Antenatal Determination of Fetal Brain Activity in Response to an Acoustic Stimulus Using Functional Magnetic Resonance Imaging

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Abstract: Functional magnetic resonance imaging (fMRI) is now a well-established technique for directly identifying adult brain activity. This study builds on earlier pilot work that showed that fMRI could provide direct evidence of fetal brain cortical activation in response to an auditory stimulus. The new work presented here aims to assess the sensitivity of this technique in a larger sample group. This article includes a specific discussion of the methodology required for fetal fMRI. Sixteen pregnant subjects were scanned between 37 and 41 weeks gestation, 12 had an auditory stimulus applied to the maternal abdomen (study group) and 4 had an auditory stimulus applied to the mother's ears (control group). Two of twelve (2/12) study-group patients experienced back pain so that the experiment was abandoned; 4/12 showed significant activation ($P < 0.005$) in one or both of the temporal lobes; 1/12 showed significant activation in the frontal lobe. A susceptibility artifact at the interface between the maternal bowel and the fetus affected 3/12 data sets. No significant activation was found in 3/4 of the control cases, and 1/4 could not be analyzed due to a susceptibility artifact. *Hum. Brain Mapping 12:94–99, 2001.*

INTRODUCTION

The evaluation of fetal brain development and function is a pressing concern for obstetricians. Currently, altered patterns of fetal movement and the associated changes in fetal heart rate are used clinically as an indirect indication of brain activity. An attempt has been made to study fetal brain function directly using magnetoencephalography [Wakai et al., 1996]; however, this technology is available in only a few centres worldwide.

Functional magnetic resonance imaging (fMRI) is an established technique for studying adult brain activation in response to a stimulus [Kwong et al., 1992]. In the work reported here the fetal fMRI response to an acoustic stimulus was determined. In the adult such a stimulus leads to a blood oxygenation level dependent (BOLD) fMRI signal change in the plane of the superior temporal gyrus in the auditory cortex. This study builds on earlier pilot work that reported the detection of fetal brain cortical activity in response to an auditory stimulus [Hykin et al., 1999]. The work presented here proposes modifications to standard fMRI data processing techniques to address the particular problems of fetal fMRI. It also aims to assess the sensitivity of this technique in a larger sample group and to discuss the challenges presented by fetal fMRI.

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MATERIALS AND METHODS

Subjects

Sixteen healthy women with apparently normal singleton pregnancies volunteered in response to posters displayed in antenatal clinics. The local ethics committee approved this study and informed written consent was obtained prior to scanning from every volunteer. Twelve women were recruited for fMRI with acoustic stimulation and four acted as fMRI controls. To reduce fetal motion, subjects were selected during the final 3 weeks of pregnancy after an initial examination demonstrated that the fetal head was engaged in the maternal pelvis. The pregnant volunteers lay in the magnet with a degree of left lateral tilt to minimize pressure on the superior vena cava and improve comfort. During imaging, fetal heart rate (FHR) and activity was monitored using a modified Doppler-ultrasound probe [Shakespeare et al., 1999]. In addition, five adult volunteers were also recruited.

MRI

EPI was carried out on a 0.5 T superconducting magnet specifically designed for echo-planar imaging. The modulus blipped echo-planar single-shot technique (MBEST) [Mansfield et al., 1990] encoding sequence was used to acquire each image in 130 ms, with the switched gradient sinusoidally modulated at 0.5 kHz. The echo time to the centre of k-space was 70 ms. All imaging planes were transverse to the mother, but because the fetal head did not generally lie axially, these planes were usually oblique to the fetus. The in-plane resolution was 5.0 mm $\times$ 5.0 mm, the slice thickness was 15 mm, and the data matrix was 128 $\times$ 128. One volume contained six slices and these were acquired with a repetition rate (T_r) of 2 Hz so that each slice was sampled every 3 sec. All scanning conformed to the National Radiological Protection Board guidelines [HMSO, 1991].

fMRI paradigm

A total of 180 EPI scans were obtained prior to presentation of the paradigm. The stimulus used was a recording of Spanish guitar music, chosen because it had a large dynamic range in both frequency and intensity. This was played to the fetus during EPI through MRI compatible headphones, strapped to the maternal abdomen. Fifteen seconds of music was presented, followed by a 15-sec rest period. This cycle was repeated 30 times using the same 15-sec sample of music. The duration of these periods were chosen to be long enough to easily accommodate the haemodynamic response to the stimulus, which has been measured as 6 sec in adults [Clare, 1997]. The volume of the music was adjusted to give 85 dB SPL at the surface of the maternal abdomen [Glover et al., 1995]. For the four controls, the same sample of music was played to the mother through the same headphones, at 70 dB SPL using a similar paradigm. For the adult volunteers, a similar paradigm was followed, with the headphones delivering 80 dB SPL, and the imaging voxel size was 4 $\times$ 4 $\times$ 15.

Data analysis

All analysis was performed blind to the experimental protocol. In analyzing this data, two potential problems result from patient movement. First, movement during image acquisition could potentially produce ghosting artifacts in the images. The short imaging time afforded by EPI meant that no such artifacts were observed. Second, movement between image acquisitions could cause misregistration of consecutive images. This caused a significant challenge for this study. In order to identify any periods of gross fetal motion, several 3-pixel regions of interest (ROI) were chosen that covered areas of high-signal contrast (e.g., brain to skull). The mean signal intensity in the ROIs was then sampled for each volume throughout the imaging period to produce a crude plot of fetal motion. This was compared to the FHR traces in order to identify cycles that were obviously affected by fetal motion. If periods of motion were evident in both plots the cycle was removed. This eliminated most larger fetal motions. All volumes were then averaged, resulting in an image with relatively high signal to noise, with the most usual position of the fetal brain clearly contrasted against the skull. An object map was then drawn with Analyze software (Mayo Foundation) to mark out this boundary. Each remaining cycle was then viewed as a movie with the object map overlaid. Cycles in which the fetus moved outside the bounds of the object map or where maternal signal crossed into the object map were then also removed.

During the studies the mother and fetus would each have displayed independent relative movement patterns, therefore it was crucial that all signal of maternal origin was masked out prior to image registration. All remaining volumes were segmented using the object map so that only the fetal signal remained. The images were then rescaled by normalizing the mean pixel intensity for each image (global normalization). Prior to image registration, each volume was interpo-

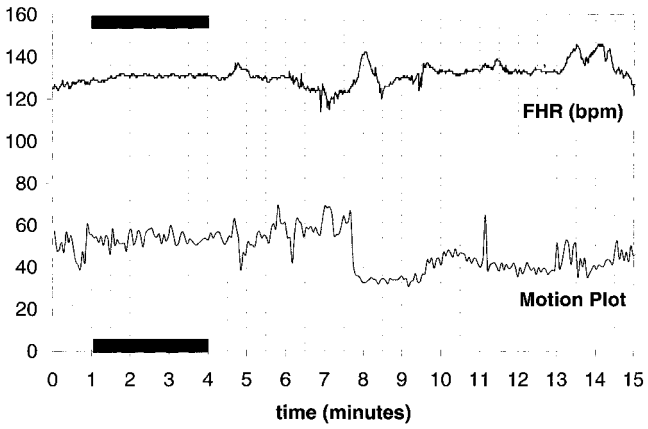

Figure 1.

The fetal heart rate recording, measuring beats per minute, was obtained using an MRI-compatible Doppler ultrasound transducer during fMRI. This is displayed with a crude motion plot obtained from an ROI drawn in a region of high contrast for the same subject. The six cycles that were free from large-scale fetal and maternal motions are highlighted by the black bar and were used during subsequent analysis.

lated to produce artificially cubic voxels. Motion correction was then carried out using automated image registration (AIR) within Medx software (Sensor Systems, Inc.), which registers all of the volumes to the same reference volume. The success of this process was checked by viewing the images as a movie. In several cases an image artifact was observed due to a susceptibility gradient between the maternal bowel and the fetal brain. In these cases, motion correction was not possible as the shape of the fetal brain in the image depended upon its position relative the mother. The motion-corrected images resulting from these subjects appeared severely distorted indicating that motion correction had failed.

The images were then filtered using in-house software [Clare, 1997]. Spatial filtering was performed on the data by convolving the images with a 2D Gaussian kernel with 4.26 mm full width, half-maximum. High-frequency noise was removed using a Gaussian temporal filter of width 1.4 s and low-frequency drift was generally removed by zeroing the six lowest frequency components in the Fourier domain. However, if less than seven cycles were unaffected by large fetal motions, then the n lowest frequency components were removed (n being the number of cycles minus one.)

The stimulus time course was convolved with an appropriate Poisson function, assuming a 6-sec, haemodynamic response, as reported in adults. This was correlated to the signal intensity for each pixel throughout the experiment. Each pixel was then assigned a correlation coefficient, which was converted to a z-score to enable assessment of the statistical significance of activated regions. This information was overlaid on to modulus images to produce functional statistical parametric maps (SPM), which were thresholded to highlight areas of possible brain activity. Using the theory of Gaussian random fields [Friston et al., 1994], a corrected p-value was assigned to activated clusters. Finally the SPMs were resliced transverse to the fetal head, using Analyze software, and the position of the activated region in the brain was identified. A similar procedure was used for the adult data.

RESULTS

Figure 1 displays typical examples of the FHR and the movement plots. The 7/30 cycles identified as having minimal movement are marked. By viewing these images as a movie it was apparent that the FHR and movement plots provided an accurate method of identifying fetal and maternal movement. For the 12 cases where fMRI was carried out, satisfactory artifact free data was acquired for seven subjects, five of which showed statistically significant activation ($p < 0.005$). The mean ($\pm$ SD) signal change measured in temporal lobe activation was 2.2 $\pm$ 0.3 %. Full details of these results are found in Table I. For the four controls, the data from one subject was affected by a susceptibility artifact and could not be analyzed. This artifact is illustrated in Figure 2. No significant activation was found in the remaining three control cases. Figure 3 is an example of a 6-cycle time course and its average respectively. Figure 4 displays the resliced transverse statistical parametric maps for subjects 1–5 (Table I) who yielded positive results.

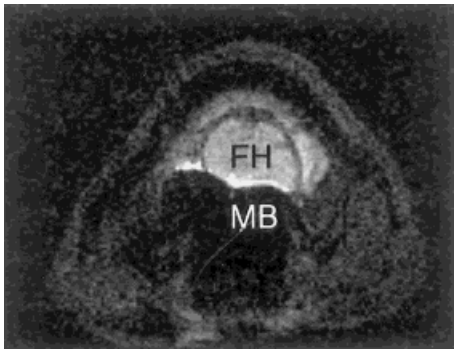

Figure 2.

A severe susceptibility mismatch is demonstrated at the interface between the maternal bowel (MB) and the fetal head (FH). This image displays the worst-case scenario.

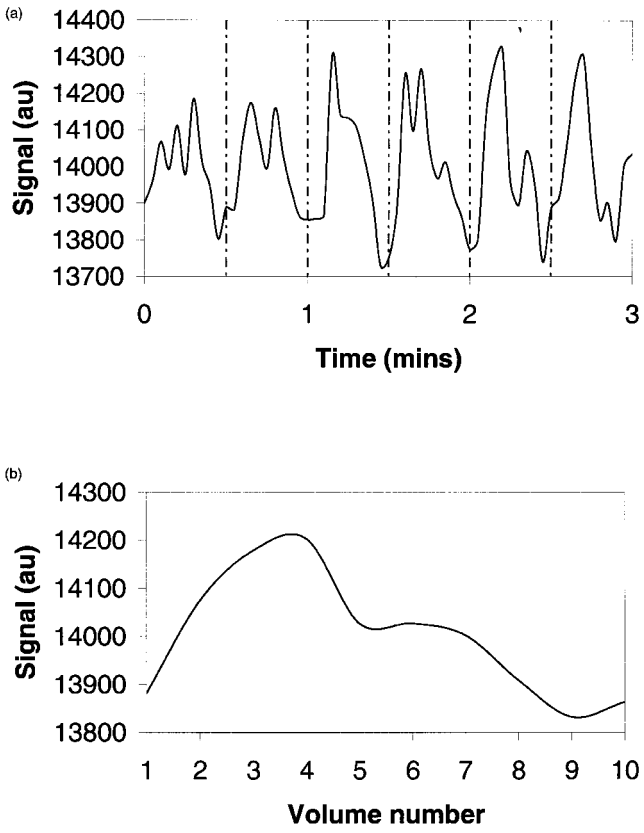

Figure 3.
(**a**) A 6-cycle time course and (**b**) the average time course for subject 2.

For the adult data, significant activation was observed in only the left hemisphere in two volunteers and in both hemispheres for one volunteer. The average signal change was 1.1% (0.14 standard deviation), and the remaining two volunteers showed no activation.

DISCUSSION

Although fetal brain development has a huge impact on the quality of later life, there has previously been little work examining the functional development of the fetal brain. This work has used fMRI to provide direct evidence of fetal brain activity in normal pregnancy in response to an auditory stimulus.

EPI has several advantages for fetal fMRI. First, it causes minimal radio frequency (RF) power deposition, which could be a concern in fetal imaging where heat dissipation might be restricted due to the separation of fetal and maternal blood circulation. This is particularly relevant to fMRI studies where many images are acquired with a short repetition rate. Second, EPI is intrinsically T_2* weighted and so is very sensitive to changes in susceptibility. This is an advantage in fMRI studies, which effectively map changes in susceptibility. However some susceptibility artifacts were observed at the interface between the fetal brain and the maternal bowel in 4/16 cases. This was probably exacerbated because pregnant women are often advised to take iron supplements.

T_2* has been measured experimentally for three subjects at 39 weeks gestation to be 161, 200, and 204 ms. In the adult brain this value was 87, 51, and 116 ms. These differences probably reflect differences between fetal and adult haemoglobin levels of blood oxygenation, the presence of air in the sinuses of the adult, architectural differences between adult and fetal brains, and relative lack of mylenation in the fetal

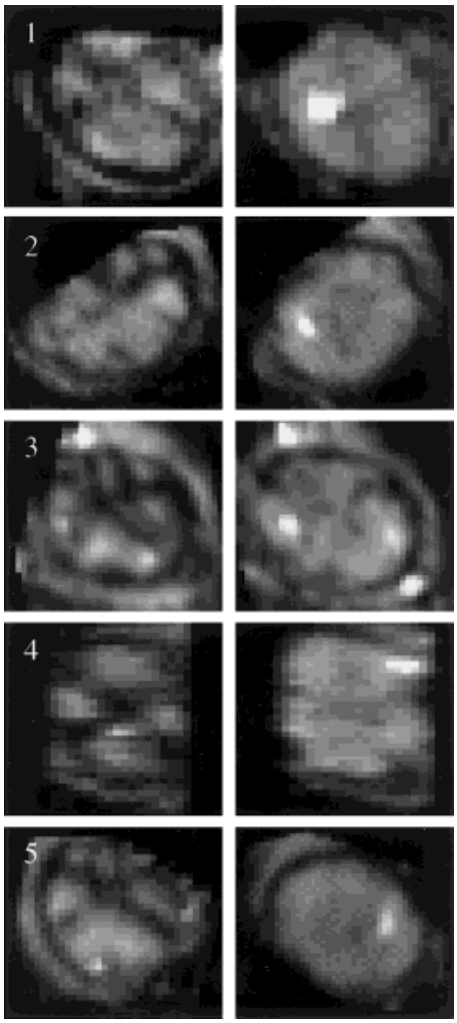

Figure 4.
Two adjacent resliced transverse slices of the statistical parametric map (SPM) for subjects 1–5.

TABLE I. A detailed summary of the results obtained for all fetal subjects

	Patient	N° cycles analysed	Activated area	Signal change (%)	*P*
Study group	1	14	Left temporal lobe	2	1×10^{-7}
	2	6	Left temporal lobe	2.5	2×10^{-4}
	3	6	Right temporal lobe	2.2	1×10^{-4}
			Left temporal lobe	2.6	2×10^{-3}
	4	7	Frontal lobe	2.6	1×10^{-5}
			Sagittal sinus	2.3	2×10^{-6}
	5	11	Right temporal lobe	1.9	1×10^{-6}
			Sagittal sinus	5.1	3×10^{-5}
	6	13	no significant activation		
	7	5	no significant activation		
	8		*Suffered back pain*	*Data not analysed*	
	9		*Suffered back pain*	*Data not analysed*	
	10		*Susceptability artifact*	*Data not analysed*	
	11		*Susceptability artifact*	*Data not analysed*	
	12		*Susceptability artifact*	*Data not analysed*	
Control	1		*Susceptability artifact*	*Data not analysed*	
	2	19	no significant activation		
	3	6	no significant activation		
	4	5	no significant activation		

brain. To obtain the maximum signal change between the active and resting state, the echo-time to the centre of k-space should be equal to the T_2* value of that tissue [Van Gelderen et al., 1994]. This suggests that greater sensitivity should be obtained in future studies by using a longer echo time.

The main disadvantage in using EPI for auditory fMRI studies is the confounding effect of scanner noise [Talavage et al., 1998], since any acoustic stimulus is superimposed on the noise of the MR scanner. EPI uses fast-switching gradients and produces a sound pressure level (SPL) of 100 dB in the 0.5 T scanner used in this work. The scanner noise was present during both the active and rest periods of the experiment. Clearly, this may have partially saturated the response of the fetal auditory cortex so that any additional stimulus noise did not solicit maximum response [Hall et al., 1999]. It is also known that the fetus moves in response to an external stimulus [Gagnon et al., 1987]. Therefore the noise created by the scanner during EPI might have triggered movement in the fetus. Subject movement would not only cause artifactual fluctuations in pixel signal time courses, but would also have affected spin history and distorted the image as the subject moved through inhomogeneities in the magnetic field [Jezzard and Clare, 1999], even at 0.5 T. These effects could have potentially obscured the subtle signal changes involved in fMRI and are very difficult to correct for. To reduce movement effects during the fMRI experiment, 180 EPI scans were acquired prior to presentation of the paradigm in an attempt to habituate the fetus to the sound of the scanner and to allow the longitudinal magnetization to reach a steady state. The music played to the fetus might also have been expected to prompt movement that was correlated to the paradigm. However, no evidence of this was recorded by the fetal heart rate monitor. At the late gestations studied, the maternal pelvis naturally restricts large fetal movements. The motion correction process should have removed any smaller scale movement that persisted. The relatively large voxel size also minimizes the probability that any activated regions will move between voxels.

In previous studies on adults, fMRI has generally been performed at higher field strengths that yield higher image signal to noise ratios (SNR) and higher contrast to noise (CNR) for the BOLD effect. However the lower field strength available for this study produced significant activation in 3/5 adult volunteers using the same protocol developed here for fetal imaging. We have restricted the static field strength because the fetus is a particularly vulnerable subject as its organs are developing rapidly, although the vast majority of studies have found no bioeffects of MRI during pregnancy [Myers et al., 1998]. Furthermore, scanner noise, which has already been identified as a confounding factor in fMRI, increases with field strength. This is particularly pertinent to fetal scanning as the fetus relies on a mismatch in

sound transfer between the air, abdominal wall, and amniotic fluid to attenuate all noise. Unlike the adult, headphones cannot be used to pipe sounds directly to the fetus whilst protecting its ears from the noise of the scanner.

This study also used a larger voxel size and collected a large number of cycles to improve SNR and CNR, respectively, and to overcome small motion effects. However, complete cycles affected by large-scale movement had to be removed which, on two occasions, left only 5/30 cycles for analysis. A disadvantage of using large voxels is that it made identification of fetal brain anatomy difficult and hindered motion correction, which requires a degree of distinctive anatomy in order to resolve small fetal movements [Woods et al., 1992]. Also, if the local volume of activated cortex is small, the CNR is reduced due to partial volume effects, rendering the activation undetectable. Future work will investigate the feasibility of using a smaller voxel size by balancing the reduction in signal to noise ratio with the benefits of improved resolution. The resulting loss in SNR could be compensated by using phased array or surface RF coils. This would improve the efficiency of motion correction. An increase in resolution would also help identify the exact position of the activated area of the brain, although it may be preferable to collect a separate anatomical image using FLASH or HASTE sequences [Levine et al., 1996].

To obtain adequate temporal resolution, scanning was performed at 2 Hz, which allowed a volume to be acquired covering most of the brain in a reasonable imaging time (6 slices in 3 sec). This T_r was chosen by balancing the benefits of increasing temporal resolution against an increase in RF power deposition, a decreased SNR due to T_1 saturation, and increasing scanner noise.

At present, only pregnant women close to term have been scanned with fMRI, as at this time the fetal head is engaged in the maternal pelvis, which is thought to reduce gross fetal movement. The logistics of scanning fetuses earlier in pregnancy requires further investigation. Future development work will also include the use of other stimuli in order to investigate ways of minimizing the effects of scanner noise and stimulus-invoked movement.

CONCLUSION

Despite the challenges presented by fetal fMRI, statistically significant activation was observed in 5/12 fetuses. This study has shown that brain activity can be detected in response to stimulation prenatally and that fMRI has the potential to become a useful tool for evaluating fetal brain development, function, and pathological influences. Further studies using other paradigms are underway.

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