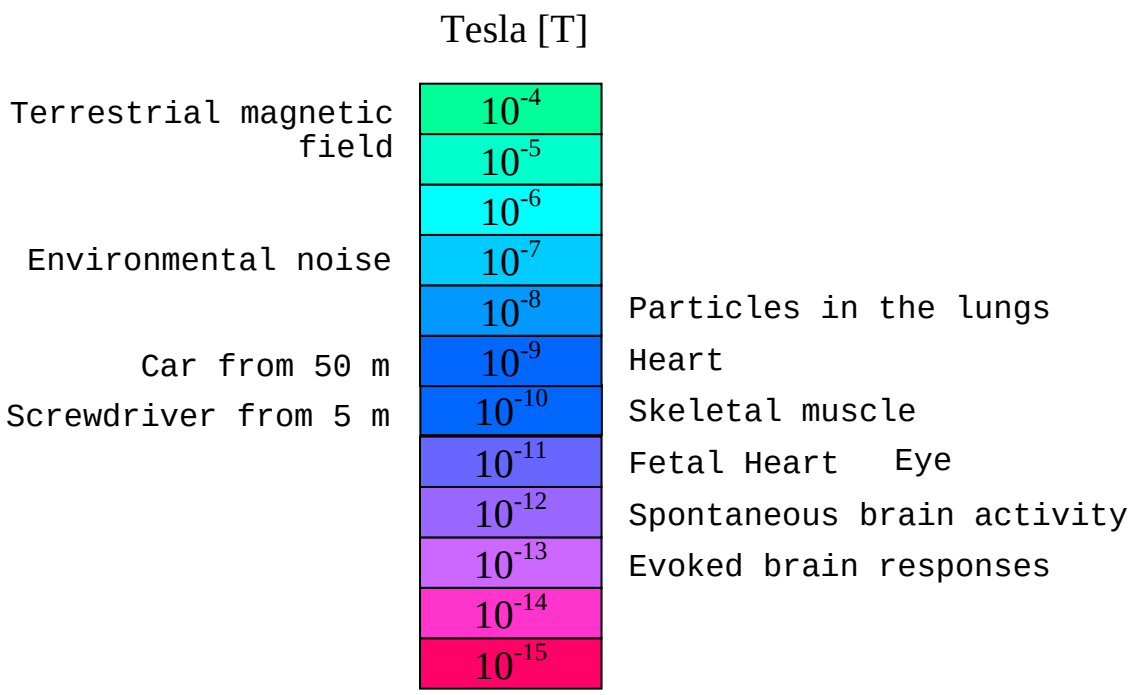
The following chapter reviews previous studies conducted with the SARA system.

1.3 Fetal Magnetoencephalography

Brain cells generate electric currents which are accompanied by magnetic fields. Initial detection of these magnetic brain signals from the surface of the scalp has been shown by Cohen (1968). Since then, MEG has been greatly improved and is now an established technology in research and clinical settings. For a more detailed description of clinical usage, see Papanicolaou, Castillo, Billingsley-Marshall, Pataraia, and Simos (2005). Current multichannel whole-head MEG systems have helped to improve the understanding of normal as well as abnormal brain functioning in adults. As yet, prenatal EEG or MEG recordings have been performed to a rather limited extent. Protected by the maternal abdomen, the fetus is quite secluded from the external environment.

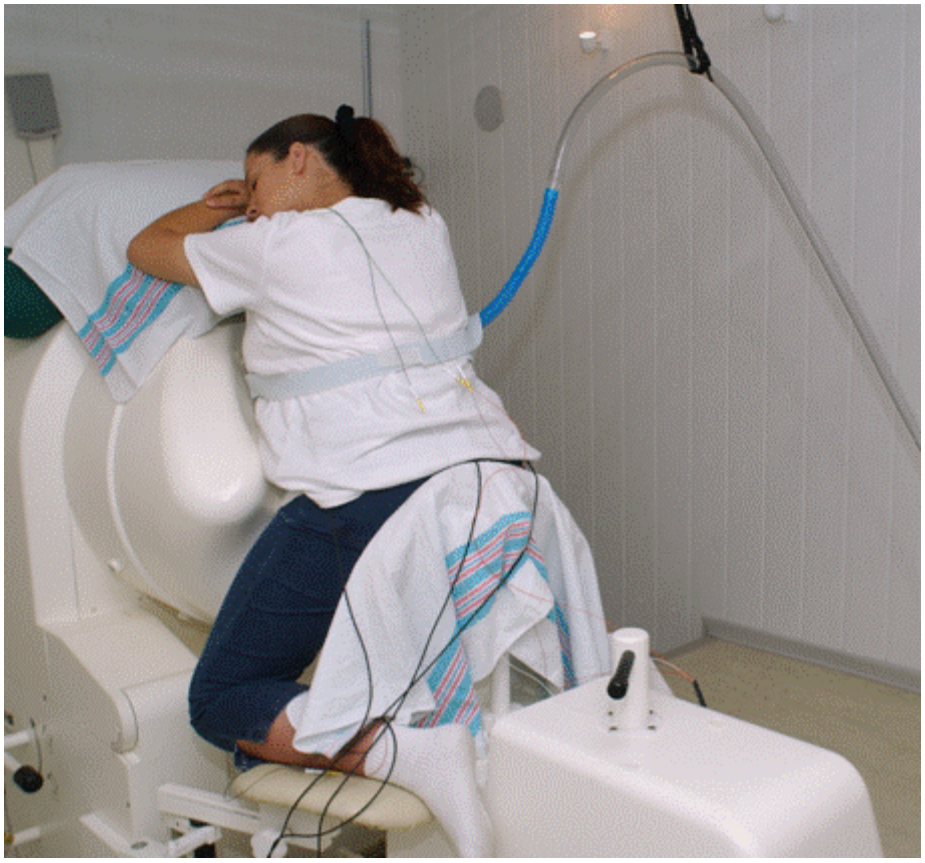
Theoretically, EEG electrodes could be placed on the mother's abdominal skin, in an attempt to detect fetal electrical brain signals. But the different electrical properties of maternal skin, muscles and water, in addition to the natural weakness of fetal cortical activity, make it impossible to record fetal brain responses directly from the abdomen. The highly sensitive SQUID sensors used in current MEG technology, on the other hand, allow the registration of fetal brain activity. Magnetic fields have the advantage to permeate through body tissue and bones with no significant distortion. However, environmental artifacts, including the magnetic field of the earth (10^{-4} Tesla), traffic, light bulbs etc. are much larger than the small magnetic fields generated by the brain (10^{-12} – 10^{-15} Tesla). Therefore, recordings with MEG technology are usually conducted in magnetically shielded rooms. An overview over the field strength of different magnetic sources is provided in Figure 1.

Figure 1. Overview over the intensity of different environmental and physiological magnetic fields



Blum, Saling and Bauer (1985) recorded fetal auditory evoked responses with MEG technology. The first MEG system built exclusively for fetal and neonatal recordings – the SARA system – was installed in the USA at the University of Arkansas for Medical Sciences (UAMS) in 2000 (Preissl et al., 2004). Figure 2 shows a mother seated on the SARA system for a fetal study.

Figure 2. A mother seated on the SARA system for a fetal recording.



The major advantages of this method are the non-invasive recording of brain signals, which allows the conduction of studies on the human fetus and the high temporal resolution of the detected cortical activity. However, MEG technology does not provide a high spatial resolution. An inherent problem of MEG/EEG recordings is the determination of the source that generated the activity at the sensor level. In general, the reconstruction of the sources, the so-called inverse problem, is possible only if an adequate model of the investigated structure is available. In case of fetal recordings, this

information is obtainable with the help of additional imaging systems, such as ultrasound (Gutierrez, Nehorai, & Preissl, 2005). Another drawback of MEG technology is its maintenance. It requires very low temperatures for proper functioning, which are sustained by an expensive helium-based cooling system. But the aforementioned advantages make MEG technology an established tool for neurophysiological assessments, especially on fetuses and neonates.

The following overview summarizes the studies previously conducted with the SARA system.

1.3.1 Auditory Evoked Fields

With fetal MEG technology, auditory evoked fields to pure tone stimuli have been detected in up to 80% of the fetuses. The responses occurred at latencies around 200 ms (Eswaran et al., 2002a) and have been shown to decline with advanced GA, suggesting a more mature brain response (Holst et al., 2005; Schleussner & Schneider, 2004).

1.3.2 Visual Evoked Fields

Moreover, visual flash evoked fields have been successfully detected with the SARA system with a fetal response rate of 80% (Eswaran et al., 2002b; Eswaran, Lowery, Wilson, Murphy, & Preissl, 2004). Figure 3 shows the setup for the presentation of the light stimulus.

Figure 3. Study setup for prenatal recording of visual evoked responses

1.3.3 Spontaneous Brain Activity

In addition to evoked brain potentials, recordings of fetal spontaneous brain activity have been conducted (Preissl et al., 2004), and characteristic activity patterns, such as trace alternates were described (Eswaran et al., 2007).

1.3.4 Fetal Behavioral State

The SARA system also allows the determination of the fetal behavioral state. By combining fetal heart rate variability with body movements, Nijhuis, Prechtl, Martin, and Bots (1982) categorized four different states: quiet asleep (F1), active asleep (F2), quiet awake (F3) and active awake (F4). Fetal state determination is important for the recording of evoked fields because it is likely for the brain responses to be influenced by the fetal state at the time of recording.

1.3.5 Fetal Magnetocardiography

Fetal magnetocardiography has been conducted with fMEG as well (Lowery et al., 2003) and it has been shown to differentiate between high- and low risk conditions (Govindan et al., 2007).

In summary, current fMEG technology has been shown to successfully detect basic evoked responses to sensory activation in fetuses as well as spontaneous brain signals. Consequently, in a next step, more advanced, higher-level cortical processes are the focus for current and future investigations. A first approach towards the assessment of cognitive performance with fMEG technology has been conducted by Draganova, Eswaran, Murphy, Huotilainen, Lowery et al. (2005). In this study, MMR has been found in 60% of the fetuses between 33 and 36 weeks GA. In a more recent study, the discriminative wave has been detected in 66% of the fetuses as early as 28 weeks GA (Draganova et al., 2007).

1.4. Fetal Habituation

Habituation is defined as the decrease of an elicited response as a result of repeated stimulation (Thompson & Spencer, 1966); and it is one of the primary phenomena utilized to assess neurological integrity of the central nervous system functioning (Leader et al., 1984; Madison et al., 1986a). Several ultrasound studies have shown fetuses to

exhibit habituation (Bellieni et al., 2005; Groome, Gotlieb, Neely, & Waters, 1993; Kuhlman, Burns, Depp, & Sabbagha, 1988; Madison et al., 1986a; Shalev, Benett, Megory, Wallace, & Zuckerman, 1989; Smith, Davis, Rayburn, & Nelson, 1991; Van Heteren, Boekkooi, Schiphorst, Jongsma, & Nijhuis, 2001b). Moreover, there is evidence that younger fetuses (below 28 weeks GA) habituate at a slower rate than those at advanced gestational ages (older than 32 weeks GA) (Doherty & Hepper, 2000; Groome et al., 1993; Kuhlman et al., 1988). However, some studies could not reproduce this gestational age dependency (Bellieni et al., 2005; Madison, Madison, & Adubato, 1986b).

In the past, habituation paradigms performed with ultrasound have been used to differentiate also between high and low risk conditions in the fetus (Hepper, & Shahidullah, 1992; Leader, Baillie, Martin, & Vermeulen, 1982a; Leader, & Baillie, 1988). Maternal conditions such as diabetes (Doherty & Hepper, 2000), depression (Allister, Lester, Carr, & Liu, 2001) and stress (Sandman et al., 2003) have been shown to affect the fetal habituation in a negative way, indicating developmental delays which may be linked to impaired function of the cerebral cortex (Morokuma et al., 2004). Therefore, the prenatal detection of adverse habituation could be used as an indicator for the re-evaluation of maternal high-risk conditions. Regarding normal brain development, the degree of habituation has been shown to be a possible predictor of

postpartum cognitive development (Gaultney, & Gingras, 2005; Madison et al., 1986b).

Previous studies on prenatal habituation used mostly vibroacoustic (Bellieni et al., 2005; Doherty, & Hepper, 2000; Gaultney, & Gingras, 2005; Groome, et al., 1993; Groome, Watson, & Dykman, 1994; Kuhlman et al., 1988; Leader et al., 1982a; Leader, Baillie, Martin, & Vermeulen, 1982b; Madison et al., 1986b; Smith et al., 1991; Van Heteren, Boekkooi, Jongsma, & Nijhuis, 2001a; Van Heteren et al., 2001b) or sound stimulation (Hepper, & Shahidullah, 1992; Shalev et al., 1989) to elicit a response. The outcome measures were mainly fetal body movements (Doherty & Hepper 2000; Groome et al., 1993; Madison et al., 1986b; Van Heteren et al., 2001a; Van Heteren et al., 2001b) or blink-startle reflex (Bellieni et al., 2005) observed by ultrasound and/or heart rate acceleration (Goldkrand & Litvack, 1991; Smith et al., 1991). These measures are based on physiological reactions and are believed to indicate cortical stimulus perception (Bellieni et al., 2005; Hepper, 1995; Morokuma et al., 2004). However, all of these responses are indirect observations of brain responses, and have to be interpreted carefully since it is not clear whether the development of fetal motor skills influences these observations (Hepper, 1997). These studies used a long-term habituation paradigm in which the stimulus was presented repeatedly until response cessation. The number of stimuli

leading to a termination of the response is called the habituation rate.

No previous study has investigated habituation in fetuses via direct detection of brain responses. In adult EEG and MEG studies, habituation has been tested with a paradigm called short-term habituation (Lasky, Maier, & Hecox, 1996; Noguchi, Inui, & Kakigi, 2004; Rosburg et al., 2006; Vanhanen et al., 1996). Lasky (1997) conducted this paradigm with auditory stimulation on newborns and adults. It differs from the above-mentioned long-term habituation as follows. First, the number of stimuli presented is fixed from the beginning, and therefore independent of the strength of an elicited response. Second, the ISI is usually shorter. Third, in order to enforce dishabituation, the train of stimuli is followed by a longer break or a deviant stimulus. Hence, the criterion for the occurrence of habituation is the decrease in response latency and/or amplitude from the first to the second stimulus rather than cessation of the response. In case a paradigm does not fulfill all the criteria to distinguish habituation from receptor fatigue (Thompson & Spencer, 1966), it is more appropriate to call the phenomenon “response decrement” (Kuhlman et al., 1988). Nevertheless, studies using this paradigm on adults have shown its usefulness in the initiation of habituation in healthy adults (Amochaev, Salamy, Alvarez, & Peeke, 1989; Lasky et al., 1996) as well as in the

differentiation between cognitively impaired and normal participants (Vanhanen et al., 1996).

1.5 Rapid Temporal Processing as a precursor for the development of speech perception

Human newborns can discriminate different syllables (Guttorm, Leppänen, Tolvanen, & Lyytinen, 2003; Molfese, 2000) and recognize their mother’s voice (DeCasper, & Fifer, 1980; Fifer, & Moon, 1989; Ockleford, Vince, Layton, & Reader, 1988) within the first days after delivery. Moreover, the auditory perception of neonates at genetic risk for language disorders, like dyslexia or specific language impairment (SLI), differs from infants without genetic predetermination. Richardson, Leppänen, Leiwo, and Lyytinen (2003) found a deficiency in the processing of speech sound duration in 6 months old infants with dyslexic parents, suggesting an early developmental impairment, long before the child can write and spell words. Such differences in auditory perception have been found as early as 36 hours after delivery and were not only evident in speech sounds such as syllables, but also in non-speech sounds (Molfese, 2000). This suggests that a more basic scheme might precede the successful perception of speech. The processing of rapidly presented (within milliseconds) stimuli might be an important precursor for successful speech perception, and therefore, language acquisition. Several studies have shown

RTP skills to be impaired in individuals at risk for, or suffering from, language disorders like SLI or dyslexia (e.g. Benasich et al., 2002; Farmer & Klein, 1995; Tallal & Piercy, 1973; Walker, Hall, Klein, & Phillips, 2006; Wright et al., 1997). In an EEG study, Benasich et al. (2006) showed that latency differences in the evoked responses of RTP tasks not only differentiate infants at risk for language impairments from a low-risk control group, but also that the response latencies predict an infant's language performance later in life, regardless of an existing family history. In a MEG study on children and adults, Oram Cardy, Flagg, Roberts, Brian, and Roberts (2005) showed differences in brain responses evoked by rapidly presented tones. Participants with language impairments responded significantly less often to the second tone of a pair than individuals with normal speech performance and vice versa.

Some authors found frequency discrimination (FD) skills or the ability to distinguish different stimulus durations to differentiate between children with SLI and their control group (Ors et al., 2002; Richardson et al., 2003; Uwer, Albrecht, & Von Suchodoletz, 2002). However, Bishop and McArthur (2005) showed abnormalities or delays in the waveforms of auditory event-related potentials (ERPs) in SLI participants that were not detected by an FD task. Also, group differences found in younger children were no longer evident in the same participants at a later age. The authors reasoned

that behavioral FD thresholds might be more sensitive to maturational processes than ERP response patterns. The above mentioned ERP study on six months old infants (Benasich et al., 2006) used electrocortical responses to rapidly presented tone pairs as well as FD to investigate differences between infants at risk for language disorders and a control group. Rapidly presented non-speech stimuli (tone pairs) were presented in two difficulties: a) the sounds were separated by a 300 ms gap (long), b) the sounds were separated by a 70 ms gap (short). Both paradigms included a frequent and a deviant tone (defined by frequency) in order to record the neurophysiological correlate of FD, the so-called MMR. The ERPs between the groups were clearly different in the more difficult short version. The low-risk babies showed faster ERP responses (shorter latencies) as well as higher amplitudes in their MMR. However, statistically significant predictions for language performance at two years of age were found only in the ERP latencies. These results seem to indicate that ERPs to rapidly presented stimuli are more useful in the prediction of later language outcome than FD.

Despite increasing support for the importance of general non-speech precursors for language acquisition, even in modalities other than auditory (Talcott, Hansen, Assoku, & Stein, 2000; Witton et al., 1998), there has been controversy in the literature. Some studies did not find a general temporal processing deficit to be crucial in the development

of language disorders (McArthur & Bishop, 2001; Mody, Studdert-Kennedy, & Brady, 1997; Studdert-Kennedy, Mody, & Brady, 2000). Rather, these authors believed a speech-specific failure in phonological representation to be the underlying factor for language disorders. The first publication to challenge the RTP theory was the study by Mody et al. (1997). Since then, the different theories have been discussed extensively in the literature. Denenberg (1999) first published a detailed critique of the study by Mody et al. (1997). He claimed that Mody's investigation did not contribute to the debate of a general deficit in RTP versus a specific impairment in phonological representation for the following reasons. Mody's experimental group consisted of children that were categorized as "poor" readers, whose reading skills were at a normal level for their grade. In opposition, the reading performance of children categorized as "language impaired" by Stark and Tallal (1981) were at least one year below their actual grade level. Moreover, Denenberg argued that Mody's sample size was too small in order to reject the null hypothesis, which led to the finding that the two groups did not differ in their auditory perception. Studdert-Kennedy et al. (2000) replied to Denenberg's critique, disapproving it as misunderstandings, and argued with studies conducted by other groups that also supported the speech-specific hypothesis rather than the general deficit (Best & Avery, 1999; Bishop, Carlyon, Deeks, & Bishop, 1999;

Bradlow et al., 1999; McAnally, Hansen, Cornelissen, & Stein, 1997; Nittrouer, 1999). In summary, the existence of a speech-specific failure in phonological representation seems to be undisputable, even by its challengers. However, even though it contributes to reading impairments, it does not explain their cause. Therefore, the phonological deficit is believed to be the result of an underlying, more general impairment (Ramus et al., 2003). In addition to the phonological and the RTP hypothesis, other major theories are prevalent in the literature. The cerebellar theory hypothesizes mild dysfunctions in the cerebellum as the biological cause for different cognitive deficits in dyslexics. The magnocellular theory integrates different hypotheses – or better – generalizes the difficulties dyslexics have, to be caused by deficiencies in multiple sensual modalities, such as visual, auditory and tactile. For a more detailed overview over these theories, see Ramus et al. (2003). Despite extensive research in the field, the results remain controversial, with supporting evidence for each theory. Many of the studies on language impairments have been conducted on children or young adults. This enables clearly distinguishable categorizations and well-defined experimental groups. However, it is likely that coping strategies as well as learning mechanisms are more prevalent with increasing age. This, in turn, might interfere with the attempt to find the underlying root of aberrant speech development (Tallal & Gaab, 2006). In fact, McArthur

and Bishop (2001) found impaired FD in younger subjects with SLI. However, 18 months later, the performance in FD of the same subjects was in the normal range. As stated above, the waveforms of ERP data in this study have been shown to be less affected by maturational processes. Therefore, the type of outcome measure as well as the study paradigm applied, might contribute to contradictory results. For a more detailed discussion see Tallal and Gaab (2006). This thesis focuses on RTP because this pre-speech paradigm is applicable to individuals at very early developmental stages, long before they begin to speak.

Some of the studies mentioned above (e.g. Benasich et al., 2002; Farmer, & Klein, 1995; Tallal, & Piercy, 1973; Walker et al., 2006; Wright et al., 1997) suggested that impaired RTP might contribute to the development of language disorders. It is believed to prevent the accurate processing of brief acoustic transitions crucial for the successful perception of speech. Therefore, adequate language learning might be impeded on a very basic level. However, the neural basis of RTP maturation is still not entirely understood. Questions regarding the development of RTP, like the minimum length of the gap between the two tones in a tone pair for certain maturational stages, as well as the onset of RTP impairments in at risk populations, have not yet been addressed. Some neuropathological studies found malformations in the brains of dyslexics which are believed to originate in the fetal stages

of life (Galaburda, & Kemper, 1979; Galaburda et al., 1985; Humphreys et al., 1990). This suggests that the underlying deficits leading to disordered language acquisition might develop during cell migration in the prenatal period. Whether such early neuroanatomical malformations lead to RTP deficits is still subject to speculation. A better understanding of the early RTP maturation in normally developing infants could be a first step towards a better insight into the roots of language impairments. As yet, the development of RTP and its neurophysiological correlates during the prenatal and early postnatal stages has not been studied.

1.6. Aims of present studies

The studies in this thesis aimed to take a first step towards an improved assessment of human pre- and early postnatal brain development with novel fMEG technology. Established study paradigms that assess cognitive functioning, such as integrative processes and speech development, were applied to neonates and fetuses. Therefore, this work might contribute to a more accurate detection of early developmental delays and the prevention of subsequent impairments.

Each study investigated a specific aspect of cognitive brain development. In the first one, response decrement as a basic indicator for higher-level processing was addressed (Sheridan et al., in press). Since there are no known reports

of neonatal response decrement to flash evoked responses assessed with MEG technology, the first aim of this study was to evaluate the results of this visual paradigm on newborns before assessing its applicability to fetuses. The application of this paradigm to the results from the same participants during their fetal stage of life was therefore investigated in a second step. The latter was planned as an observational study.

The second investigation applied rapidly presented auditory stimuli to neonates and fetuses. Pairs of standard and deviant non-speech tones were applied in two difficulties – short and long. The main goal of this study was to investigate the applicability of RTP tasks to neonates and fetuses in order to assess precursors of speech perception at very early developmental stages. Moreover, possible maturational changes in the cortical response patterns were investigated. Here as well, data analysis started with the neonatal data going backwards, from the more- to the less mature developmental stages.

The expected results are listed below.

1.6.1 The application of established study paradigms with fMEG technology

1.6.1.1 Response Decrement (Study 1)

Previous studies with the SARA system have shown the successful detection of visual evoked responses (Eswaran et

al, 2004). Therefore, it is expected that response decrement of flash evoked responses in newborns and possibly even in fetuses can be conducted with fMEG technology.

Moreover, the first-time use of the response amplitude as an outcome measure is expected to be successful. As yet, the response amplitudes have not been used as primary outcome measures for prenatal investigations because the amplitudes are affected by the distance between the source and the sensors. In fetal recordings, this distance is not controllable. With the short-term habituation paradigm, however, the peak amplitudes are analyzed separately for each participant in order to investigate the response decrement over the four light flashes. The intraindividual comparison is enabled because all four stimuli are equally affected by changes in the fetal position.

1.6.1.2 Rapid Temporal Processing (Study 2)

RTP has been successfully recorded with adult whole-head MEG technology (Oram Cardy et al., 2005). Moreover, EEG studies have shown the assessment of RTP skills in infants (e.g. Benasich et al., 2006). Therefore, it is expected that RTP skills in newborns and possibly even in fetuses can be assessed with fMEG technology.

1.6.2 Course of development over age

The applied paradigms were applied to assess the course of brain development, from the fetal until the early postnatal stages of life.

Study 1: Response Decrement

Findings of previous long-term habituation studies have shown fetuses to habituate as early as 22 weeks GA (Leader et al., 1982b). Short-term habituation paradigms have been successfully applied on adults (e.g. Lasky et al., 1996). Based on these findings, the observation of a decrease in response amplitude from the first to the last stimulus in this study is expected to be successful in neonates and – by means of fMEG technology – even in fetuses.

Moreover, recent psychophysiological studies on adults have shown the response latency to decrease with repeated stimulus presentation (Rosburg et al., 2006). Therefore, the latency of the response to the first stimulus in this study is expected to be shorter than to the last one.

Study 2: Rapid Temporal Processing

In an EEG study, Benasich et al. (2002) showed six months old babies to respond with two peaks to a tone pair with a 300 ms gap, whereas the short tone pair resulted in one peak. Adults with language impairments have been shown to respond less often to the second tone in a pair than their control group (Oram Cardy et al., 2005). However, no study has

assessed tone pair response patterns to gaps of 300ms or less in newborns below 6 months of age. Therefore, the aberrant perception is reflected by only one response to two tones. It is likely that an immature response is represented similarly by only one peak. Based on this hypothesis, the observation of a trend from one response in younger participants to two responses in the older ones is expected in this study. Depending on the cortical processing capabilities, this trend is assumed to be significant in either the short or the long paradigm: (1) If the short version is too difficult, and even the oldest babies respond with only one peak (ceiling effect), an age trend is evident in the longer (easier) tone pair. (2) On the other hand, if all the infants respond with two peaks to the long version (floor effect); an age trend is detectable in the more difficult, short tone pair.

1.6.3 Response rate

Another goal of this study was to address the impact different stimulus modalities and presentations have on the detected response rates.

Study 1: Response Decrement

Based on previous studies with the same light stimulation (Eswaran et al., 2004) a fetal response rate of about 80% is expected.

Study 2: Rapid Temporal Processing

A previous study on auditory brain responses with similar length and stimulus duration (Draganova et al., 2005, 2007), indicates that a fetal response rate between 60% and 66% to one of the two tones in the pair can be expected in both paradigms.

In an MEG study on neonates, Sambeth, Huotilainen, Kushnerenko, Fellman, and Pihko (2006) found the deflection of the responses to be larger to the novel and deviant stimuli than the standard one. Therefore, the responsiveness to the stimulus that is presented more frequently is expected to be lower, whereas the infrequent one is expected to cause a higher response rate. In this study, the neonatal response rate is expected to be higher for the deviant than the standard tone pair.

2. Methodology

2.1. Participants

The participants were recruited at the fetal stage of life in order to investigate the newborns and further assess the applicability of the paradigms on the fetuses. They were healthy pregnant women with no known risks or complications, and all the newborns were delivered in term.

The studies were approved by the local Institutional Review Board and each mother or their legal representative signed an informed consent form.

2.1.1 Postnatal recordings

All of the participating mothers were part of the corresponding antecedent fetal study described below. For the postnatal follow-up recordings, they were advised to bring the newborns in for the neonatal study within three weeks (in study 1) or within the first six weeks (in study 2) after delivery.

2.1.2 Prenatal recordings

The fetal recordings were conducted between 29 to 38 weeks GA and some mothers completed up to three prenatal studies.

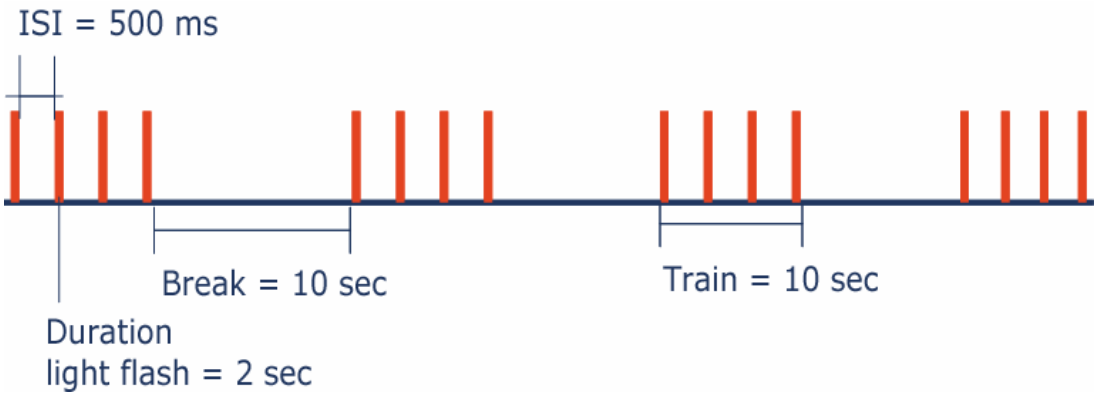
2.2 Stimulation Paradigm

In both studies the stimulation paradigm was the same for the pre- and postnatal studies, except in study 1 the duration of the recording was shorter in the follow-up.

2.2.1 Response decrement (study 1)

This paradigm consisted of a train of four light flashes (duration 500 ms) with an ISI of 2 s, followed by a 10 s break (Figure 4).

Figure 4. Study paradigm for study 1: response decrement.



In the neonatal study, this sequence was repeated 60 times (three times per minute). This added up to a 20-minute recording with a total of 240 stimuli, or 60 for each of the four light flashes. For the prenatal study, the duration of the recording was increased to 30 minutes. This led to a total

of 360 stimuli or 90 presentations of each one of the four light flashes.

2.2.2 Response decrement (study 2)

In this study, auditory, non-speech tone pairs were presented. They were divided into two difficulties, which were recorded separately. The more difficult paradigm had shorter time gaps (70 ms) between the two tones of a tone pair, whereas the easier one had longer time gaps (300 ms). The duration of all tones was 70 ms. The stimulus onset asynchrony (SOA) – the interval from the onset of the first stimulus in a tone pair to the onset of the first stimulus in the next tone pair – was 915 ms in the short paradigm and 1140 ms in the long paradigm. The recording time for the short study was 11 minutes, resulting in about 721 stimulus presentations, whereas the total duration of the long paradigm was 14 minutes, in which 735 tone pairs were presented. The two paradigms were applied consecutively in random order. Both versions included a standard (85%) and a deviant (15%) tone pair. The standard tone pair consisted of two 500 Hz tones; whereas the deviant tone pair had the same 500 Hz tone followed by a second tone with a frequency of 750 Hz. Figures 5 a) and 5 b) show the stimulus presentation. It was based on a study conducted by another group that reported the processing of tone pairs in older infants (Benasich et al., 2006).

Figure 5 a). Study paradigm – long – for study 2 (rapidly presented auditory stimulation): The deviant tone pair was presented in 15% of the stimuli and is indicated by the red bar in the second tone of the pair.

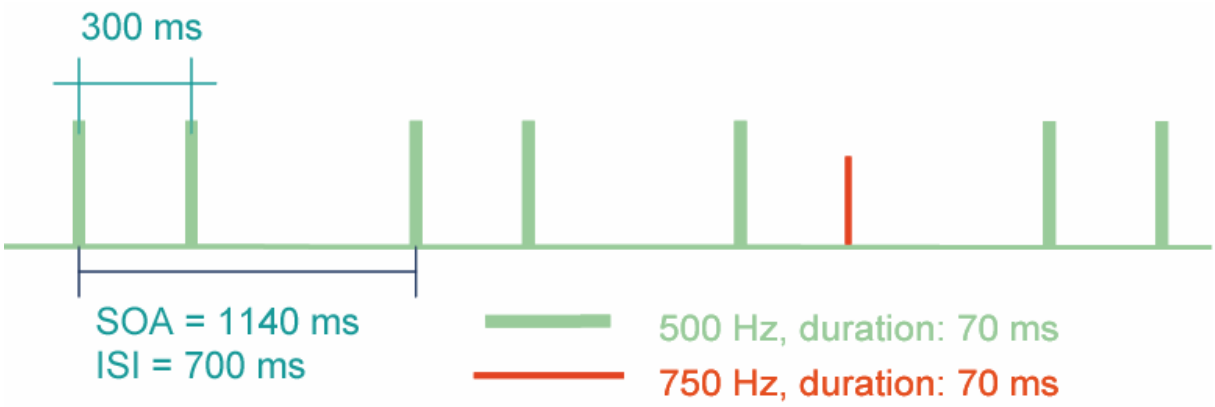
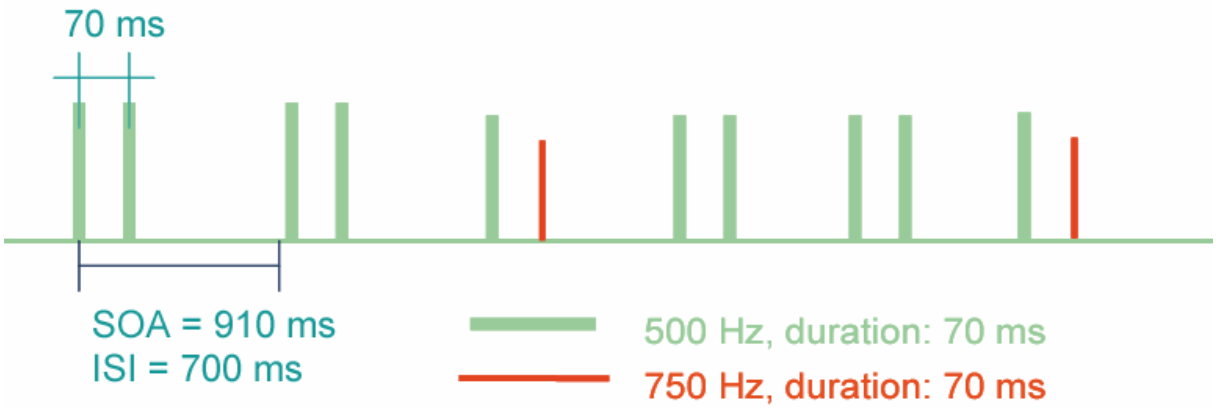


Figure 5 b). Study paradigm – short – for study 2 (rapidly presented auditory stimulation): The deviant tone pair was presented in 15% of the stimuli and is indicated by the red bar in the second tone of the pair.



2.2.3 Stimulation setup

The light source (study 1) as well as the speaker that generated the sound (study 2), were located outside the magnetically shielded room (MSR) (Vacuumschmelze, Germany) in which the recordings were performed.

In both studies, stimulus presentation was controlled by software called STIMPRO (UAMS, Little Rock, AR). The light-stimulus system in study 1 consisted of a 3.4 m fiber optic cable which terminated in a 3 x 5 cm woven panel (Stocker Yale, Salem). The input end of the cable was split into four sub-bundles illuminated by individual high-power LED (light emitting diode) arrays. A light output of 35 mW at 630 nm (red) was measured at the panel. No artifacts from the LEDs have been observed in our previous studies with the same light stimulus (McCubbin et al., 2007a). For the neonatal recordings, the woven panel was fastened to the ceiling at a distance of at least 75 cm above the infant’s head. For the prenatal study, it was placed on the surface of the maternal abdomen. The mother then leaned forward on the woven panel, covering the flash from her own view.

The tones in study 2 were transmitted to the inside of the MSR through a plastic tube whose distal end consisted of an inflated plastic bag. For a detailed description of the stimulus delivery see Draganova et al. (2005). The sound intensity for the neonatal study was 80 dB. Taking into

account the sound attenuation of the maternal abdomen (Ockleford et al., 1988), the intensity for the prenatal study was adjusted accordingly in order to ensure that a similar audibility reaches the fetus (100 dB). In our previous studies with the same sound presentation no artifacts from the auditory stimulus have been observed.

2.3. Data acquisition and recording

In both studies, the recordings of the evoked responses were performed with the SARA system, which consists of an array of 151 primary magnetic sensors (Lowery, Eswaran, Murphy, & Preissl, 2006).

2.3.1 Neonatal recordings

For the neonatal studies, a cradle attachment on the SARA system enables the newborn to lie with the head resting on the lower center of the sensor array. Figure 6 shows a neonate during a recording.

Figure 6. Neonatal recording

In study 1, all babies were lying on their back and facing the light panel above their head. This position minimized the distance of the infant’s occipital lobe to the SQUID sensors. A marking system based on four localizing coils was used to indicate the location of the newborn’s head. The coils were fixed to a cap in order to facilitate study preparation and minimize disturbance of the infant. One of the coils was located at the baby’s forehead and the three reference coils marked the right, back and left side of the head. Before the recording was conducted, these coils were activated at a certain frequency to compute their location in relation to the MEG sensors. The sound bag in study 2 was attached to the ceiling, about 75 cm above the neonate’s head.

Since some of the babies in study 2 were older than in study 1, it was more challenging to keep them in a quiet state. Therefore, the position of the babies in this study varied depending on what caused the baby to be most comfortable and calm. Moreover, the marking system was no longer used in the neonatal recordings of study 2. This was justified by the experiences with the neonates in the first study. The newborn’s brain responses were clearly detectable and the marking system did not provide additional information for the analysis. Also, attaching the reference coils caused fussiness in some babies, which was avoided by not using the marking system. In both studies, the infant’s mother or father was seated next to the cradle inside the shielded room, and

the recording session was monitored by a camera. This allowed the investigator to note down the neonate’s state, movements and parental intervention in a qualitative way. The recording session was discontinued when the babies started to cry or if extensive movements occurred.

In both studies, the mother or father was told to sit as still as possible and to intervene only if the baby became agitated.

2.3.2 Fetal recordings

The cradle attachment was replaced by a seat. From a sitting position, the mother leaned forward into the sensor array, which is shaped to fit the gravid abdomen. Figure 2 (page 11) shows a mother seated on the SARA system for a fetal recording. The sensors are arranged so that during the prenatal recordings, the maternal abdomen is covered from the pubic symphysis to the costal arch. Prior to prenatal data collection, an ultrasound was performed in order to estimate fetal weight, size, position and level of activity. The mother was then taken to the MSR for the fMEG measurement. After seating her comfortably in front of the sensor array, a portable ultrasound exam was performed to determine fetal position so that the fiber optic light stimulus panel (in study 1) or the speaker bag (in study 2) could be placed on the maternal abdomen directly over the fetal head. In most cases, the stimulus was placed right above one or both of the

fetal eyes (in study 1). However, in some cases the eye was not visible from the maternal abdomen due to the fetal position, e.g. if the fetus was facing towards the mother's back. If this was the case, the stimulus was placed over the part of the head that was closest to the fetal eye. A notation was made of the orientation of the fetal head. Three localization coils were then attached around the maternal waist and one localization coil over the light panel (in study 1) or underneath the sound bag (in study 2). This marking system recorded the position of the mother and the fetal head in relation to the MEG sensors at the start and the end of the fMEG measurement and allowed the location of the fetal head to be indicated in the dataset. Upon completion of the study, the ultrasound exam was repeated to determine if the fetal head position had changed during the recording.

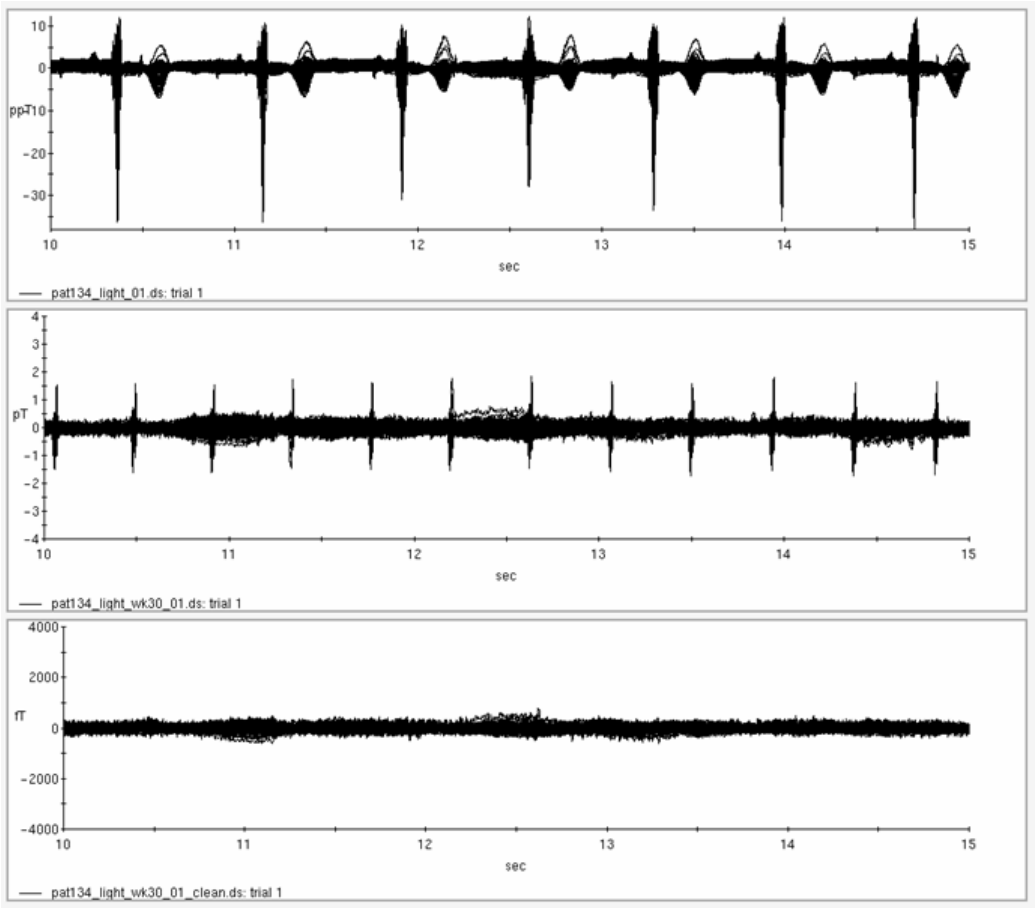
In both studies, participants were instructed to sit as still as possible. The mother was told to communicate any discomforts immediately through the speaker system located in the MSR.

2.4. Data analysis

The magnetic field signals were recorded with a sampling rate of 312.5 Hz in continuous mode in both studies. After attenuation of the neonatal or the maternal and fetal heart signals by orthogonal projection (McCubbin et al., 2006; Vrba

et al., 2004), respectively (see Figure 7), the dataset was split into separate trial classes.

Figure 7. Heart Removal via orthogonal projection



2.4.1 Response decrement (study 1)

For this study, the trial classes were based on the sequence number of the stimulus (first, second, third and fourth). Each segment had a window length of 1 s pre- and 1 s post stimulus interval. Automatic threshold detection was applied in order to mark trials containing artifacts (MEG amplitude single channel threshold 2 pT), which were excluded from further analysis. The datasets were then filtered between 1 Hz and 10 Hz in order to exclude any further residual artifacts, such as maternal and fetal cardiac activity, movements and breathing.

Averages for the different trial classes were computed. In order to identify an elicited brain response, the activity in the 1 s prestimulus interval was visually compared to the activity after stimulus onset. A wave form was considered a cortical response, if all of the following conditions were met (1) the baseline activity was smooth, (2) a peak of at least 8 fT occurred between 150 ms and 600 ms after stimulus onset, and (3) no such peak was visible in the plus-minus average. This manual comparison between the conventional and the plus-minus averaging estimates the signal to noise ratio and thus the reliability of the signals. In addition, if a peak on one of the subsequent triggers appeared 100 ms earlier or later than the response to the first trigger; it was considered a different component and therefore disregarded. The responses

for each one of the four stimuli were determined separately as described below. Based on the response to the first light flash, six channels with the best signal to noise ratio were selected, and latency as well as peak amplitude of their signal was determined. In MEG data, those peaks can either be of positive or negative value. This polarity is based on the dipole pattern of magnetic fields and therefore cannot be interpreted by means of different positive or negative components. To quantify the peak amplitude, we calculated the root mean square (RMS) over all six channels at the peak latency. Based on the assumption that the evoked responses to each one of the four light flashes are elicited in the same brain region, the same channels were selected for all the triggers.

2.4.2 Rapid temporal processing (study 2)

In this study, the dataset was split into separate trial classes based on the kind of stimulus. The deviant tone pairs were one category; and the standard tone pairs, that were presented right before a deviant one (predeviant standard tone pairs), constituted the other category. The latter was chosen because it ensured the number of stimuli for the deviant and standard tone pair to be equivalent. Each segment had a window length of 200 ms pre- and 1 s post stimulus interval in the long version and 100 ms pre- and 800 ms post stimulus interval in the short paradigm. Automatic threshold detection was

applied in order to mark trials that contained artifacts (MEG amplitude single channel threshold 2 pT) as “bad” and exclude them from further analysis. The datasets were then filtered between 1 Hz and 10 Hz in order to exclude any further residual artifacts, such as maternal and fetal cardiac activity, movements and breathing. Averages for the different trial classes were computed.

For the identification of an evoked brain response a simple statistical test was included. In the first step, trigger markers were placed at random times in the recording, and the prestimulus noise distribution was determined based on the prestimulus averages with the random trigger points. The evoked activity for the “real” stimulus was compared to this noise estimate. If the difference was significant on the second percentile, the dataset entered the next step for analysis. All other datasets were disregarded from this point on and categorized as “non-responders”.

After this preselection, the datasets had to pass a second stage of visual analysis in order to be considered for evoked responses. Responses were defined a) If the channels showing evident peaks in the poststimulus area were in the location of the fetal/neonatal head and b) If the peak occurred later than 100 ms after onset of the first stimulus in the pair. This latency criterion was defined based on the findings in our previous studies on auditory evoked responses (Eswaran et al., 2002a; Holst et al., 2005; Preissl, et al., 2004). In 25% of

the cases additional peaks, not picked as significant by the preselecting analysis in the first step, were still selected as responses: if one peak was significant, but an additional peak (that did not reach statistical significance) occurred in that same channel within the aforementioned time frame. This incongruity in the significance level of two peaks can be explained by a methodological issue: the screening with random triggers did not include trial rejection, which could have caused a higher noise level in the calculation of the noise estimate. Therefore, in these cases the comparison between pre- and poststimulus interval did not reach statistical significance even though a second peak was clearly visible in the same channel.

The responses for each one of the two paradigms (long and short) as well as for the different stimuli (predeviant standard and deviant) were determined separately. Data analysis, as explained above, was the same for all study conditions. The neonatal data were analyzed first, due to the lower noise level and less artifacts. Having a first impression on the response patterns in neonates facilitated the analysis of the fetal datasets.

2.5. Statistical Analysis

All statistical analyses were performed using SAS version 9 (The SAS Institute, Cary, NC) and SPSS (Version 11.0).

2.5.1 Response decrement (study 1)

The raw amplitude is strongly affected by the distance between the head and the MEG sensors. Since the amplitude was the main outcome variable in study 1, the neonatal amplitude data were analyzed three ways in order to adjust for this:

(1) For each recording session, Spearman's coefficient of rank correlation between amplitude and flash number was calculated; for this, amplitudes below detection threshold were treated as left-censored at 8 fT. The distribution of the rank correlation coefficients across sessions was summarized as the median, quartiles, and range. A non-parametric 95% confidence limit on the median rank correlation was also calculated.

(2) The raw amplitudes from each recording session were analyzed via mixed-models repeated-measures ANOVA. In order to minimize bias in this parametric procedure, amplitudes below detection threshold were treated as missing. ANOVA post-hoc testing was conducted via orthogonal polynomial contrasts (polynomial-contrast analysis) designed to decompose trends with flash number into linear versus non-linear (higher-order polynomial) components.

(3) In each recording session, the amplitudes for second, third, and fourth flash were divided by the first-flash amplitude, and the resulting relative amplitudes were subjected to mixed-models analysis for calculation of repeated-measures-adjusted means and 95% confidence limits. Latencies from each recording session were also analyzed via mixed-models repeated-measures ANOVA, and also analyzed in post-hoc testing via polynomial-contrast analysis. For both amplitudes and latencies, covariance structures for the repeated measures were fit to the data using the Akaike Information Criterion.

The fetal response rate was too low to conduct any statistical analyses. In case a response was not evident in one or more of the subsequent stimuli, the last observation was carried forward, meaning the amplitude was determined at the same latency point as the peak value in the previous trigger. In this respect the RMS value can be regarded as a noise estimate.

2.5.2 Rapid temporal processing (study 2)

The infant’s tone pair responses were analyzed as a function of chronological age and the number of responses (zero, one or two) with a smoothed trend estimate. In addition, the 15 participants were divided in three age groups and a trend test with tertiles of age was conducted. The group called “low” consisted of all the newborns between two and 13

days of age, the “medium” group was aged between 14 and 22 days and the category “high” contained the infants between 25 and 38 days of age.

The fetal data did not qualify for a trend estimate or separation into groups because the majority showed only one response.

In order to test differences in response patterns between fetuses and neonates, significance was tested with Chi square, using SPSS.

3. Results

3.1. Decrement of flash evoked responses

A total of 12 neonatal measurements from 12 newborns were recorded and 48 fetal studies from 25 participants were conducted between 29 and 37 completed weeks GA. One of the 12 newborn datasets was excluded due to technical interference during the study. Two other newborn recordings were terminated before the half-way point because of infant crying. These datasets were also excluded. All of the nine remaining neonates responded to the light flash. Fetal MEG measurements were conducted between one and three times per participant at different GA's. Nine fetuses were recorded once, nine twice, and seven of them three times. The interval between studies on the same participant was at least two weeks, except for one participant who was recorded at a GA of 35 weeks and again at 36 weeks. In summary, forty-eight datasets were collected from 25 participants. Ten out of the 48 fetal recordings were excluded from the analysis because the study was stopped before the last trial was finished. The reasons for early termination included increased maternal heart rate and/or discomfort. Overall, nine recordings from nine neonates and 11 recordings from seven fetuses showed responses to, at least, the first light flash.

3.1.1 Neonatal Results

In order to show the response decrement from the first to the last stimulus, the latencies of the responses, the RMS values of the response amplitudes and their rank correlations with flash number for the neonatal data are shown in Tables 1 a) and 1 b).

Table 1 a).

Neonatal Response Latencies

Patient ID	Age	Tri	Lat 1st	Lat 2nd	Lat 3rd	Lat 4th
Subject 06	22	60	0.301	0.318	0.314	0.316
Subject 08	16	57	0.416	0.43	0.451	0.444
Subject 09	14	60	0.307	0.318	0.329	0.319
Subject 10	12	54	0.286	0.285	0.283	0.318
Subject 11	12	30	0.355	0.404	NER	NER
Subject 12	17	24	0.441	0.477	0.44	NER
Subject 16	14	60	0.218	0.234	0.230	NER
Subject 22	9	30	0.242	0.244	0.266	0.262
Subject 23	6	60	0.24	NER	0.242	NER

Note. Neonatal age (“Age”; in days), number of triggers (“Tri”) recorded, and latencies (“Lat”; in ms) with each numbered flash.

Table 1 b).

Neonatal Response Amplitudes and Correlations

Patient ID	Age	Tri	Amp 1st	Amp 2nd	Amp 3rd	Amp 4th	RankCorr[b]
Subject 06	22	60	111.70	96.78	134.60	92.55	-0.400
Subject 08	16	57	66.00	49.52	36.83	20.18	-1.000
Subject 09	14	60	112.82	90.30	95.33	89.75	-0.800
Subject 10	12	54	27.03	16.07	13.02	15.00	-0.800
Subject 11	12	30	26.12	18.59	<8[a]	<8[a]	-0.949
Subject 12	17	24	44.28	19.27	24.10	<8[a]	-0.800
Subject 16	14	60	17.05	13.88	12.04	<8[a]	-1.000
Subject 22	9	30	118.84	92.62	95.44	36.86	-0.800
Subject 23	6	60	18.68	<8[a]	12.46	<8[a]	-0.632

Note. Neonatal age (“Age”; in days), number of triggers (“Tri”) recorded, response amplitudes (“Amp”; RMS values in fT) with each numbered flash, and Spearman’s rank correlations (“Rank Corr”) of amplitude with flash number.

[a]Amplitudes below the detection threshold of 8 fT were left-censored at 8 fT.

[b]Rank correlations were calculated using averaging for ties by treating left-censored amplitudes as equal to the left-censoring value of 8 fT.

The rank correlations between VER amplitude and flash number were uniformly negative, ranging from –1.00 to –0.40, and had a median of –0.80, and lower and upper quartiles of –0.949 and –0.800, respectively. The non-parametric 95% confidence limits on the median stretched from –1.0 to –0.632, showing that the median rank correlation was significantly below –0.5. Together, these observations strongly indicate that the newborn VER amplitude decreased with successive light flashes.

The behavior of the amplitude decreases were examined in more detail via repeated-measures analysis both before and after normalizing to the amplitude of the first flash: Figure 8 a) shows the averaged evoked response (19 trials) to each of the four stimulus trains on a 16 day old (41 weeks GA) newborn. An overlay of the selected channels over the head is shown. Also, the corresponding isofield map (Figure 8 b)) of the magnetic field and the head location are illustrated. Figure 8 c) shows an overlay of single channel response for all the four stimuli. From this figure the relative decrease in the amplitudes of the response from the first (-102.5 fT) to the fourth flash (-33.9 fT) is clearly observable.

Figure 8 a). Averaged evoked response (19 trials) to each of the four stimulus trains on a 16 day old (41 weeks GA) newborn.

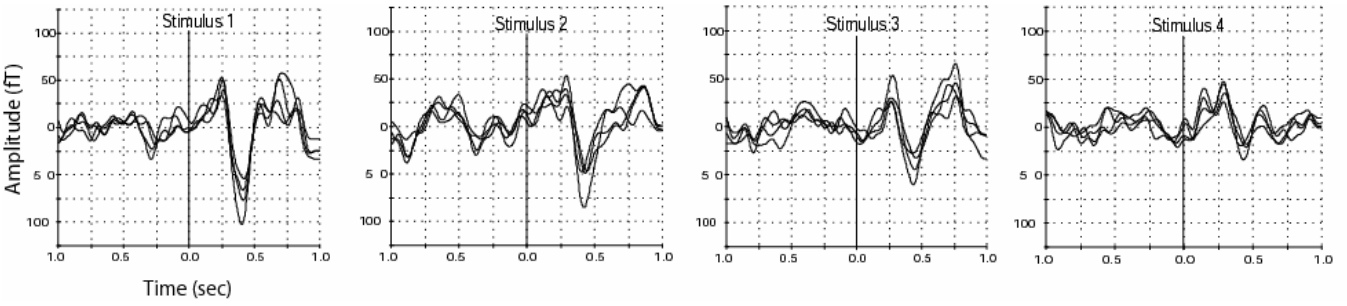


Figure 8 b). corresponding isofield map of the magnetic field and head location.

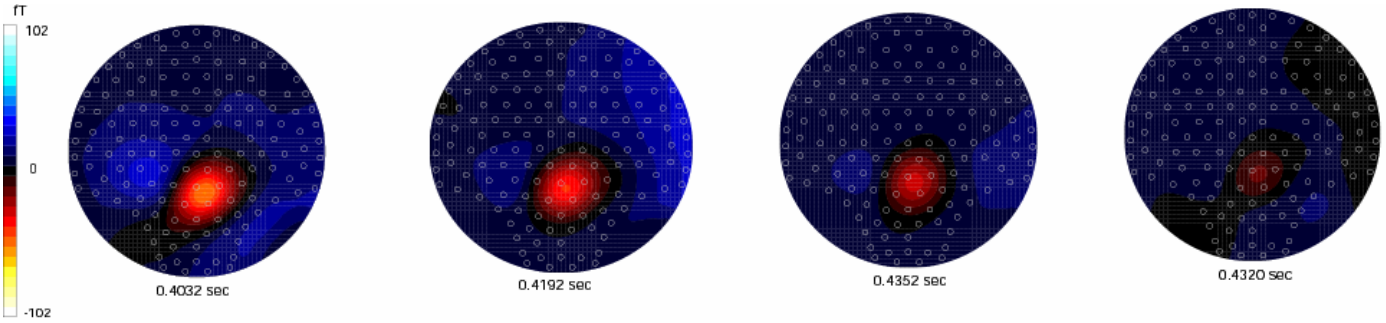
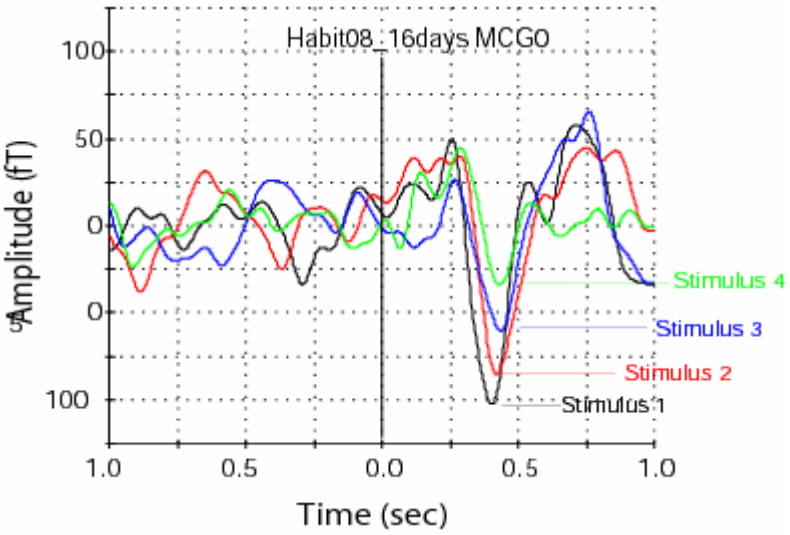


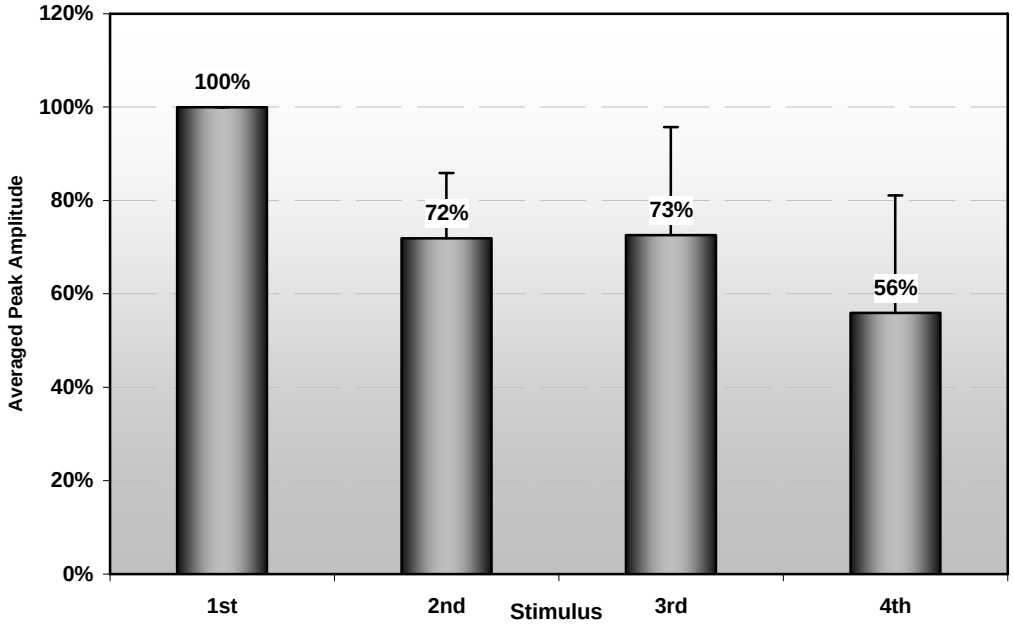
Figure 8 c). overlay of single channel response for all the four stimuli.



Raw amplitudes for neonates were analyzed via repeated-measures ANOVA using a mixed model with heterogeneous autoregressive covariance structure for the repeated flashes. The fitted model yielded means (95% confidence limits) in fT of 60.3 (31.7–88.8) for the first flash, 45.7 (20.3–71.0) for the second flash, 48.7 (14.3–83.0) for the third flash, and 30.1 (0.5–59.6) for the fourth flash; the first-order autoregressive correlation coefficient was 95% between amplitudes measured on adjacent flashes. Polynomial-contrast analysis of the downward trend in raw amplitude with flash number disclosed that the trend was statistically significant for a linear component (linear contrast F=9.74, DFs=(1,18); *P*=0.0059), with non-significant evidence for deviation from linearity (higher-order-polynomials contrast F=2.42, DFs=(2,18); *P*=0.12). After normalizing each participant’s

subsequent amplitudes to their first, the resulting relative amplitudes were also analyzed via repeated-measures ANOVA. In all neonates, the second, third, and fourth amplitudes were significantly smaller than the first, with means (95% confidence limits) relative to first flash of 72% (56%–87%) for the second flash, 73% (57%–88%) for the third flash, and 53% (34%–71%) for the fourth flash. The autoregressive correlation coefficient was 54% between relative amplitudes calculated on adjacent flashes. Figure 9 illustrates the persistence of the downward trend in relative amplitude after the first flash. Polynomial-contrast analysis on the downward trend among normalized, relative amplitudes through all four flashes yielded strong significant evidence for a linear component (linear contrast F=26.42, DFs=(1,10); P=0.0004), along with a non-significant deviation from strict linearity (higher-order-polynomials contrast F=3.27, DFs=(2,10); P=0.081). These results confirm the decrease in response amplitude from the first to the last stimulus.

Figure 9. Decrease of relative response amplitude in neonates.



In order to assess a possible effect on the response latencies in the neonates, the latencies were analyzed via repeated-measures ANOVA using a mixed model with compound-symmetry covariance structure for the repeated flashes. The fitted model yielded means (95% confidence limits) in ms of 312 (254–370) for the first flash, 329 (271–387) for the second flash, 327 (269–385) for the third flash, and 335 (276–394) for the fourth flash; the compound-symmetry correlation was 98% among repeated latency measures from the same newborn. Under polynomial-contrast analysis, the trend toward longer latency with later flashes was statistically significant for a linear component (linear contrast F=9.28, DFs=(1,18); *P*=0.0069), but statistically insignificant for non-linear components (higher-order-polynomials contrast

F=1.80, DFs=(2,18); *P*=0.19), which therefore confirmed the expected increase in latency from the first to the last stimulus.

3.1.2 Fetal Results

Out of the 38 fetal recordings that were included in data analysis, eleven responded at least to the first light stimulus. In the remaining 27 datasets no evident response could be detected either to the first, second, third or fourth trigger, leading to a response rate of 29%. The expected response rate of 80% could therefore not be reached.

In order to further address the course of development of the decrement of evoked responses in fetuses, a representative response from a 36 week GA fetus is shown in Figure 10 a). It shows an averaged response of 30 trials for each of the four triggers. The corresponding isofield maps are shown in Figure 10 b). The decrease in amplitude after the first trigger can be described as response cessation after the initial response, since the responses to the second, third and the fourth stimulus are not evident, and are possibly below the noise level of the instrument (5 fT). This is also observable in Figure 10 c), which illustrates an overlay of single channel tracings of all four triggers. The fetal head position as determined by ultrasound is demonstrated in Figure 10 d).

Figure 10 a). representative response from a 36 week GA fetus

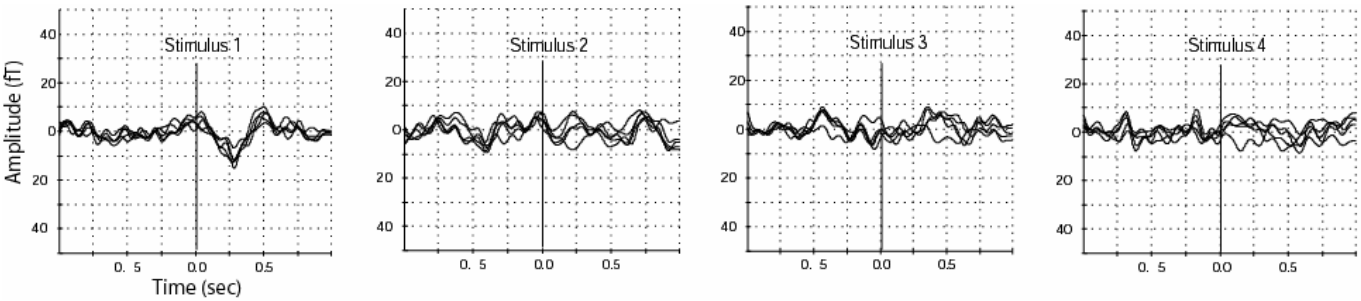


Figure 10 b). corresponding isofield maps

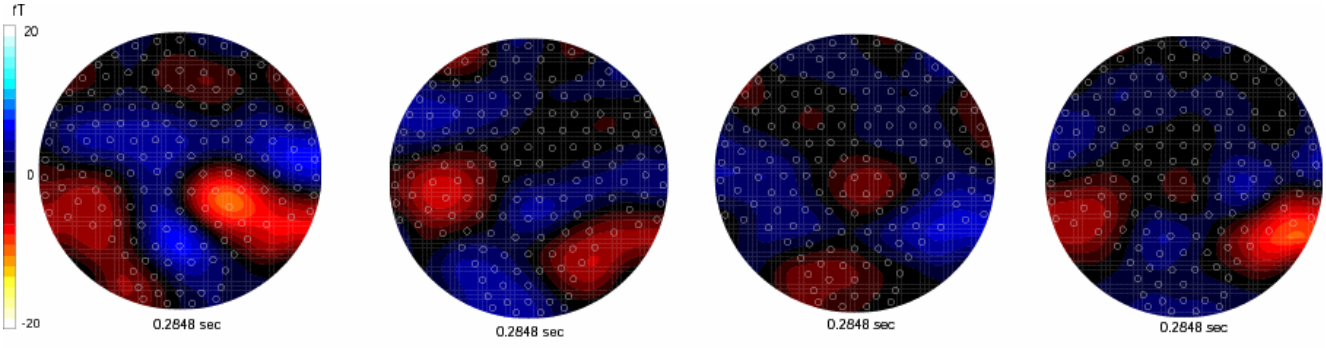


Figure 10 c). illustrates an overlay of single channel tracings of all four triggers

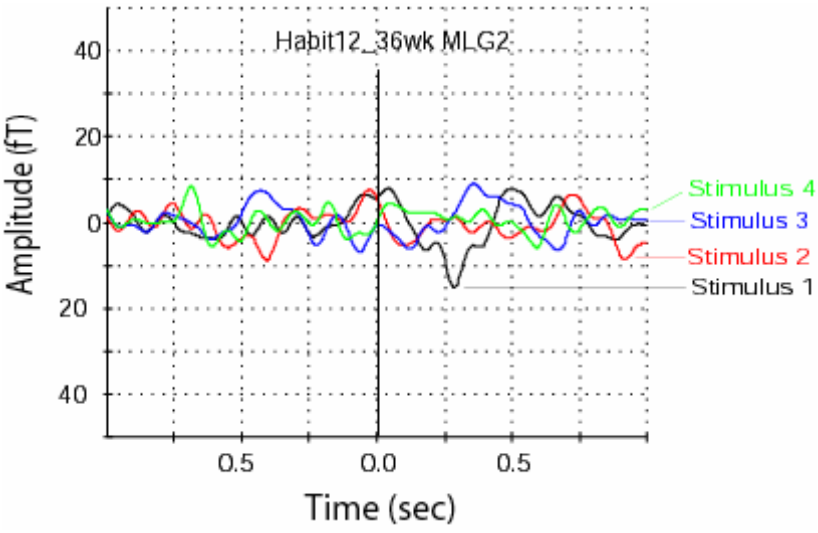
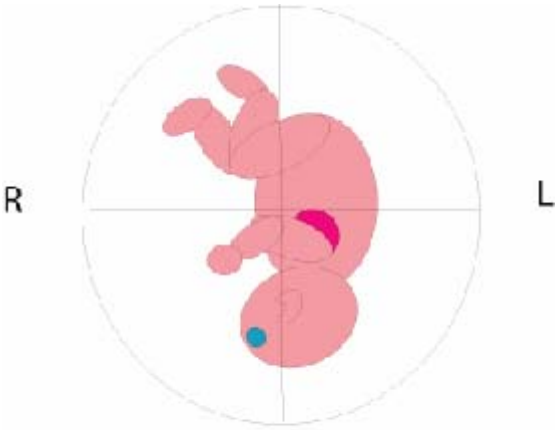


Figure 10 d). fetal head position as determined by ultrasound



The RMS values of the latencies and the peak amplitudes of the responses are shown in Table 2 a) and b). The peak amplitudes of fetal VERs with an average of 15.35 fT (range: 8.85 fT – 28.22 fT) were small compared to the neonatal averages. In only two cases we were able to observe a response to the second light flash. In both cases, the peak amplitude to the second stimulus was smaller than the first response. In the remaining nine fetal recordings, a response could only be detected at the first light stimulus. The other fields in the table show the noise estimate, which is marked by an asterisk. Because of the small response rate and the high number of missing values in the prenatal study, no further statistical analysis was conducted. Nevertheless, the fact that most of the fetuses responding to the first light flash failed to do so at the subsequent ones indicates a possible response decrement into the noise level of the instrument.

The response to the first light flash occurred at an average (range) latency of 350 ms (190 ms – 516 ms) after stimulus onset. Latencies for subsequent stimuli have not been calculated due to the high number of missing values.

Table 2 a).

Fetal Response Latencies

Patient ID	GA	Lat 1st	Lat 2nd	Lat 3rd	Lat 4th
Habitlgt01	32	0,516	0,483	0,483[a]	0,483[a]
Habitlgt01	34	0,190	0,190[a]	0,190[a]	0,190[a]
Habitlgt01	36	0,338	0,338[a]	0,338[a]	0,338[a]
Habitlgt11	30	0,316	0,316[a]	0,316[a]	0,316[a]
Habitlgt12	34	0,368	0,368[a]	0,368[a]	0,368[a]
Habitlgt12	36	0,281	0,281[a]	0,281[a]	0,286[a]
Habitlgt17	32	0,465	0,465[a]	0,465[a]	0,465[a]
Habitlgt18	35	0,437	0,412	0,412[a]	0,412[a]
Habitlgt18	36	0,476	0,476[a]	0,476[a]	0,476[a]
Habitlgt21	34	0,291	0,291[a]	0,421	0,338
Habitlgt22	36	0,209	0,209[a]	0,209[a]	0,209[a]
Avg (N=11)		0,35	0,35	0,36	0,35

Note. Fetal GA (“GA”; in weeks), response latencies (“Lat”; in ms) and their averages (“Avg”).

[a]In case a channel did not reveal an evident response in the subsequent stimulus, the last observation was carried forward, meaning the latency was the same latency as in the previous trigger.

Table 2 b).

Fetal Response Amplitudes and Correlations

Patient ID	GA	Amp 1st	Amp 2nd	Amp 3rd	Amp 4th	Corr
Habitlgt01	32	12,63	9,49	5,77[a]	8,18[a]	-0,771
Habitlgt01	34	10,28	7,08[a]	3,08[a]	2,96[a]	-0,952
Habitlgt01	36	17,05	3,49[a]	4,63[a]	12,7[a]	-0,239
Habitlgt11	30	27,21	9,06[a]	7,85[a]	4,89[a]	-0,869
Habitlgt12	34	11,80	4,15[a]	4,06[a]	4,26[a]	-0,767
Habitlgt12	36	11,86	3,72[a]	1,73[a]	3,36[a]	-0,781
Habitlgt17	32	10,59	5,03[a]	1,61[a]	3,37[a]	-0,833
Habitlgt18	35	10,12	6,40	2,62[a]	3,60[a]	-0,896
Habitlgt18	36	20,25	6,75[a]	4,06[a]	6,61[a]	-0,768
Habitlgt21	34	28,22	11,1[a]	27,67	16,62	-0,279
Habitlgt22	36	8,85	4,00[a]	2,30[a]	4,40[a]	-0,695
Avg (N=11)		15,35	6,39	5,94	6,45	-0,714

Note. Fetal GA (“GA”; in weeks), response amplitudes (“Amp”; RMS values in fT), correlation coefficients (“Corr”) and their averages (“Avg”).

[a]In case a channel did not reveal an evident response in the subsequent stimulus, the last observation was carried forward, meaning the amplitude was determined at the same latency as the peak value in the previous trigger.

3.2. Responses to rapidly presented auditory stimuli

A total of 15 neonatal measurements were conducted from 15 newborns between two and 38 days chronological age. All of the babies completed both, the short and long paradigm. In the prenatal study, a total of 34 recordings were performed on 22 fetuses at gestational ages between 29 and 38 weeks. Twelve mothers came back for a second prenatal study, at least two weeks after their first visit. In five fetal recording sessions, only one of the two paradigms (long or short) was recorded due to maternal discomfort. These incomplete recordings were excluded from data analysis, leaving 29 datasets from 20 participants for further processing.

3.2.1 Neonatal recordings

The data from each recording session were analyzed four times: the long paradigm, the short paradigm, the deviant and the standard stimuli. Therefore, the results are reported in four different sections, each representing the findings for the different study conditions. The last section summarizes and relates them to one another. The neonatal response patterns are shown in Table 3 a) and Figure 11 a) and 11 b) show examples for neonatal responses to the different paradigms.

Table 3 a).

Number of Responses in Neonates (N=15)versus Different Stimulus Conditions

Number of Responses	Long Paradigm		Short Paradigm	
	Deviant	Standard	Deviant	Standard
Zero	0	4	0	4
One	7	4	14	3
Two	8	7	1	8
Total	15	11	15	11
Response Rate	100%	73%	100%	73%

Figure 11 a). Example for responses from a 38 day old neonate: this subject responded with two peaks to both long paradigms (on the top level) and with one peak to both short paradigms (on the bottom). The peaks to the deviant tone pair are on the left side and the ones to the standard tone pair on the right side. Onset of the first tone of the pair is at 0.0 s and the peaks are indicated by the red bar.

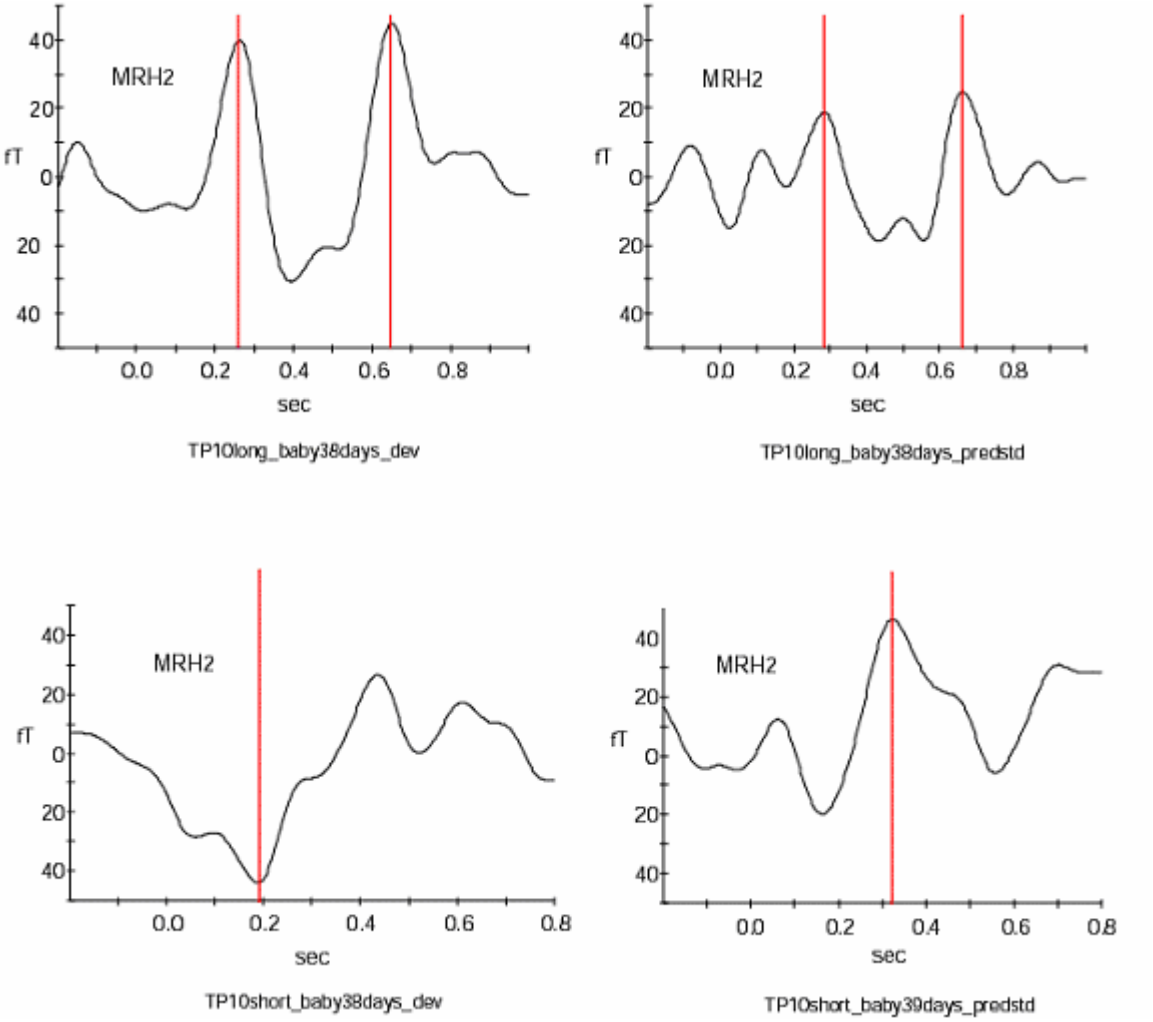
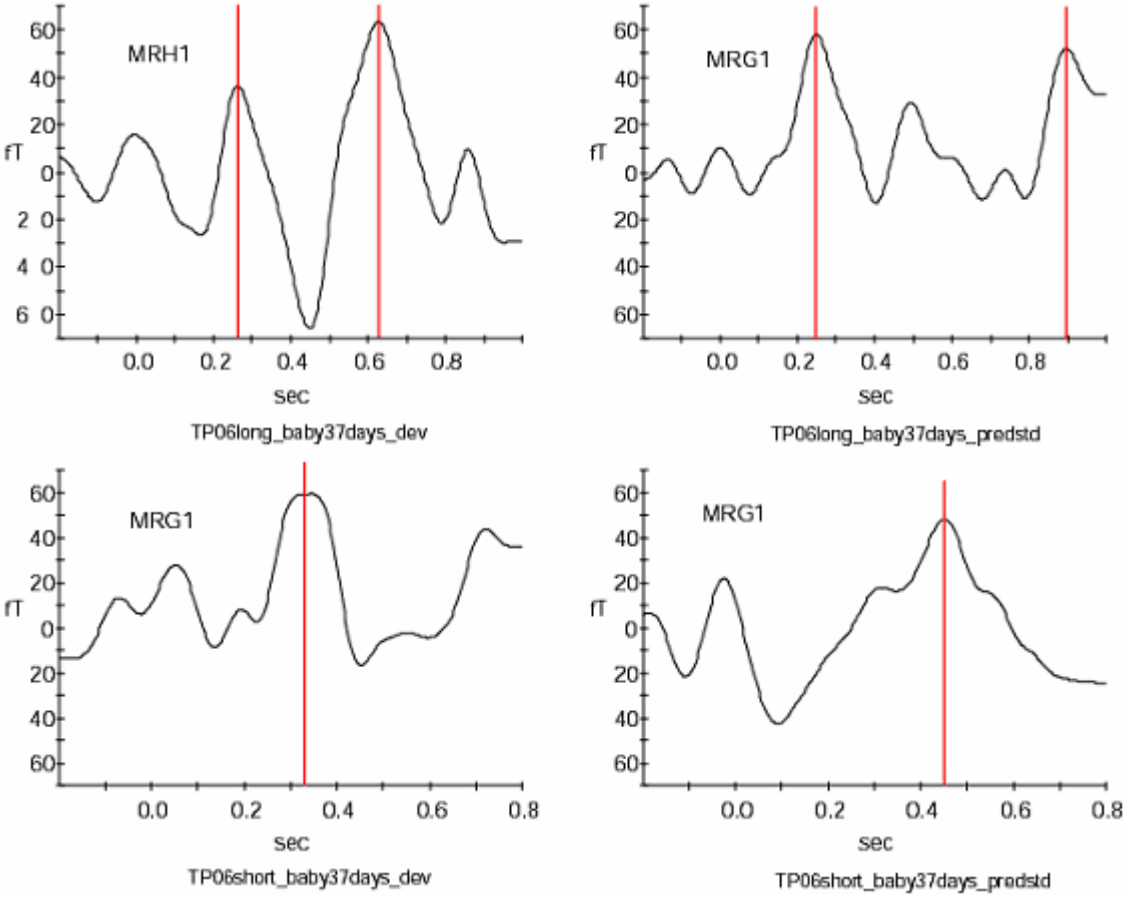


Figure 11 b). Example for responses from a 37 day old neonate: this subject responded with two peaks to both long paradigms (on the top level) and with one peak to both short paradigms (on the bottom). The peaks to the deviant tone pair are on the left side and the ones to the standard tone pair on the right side. Onset of the first tone of the pair is at 0.0 s and the peaks are indicated by the red bar.



a) Long Paradigm, Deviant Tone

All the babies responded to the long deviant tone pair with either one or two peaks. When dividing the neonates into three age groups, a statistically significant trend (P=0.0221) for number of responses by the three age groups was evident (Table 4 a)). This suggests that only the older babies showed two peaks in response to the long deviant tone pair.

Table 4 a).

Neonatal Number of Responses by three Age Groups for the Long Deviant Paradigm

Number of Responses	Age Tertiles			Total
	Low	Medium	High	
Zero	0	0	0	0
	00.00	00.00	00.00	
One	4	3	0	7
	80.00	50.00	0.00	
Two	1	3	4	8
	20.00	50.00	100.00	
Total	5	6	4	15

Note. A statistically significant trend (P=0.0221) for number of responses by the three age groups was evident.

The trend estimate of zero, one or two responses as a function of neonatal age (in days) are shown in Figure 12. There was a trend to two peaks with increasing age. The newborns responding twice were between 12 and 38 days old, whereas the one-time responders were aged between two and 22 days. Therefore, an overlap between 12 and 22 days was evident – some newborns responded twice, the others only once. However, all the neonates older than 22 days responded with two peaks, whereas the ones younger than 12 days had only one detectable peak. In summary, seven babies responded once and eight twice. The latencies for single responses were between 205 ms and 806 ms (mean 474 ms) after onset of the first tone in the pair. In the dual responses the first peak occurred at latencies between 102 ms and 381 ms (mean 213 ms) and the second peak was observed between 522 ms and 912 ms with a mean of 677 ms.

b) Long Paradigm, Standard Tone

The response rate in the long standard tone pair paradigm was 73%. Four neonates did not show an evident response. These newborns were between 12 and 22 days old. The remaining 11 babies responded with either one or two peaks. This was affected by chronological age: A statistically significant trend (P=0.0435) for number of responses by the three age groups was evident (Table 4 b)).

Table 4 b).

Neonatal Number of Responses by three Age Groups for the Long Standard Paradigm

Number of Responses	Age Tertiles			Total
	Low	Medium	High	
Zero	2	2	0	4
	40.00	33.33	0.00	
One	2	2	0	7
	40.00	33.33	0.00	
Two	1	2	4	8
	20.00	33.33	100.00	

The trend to two peaks with increasing age was observed here as well (Figure 12). Six of the seven babies, that exhibited two responses, were between 21 and 38 days old. One of them was 6 days of age. The one-time responders were between 2 and 22 days old. All the neonates older than 22 days responded with two peaks and the ones younger than 21 days showed one peak, except for the 6 day old outlier. Altogether, four newborns responded once, seven twice and four were non-responders. The single responses occurred at latencies between 141 ms and 480 ms (mean 292 ms). The first peaks of the dual responses were found between 154 ms and 288 ms (mean 223 ms)

and the second ones between 358 ms and 906 ms (mean 591 ms) after onset of the first tone.

Figure 12. Neonatal responses (zero, one or two) as a function of chronological age (in days), with smoothed trend estimate.

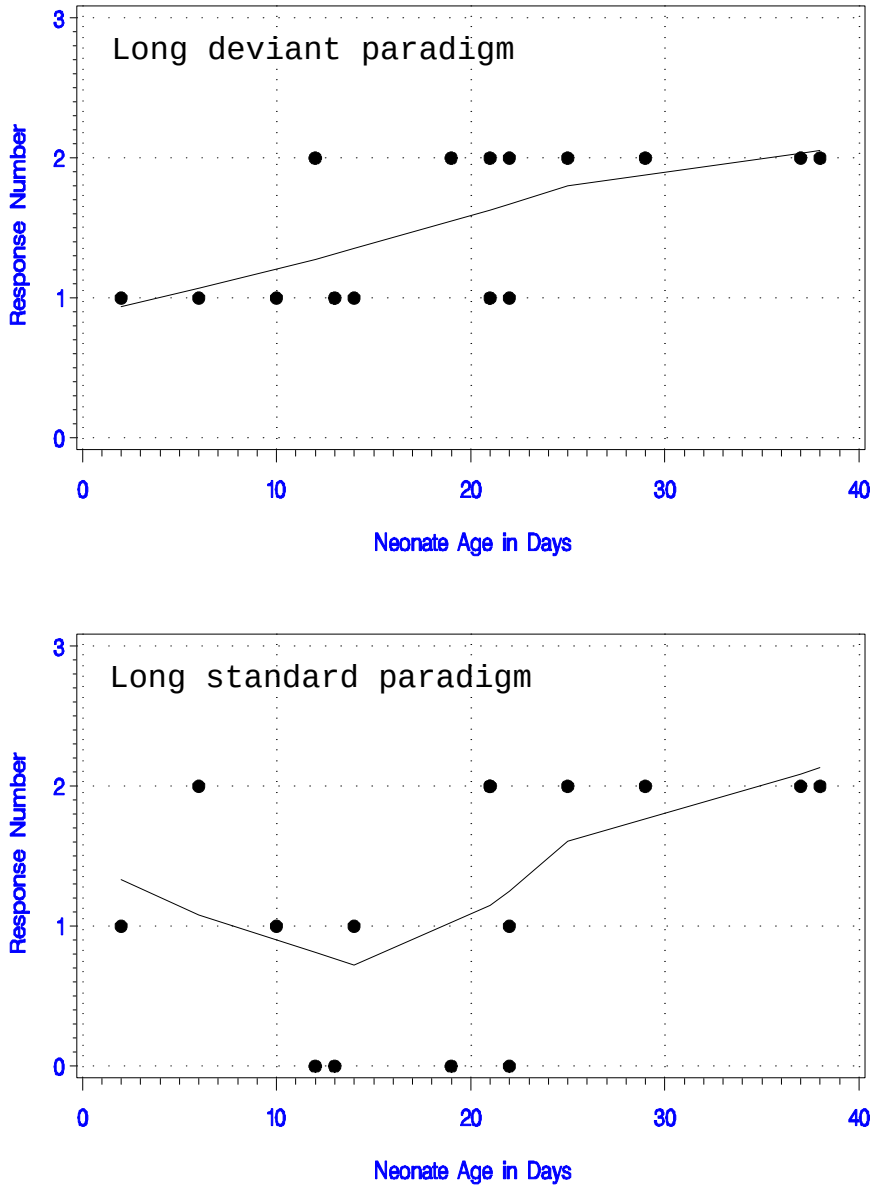
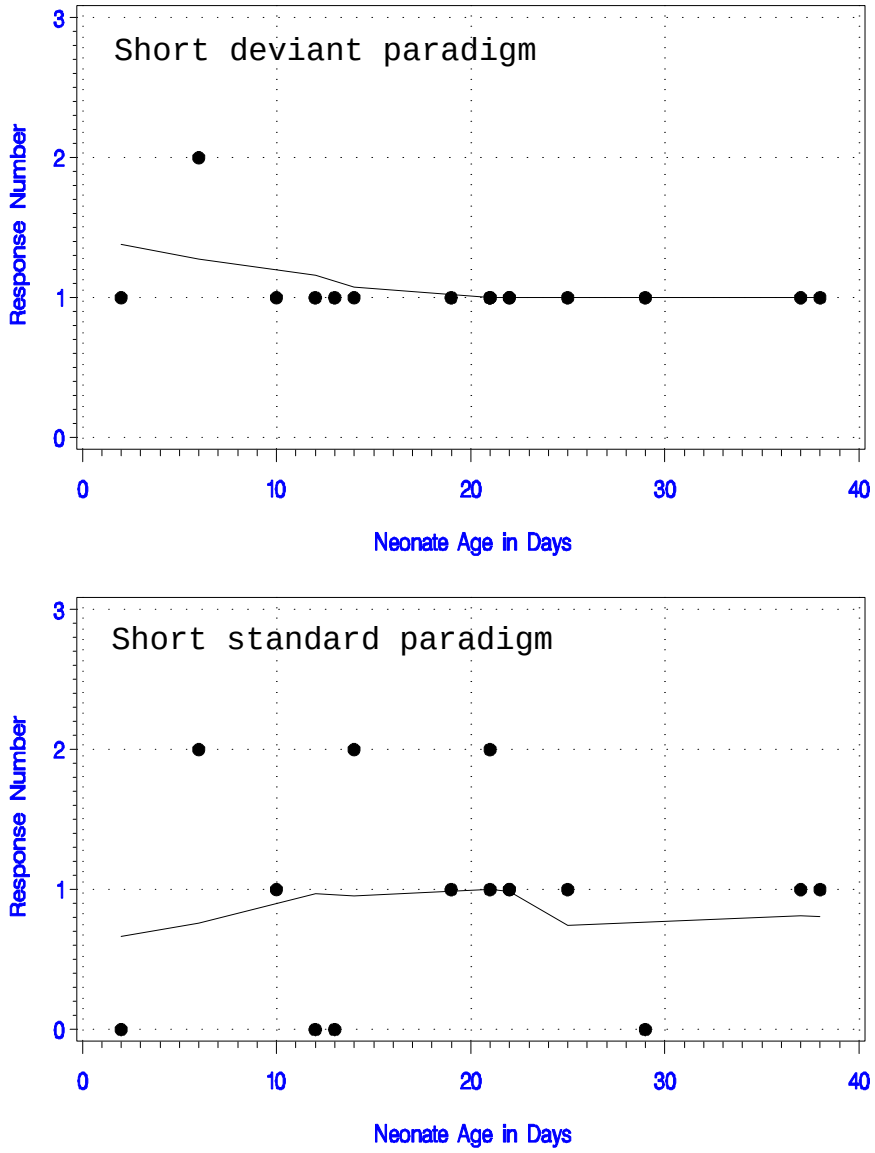


Figure 12 - continued. Neonatal responses (zero, one or two) as a function of chronological age (in days), with smoothed trend estimate.



c) Short Paradigm, Deviant Tone

In the short paradigm, the response rate to the deviant tone pair was 100%. Only one participant showed two responses at latencies of 342 ms and 451 ms. This was the same six day old baby that was called “outlier” in the previous paragraph. All the other 14 infants responded once. The latencies of the single peaks varied between 176 ms and 667 ms with a mean of 394 ms. Figure 12 shows the trend estimate for this paradigm as well.

d) Short Paradigm, Standard Tone

Four neonates did not show an evident response to the short standard tone pair, leading to a response rate of 73%. These newborns were between 2 and 29 days old. Out of the remaining 11 babies three responded with one peak and eight had two responses. No trend of age with number of responses was observable (compare Figure 12). The one time responders were aged between 10 and 38 days and the babies with two peaks were 6, 14 and 21 days old. The single responses had latencies between 214 ms and 451 ms with a mean of 358 ms. In the dual responses the first peak was observed at latencies between 102 ms and 205 ms (mean 150 ms), and the second peak occurred between 349 ms and 653 ms (mean 566 ms) after stimulus onset.

e) Summary

The response rate in both deviant paradigms (long and short) was higher than in the standard ones. Neither of the short paradigms (deviant and standard) showed a trend of

response pattern with age. However, in the long tone pair paradigm the trend to an increasing number of two responses with increasing age was observable in both, the deviant and the standard paradigm.

3.2.2 Fetal recordings

The data from each recording session were separated into the same four categories as the neonatal data. The fetal response patterns are summarized in Table 3 b) and Figure 13 a) and 13 b) show examples for fetal responses.

Table 3b).

Number of Responses in Fetuses (N=20[a])versus Different Stimulus Conditions

Number of Responses	Long Paradigm		Short Paradigm	
	Deviant	Standard	Deviant	Standard
Zero	4	7	6	6
One	21	19	23	23
Two	4	3	0	0
Total	25	22	23	23
Response Rate	86%	76%	79%	79%

[a]29 recordings were conducted on 20 fetuses.

Figure 13 a). Example for responses from a fetus at 36 weeks GA: this subject responded with one peak to the long deviant tone pair (on the top left side), with two peaks to the long standard tone pair (on the top right side) and with one peak to both short paradigms (bottom left and right side). The peaks to the deviant tone pairs are located on the left side and the ones to the standard tone pair on the right side. Onset of the first tone of the pair is at 0.0 s and the peaks are indicated by the red bar.

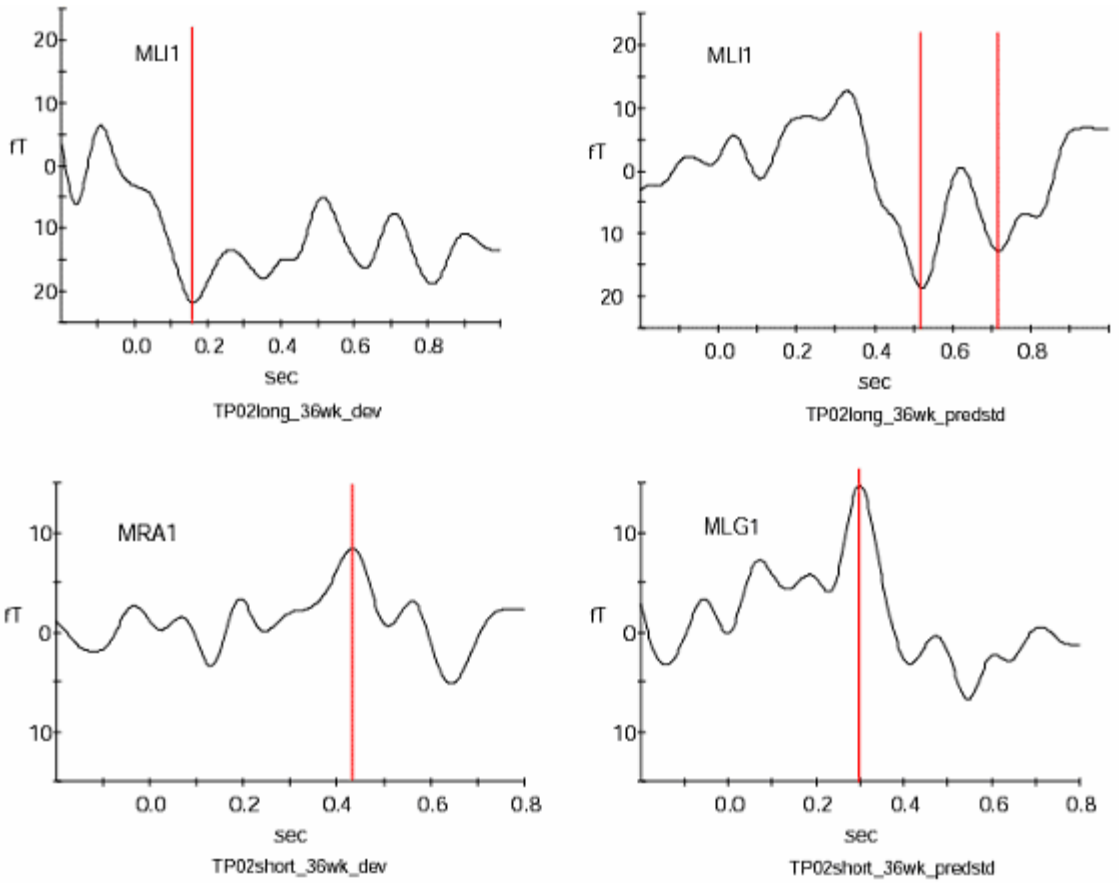
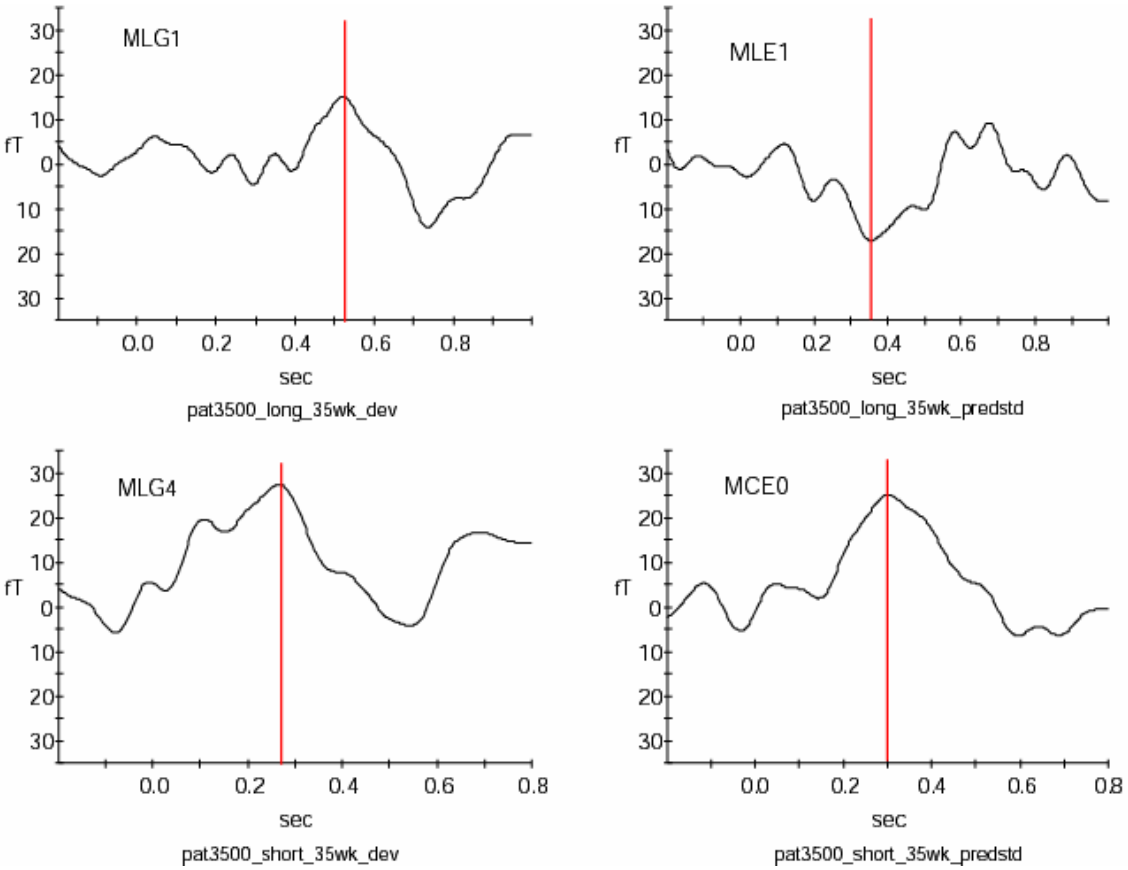


Figure 13 b). Example for responses from a fetus at 36 weeks GA: this subject responded to all paradigms with one peak. The peaks to the deviant tone pairs are located on the left side and the ones to the standard tone pair on the right side; the long paradigm is on the top level and the short one on the bottom. Onset of the first tone of the pair is at 0.0 s and the peaks are indicated by the red bar.



a) Long Paradigm, Deviant Tone

Out of the 29 recordings, 25 fetuses (86%) responded to the long deviant tone pair. The majority of those responders had one detectable brain response at latencies between 100 ms and 680 ms, with a mean of 370 ms. Four participants, aged either 35 or 37 weeks GA, responded with two peaks. They showed the first brain responses between 170 ms and 230 ms (mean 200 ms) after onset of the first tone in the pair and the second one at latencies between 640 ms and 915 ms (mean 745 ms).

b) Long Paradigm, Standard Tone

The response rate for the long paradigm and the standard tone pair was 76%. Seven fetuses, aged between 35 and 38 weeks GA, did not show an evident response. Out of the 22 responding fetuses, three (33, 36 and 37 weeks GA) had two peaks. Their first responses were recorded at latencies between 350 ms and 520 ms (mean 410 ms) and the second peak was detected between 430 ms and 710 ms (mean 550 ms) after onset of the first tone. The latencies for the single responses varied between 100 ms and 630 ms with a mean of 340 ms.

c) Short Paradigm, Deviant Tone

The response rate for the short deviant tone pair was 79%. All of these participants showed one peak at latencies between 115 ms and 640 ms (mean 360 ms) after onset of the first stimulus. The six fetuses that did not show a detectable response were aged between 29 and 36 weeks GA.

d) Short Paradigm, Standard Tone

In six recordings on five participants no peak was evident in the short standard tone pair, leading to a response rate of 79%. No second peaks were detected. The latencies of the single responses occurred between 140 ms and 590 ms (mean 290 ms) after onset of the initial tone in the pair.

3.2.3 Summary

The fetal recordings revealed mostly single responses in all of the paradigms. A few recordings showed two peaks in the long versions, but no dual responses were observed in the short ones. In comparison, the neonates responded significantly more often with two peaks than the fetuses (P=.004).

No significant difference was found between the response rate of the deviant and standard tone pairs, as opposed to the neonates. However, in the long version of the paradigm the fetal response rate was slightly higher for the deviant tone than for the standard stimulus (76% versus 86%). The fetal results exceeded the expected response rate of 60%.

4. Discussion

4.1. The application of established study paradigms with fMEG technology

Both study paradigms were applied to newborns and successfully recorded with novel fMEG technology. The fetal recordings have limitations, but do provide implications for future investigations. Therefore, these prenatal studies are a first step towards a better insight into the development of fetal cortical functioning.

4.1.1 Response decrement (study 1)

One novelty of this study was the use of amplitudes on data collected with the fetal MEG system. The amplitude is influenced by the distance from the signal source to the sensors as well as by the fetal position. Therefore, an interindividual assessment of amplitudes has not been possible in the past. In this paradigm, the four triggers were presented within a short time frame. Even if the fetus changed its position during the recording – and therefore the distance between the source and the sensors, the amplitude of the four triggers would be affected equally. However, the main interest of this study was response decrement. But the assessment of the response decrement was possible only by the use of peak amplitudes and their intraindividual comparison. Therefore,

this investigation shows that current technology enables the direct recording of response decrement.

It is important to note, that this study did not encompass the task of identifying which part of the response decrement occurred due to habituation versus receptor fatigue. In order to investigate this, a criterion called dishabituation (Thompson & Spencer, 1966) has to be shown. But this study was conducted in order to evaluate a simpler paradigm as a first step. This provides the basis for a subsequent study paradigm that tests dishabituation as well. By applying the same alterations to the paradigm as described above, dishabituation could be proved and therefore receptor fatigue determined. Until this is investigated in a future study, the phenomenon observed here was called response decrement instead of habituation.

4.1.2 Rapid temporal processing (study 2)

The direct recording of RTP with fMEG technology has been successful in newborns. However, the fetal results indicate large interindividual differences. Therefore, these datasets are not interpreted. In future studies, the gaps between the two tones in a pair could be increased in order to reduce the difficulty. This might reveal better insight in fetal response patterns.

4.2. Course of development

4.2.1 Response decrement (study 1)

All the neonates with a detectable response revealed a decrease in response amplitude from the first to the last stimulus, as shown by the rank correlations and by repeated-measures ANOVA on both, raw and relative amplitudes. Post-ANOVA polynomial-contrast analysis showed that the decrease had a significant linear component.

Moreover, the latency increased significantly over the four light flashes. This is in concordance with findings in previous studies (Rosburg et al., 2006).

The fetal data revealed response cessation after one stimulus rather than continuous response decrement. This might be due to the low number of stimuli available for averaging, combined with the naturally small amplitude of fetal brain responses. In fact, our previous VER studies averaged 180 light flashes, whereas this paradigm adds up to only half as many stimulus presentations. A lower number of averaged stimuli leads to a decreased signal to noise ratio. Considering the naturally small amplitude of fetal brain responses, the number of detectable responses could be affected even by slight changes in the signal to noise ratio. The fetal evoked response to the first stimulus starts with a low peak amplitude. Therefore, any reduction of response amplitude might not be detectable because it is below the

noise level of the device. But despite their limitations, the fetal datasets might indicate a decrease in response amplitude as well. In future studies, the 10 s interval between the light flash sequences could be replaced by a train of deviant stimuli to increase the stimulus number and to keep the recording time constant at 30 minutes.

4.2.2 Rapid temporal processing (study 2)

In this study, the expected trend over age was observed: older neonates responded more often with two peaks than younger neonates and fetuses. Between six and 22 days of age some infants responded with one, others with two peaks. This overlay might reflect a time frame in which the maturation of the brain differs interindividually. Genetic predetermination and/or exposure to and experience with crucial stimuli might cause these differences between the first and second week after delivery. But by 22 days of age all newborns showed dual brain responses to the longer tone pair, which was separated by a 300 ms gap. However, no such trend was evident in the short paradigms. This might indicate that the short gap between the tones (70 ms) may be too difficult for the babies to process, whereas the longer condition could be successfully processed starting at about three weeks of age. The reasons for the trend over age in the longer paradigm remain speculative, but might indicate a critical stage for the processing capabilities of rapid, successive stimuli within

the first three weeks after delivery. This needs to be investigated in future studies with a larger number of participants in different age groups and longer gaps (> 300 ms) between the two tones.

In the short paradigm, the majority (eight out of 11 responders) of the babies showed two peaks in response to the standard tone. However, this was not confirmed for the deviant tone pair. Here, only one infant responded with a dual response. In the short paradigms, the neonatal response patterns (zero, one or two peaks) did not show a trend over age. In adults, the brain responses to rapidly successive stimuli with the same ISI merge into one peak, and the same has been shown for six months old infants (Benasich et al., 2002). The results of the current study show two peaks in the short paradigm in some cases. This might reflect an inability to successfully process this rapid succession of stimuli. However, this remains unclear until addressed in future studies with a larger number of participants, including different age groups and varying ISI's between the tones in a pair.

4.3 Fetal response rate

4.3.1 Response decrement (study 1)

The fetal response rate of 29% in this study is much lower than the findings in our previous fMEG studies with the same light stimulus (Eswaran et al., 2004). Therefore, the expected

response rate of 80% could not be reached. This is most likely due to the low number of averaged stimuli, leading to a reduced signal to noise ratio, as explained in section 4.2.1. Therefore, the same implications for modifications of the paradigm might help to address the low fetal response rate.

4.3.2 Rapid temporal processing (study 2)

The expected response rate of 56% was exceeded. The tone pair paradigm increased the auditory fetal response rate to the standard tones from 56% in a similar paradigm (Draganova et al., 2007) to between 76% and 79% in this study. This might be due to the overall longer duration of the tone pair compared to a single tone. But this needs to be investigated in a separate study that compares single tones and paired tones directly.

Another question that needs further investigation is the dual responses of some fetuses as early as 32 weeks GA in the long paradigm. One explanation might be the fetal state at the time of recording. The processing of rapidly presented stimuli might differ depending on whether the fetus was asleep or awake. But this question needs to be addressed in a longitudinal study with more participants.

As expected, the neonatal response rate for the deviant stimulus was higher in the long and the short paradigm. Habituation processes, as explained in study 1, might have

lowered the responsiveness to the more frequently (85% of the time) presented standard tone pair.

5. Conclusions

Both studies investigated the use of cognitive paradigms that have not been applied on fetuses and newborns before. The SARA system allowed a direct detection of elicited brain responses and contained no risk for mother or baby due to non-invasive recordings.

This was the first time a visual short-term habituation paradigm has been applied on human fetuses and neonates. The study has shown that it is a promising approach for the detection of response decrements in newborns and – with limitations – even in fetuses.

In a next step, the effectiveness of the fetal recordings needs to be addressed. Adjustments in the study paradigms, as suggested in the discussion, might result in a higher response rate and therefore better interpretability of the prenatal datasets. In addition, technological improvements, such as more rigorous peak determination methods based on statistical analysis(McCubbin et al., 2007b) or source reconstruction methods (e.g. beamformer)(McCubbin et al., 2007a) might contribute to a more efficient data analysis.

Despite its challenges, fetal habituation is worth further study. Maternal diabetes (Doherty & Hepper, 2000), depression (Allister et al., 2001) and stress (Sandman et al., 1995) have been shown to affect fetal habituation in a negative way. Therefore, a prenatal detection of adverse habituation could

be used as an indicator for the re-evaluation of maternal high-risk conditions. A diabetic mother might need to improve her blood sugar control; the symptoms of maternal depression might need to be better evaluated or addressed with a multi-faceted treatment that includes behavioral as well as other interventions. A mother with a high level of stress in her life might need to address this (e.g. seek social support for her everyday life and learn relaxation techniques) in order to avoid adverse effects through high maternal ß-Endorphin levels on her baby. Obviously, these suggestions are speculative and need to be addressed in futures studies. But knowing about abnormal habituation performance before delivery might encourage more research that focuses on possible prenatal interventions.

The recording of evoked responses to rapidly presented auditory stimuli was conducted successfully. The SARA system allowed the direct detection of brain responses at early developmental stages. In future studies, the determination of a critical time frame for rapid, successive evoked responses in a larger sample of normal participants should be addressed. This could be an important step towards an increased knowledge of processing capabilities and help with the detection of language impairments at early developmental stages. It also might contribute to the development of intervention methods that improve delayed auditory processing and therefore prevent further deficits in speech development.

Longitudinal studies in high- and low risk conditions have to be conducted in order to determine the predictive value of fetal MEG recordings.

Another important step towards more reliable fetal recordings might be the determination of fetal state for every dataset. It is possible that much of the variability in the prenatal results can be explained by variations in the fetal state.

Even though both studies have their limitations, especially regarding the fetal data, the usefulness of fMEG technology for these paradigms has been shown. Moreover, these investigations entailed implications for modifications in the paradigms that might improve the results of future studies; and therefore yield to greater insight into the course of cognitive maturation and speech development in uterus.

All of these suggestions lead to one conclusion: Fetal MEG technology is still in the beginning stages and more studies need to be conducted in order to improve current technical and methodological challenges. But nevertheless, the studies conducted so far indicate the potential of this device to increase knowledge for early human brain development.

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