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Development of auditory evoked fields in human fetuses and newborns: A longitudinal MEG study

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Abstract

Objective: To investigate the maturation of the auditory cortex by non-invasive recording of auditory evoked magnetic fields in human fetuses and newborns with the relatively novel and completely non-invasive technology of MEG.

Methods: Serial recordings were performed every 2 weeks on 18 fetuses beginning from week 27 of gestational age until term with a follow-up recording on the newborn. Auditory stimulation consisted of tone bursts in an oddball design with standard tones and deviant tones.

Results: In 52 of 63 fetal and in all of the neonatal recordings an auditory evoked magnetic field was obtained. A decrease in latency with increasing age of the subjects was observed in the combined analysis of fetuses and neonates.

Conclusions: With advanced study using MEG, 83% of the measurements showed auditory evoked fields in fetuses that correspond with existing literature in electrophysiology in the past. These findings indicate that MEG is a technique that can be used to investigate maturation of the auditory cortex based on auditory evoked fields in fetuses and neonates.

Significance: Maturational changes have been examined in the past. With the use of this novel technique, applied to a serial study, it is possible to trace the development of auditory responses in utero and newborns.

Keywords: fMEG (fetal MEG); nMEG (neonatal MEG); AEF (auditory evoked field); fAEF (fetal auditory evoked field); nAEF (neonatal auditory evoked field), response latency

1. Introduction

Fetal magnetoencephalography (fMEG) is a non-invasive method designed to detect human fetal brain activity by acquisition and recording of neuromagnetic fields. Since the first fMEG report of fetal auditory evoked fields (fAEF) by Blum et al. (1985), several fetal AEF studies have been performed to verify the cortical origins of these signals and to effectively improve recording and analysis procedures (Eswaran et al., 2000, 2002; Lengle et al., 2001; Preissl et al., 2001a,b; Schneider et al., 2001; Vrba et al., 2003). Other studies have shown that changes in fetal cortical sensory processing correlate across gestational age (Eswaran et al., 2002, 2004; Lengle et al., 2001; Schneider et al., 2001). In addition to the investigations of the auditory sensory system, it has been established that visual evoked fields (Eswaran et al., 2001) can be recorded in the fetus.

The development of the brain and the specialization of different brain areas have to be regarded as a dynamical process. This process includes neuron generation and elimination and the generation of nerve cell complexes, which mainly take place during gestation. During gestation

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and after birth, the connections of neurons in the brain are highly changeable. With regard to the functional development, the myelination of the intracortical neuropil is still in progress at birth and is ongoing for two decades or more (Herschkovwitz, 1988). Based on the dynamical processes of neural maturation, it is known that cortical auditory responses can be obtained with different methods from the fetus in the third trimester. Until recently it was only possible to determine the maturation of cortical brain responses related to auditory processing in newborns.

For investigation of maturational changes, MEG is currently the only technology to determine the development of auditory responses in both fetuses and in newborns. The major advantage of MEG is that studies on the development of auditory systems can be performed non-invasively. In 1996, Wakai et al. recorded one hundred 0.5 s trials each from a group of fetuses and newborns with the use of MEG. The group detected cortical AEF in 4 of 14 fetal subjects with response latency around 200 ms, and other components with longer or shorter latencies which were not consistent throughout the subjects. They also conducted measurements on 4 neonates which resulted in AEF similar to those detected in the fetuses.

In their study to improve quality of the measurements of fAEF, Lengle et al. (2001) conducted multiple recordings on 1 or 2 days on fetuses at gestational ages of 29–40 weeks, and two recordings on neonates. They showed that the peak response latency to an auditory stimulus decreased with age in fetuses and newborns. Schleussner et al. (2001) confirmed an effect of age on response latency in fetuses with a decrease from about 300 ms in week 29 of gestation to nearly 150 ms at term of pregnancy. Both studies used a cross-sectional approach.

In an earlier EEG study on neonates conducted by Pasman et al. (1992), maturational changes in cortical auditory evoked responses (AER) were shown. AER latencies of components Na, N1, P2 and N2 were longer in term infants at a conceptional age of 40 weeks than at an age of 52 weeks.

For the investigation of developmental changes in fetuses and neonates a longitudinal study approach is preferable. To our knowledge, there have been no systematic longitudinal studies of the maturational changes beginning in the fetal stage and continuing in the newborn period.

One has to be aware, however, of the difficulties encountered in this approach. The compliance of the mothers to attend several sessions in an experimental protocol may be low and the detection probability of fAEF response for a single recording session is around 50%. Eswaran et al. (2002), however, showed that the detection rate could be enhanced substantially through serial recordings. In our study we used a longitudinal design to serially record fAEF, beginning at 27 weeks gestation inclusive of one newborn follow-up recording, to determine the maturational effect on AEF latency. We were especially interested in whether this could be regarded as a continuous process, or whether there are abrupt changes at birth. Investigation of age-related development of the fetal auditory system could possibly help in determination of the functional differences between normal and abnormal fetal development.

2. Methods

Eighteen healthy women in the last trimester of a singleton pregnancy participated in the study. The criteria for the participation were an uncomplicated pregnancy and serial participation in at least two different sessions from week 27 of gestational age to the term of pregnancy (ideally every 2 weeks) and once after delivery. The study was approved by the local Institutional Review Board and written informed consent was obtained from all participants prior to the study.

Before the MEG recordings an ultrasound examination (GE Voluson 730) of the pregnant woman was conducted outside of the magnetically shielded room in order to define the general location of the fetus and furthermore to determine the fetal head position. Immediately afterwards, an fMEG study was performed in a magnetically shielded room (Vacuumschmelze Hanau, Germany) using the SARA (SQUID Array for Reproductive Assessment) system (VSM Med Tech Ltd, Canada). The participants were placed in a seated position with their abdomen leaning against the covered sensor array (Fig. 1). Once seated, the fetal head position was reconfirmed with a portable ultrasound (GE Logio a 100 MP). The recorded head position was later compared to the location of the evoked fields. For the neonatal studies, we used a cradle attachment specially designed to fit the SARA system sensor array. This allowed us to position the newborn so that the head rested against the sensor array housing (Fig. 2). The evoked fields were visible in 3–10 channels in fetuses and newborns, depending on the distance between the sensor array and the fetal head.

Fig. 1. Study participant is seated on SARA. The pipe transmits sounds to the external maternal abdomen to stimulate the fetus.

Fig. 2. A newborn is positioned in the cradle that is attached to the SARA MEG system.

The auditory stimuli were produced using the STIM software (Neurosoft, El Paso, TX). A speaker was mounted outside the magnetic shielded room and sound for fetal stimulation was transferred to the maternal abdomen by means of plastic tubing with an inflated balloon attached at the distal end. For recording, the balloon was placed on the superior aspect of the maternal abdomen in order to deliver stimuli over the fetal head. For additional details see Eswaran et al. (2000). For the newborn studies, the balloon was suspended in the midline over the cradle above the newborn's head.

Tone bursts of 500 Hz frequent stimuli (80%) and 700 or 1000 Hz rare stimuli (20%) were presented in a random sequence. We used this oddball paradigm to minimize possible habituation effects. The sound intensity for the stimulation of the fetuses was 120 dB at the mother's abdomen, and for newborns the intensity was set at 80 dB at the end of the stimulation device over the cradle. The newborns were stimulated at each ear separately by turning the infant appropriately with one ear exposed to the direction of the sound to create as nearly as possible a monaural stimulation. The duration of the tones bursts was 500 ms and the inter-stimulus interval (ISI) was 2 s. Auditory evoked field signals were recorded with a sampling rate of 312.5 Hz by 151 magnetic sensors for 6 epochs lasting 1 min each. Except for the sound level, all other stimulation and recording parameters were identical for fetuses and newborns.

The fetal behavior was determined by observation of wakefulness or sleep, based on ultrasound prior to the study. Breathing, gross body movements, eye movements and extremity movements were recorded. If all of the possible activities were absent in the fetus the state was considered sleep. The sleep and wake states in the neonates were determined and noted by observing them through a video camera.

2.1. Signal analysis

Maternal and fetal/newborn heart signals were extracted from the recordings using the orthogonal projection algorithm described by Vrba et al. (2004a,b). The continuous 6 epochs, each lasting 60 s, were split into single trials depending on the trigger generated at the onset of the auditory stimulation. The single trials started 200 ms before trigger and ended 800 ms after the trigger. For artifact removal, all single trials showing deflections larger than 2 pT were excluded from further analysis. A stimulus triggered average waveform was determined for each single subject. The results were filtered with a high pass of 0.5 Hz and a low pass of 10 Hz. The most prominent peak between 100 and 450 ms with either a positive or negative polarity was considered as an evoked field. The prominent peak had to be identified in at least 3 channels, each in either positive or negative deflection. In addition, we calculated the plus/minus average as difference of the averages of all even and odd trials. The plus/minus average is a valid method to estimate the noise for time locked activity (Chiappa, 1990). Our requirement for determination of the evoked response was that no peak was observable in the plus/minus average at the same latency as the putative evoked response.

2.2. Statistical analysis

The statistical computations were performed using SAS (SAS Institute, Inc., USA). In order to evaluate gestational changes in response latencies in fetuses and newborns, we performed a repeated-measures regression analysis that included both types of sessions. The dependent variable was latency, and the independent variables were continuous gestational age (fixed effect) and patient (random effect). In addition to the analysis, which combined fetuses and newborns, separate regression analyses were also performed for fetuses and newborns. The fetus-only analysis was identical in form to the combined analysis. The neonate-only analysis, however, was purely across-subjects, not longitudinal, because there was only one postnatal observation per subject. Effects with $P<0.05$ were regarded as statistically significant. For newborns, it was possible to test whether latencies differed between the left and right ears. This factor was not found to be significant, so the average latency from the two ears was used in the final model. We also averaged response latencies in 3 different age groups on fetuses (27–31, 32–35 and 36–39 weeks of gestational age) and the response latencies in neonates.

3. Results

Out of 18 subjects, 16 completed the study successfully. We excluded two subjects from the study. One of these participants did not meet the criteria for a serial study, since she came only once and did not come to the follow-up study with her newborn. Another subject was excluded after two recordings due to hardly detectable fetal heart signals, which makes the data processing impossible. Two other patients did not bring the newborns back, but were still

Table 1
Number of times each subject participated, and number of responses

Pat ID	Weeks of gestational age fetus and neonate* recorded	*n* fetal sessions	*n* fetal responses	Neonate responses
400	30, 32, 34, 35, 37, 39, 42*	6	6	1
401	28, 30	2	0	
402	32, 34, 38*	2	1	1
403	30, 32	2	2	
404	27, 29, 31, 33, 35, 37, 39, 41*	7	5	1
405	34, 39*	1	1	1
407	27, 29, 31, 33, 35, 37, 40*	6	4	1
408	27, 29, 31, 33	4	3	
409	27, 29, 31, 34, 35, 38, 40*	6	5	1
410	28, 30, 32, 42*	3	3	1
412a	30, 35, 37, 39, 44*	4	4	1
412k	30, 32, 36, 38, 39*	4	4	1
415	30	1	0	
416	29, 33, 35, 37, 39, 41*	5	3	1
417	29, 31, 37, 41*	3	3	1
418	31, 33, 41*	2	2	1
419	30, 32, 40*	2	2	1
420	27, 29, 31, 33, 35, 41*	5	4	1

*Neonate.

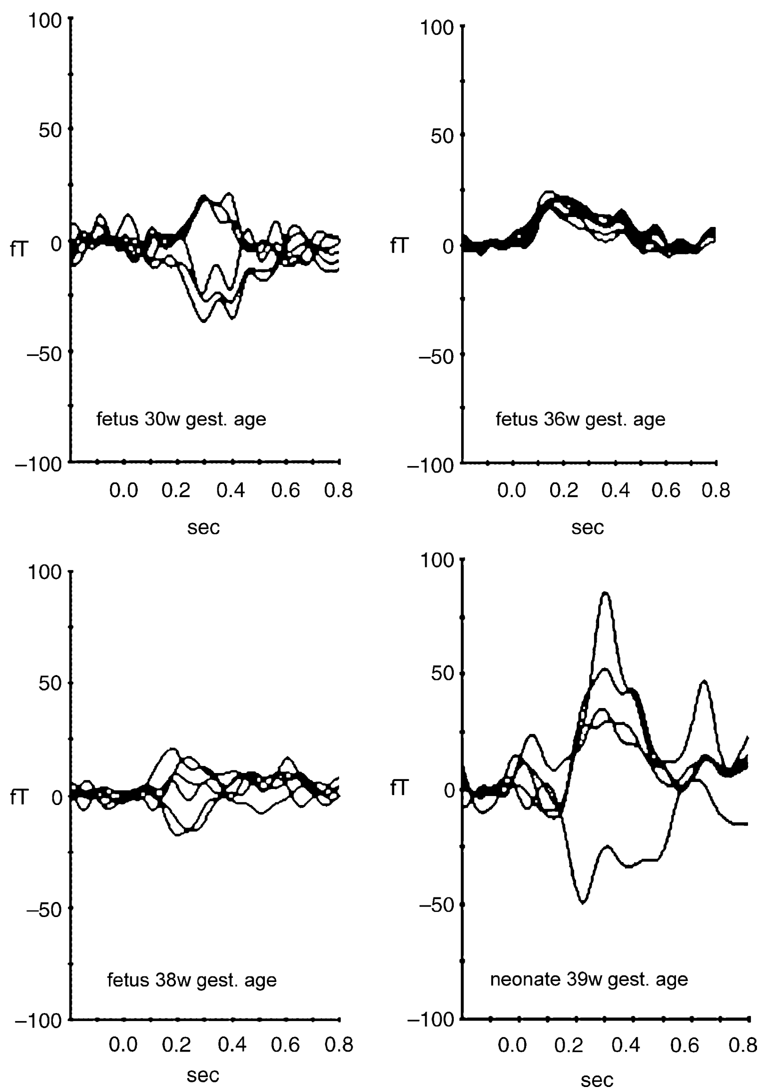

Fig. 3. AEF traces of a fetus of 3 different gestational ages (30, 36 and 38 weeks) and the newborn (39 weeks gestational age).

Table 2
Average response latencies across individuals, SD split into 3 different gestational age groups of fetuses and two different age groups of neonates for both ears

Gest. age (weeks)	Latency (ms)	SD
Fetus		
27–31 ($n=22$)	288	90.5
32–35 ($n=19$)	251	86.0
36–39 ($n=11$)	197	50.7
Neonates		
38–44 (left/right avg.) ($n=14$)	234	51.0

included since they fulfilled the criteria for a serial study. Table 1 shows the number of sessions in which each subject participated.

Sixty-three fetal recordings were made of which 8 were excluded from the analysis due to artifacts resulting from deep breathing or other movements, 3 were excluded because a response was not clearly identifiable, and 52 (83%) had evident responses based on the most prominent peak (Fig. 3). Signals of amplitude 7 fT or higher were detected. The mean latency in fetuses of gestational ages including 27–31 weeks was 288 ms. In fetuses of 32–35 weeks the average latency was 251 ms and in fetuses from 36–39 weeks the average latency was 197 ms (see Table 2). The distribution chart (Fig. 4) displays the decrease in response latency in fetuses as well as the follow-up recordings after birth.

In fetuses, the average amplitude was 23.4 fT, with a minimum of 7.2 fT and a maximum of 63 fT. For the neonates, the average amplitude was 69.4 fT for the left ear stimulation, with a minimum of 11.4 fT and a maximum of 177 fT. For the right ear stimulation, the average amplitude was 57.7 fT with a minimum of 11.8 fT and a maximum of 117 fT (see Table 3).

The average distance between the maternal abdominal surface and the head of the fetus was 49 mm. There was no significant correlation ($F=0.2$) between amplitude and distance.

Of the 52 fetal recordings with evident responses, 44 (83%) occurred during a wakeful state, 7 (13%) during an undetermined state (ultrasound was not performed in these few cases), and 2 (4%) during sleep state.

In the study on the neonates, all of the 14 individuals showed nAEF (100%) after auditory stimulation of each ear with amplitudes of 39 fT or higher. Fifty-four percent of the neonatal recordings were made during sleep state, 21% during quiet wake state, 14% of the neonates were in a mixed state of sleep and quiet wakefulness, and in 11% the state could not be determined. The average of the response latencies from left and right ear stimulation was used since there was no significant difference in latency after left and right ear stimulation ($F(1,25)=0.05$, $P>0.5$).

The repeated measure regression analysis across fetuses and neonates revealed a significant decrease of AEF latencies ($F(1,49)=6.31$, $P<0.05$) at a rate of 5.5 ms per week (95% confidence interval: [1.1, 9.8]) (Fig. 4).

Analyzing the group of fetuses and the group of neonates separately, we found a significant effect of age on response latency among fetuses ($F(1,35)=8.03$, $P<0.01$), but not in newborns ($t=0.92$, $P=0.37$). In the group of fetuses, the decrease was at a rate of 9.6 ms per week (95% confidence interval: [2.7, 16.5]).

4. Discussion

For this study we recorded AEF serially on a group of fetuses approximately every 2 weeks until delivery, and once again after birth. For the neonatal sessions, the subjects ranged in gestational age from week 38 to week 44, and in chronological age, from 6 days to 6 weeks with an average chronological age of 2 weeks. To our knowledge, this is

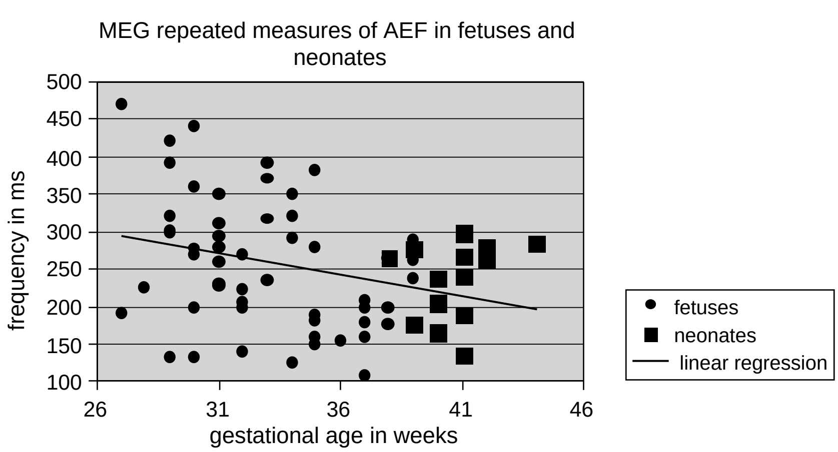

Fig. 4. Latency of the first prominent peak of the auditory evoked field for fetuses and newborns. Each mark corresponds to a single recording. The trend line shows a statistically significant decrease in latency with age.

Table 3
Average response amplitudes across individuals (fT, femto tesla)

Amplitudes in fT	Fetus	Neonate	
		Left ear	Right ear
Average	69.4	69.4	57.4
Lowest	7	11.4	11.8
Highest	63	177	117

the first longitudinal study on fetuses with a continuation after delivery.

In this study we obtained around 100 averages per recording. This number is lower than in our previously published work since we used an ISI of two to avoid possible overlap of evoked fields. Also, considering the subject's comfort, the risk of maternal movement, and the desire to maximize the number of trials, we decided that the optimum recording time was 6 min.

In the 62 fetal recordings the rate of detection of fAEF was 83% and in the 14 neonatal recordings it was 100%. For the identification of AEF we chose the largest peak. In agreement with Lengle et al. (2001), we found there was a significant decrease in latency over increasing age for normal fetuses and newborns, indicating a continuous development of auditory processing capabilities. From the confirmation by Schleussner et al. (2001) we found a decrease in response latency in fetuses at gestational age of 29 weeks to term. Their research group showed a decrease from 300 ms at the earlier stage to 150 ms at term whereas our study revealed an average latency of 288 ms at 29 weeks and 197 ms at term. In a separate analysis for the newborns 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 is certainly a factor that should be investigated in further studies.

Our findings on the development of auditory development in fetuses were consistent with existing literature showing results with EEG on neonates. Intra-individual variability in the group of fetuses is the source of the wide latency distribution, which ranged from 470 ms (in one 27 week old fetus) to 109 ms (in one 37 week old fetus). Pasman et al. (1992) measured latencies for N1 of approximately 140 ms, P2 of 200 ms and N2 around 344 ms in term infants at term with EEG. In their MEG study, Wakai et al. (1996) detected response latencies in fetuses and neonates at approximately 200 ms, although they observed components with latencies longer or shorter than 200 ms that were inconsistent across subjects. In our study only one component was observable in most cases in fetuses and neonates. Therefore, we decided to use the most prominent peak for the analysis. Since, only the most prominent peak was identified, the latency component may not correspond across all recordings because variation in fetal position can cause one component to be dominant over another. In the neonatal MEG measures of AEF, both ears have been stimulated separately and the latency responses of both sides have been averaged, because no significant latency difference in the sides was observed.

5. Conclusion

In our longitudinal study using MEG, an age related decrease in response latency in fetuses and newborns could be confirmed. Consistent with our current knowledge about developmental changes in the auditory system of newborns, we see a decrease of the latency between the 29th week of gestation and several weeks after birth. This gradual decrease clearly indicates a continuous development of the auditory system and the related brain processes. The functional significance of this intrauterine development has to be investigated further, since it may be an important indicator for normal brain development. For subjects with a higher risk of brain abnormalities, the fMEG can be an important non-invasive screening tool.

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