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Research Report

Fetal auditory responses to external sounds and mother's heart beat: Detection improved by Independent Component Analysis

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ABSTRACT

In this paper, we present a magnetoencephalographic study of the fetal auditory response to external stimuli and to the sound of the mother's heartbeat. We describe how an *ad hoc* functional selection procedure allowed us to isolate the sources in the fetal brain responding to sounds only, after the application to the recorded data of a standard Independent Component Analysis algorithm. In our experiment, acoustic stimuli were delivered to twelve healthy women with uncomplicated pregnancies at a time between 36 and 40 weeks gestational age, with their fetuses in breech presentation. Ultrasound images allowed determination of the region over the women's abdomen nearest to the fetal head, over which both the acoustic stimulator and the MEG sensors were subsequently placed. In 8 out of the 12 cases, our analysis provided consistent evidence of a fetal response both to the mother's heartbeat and to the external auditory stimulation; both were characterized by a clear prominent component at around 200 ms latency, which is widely accepted as the marker of the fetal response to auditory stimuli.

1. Introduction

Studies of fetal heart rate variations caused by sounds (Grimwade et al., 1971) and, more recently, reports on fetal responses to external acoustic stimulation (Blum et al., 1985, 1987) have demonstrated that the sense of hearing is at work already in the last 2 months of intrauterine life. For this reason, hearing has become the most studied neurosensory activity in human fetuses (Querleu et al., 1988; Lutz, 1991; Johansson et al., 1992; Gerhardt and Abrams, 1996; Zappasodi et al., 2001; Eswaran et al., 2002).

The intrauterine acoustic environment of the human fetus is made of frequent cardiovascular, respiratory, and intestinal sounds punctuated by isolated, short bursts during maternal body movements and vocalization (Qurleau et al., 1988; Abrams and Gerhardt, 2000). All these sounds are an important

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component of prenatal development since they provide a memory-linked foundation for later learning and behavior. Studies evaluating signals recorded by a microphone placed in the mother's vagina near the time of delivery have established that the sound of the maternal heart contributes to fetal imprinting by dominating the intrauterine acoustic environment (Salk, 1962; Bench, 1968; Murooka et al., 1976; Rosner and Doherty, 1979; DeCasper and Sigafoos, 1983). This is the reason why the reproduction of the maternal heart sound is often used for quieting newborns (Smith and Steinschneider, 1975; Rosner and Doherty, 1979).

In this paper, we present a magnetoencephalographic (MEG) study of the fetal auditory response to sounds. We applied Independent Component Analysis (ICA, fastICA: Hyvärinen, 1999) to MEG data recorded from twelve fetuses during acoustic stimulation. In particular, we used an *ad hoc* functional selection procedure to detect the independent components corresponding to the sources in the fetal brain responding both to the external stimuli and to the sound of the mother's heartbeat.

Magnetoencephalography is a non-invasive technique that detects neuromagnetic fields generated by synchronous postsynaptic currents both during spontaneous cerebral activity and in response to external stimulation. Body tissues are virtually transparent to magnetic fields so that MEG signals can be detected outside the body without distortion (see Del Gratta et al., 2001 for a review).

After the pioneering work of Blum et al., 1985, 1987, fetal MEG (fMEG) has made significant progress through the employment of multisensor systems: auditory fMEG represents today a major field of research (Wakai et al., 1996; Preissl et al., 2001; Schneider et al., 2001; Lengle et al., 2001; Zappasodi et al., 2001; Eswaran et al., 2000, 2002, 2005; Holst et al., 2005) and a multichannel system specifically designed for fetal application has been realized (SARA, Robinson et al., 2001). Even fetal cortical components associated with discriminative and memory functions have been recently recorded (Huotilainen et al., 2005; Draganova et al., 2005; see Preissl et al., 2004 for current progress and trends in fMEG). The major problems in recording cerebral fetal responses are related to the non-optimal position of the fetal head with respect to the detecting sensors, several centimeters away from the fetal cortex. Moreover, the cerebral fetal electromagnetic signals are several orders of magnitude lower than signal generated by environmental noise and other biological sources (mainly cardiac activity).

Current techniques to monitor neuronal fetal well-being are indirect (i.e., a proxy is used, as for example the cardiac signal, to evaluate brain functionality). Fetal MEG provides instead a direct measure of the fetal brain's activity at rest or in response to external stimuli. This is of paramount importance in gathering prenatal information on cerebral functionality, both in the healthy and in the disease, to deeply understand maturation phenomena and to early individuate dysfunctionalties.

Independent Component Analysis is a statistical technique that, under certain assumptions, separates the different sources contributing to a signal (Comon, 1994; Hyvärinen, 1999). This technique has proven to be very effective in the identification and elimination of artifacts and noise from biological signals (Barbati et al., 2004) and has been also successfully used for the identification of fetal magnetic cardiac activity (Lathauwer et al., 1995; Cardoso, 1944; Comani et al., 2004; Salustri et al., 2005; Theis, 2005).

The strength of ICA lies in the fact that it does not require an *a priori* knowledge of the nature of sources and interferences. Moreover, it gives the possibility to select a limited set of independent components which describes the activity of interest and to reconstruct the biological signal as if it were generated only by the sources of that activity (Makeig et al., 2004; Tang et al., 2004).

The ICA of the fetal data is based on the assumption that the signal detected by the MEG sensors is a linear mixture of stochastically independent contributions coming (i) from the fetal brain, which is what we want to investigate, (ii) from unwanted biological near-field sources, as for example, maternal and fetal hearts, gastric and uterine muscle contractions, motion artifacts, etc. and (iii) from the external environment.

2. Results

2.1. *Reliability of the fetal auditory source identification*

As shown in Fig. 1, the magnetic field signals obtained by removing the ICs corresponding to the maternal and fetal cardiac activity (Fig. 1, panel c) are not sufficient to identify latency and amplitude of the fetal auditory responses. On the contrary, by applying the above described procedure to isolate the ICs describing only the cerebral sources responding to sounds, a clear component at around 200 ms in the cortical responses to external acoustic stimulation ($fAEF_{ext}$) time course (Fig. 1, panel d, column 2) can be recognized, showing a satisfactory morphology.

Identifiable $fAEF_{ext}$ were found in 8 out of the 12 subjects.

2.2. *Fetal auditory responses to external sounds and mother's heart beat*

In all subjects with reliable $fAEF_{ext}$, a response to the maternal cardiac sound ($fAEF_{mh}$) was also found.

$fAEF_{ext}$ and $fAEF_{mh}$ showed comparable morphologies (Fig. 2). The two-tailed paired Wilcoxon test of the latencies across subjects showed that the latter were longer for $fAEF_{mh}$ than for $fAEF_{ext}$ ($P = 0.012$). The latency difference between $fAEF_{mh}$ vs. $mMCG_{mh}$ and $fAEF_{ext}$ vs. $mMCG_{mh}$ resulted strongly variable across subjects (Fig. 2). Moreover, $fAEF_{mh}$ amplitudes across subjects were higher than the amplitudes of the $fAEF_{ext}$ (Table 1, $P = 0.012$).

A two-tailed paired Wilcoxon test corrected for spatial autocorrelation of the magnetic field spatial distributions showed non-different values for FSD_fAEF_{ext} versus FSD_fAEF_{mh} at the latency of maximal response ($P > 0.500$ in all fetuses, Fig. 2). On the contrary, although the latencies of the auditory response peak ($fAEF_{ext}$ and $fAEF_{mh}$) and of the $mMCG_{mh}$ were quite comparable, at these latencies, the two-tailed spatial corrected Wilcoxon test delivered a clear difference between the spatial distribution of the signals generated by the fetal auditory sources (FSD_fAEF_{ext} and FSD_fAEF_{mh}) and the

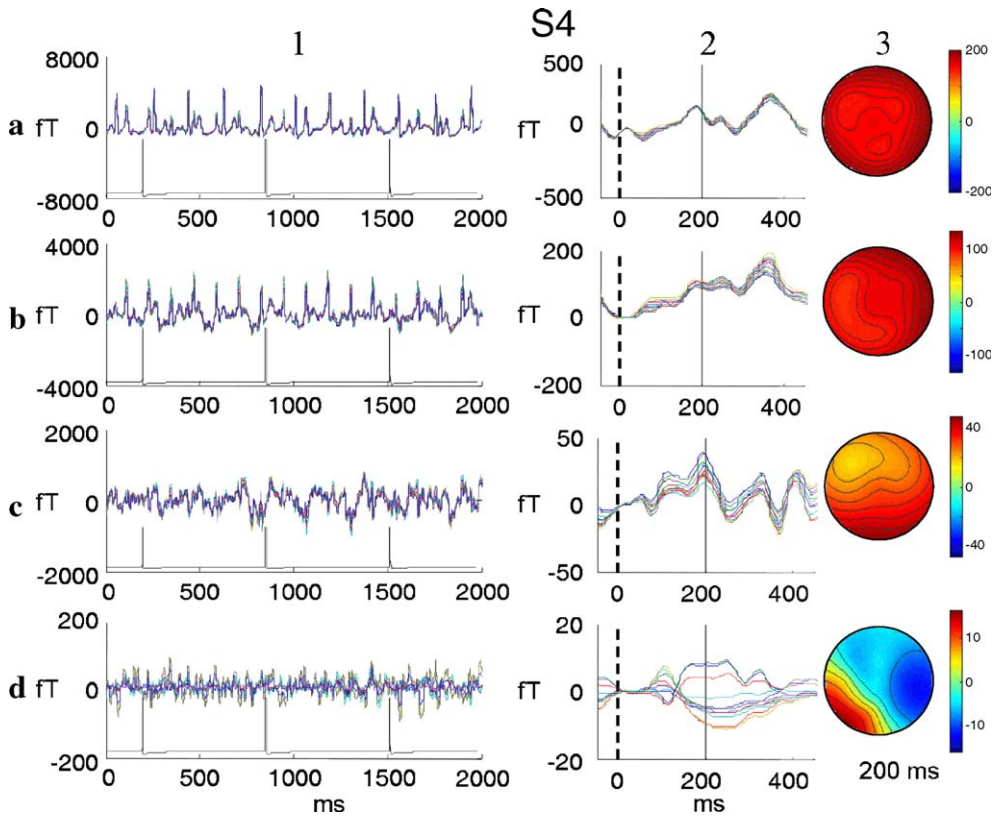

Fig. 1 – Effects of ad hoc functional selection IC procedure. All MEG sensors are shown superimposed. Column 1: 2 s time course; column 2: average on the external sounds (dotted vertical line); column 3: spatial magnetic field distribution at the latency of the main component (solid vertical bar). (a) Filtered original MEG signals: maternal and fetal cardiac peaks are evident in the trace. (b) Retroprojection of all ICs but those describing the maternal cardiac activity. (c) Retroprojection of all ICs but those describing the maternal and fetal cardiac activity; averages (c2) still show a not good quality of morphology, and auditory response latency and amplitude are not identifiable. (d) Retroprojection of only the ICs describing the auditory response, according to the procedure described in the Experimental procedures section. Latency and amplitude of the auditory response are now clearly identifiable (d2). In this case, the field distribution (d3) shows a dipolar-like shape, indicating a good positioning of the system with respect to the fetus head.

components representing the maternal heart activity (FSD_mMCG_{mh}) ($P < 0.0004$: FSD_fAEF_{ext} vs. FSD_mMCG_{mh}; FSD_fAEF_{mh} vs. FSD_mMCG_{mh}).

3. Discussion

As already mentioned in Introduction, the sense of hearing is already functioning in the last 2 months of intrauterine life and it is now widely accepted that healthy fetuses respond to a 1000 Hz stimulus after the 33rd week of gestational age. We have used 1000 Hz tone bursts with ISIs above 1 s since the aim of our study was to obtain a response cortical in origin, similar to the one observed in adults, i.e., around 100 ms after the stimulus onset: this type of external stimulus does not elicit responses from cochlea, brainstem and thalamus. To obtain responses from these areas, both in full-term and preterms, published literature recommends clicks containing very high frequencies. Moreover, the latencies of the responses we have obtained support the notion that they originate in the fetal auditory cortex.

3.1. *Fetal auditory response to external sounds*

Previous fMEG studies have reported successful fetal auditory response detection in about 50% of the examined fetuses (Eswaran et al., 2002; Lengle et al., 2001; Zappasodi et al., 2001; Preissl et al., 2001, 2004). Some analyses, utilizing data collected from the same fetuses in multiple sessions at different times, have reached levels up to 80%, but this figure returns to an actual 50% when number of successful detections is normalized to the total number of recordings (see Preissl et al., 2004 for a discussion on this issue). All these studies agree that these detected responses come from the fetal cortex and are equivalent to the middle/long latencies auditory responses observed in children and adults.

Our ICA-based procedure, applied to one-time recordings only, delivered clear evoked responses from 8 fetuses out of 12, i.e., 67% of the cases. Moreover, all the responses to external sounds showed on average an 83 fT peak at latencies around 206 ms. A component characterized by this amplitude and latency is widely interpreted as the marker of the fetal response to auditory stimuli (Preissl et al., 2004).

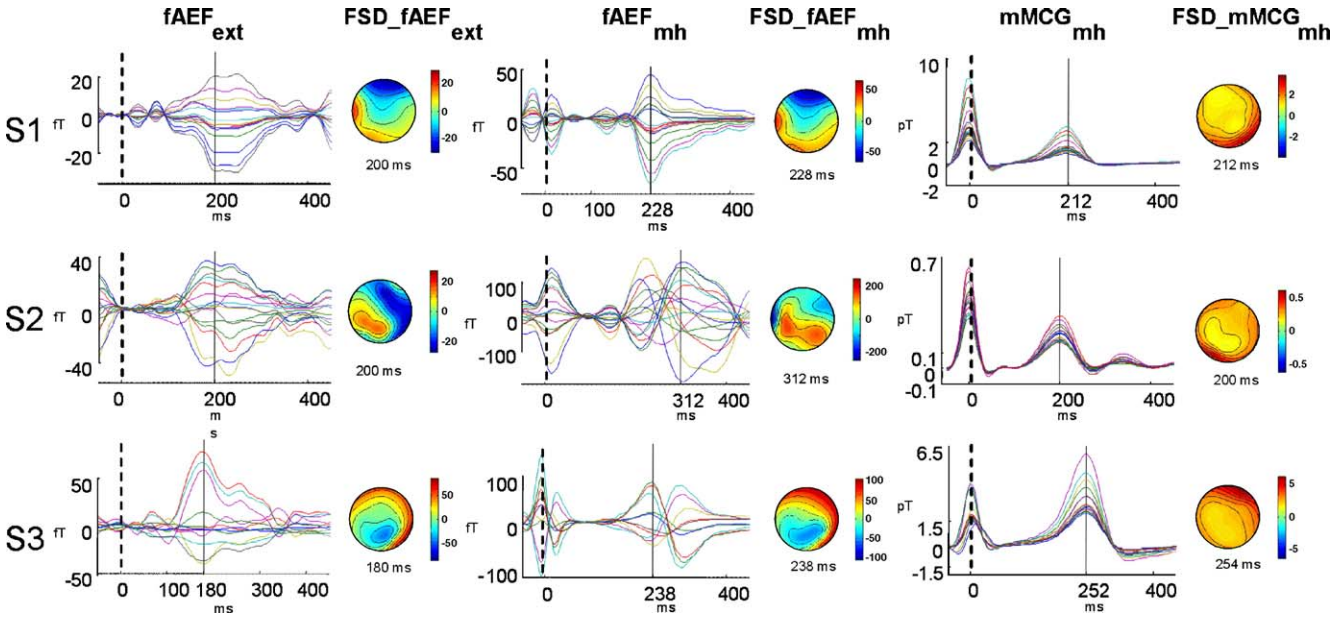

Fig. 2 – Fetal auditory responses to external sounds and maternal heart beat. Signals obtained retroprojecting specific ICs (y_{select}) and their spatial magnetic field distribution at the indicated latencies (solid vertical bar) with respect to trigger (dashed vertical bar), shown for three representative subjects (S1, S2, S3). $fAEF_{ext}$: retroprojection of y_{select} averaged on the external acoustic stimuli. $fAEF_{mh}$: retroprojection of y_{select} averaged on the maternal heart R-wave; in this case, the residual artifact on the y_{select} is visible around the dashed vertical bar, i.e. the R-wave. Note that the field spatial distributions (FSD) are strongly asymmetric in agreement with the difficult MEG system positioning with respect to the fetal head. $mMCG_{mh}$: retroprojection of the ICs representing the maternal heart, averaged on the maternal heart R-wave.

3.2. *Fetal auditory response to mother heart beat sounds*

An interesting finding of our analysis is that the same cerebral sources that responded to external sounds also responded to the sound of the maternal heartbeat, in agreement with the fact that the latter is itself an acoustic stimulus. Heart sounds are low frequency transient signals generated by the vibrations produced during the closure/opening of the heart valves (Tilkan and Conover, 1984). In healthy subjects, they correspond to two prominent acoustic events: a first one, caused by the closure of the mitral and the tricuspid valves, and a second one, around 300 ms later, caused by the closure of the aortic and the pulmonary valves. The start of the first event can be estimated by the onset of the R-wave in the ECG (Lehener and Rangayyan, 1987), corresponding to the left ventricular depolarization.

We used the R-wave as a trigger to obtain the $fAEF_{mh}$ and $mMCG_{mh}$. In some of the fetuses, also the auditory response to the subsequent maternal cardiac event could be spotted, probably due to a more precise timing between the two events (Fig. 3). The high variability in latency of the $fAEF_{mh}$ could be attributed to the fact that the mechanical actions producing the cardiac sounds are not exactly the same in all subjects.

Table 1 – Characteristics of the main component of the auditory response to the external stimuli ($fAEF_{ext}$), the maternal heart beat ($fAEF_{mh}$) and the latency of the maternal cardiac T-wave (lat T), as identified in the signal averaged on the retroprojected R-wave ($mMCG_{mh}$)

	# IC	$fAEF_{ext}$			$fAEF_{mh}$			# IC	$mMCG_{mh}$		
		# ave	lat (ms)	amp (ft)	# ave	lat (ms)	amp (ft)		# ave	lat (ms)	amp (ft)
S1	2	140	200	52	400	228	115	2	800	212	2600
S2	2	150	200	82	1000	312	288	1	750	200	500
S3	3	200	180	120	668	238	138	3	668	252	245
S4	2	219	200	22	800	248	70	2	849	200	2400
S5	4	180	200	107	600	220	168	2	600	252	1045
S6	2	130	224	95	750	232	220	1	800	228	1400
S7	2	227	196	67	850	232	137	1	800	236	1350
S8	2	320	248	116	1000	352	260	1	1400	252	653
MEAN ± SD			206 ± 21	83 ± 34		258 ± 48	175 ± 75			229 ± 23	1274 ± 857

#IC = number of y_{select}; #ave = number of trials averaged; lat = latency; amp = amplitude.

The spatial distributions of the signals generated by the fetal cerebral sources were very similar when responding to the maternal heartbeat (FSD_fAEF$_{mh}$) and to the external stimuli (FSD_fAEF$_{ext}$) at the latencies of the prominent component. This distribution was different from the spatial distribution of the signal generated by the components representing the maternal heart activity during the T-wave (FSD_mMCG$_{mh}$), although the latencies of auditory response peak and of the maternal heart T-wave were comparable. These differences are key to understanding the origin of the signals obtained.

3.3. *Comparison between responses to external and mother heart beat sounds*

The observed difference in latency between fAEF$_{ext}$ and fAEF$_{mh}$ could be due to a delay in the transmission of the cardiac sound to the uterine environment.

fAEF$_{mh}$ showed greater amplitudes than fAEF$_{ext}$: this difference was probably due to different ISIs. In fact, it has been reported that the amplitude of the healthy children's 200–250 ms component is high for ISIs under 1 s but decreases rapidly for longer ISIs (Takeshita et al., 2002). In our case, the amplitude of the 200 ms component was higher in response to the maternal heart than to the external sound. This was probably due to the fact that the external acoustic stimulation ISI was greater than 2 s, whereas the maternal R–R distance averaged approximately 700 ms. We believe that the amplitude differences were not due to the different intensities of the acoustic stimuli. In fact, measurements of uterine basal noise show a weighted SPL of 28 dB away from the placenta, while the system's basal noise reached about 15 dB, and maternal vascular noises can reach 25 dB above the basal noise (Querleu et al., 1989). On the other hand, the amniotic fluid attenuates frequencies below 500 Hz less than 5 dB and frequencies above 500 Hz up to 30 dB, resulting in a loss of external stimulus intensity. As a result, in our experiment, the external stimulus and cardiac sound intensities could be assumed similar. Moreover, differences between temporal morphology and/or field distribution associated to fAEF$_{mh}$ and fAEF$_{ext}$ could be caused by the fact that the mechanical displacement of amniotic fluid due to maternal heartbeat evokes somatosensory responses in addition to the auditory ones.

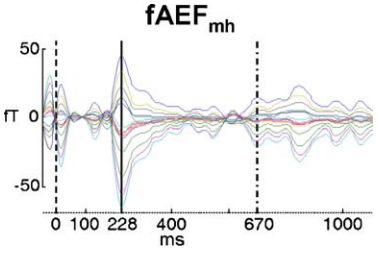

Fig. 3 – Fetal response to maternal heart sounds. Signals obtained by retroprojecting y_{select} in the case of subject S1 and averaging on the maternal heart R-wave. After the auditory response to the maternal first heartbeat sound (solid vertical bar), a component is visible which possibly represents the auditory response to the second heartbeat sound.

The continuous production of the maternal sound beat might induce some habituation effect. Habituation phenomena were observed at primary auditory cortex level, with evoked response amplitude decrease (healthy adults' M100, Hari, 1991; Woody et al., 2000; Rosburg et al., 2002) and a decrease in the receptive field which is strictly frequency-specific (Weinberger, 1998), but they accompanied strictly repetitive sounds presentation with fixed frequency content, occurring at fixed inter-stimuli. While the sound from the maternal heart reaches the fetus, many variations occur, as for example those induced by the fetus' movements which, by changing orientation and distance from the mother heart, change in practice the characteristics of the stimulus; as well, the interstimulus interval is far from fixed. In these respects, the habituation effects could be expected not to significantly affect fetal responses to the mother heart beat from primary auditory areas.

3.4. *Methodological considerations*

The sole removal of dominant artifacts (i.e., maternal and fetal cardiac activities) was not enough to reach a signal quality sufficient to identify the fetal cerebral auditory responses. To face this problem, we have passed the data through an ICA algorithm with an ad hoc procedure which selects the ICs representing the fetal auditory responses using functional properties. Our approach was based on a direct study of the signal morphology in terms of amplitude and field distributions of the retroprojected ICs, hence eliminating the intrinsic indeterminacy of all ICA-based procedures (i.e., amplitude and sign, Hyvärinen et al., 2001; Cichocki and Amari, 2002; Meinecke et al., 2002) and on using prior information about the nature of the fetal brain source of interest.

Our results prove that the post-extraction functional selection procedure in the ICA algorithm allows the identification of fetal cerebral sources. The present work can be considered a further extension of the already broad ICA applications spectrum.

In conclusion, the ICA-based extraction procedure allowed characterizing fetal acoustic activity in 67% of the cases. The auditory responses were clearly related to both external and maternal cardiac sounds. This confirms that fetal MEG is a neurophysiological technique suitable for testing fetal cerebral activity and for gathering information about brain functionality.

4. Experimental procedures

4.1. *Subjects*

Twelve healthy women with uncomplicated pregnancies and fetuses in breech presentation were examined. Preliminary ultrasound fetal analysis was carried out to confirm a gestational age between 36 and 40 weeks to exclude brain malformations and to estimate volume of amniotic fluid and fetal weight (required to be at least 2500 g to qualify). Resistance in the umbilical arteries, fetal middle cerebral artery and fetal abdominal aorta was evaluated by means of

echo-fluximetry. Tococardiography, including fetal heart and maternal uterine contraction monitoring (Smith and Onstad, 2005), was collected a few days before and after the MEG recordings to assess fetal central nervous system integrity and fetal well-being.

4.2. MEG recordings

MEG recordings were performed by means of a 28-channel system (16 internal axial gradiometers and 9 peripheral magnetometers, 3 balancing magnetometers being devoted to noise reduction; gradiometers and magnetometers are characterized by a noise of 5–6 $fT/Hz^{1/2}$ and 7–9 $fT/Hz^{1/2}$ respectively; Foglietti et al., 1991). The 25 measuring sites are regularly distributed on a spherical surface, covering an area of about 180 cm^2. The entire system is located inside a magnetically shielded room (Vacuumschmelze GMBH). A single electrical derivation was used to record the mother's electrocardiogram (ECG).

All women sat comfortably in a semi-reclining position; the presentation of the fetus and the region of the mother's abdomen nearest to the fetal head were determined by ultrasound in order to place the MEG sensor array as close to the fetal brain as possible. The distance of the fetal head from the maternal abdomen was also evaluated echographically and resulted from 1.5 to 3.5 cm.

Magnetic fields were continuously acquired at 250 Hz sampling rate. Off-line bandpass forward–backward filtering between 1 and 15 Hz (Butterworth second order filter) was applied.

4.3. Stimuli

All the fetuses were stimulated by 1000 Hz tone bursts, except 2 that were stimulated at 500 Hz. Each stimulus lasted 500 ms, with an intensity of 103 dB SPL. The interstimulus interval (ISI) was 2631 ms. Stimuli were delivered via a plastic tube to the area of the maternal abdomen closest to the fetal head. From 200 to 350 stimuli were delivered. The entire procedure lasted about 1.5 h for each subject. The recorded data were inspected visually, and the trials heavily contaminated by fetus movements were discarded.

4.4. ICA

Our objective was to extract signals containing only the fetal cerebral sources responding to acoustic stimuli. To this purpose, we assumed the set of observed fMEG signals to be generated by the mixing model:

$$\mathbf{x}(t) = \mathbf{A}\mathbf{s}(t) \tag{1}$$

where $t = 0, 1, 2, \ldots$ is the discrete sampling time; $\mathbf{x}(t) = [x_1(t), \ldots, X_m(t)]$ is the m-dimensional vector of the observed signal recorded by m sensors; $\mathbf{A}$ is an $m \times n$ (with $n \leq m$) unknown full-rank mixing matrix; $\mathbf{s}(t) = [s_1(t), \ldots, s_n(t)]^T$ is the n-dimensional unknown vector of the sources. It is assumed that the vector $\mathbf{s}(t)$ can be partitioned in a subset of 'interesting' sources (i.e., the fetal brain activity) and a subset of 'uninteresting' sources (i.e., maternal and fetal cardiac activity, fetal movements, instrumental noise, …).

Before applying ICA, a first phase of data 'whitening' was performed to conveniently transform the original signals into a new set of uncorrelated signals having zero mean and unit variance (Hyvärinen et al., 2001). This was done by applying Principal Component Analysis (PCA), but no dimension reduction was performed to avoid the possibility of excluding interesting fetal cerebral sources, possibly several order of magnitude lower than the artifact components (mainly cardiac interferences) and background noise. Consequently, in our application, $m = n$.

In this frame, for each subject, sensor signals were processed by an ICA demixing system described by the general model:

$$\mathbf{y}(t) = \mathbf{W}\mathbf{x}(t) \tag{2}$$

where $y(t) = [y_1(t), \ldots, y_n(t)]^T$ is the n-dimensional vector of the estimated ICs and $\mathbf{W}$ is the separation matrix, i.e., the estimate of the inverse of the unknown mixing matrix $\mathbf{A}$, up to permutation and scaling:

$$\mathbf{W} = \hat{\mathbf{A}}^{-1} \tag{3}$$

We used the fastICA algorithm proposed by Hyvärinen (1999).

4.5. Functional selection procedure

In concordance with the improvements of the standard ICA model, introduced for example in the context of fMRI (Beckmann et al., 2004), we developed the following procedure to select components of interest in fMEG data.

The outputs of the ICA demixing system resulted in a number of ICs representing both 'interesting' and 'uninteresting' artifactual sources. As a first step, in order to identify the cerebral sources which only responded to auditory stimuli, we used a 'functional' criterion to mark, among all estimated ICs, those contributing to the acoustic cerebral responses and excluding all the others. To this aim, each k-th y_k ($k = 1, \ldots, n$) was retroprojected by $\mathbf{W}_k^{-1}$, obtaining in this way retroprojected signals with the only contribution by the single k-th y_k.

$$x_{Rec_k} = \mathbf{W}_k^{-1} y_k \tag{4}$$

where $\mathbf{W}_k^{-1}$ denotes the corresponding selected k column of the inverse of $\mathbf{W}$.

Since we looked for components representing an acoustic response elicited around 200 ms after the auditory stimulus, which is usually interpreted as the counterpart in infants of the M100 in adults, each x_{Rec_k} was separately averaged on the external acoustic stimulus and on the maternal cardiac R-wave.

Only ICs with evolution of the corresponding retroprojected field averages showing a prominent peak between 150 and 300 ms and an appropriate morphology were visually selected, while the others were discarded. Note that averages on either external acoustic stimulus or on the maternal cardiac R-wave provided the same selected ICs; in one single case (S2), two ICs responded only to one of the two auditory stimuli, and they were all included among the selected ones. All these chosen ICs described the whole fetal cerebral sources responding to auditory stimuli (y_{select}).

As a second step, these selected ICs were passed through the inverse system eq. (4) to obtain the fetal cerebral acoustic response:

$$x_{Rec_{select}} = \mathbf{W}^{-1}_{select} y_{select} \quad (5)$$

where the subscript 'select' stresses that only a part of the estimated ICs contained the fetal response and $\mathbf{W}^{-1}_{select}$ denotes the columns of $\mathbf{W}^{-1}$ corresponding to the selected ICs.

Once we isolated in this way the magnetic field generated only by the fetal cerebral sources responding to auditory stimuli ($\mathbf{X}_{Rec_{select}}$), we averaged this field both on the external stimuli ($fAEF_{ext}$) and, in order to estimate the acoustic response to the maternal heart beat, on the maternal R-wave ($fAEF_{mh}$). $fAEF_{ext}$ and $fAEF_{mh}$ latencies were defined as the first prominent peak.

In order to check that the estimated response was not a 'residual' cardiac artifact, we then selected all the ICs describing the maternal heart and averaged the signals obtained by retroprojecting them on the R-wave ($mMCG_{mh}$).

In this way, we compared across subjects vectors pairs of latencies of $fAEF_{ext}$, $fAEF_{mh}$ and $mMCG_{mh}$ by means of the non-parametric paired ($fAEF_{ext}$ vs. $fAEF_{mh}$) and simple ($fAEF_{ext}$ vs. $mMCG_{mh}$ and $fAEF_{mh}$ vs. $mMCG_{mh}$) Wilcoxon test that do not assume gaussianity of the vectors distribution across subjects. $fAEF_{mh}$ and $fAEF_{ext}$ amplitudes across subjects were also compared.

Moreover, for each subject, vectors pairs of the field spatial distribution at the selected latencies of $fAEF_{ext}$, $fAEF_{mh}$ and $mMCG_{mh}$ were compared by means of the non-parametric paired (FSD_fAEF_{ext} vs. FSD_fAEF_{mh}) and simple (FSD_fAEF_{ext} vs. FSD_mMCG_{mh} and FSD_fAEF_{mh} vs. FSD_mMCG_{mh}) Wilcoxon test, corrected for the presence of spatial autocorrelation; in fact, since topographic maps vectors had a significant spatial autocorrelation, tested by means of the Moran's I statistic (Cliff and Ord, 1981), a Bonferroni-type correction has been applied, making an adjustment to Wilcoxon P values based on the number of neighbors (+1) of each sensor rather than the total number of sensors (Haining, 2003). In this way, the standard significance threshold of $P = 0.05$ has been lowered at $P_{SP\_Corr} = 0.0004$.

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