FETAL MAGNETOENCEPHALOGRAPHY: DEVELOPMENTAL CHANGES IN THE AUDITORY SYSTEM

H. Preissl[1,2], M. Holst[1,2], R. Draganova[1], P. Murphy[1], C.L. Lowery[1], H. Eswaran[1]

[1] SARA Research Center, Department of Ob/Gyn, UAMS, Little Rock, USA
[2] MEG Center, University of Tübingen, Tübingen, Germany

preissl@uams.edu

Abstract: In our serial studies we investigated sensory auditory processing in human fetuses in utero and newborns, which can be determined by auditory evoked field studies using magnetoencephalography (MEG). The aim of our studies were the perinatal investigation of the development of the auditory system by measuring the response latencies. In two parallel conducted serial studies we recorded (a) auditory evoked fields (AEF) in 18 normal developed fetuses and 14 neonates to investigate the development of the auditory cortex, and (b) for the investigation of cognitive discriminative ability in the fetal auditory cortex, the mismatch negativity in 25 fetal and 10 newborn studies. We used response latencies of the auditory evoked fields for the analysis of auditory processing over gestational age. We found in study (a) that 52 of 55 recordings (94.5%) and all neonate studies (100%) showed an evoked response and in study (b) that the detection rate to sound changes was 60% in the fetal data and 80% in neonates. With the novel technique of MEG, used for serial studies, it is possible to trace the development of auditory responses of fetuses in utero and newborns.

Introduction

The development of the specialization of the different brain areas is an ongoing dynamical process, which includes neuron generation and migration, and elimination of previously existing neurons and synapses during the fetal development. During the perinatal period the connections of neurons in the brain are highly changeable and also the myelination of the axons is still in progress at birth and up to 20 years after birth [1]. The auditory system, is functional at roughly 20 weeks (term is 40 weeks) of gestational age (GA) in the human fetus. Even at this early stage the auditory cortex is functional. Different paradigms have been designed to study cortical function by measuring latencies of evoked fields elicited by auditory stimuli. In our study we were interested in the maturation of the development of responses to standard stimuli and, parallel in the maturation of cognitive ability by using auditory stimuli in an oddball paradigm to determine, whether the fetus is able to generate a mismatch negativity (MMN).

Blum et al. [2] demonstrated in 1985 for the first time auditory evoked fields (AEF) of the cortex in human fetuses in utero, recorded with magneto-encephalography (MEG). Until recently, MEG was used to detect neuromagnetic fields mostly in adults. The advantage of MEG is that the recordings are completely non-invasive for the detection and quantification of auditory evoked cortical fields and other brain related activations. Blum and his group showed cortical magnetic fields after auditory stimulation in fetuses with response latencies around 250ms in two fetuses, one recorded at 34, the other at 35 weeks GA. In a one time follow-up study on the newborns, latencies of around 135 ms could be identified. Several studies have been conducted since then and the technology has been improved.

Wakai et al. [3] recorded one hundred trials of 0.5 seconds each from a group of 14 fetal subjects. The detection rate was 29 %. The response latencies were around 200ms but also other components with shorter or longer latencies were apparent but not consistent throughout the subjects. Multiple recordings were conducted by Schleussner et al. [4] and Lengle et al. [5] on one or two days on fetuses, both studies included fetuses from a GA of 29 weeks to term of pregnancy. Both groups reported a decrease in response latency to an auditory stimulus with age in a cross sectional approach and an average detection rate of about 50%. Eswaran et al. [6] performed multiple recordings per week between 30 to 35 weeks GA in 10 subjects. They showed that multiple sessions could improve the detection rate for single subjects

In adults, auditory cognitive functions are typically investigated by using sound discrimination tasks. In the so called oddball paradigm, sounds are presented in a sequence of a standard (frequent) sound intermixed with a deviant (infrequent) sound of different frequency, duration or intensity. Näätänen [7], showed that the difference waveform obtained by subtracting the evoked responses to the standard from those of the deviant tones exhibits a specific component. This component is called mismatch negativity (MMN) because it appears as a negative deflection in electroencephalographic (EEG) recordings of adult subjects. The application of the oddball paradigm in neonates and fetuses is appropriate due to the fact that the MMN response is elicited to unattended stimuli [8] and it is a prerequestite for language development.

In order to investigate the fetal and neonatal development of the auditory cortex it is necessary to conduct a serial study. Since MEG is completely non-

ISSN: 1727-1983

invasive the technique can be used for multiple recordings.

Materials and Methods

We performed measurements with a 151-channel SQUID fMEG system (SARA) [9] especially designed for fetal investigations. SARA is a stationary, floor-mounted instrument where the mother sits and leans her abdomen against an anatomically shaped sensing surface (Fig 1). This design is inherently safe. The mother is comfortable, and can gain easy access to or dismount from the system. To attenuate the influence of external magnetic fields, SARA is installed in a magnetically shielded room (Vakuumschmelze, Germany).

Figure 1: Left side: The fMEG System installed in the shielded room. The sensor array contains 151 primary sensors. Right side: Mother leaning over the sensor array. The auditory stimulation is attached and sound is delivered via the tube, and the biomagnetic signals generated in the abdomen are recorded.

An array of 151 SQUID sensors covers the mother's anterior abdominal surface, from the perineum to the top of the uterus (in late gestation). The primary sensor flux transformers are axial 1st-order gradiometers, with 8 cm baselines. The nominal SQUID sensor noise density is 4 fT/√Hz. A set of 29 reference SQUID sensors is incorporated for attenuation of environmental and vibrational noise caused by internal physiological processes or small movements from the mother. The reference sensor array could also be used for the calculation of higher-order gradiometers. The primary sensor array is curved to fit the pregnant abdomen, covering a region of ≈45 cm high and ≈33 cm wide, with an area of ≈1300 cm2 and inclined at ≈45 deg. The mother sits and leans forward against the smooth surface of the array. In order to determine the position of the fetal head in relation to the observed MEG signal we utilized a fiduciary marking system which consisted of four coils. Three coils were attached to the mother's left and right sides and on the back. The fourth coil was positioned over the fetal head whose location was confirmed using a portable ultrasound in the shielded room prior to the study. Before each recording, the location of the coils was determined by activating them at a certain frequency to compute their coordinates in relation to the SQUID sensors. Using a custom made cradle which can be attached to the fMEG system it is also possible to record brain responses from neonates, with the limitation that only one hemisphere of the neonate can be recorded during a single session.

The fMEG is measured in the presence of environmental noise and various near-field biological signals and other interference: e.g., maternal magnetocardiogram (mMCG), fetal magnetocardiogram (fMCG), uterine smooth muscle (magnetomyogram) [10], intestinal movements and motion artifacts. After cancellation of environmental noise, the maternal magnetocardiogram (mMCG) and fetal magnetocardiogram (fMCG) are usually the most dominant artifacts and must be removed in order to observe fMEG. The magnitude of the averaged evoked fMEG signals is typically in the range from 10 to 80 fT [5] [11] while the fMCG and mMCG at the fetal thorax location can both attain amplitudes as large as 10 pT. Closer to the maternal heart, the mMCG can be as large as 100 pT.

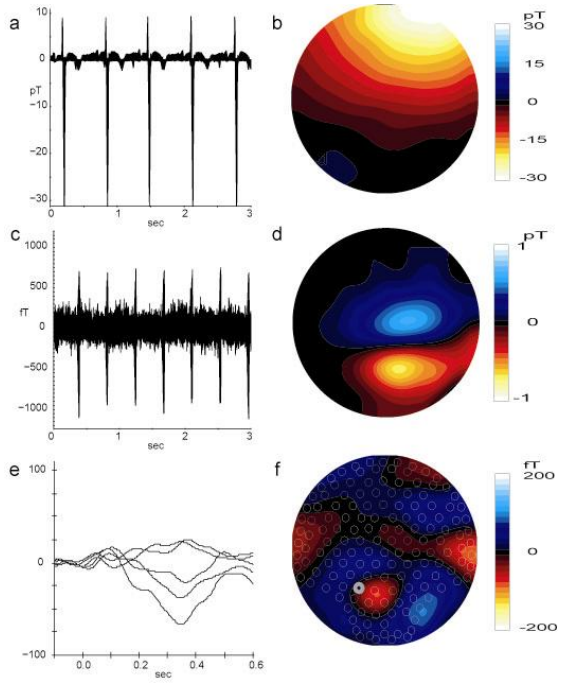

Figure 2: Example for the data analysis of fMEG recordings. a) raw signal. c) signal after extraction of the maternal heart signal, the main component is the fetal heart signal. e) evoked fetal auditory fields. The time traces for the channels over the fetal head are shown. b) field distribution for the R- wave of the maternal heart signal. d) field distribution for the R-wave of the fetal heart signal. f) field distribution of the maximum of the evoked field. The small circle indicates the single sensors. The grey ring indicates the fetal head position as determined with the fiduciary marker system.

We removed the mMCG and fMCG by orthogonal projection of the interfering signals [12]. The interference elimination by orthogonal projection was found to be robust and relatively easy to automate. The projection operators are constructed from signal space

ISSN: 1727-1983

vectors corresponding to the interfering signal-space components. For MCGs, these vectors are determined by template matching, averaging, and orthogonal construction. The projection operator application, however, redistributes fMEG signals among sensors, even to the sensor array regions distant from the fetal head location where the fMEG signal should normally not be present. Such signal redistribution does not affect the fMEG signal analysis because the effect of projection can be included in the forward solution. However, redistribution makes it difficult to interpret the fMEG signal maps visually. Since such visual interpretation is often useful, we have developed a procedure for correction of the redistributed fMEG signal topography [12].

The orthorgonal projection and redistribution corection is currently state of the art for the analysis of the fMEG analysis (Fig 2). But, as indicated above, this approach mainly results in the interpretation of waveforms and distribution of the brain signals over the array. Currently, we are working on better models for the brain activation based on 3-D ultrasound which may result in better analysis procedures [13].

Subjects AEF study: Eighteen healthy women of a singleton pregnancy from week 27 GA. The criteria for the participation were an uncomplicated pregnancy and serial participation in at least 2 different sessions.

Subjects MMN study: Twelve pregnant women between 33 and 36 weeks of gestation participated in a recent study [14]. Five of the women returned within 2 weeks after delivery for neonatal studies. The stimulation paradigm was equivalent for fetuses and newborns.

Both groups of participants were recruited from a population of patients of the UAMS-hospital and reimbursed for their participation. The studies were approved by the local Institutional Review Board and written informed consent was obtained from all subjects.

Stimulation AEF study: In a serial study starting at 27 weeks GA including a follow-up on the newborn we used an oddball paradigm of 100 ms duration stimuli consisting of 500Hz frequent stimuli (80%) and 700Hz rare stimuli (20%) in a random sequence with an inter stimulus interval (ISI) of 2 seconds +/- 0.5. We used the oddball paradigm to minimize possible habituation effects. For the stimulation of the fetuses the sound intensity was 120 dB and for the neonates 80 dB. Each recording lasted 6 minutes.

Stimulation MMN study: A sequence of two complex tones was presented to the subjects in an oddball protocol. The frequency of the "standard" frequent tone (probability of 88%) was 500 Hz with additional harmonics at 1000 Hz and 1500 Hz. The frequency of the "deviant" infrequent tone (probability of 12%) was 750 Hz with harmonics at 1500 Hz and 2250 Hz. The stimuli were generated as tone bursts with duration of 100 ms (including 10 ms rise and fall times). The ISI varied between 500 ms and 1100 ms in the randomized condition. In the second, non randomized condition, the ISI was fixed at 800 ms. The recordings were performed in two consecutive measurements of 8 minutes each. We used a fixed order sequence starting with the randomized stimulation and continuing after a short break with the non randomized condition.

The sound transmitting system for our studies consisted of a speaker that was mounted outside of the magnetically shielded room (Vakuumschmelze Hanau, Germany) and sound for fetal stimulation transferred to maternal abdomen by means of plastic tubing with an inflated balloon attached at the distal end. The balloon was placed on the on the superior aspect of the maternal abdomen in order to deliver stimuli to the fetal head. For the newborn studies, the balloon was installed in the midline above the cradle. Each ear was stimulated separately in the newborns by turning the infant to the side and magnetic fields of the opposite hemisphere have been recorded (for additional details see [15]).

Signal–analysis

Maternal and fetal magnetocardiogram signals were attenuated by an algorithm using orthogonal projection. Amplitude sensitive threshold detection was applied and all trials with amplitude higher than 2 pT were rejected. The continuous epochs were split into single trials dependent on the trigger generated at the onset of auditory stimulation. The responses to the tones were averaged with 200 ms pre- and 800 ms poststimulus for the AEF study and 100 ms pre- and 600 ms post stimulus in the MMN study and filtered between 0.5 Hz and 10 Hz. The response was validated to following criteria: (i) a response with similar latency was manifested after averaging the even and odd trials, (ii) the averaged magnetic field response was higher than noise level – as computed by plus/minus average (iii) the magnetic field distribution activity corresponds to the area around the head coil location.

Results

A total of 63 fetal recordings with pure tone stimulation were performed. Eight recordings were excluded from further analysis based exclusion criteria, like extensive fetal movement. 52 (94.5%) of the remaining 55 recordings had evident responses based on the most prominent peak in the AEF study. Signals of amplitude 7fT or higher were detected. The mean latency in fetuses GA including 27 to 31 weeks was 288 ms. In fetuses of 32 to 35 weeks GA the average latency was 251 ms and in fetuses including 36 to 39 weeks the average latency was 197 ms. The repeated measure regression analysis across fetuses and neonates revealed a significant decrease of AEF latencies ($F(1,149) = 6.31$, $p<.05$) at a rate of 5.5 ms per week.

In the second study a total of 25 fetal and 10 newborn recordings were performed. A response corresponding to detection of sound changes was found in 60% of the fetal data and 80% of the neonatal data. In Fig. 2 (e,f), an MMN response was demonstrated as channel overlay

ISSN: 1727-1983

of the magnetic fields under the head coil, (Fig. 2e) and as magnetic field distribution, Fig.2(f) In some of the recordings, except MMN, a late component was observed with latency above 400 ms, called late descriminative negativity (LDN). The average MMN latency in fetuses and newborns were 321 ms ± 31 ms and 307 ms ± 39 ms, respectively. The average LDN latency was 458 ms ± 38 ms in fetuses and 479 ± 37ms for newborns. The latency of fetal response to the standard tone calculated across all measurements was 260 ± 61ms and 206 ms ± 52 ms in newborns. No significant difference was found for the responses from randomized versus non randomized condition. However, the grand average latency of the responses across the subjects to the standard and to the deviant tones differed with statistical significance ($p<0.05$).

Discussion

In our first serial study a clear decrease in latency of auditory evoked fields during the gestational range starting at 28 weeks until delivery could be shown in confirmation to [4] and [5]. Even if a continuous decrease in response latency could be observed, the interpretation of latency changes over gestational age has to be made with cause, because it may be possible that different components may have different detection rates on different gestational ages, e.g. late components during early gestational age and early components during late gestational age, this could be interpreted as a latency shift over gestation. In a separate analysis for the neonates alone, a significant change could not be confirmed. This may be related to the low number of recordings and, also, the dramatic change in the acoustical environment in the extra uterine life. This factor should be investigated in further studies. To our knowledge, this was the first longitudinal study on fetuses with a continuation after delivery.

The second study demonstrated that it is feasible to record cortical responses in fetuses (between 33 and 36 week of gestation) in respect of sound discrimination. The successful rate of 60 % from 25 records showed, that oddball paradigm could be used in investigations of cognitive function in fetuses.

Because the fetal brain is not completely developed, we cannot interpret the results presented in this paper as analogous to those of adult and children studies. The results demonstrated specific morphology and latencies of two discriminative responses in fetuses. They confirmed preliminary findings that except MMN, another late discriminative response (LDN) was defined as part of the auditory discriminative process in this early stage of human cognitive-function development. It was shown that the randomized ISI does not disturb the sensory memory and remains appropriate for perceiving the sequence of tones thus avoiding the refractory process.

Whether or not the fetus is able to detect changes in sounds is a question of great scientific interest since this basic capability is prerequisite to the development of a functional auditory system. The elicited MMN response is well described in the literature for adults (see reviews, [16], [17], [18]. Of special interest is the underlying mechanism of the discriminative ability of the auditory system and its relation to clinical aspects of auditory disorders and language learning. This is important in adult research as well as in that of children and infants. The application of the oddball paradigm in fetal auditory studies provides a tool to assess sound change detection capabilities and investigate possible central auditory processing disorders at a very early stage.

Conclusions

Since the interpretation of the response latency in regards to the gestational age is complicated it is necessary to investigate the component structure of the evoked responses in greater detail, possibly by improved analysis techniques. Differences in amplitudes can currently not be used for comparison, because the strength can be severely affected by analysis methods and so that a comparison between different research groups is not possible. In addition, the strength is severely affected by the distance of generating source from the abdomen and as stated below, research in this area just started.

Studying sound processing and discrimination ability in fetuses and their related cortical activity can help to determine deficits, caused by central processes in the auditory system in very early stage. The combination of advanced biomedical instrumentation and signal analysis allows a detailed description of the development of the auditory system in the human fetus in utero. The conduction of a serial study provides opportunity for a detailed investigation of a continuous process.

Acknowledgments

The work on this paper was partially supported by grants from the National Institutes of Health, 1R33 EB00978-01 (NIBIB) and 5R01 NS36277-03 (NINDS), USA and DFG (BI195/50).

References

[1] Herschkovwitz N. (2001): 'Brain development in the Fetus, Neonate and Infant'. *Biol Neonate* , 54, pp.1-19.

[2] Blum T. Saling E, Bauer R. (1985): 'First Magneto Encephalographic Recordings of the Brain Activity of a Human Fetus', *Br J Obstet Gynaeco,l* 2, pp. 1224-1229

[3] Wakai R, Leuthold A, Martin C. (1996): 'Fetal Auditory Evoked Responses Detected by Magnetoencephalography'. *American Journal of Obstetrics and Gynecology*, 174 (5), pp.1484-1486.

[4] Schleussner 2001 E, Schneider U, Kausch S. (2001): 'Fetal Magneto Encephalography A Non-

ISSN: 1727-1983

Invasive Method for the Assessment of Fetal Neuronal Maturation', *British Journal of Obstetrics and Gynecology,* 180,pp.1291-1294.

[5] Lengle JM, Chen M, Wakai RT (2001):, 'Improved Neuromagnetic Detection of Fetal and Neonatal Auditory Evoked Responses', *Clinical Neurophysiology*, 112, pp. 785-792.

[6] Eswaran H, Preissl H, Wilson, J. (2002): 'Short-Term Serial Magnetoencephalography Recordings of Fetal Auditory Evoked Responses', *Neuroscience Letters*, 331, pp.128-132.

[7] Näätänen, R., (2001):, 'The Perception of Speech Sounds by the Human Brain as Reflected by the Mismatch Negativity (MMN) and its Magnetic Equivalent (MMNm)'. *Psychophysiology,* 38, pp.1-21.

[8] Alho K., Woods, D.L., Algazi, A., Näätänen, R., (1992); 'Intermodal Selective Attention. II. Effects of Attentiional Load on Processing of Auditory and Visual Stimuli in Cebral Space', *Electroeceph. Clin. Neurophisiol*., 82, pp.356-368.

[9] Vrba J., Robinson S.E., McCubbin J. (2004b): 'Fetal Meg Redistribution by Projection Operators', *IEEE Transactions on Biomedical Engineering*, 51 (7), pp.1207-1218.

[10] Eswaran, H., Preissl, H., Wilson, J.D. (2002a): 'First Magnetomyographyc Recordings of Uterine Activity with Spatial-Temporal Information using a 151 Channel Sensor Array', *Am J of Ob/Gyn,* 187, pp. 145-151.

[11] Eswaran, H., Preissl, H., Wilson, J.D. (2002): 'Short-Term Serial Magnetoencephalographic Recordings of Fetal Auditory Evoked Responses, *Neuroscience Letters,* 331, pp. 128-132.

[12] Vrba J., Robinson S.E., McCubbin J. (2004a): 'Human Fetal Brain Imaging by Magnetoencephalography: Verification of Fetal Brain Signals by Comparison with Fetal Brain Models', *Neuroimage,* 21, pp. 1009-1020

[13] Gutierrez, D. Nehorai, A., Preissl, H. (2005): 'Ellipsoidal Head Model for Fetal Magnetoencephalography: Forward and Inverse Solutions, *Physics in Medicine and Biology,* 50 (9), pp. 2141-57.

[14] Draganova R., Eswaran H., Murphy P., (2005): 'Sound Frequency Change Detection in Fetuses and Newborns, a Magnetoencaphalographic Study', *Neuroimage*, in press.

[15] Holst M, Eswaran H, Lowery L (2005): 'Development of Auditory Evoked Fields in Human Fetuses and Newborns: A Longitudinal MEG study', *Clinical Neurophysiology*, 116, pp.1949-1955.

[16] Kraus, N., McGee, T.J., (1994): 'Mismatch Negativity in the Assessment of Central Auditory Function'. *American Journal of Audiology,* 2, pp. 39-51.

[17] Näätänen, R., (1992), 'Attention and Brain function'. Hillsdale NJ: Lawrence Erlbaum.

[18] Picton, T.W. (2000): 'Mismatch Negativity: Different Water in the Same River', *Audiol. Neurootol.*, 5, pp.111-139.

ISSN: 1727-1983