Short-term memory functions of the human fetus recorded with magnetoencephalography

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Studies in fetuses and in prematurely born infants show that auditory discriminative skills are present prior to birth. The magnetic fields generated by the fetal brain activity pass the maternal tissues and, despite their weakness, can be detected externally using MEG. Recent studies on the auditory evoked magnetic responses show that the fetal brain responds to sound onset. In contrast, higher-level auditory skills, such as those involving discriminative and memory functions, were not so far studied in fetuses with MEG. Here we show that fetal responses related to discriminating sounds can be recorded, implicating that the auditory change-detection system is functional. These results open new views to developmental neuroscience by enabling one to determine the sensory capabilities as well as the extent and accuracy of the short-term memory system of the fetus, and, further, to follow the development of these crucial processes. *NeuroReport* 15:000–000 © 2004 Lippincott Williams & Wilkins.

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INTRODUCTION

The auditory system of the human fetus develops during the last trimester of pregnancy. The perceptual accuracy and memory functions of the fetal auditory system have been studied with vibroacoustic stimulation using habituation paradigms [1,2]. The results of these studies suggest that simple discrimination and memory exist, and further, studies of neonates show that auditory material repeatedly heard during the fetal period is recognized after birth [3–5].

Recording the electric brain activity of the human fetus is becoming possible using MEG [6], which detects the magnetic field produced by the active neurons in the fetal brain tissue from above the mother's abdomen. The maternal tissues have no effect on the magnetic fields. Modern artifact-suppression methods [7–9] can separate the fetal brain signal from those of the maternal and fetal hearts and other sources. With MEG, fetal brain responses to sound onset have been reported by many groups working with different types of instruments [10–13]. The statistical analysis of the responses of each individual fetus is important in verifying the results [14]. The detection rates of the responses can be raised by repeating the recordings in the same fetus during varying behavioural states and on different days [15].

When repetition of a single sound in a sound stream is violated, and a deviant sound is presented amongst the repetitive standard sounds, responses related to sound feature discrimination, short-term memory functions, distraction from and re-orientation to the original task, and attention-switching are observed in adults. These include, among others, the mismatch negativity (MMN), the P3a and P3b, forming the P300 response, and re-orienting negativity (RON). The magnetic counterpart of the MMN, the MMNm, was recently reported in healthy full-term neonates [16,17]. The MMN and MMNm provide a measure of both auditory sensory accuracy and short-term memory duration. Obtaining MMNm in the fetus would open the possibility to study noninvasively the perceptual accuracy of the fetal auditory system to external sounds, and to reveal the temporal characteristics of the fetal auditory short-term memory, which can be considered as an important step of auditory information processing prior to long-term memory and learning.

We aimed at recording the MMNm in 17 healthy fetuses by using a flat-bottom MEG instrument with 99 channels in response to harmonic tones that occasionally changed from 500 to 750 Hz. This relatively large frequency change produces an MMNm in neonates [16].

SUBJECTS AND METHODS

Auditory short-term memory of the fetus was probed using the odd-ball paradigm, in which one sound is frequently (standard) and others infrequently (deviants) presented in a random order. In this paradigm, the mismatch negativity (MMN) [18,19] event-related potential can be recorded in response to deviants as long as deviants are discriminable from standards. The MMN and its magnetic counterpart (MMNm) were obtained even in neonates in response to tone-frequency changes [20] and to changes of vowels [17].

We recorded the magnetic fields produced by fetal brain activity with a 99-channel magnetometer (Elekta Neuromag Oy, Helsinki, Finland). The sensors sample the magnetic field with 165 pickup loops at distinct locations above the abdomen, arranged on a slightly curved spherical surface [21,22]. Of these loops, 132 are coupled to form 66 planar gradiometers, organized in orthogonal pairs. The midpoint of each pair coincides with the locus of a magnetometer loop. Altogether, 99 independent signals are thus provided as inputs for fetal MEG data analysis.

Seventeen healthy women (19–34 years, fetus gestational age (GA) 35–40 weeks, mean 37 weeks 5 days) with uncomplicated singleton pregnancies volunteered to participate to the study. The well-being of the fetuses was regularly monitored, and cardiotocography was performed typically 1–3 days prior to and after the experiment. The study was approved by the Coordinating ethical committee of the Helsinki University Central Hospital. An informed consent was signed by each participant.

The locus of the fetal head was determined by ultrasound and/or palpation immediately prior to the MEG recording. The mothers were placed in a reclining position on the examination bed, with the magnetometer being placed tightly against the mother's abdomen above the fetal head. During the recording, the fetal head position was monitored by asking the mothers to report when the fetus moved its head. At that point, the data collection was interrupted.

Sounds were delivered to the fetus by installing a plastic funnel tightly against the mother's abdomen and by conducting sounds to it via a plastic tube from an acoustic driver located outside the magnetically shielded room. The frequency response of the tube-funnel system was measured to determine the distortion caused by attenuation and reverberation inside the system. During the MEG recording, the frequency response was equalized on-line by a digital signal processor to an accuracy of 5 dB within the frequency range of the stimuli (500–2250 Hz). The sounds were delivered at 85–90 dB SPL (measured with the funnel end in open air).

Sounds of 100 ms in duration (including 5 ms rise and fall times) were presented with 800 ms sound onset asynchrony (SOA). The standards (p=80%) had a fundamental frequency of 500 Hz, with harmonics of 1000 and 1500 Hz presented with 3 and 6 dB attenuation, respectively, relative to the fundamental. The deviants (p=20%) had a fundamental frequency of 750 Hz, and the harmonics of 1500 Hz and 2250 Hz were attenuated similarly as for the standard. The deviants were pseudorandomly presented among the standards, so that each deviant was always preceded by at least one standard.

The MEG signals were filtered with a pass-band of 0.1–90 Hz and then sampled at 600 Hz. Epochs starting 150 ms before and ending 600 ms after the sound onset were online-averaged. The data collection was started when >40 deviants (~160 standards) had been presented. This was done under the assumption that after 2–3 min the fetus would move less and the behavioral state would be more stable. After rejecting the epochs in which the signal was >3000 fT on any of the magnetometer channels, average evoked responses to standards and deviants of each fetus were calculated. In addition, the individual epochs of standard sound responses were divided into 9–36 sub-averages of 50 accepted events to be used in the statistical analysis.

The recordings were influenced by magnetic disturbances from the surroundings as well as from the cardiac activities of the mother and the fetus. The signals originating in the fetal brain were extracted from the recordings by a novel analysis method, the signal space separation (SSS [9]), based on the fact that the sensor array lies in a current-free space between the neural current sources and those of external disturbance. The signals can therefore be divided by means of two independent harmonic function expansions into separate components: one containing the fetal brain signals and the other containing the signals from the cardiac and environmental sources. In this study, the MEG responses were separated into the two components after averaging, and only the component originating from the fetal brain was used in the analysis. The data were thereafter digitally filtered with a band-pass of 1–20 Hz and corrected with respect to a baseline of -100 to 0 ms.

Difference signals were formed separately for each fetus by subtracting the response to the standards from that to the deviants. When >150 accepted trials were acquired for the deviants, the data were split into two sets of standard and deviant responses for visualization purposes (Fig. 1) by separating half of the standards and half of the deviants. Additionally, sub-averages of the difference signals were formed by subtracting each standard sub-average from the deviant response.

The presence of a statistically significant response was determined in each fetus using a t-test on both the sub-averages of the standards and the sub-averages of the difference signals. The largest peak between 0 and 600 ms was determined on the magnetometer channel showing the largest response to the standard sound. The amplitudes of the sub-averages of the standard at the peak latency were statistically compared with the baseline noise to determine the significance of the standard-stimulus response. Similarly, the largest peak in the difference signal between 0 and 600 ms was determined, and the sub-averages of the difference signal were compared with the baseline noise to investigate the significance of the difference signal. In all statistical testing, the significance level was set to 0.05.

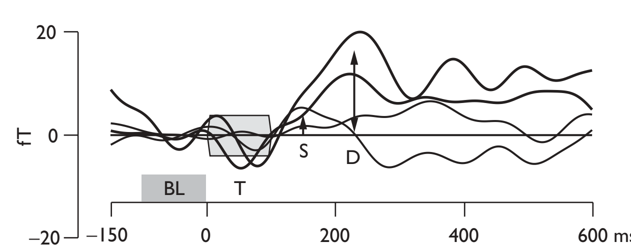

Fig. 1. MEG responses to standard (S, 500 Hz) and occasional deviant (D, 750 Hz) tones from fetus S1 are presented with thin and thick lines, respectively. The responses are divided into two sets to visualize the replicability of the recording. The baseline (BL) of 100 ms prior to the presentation of the tone (T) is used. The responses are statistically compared to zero for the repetitive standard sound (S) at the largest peak of the response and between the standard and deviant sound and the largest peak of the difference signal (D).

Table I. Peak amplitudes (in a 40-ms window centered around the peak latency) and peak latencies of individual fetal responses found in the responses to the standard sounds and in the difference signals between the standard and deviant sounds.

	GA	Responses to standard sounds latency (in ms), amplitude (in fT)	Difference signal	
			Latency (in ms)	Amplitude (in fT)
S1	39 + 5	143, 4.69	233	19.40
S2	39 + 2	N.S.	289	N.S.
S3	36 + 2	124, 6.37	287	15.00
S4	35 + 2	N.S.	193	18.10
S5	38 + 2	N.S.	337	N.S
S6	36 + 4	N.S.	308	N.S.
S7	37 + 2	N.S.	322	17.14
S8	36 + 1	94, 23.61	174	N.S.
S9	38 + 0	N.S.	441	N.S.
S10	37 + 1	406, 20.63	432	38.48
S11	39 + 2	210, 7.76	233	36.54
S12	37 + 3	465, 15.52	420	31.67
S13	38 + 3	N.S.	428	44.94
S14	37 + 4	N.S.	476	37.51
S15	40 + 1	N.S.	298	38.48
S16	36 + 0	N.S.	367	25.87
S17	40 + 0	171, 5.49	298	27.16
Mean of all detected responses	37 + 5	Not determined	326	Not determined
Mean of significant responses		230, 13.1	332	29.2

RESULTS

SSS [9] was successful in removing the fetal and maternal cardiac and external artefacts in all 17 cases. After SSS, auditory responses were observed in the magnetometer channels but were not clearly visible in the gradiometer channels, most probably due to the fact that the gradiometers are optimized for sources closer to the instrument than those originating from the fetal brain.

The typical field patterns observed in the 33 magnetometer channels suggested dipolar sources both for the responses to the standard sounds and for those found in the difference signals. A significant response to the 500 Hz standard sounds was found in 7 out of 17 fetuses at an average latency of 230 ms and an average amplitude of 13.1 fT (Table 1). A significant difference between the responses to the deviant and standard sounds was detected in 12 out of 17 fetuses at an average latency of 332 ms and an average amplitude of 29.2 fT. Of these 12 fetuses, six also showed a significant response to the standard sounds. The responses of all fetuses to standard and deviant sounds are presented in Fig. 2.

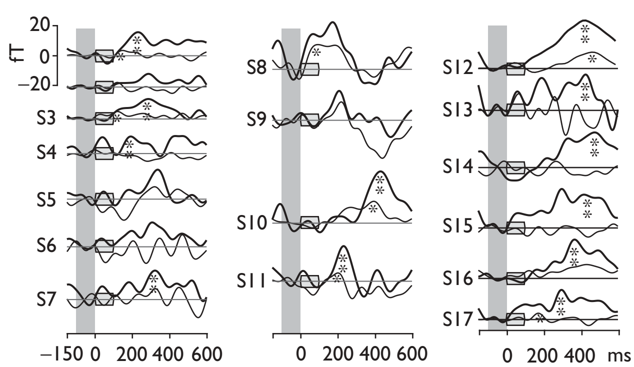

Fig. 2. Responses of all fetuses to standard (thin line) and deviant (thick line) sounds are presented from the channel showing the largest responses. The standard responses differing significantly from zero in 7 fetuses are marked with *, whereas the significant differences between the standard and deviant responses in 12 fetuses are marked with **.

DISCUSSION

We were able to record fetal auditory evoked magnetic responses to repeated tones (standards) with similar detection rates, amplitudes, and latencies as the previous groups [15]. The decreased response amplitudes may be a result of a relatively fast presentation rate, which, in turn, may lead to increase in the responses of the deviants compared to the standards, as was found in this study.

The behavioral states of the fetus [23] were not taken into account in this experiment. In general, during the preparation for the experiment, the fetus became responsive. The slight movements of the fetuses were most prominent during the first few minutes of the experiment, and also at the end of the experiment. Since several studies reported differences in neonatal responses according to sleep stages [24,25], it is possible that in these data, responses of several types were collapsed, thus decreasing the statistical power. In the future, it would be important to take into account the behavioral state of the fetus during the recording.

Most importantly, we found a discriminative response, a possible fetal analogy of the adult MMNm, to be significant in 12 out of 17 fetuses. This is consistent with previous behavioral work [1,2], suggesting that frequency-change detection is possible. In the present work, elicitation of the MMNm reflecting auditory change detection was found for the first time in fetuses. The time scale of this discriminative brain response resembled those reported in neonates [16,17,20].

CONCLUSION

Using noninvasive magnetoencephalography, we recorded apparent analogs of adult mismatch negativity from a group of healthy fetuses as a response to a frequency change. We believe that monitoring fetal auditory change-detection and auditory memory with varying stimulus setups will bring new and interesting information on the development and capabilities of the fetal auditory system.

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