

Delays in Auditory Processing Identified in Preschool Children with FASD

Julia M. Stephen, Piyadasa W. Kodituwakku, Elizabeth L. Kodituwakku, Lucinda Romero, Amanda M. Peters, Nirupama M. Sharadamma, Arvind Caprihan, and Brian A. Coffman

Background: Both sensory and cognitive deficits have been associated with prenatal exposure to alcohol; however, very few studies have focused on sensory deficits in preschool-aged children. As sensory skills develop early, characterization of sensory deficits using novel imaging methods may reveal important neural markers of prenatal alcohol exposure.

Materials and Methods: Participants in this study were 10 children with a fetal alcohol spectrum disorder (FASD) and 15 healthy control (HC) children aged 3 to 6 years. All participants had normal hearing as determined by clinical screens. We measured their neurophysiological responses to auditory stimuli (1,000 Hz, 72 dB tone) using magnetoencephalography (MEG). We used a multi-dipole spatio-temporal modeling technique (CSST—Ranken et al., 2002, MEG/EEG forward and inverse modeling using MRVIEW, in Proceedings of the 13th International Conference on Biomagnetism) to identify the location and timecourse of cortical activity in response to the auditory tones. The timing and amplitude of the left and right superior temporal gyrus sources associated with activation of left and right primary/secondary auditory cortices were compared across groups.

Results: There was a significant delay in M100 and M200 latencies for the FASD children relative to the HC children ($p = 0.01$), when including age as a covariate. The within-subjects effect of hemisphere was not significant. A comparable delay in M100 and M200 latencies was observed in children across the FASD subtypes.

Discussion: Auditory delay revealed by MEG in children with FASD may prove to be a useful neural marker of information processing difficulties in young children with prenatal alcohol exposure. The fact that delayed auditory responses were observed across the FASD spectrum suggests that it may be a sensitive measure of alcohol-induced brain damage. Therefore, this measure in conjunction with other clinical tools may prove useful for early identification of alcohol affected children, particularly those without dysmorphia.

Key Words: Magnetoencephalography, Fetal Alcohol Spectrum Disorders, Auditory, Preschool children.

THE TERATOGENIC EFFECTS of alcohol on the developing fetus were first described in the literature approximately 4 decades ago (Lemoine et al., 1968). Jones and Smith (1973) coined the term “fetal alcohol syndrome (FAS)” to denote a pattern of birth defects and developmental delays observed in children born to alcoholic mothers. It is now recognized that alcohol-exposed children *without* physical dysmorphia also exhibit cognitive deficits related to prenatal alcohol exposure (Mattson et al., 1997) and only a small proportion of children show the dysmorphic features of FAS. Although not a diagnostic term, fetal alcohol

spectrum disorder (FASD) is now used to describe this broader spectrum of physical and behavioral outcomes. Streissguth and colleagues (2004) further determined that these functional disabilities persist into adulthood, yet children who received early intervention had better life outcomes than those who did not. Because of co-morbid risk factors (e.g., poverty, malnutrition, abuse) also negatively impacting development (Farah et al., 2006), identification of a *unique* pattern of cognitive and behavioral problems is challenging. Therefore, the development and validation of tools for early identification of children affected by prenatal alcohol exposure without dysmorphia at a young age are of considerable clinical significance.

Over the last 2 decades, there has been increased use of structural and functional methods to characterize alcohol-induced brain damage. Wide-ranging changes in brain structure associated with alcohol-induced cognitive deficits and behavioral difficulties have been identified through both animal and human research [for review see (Lebel et al., 2011; Schneider et al., 2011)]. Animal studies have confirmed both gross microcephaly and more subtle changes, such as decreases in cell count and abnormal connectivity in sensory

From the Network of Mind Research (JMS, LR, AMP, NMS, AC, BAC), Albuquerque, New Mexico; Department of Pediatrics (PWK, ELK), University of New Mexico Health Sciences Center, Albuquerque, New Mexico; and Department of Psychology (BAC), University of New Mexico, Albuquerque, New Mexico.

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Reprint requests: Julia M. Stephen, PhD, The Mind Research Network, 1101 Yale Blvd. NE, Albuquerque, NM 87106; Tel.: 505-504-1053; Fax: 505-272-8002; E-mail: jstephen@mrn.org

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cortex and other cortical and subcortical regions, resulting from a wide range of alcohol exposures (e.g., (Goodlett et al., 1990; Miller, 2006; Oladehin et al., 2007; Parnell et al., 2006). While microcephaly has been recognized as one of the characteristics of children with FAS (Jones and Smith, 1973), deficits in specific brain areas have also been identified in children and adults with FASD without gross microcephaly [e.g., hippocampus, cerebellum, corpus callosum (Bookstein et al., 2006; Riley et al., 1995; Sowell et al., 2001)].

Furthermore, functional deficits have been identified both in the presence and in the absence of anatomical differences (e.g., Mattson et al., 1997; Pettigrew and Hutchinson, 1984). To search for *functional* biomarkers of FASD at the youngest age, one must turn to pre-linguistic functions, such as sensory-motor skills, because such functions rapidly develop in the first few years of life. While it is known that sensory deficits extend into adulthood in individuals with FASD (Streissguth et al., 1998) relatively little is known about the nature and extent of these problems in young children. A limited body of animal and human literature shows that basic sensory functions, including deficits in the auditory pathway, are affected by prenatal alcohol exposure (Church, 1987; Church and Gerkin, 1988; Church et al., 1997; Kaneko et al., 1996; Medina et al., 2005; Oladehin et al., 2007; Pettigrew and Hutchinson, 1984; Rossig et al., 1994; Slawewski et al., 2004). However, the human work has focused either on infants who have not yet been identified as having an FASD or on older children who may present different symptomatology than young children (Mattson et al., 1998). In addition to these physiological studies, Franklin and colleagues (2008) found that children with FASD were rated by their caregivers as having increased sensory and behavioral problems. However, no systematic study of sensory processing using objective measures in preschool-aged children with FASD has been reported.

One noninvasive functional neuroimaging method suitable for studying young children is magnetoencephalography (MEG), which has not previously been used to assess neural functioning in children with FASD. The merits of MEG include excellent temporal resolution with millisecond precision (similar to EEG) and good spatial resolution without any distortion of spatial information from the skull. EEG signals, on the other hand, are distorted by the skull (Flemming et al., 2005). Therefore, one can more precisely infer the location and timing of possible electrophysiological deficits associated with FASD while employing simpler head models during MEG source modeling. Conversely, functional MRI measures changes in deoxyhemoglobin associated with changes in blood flow in response to neuronal activity while PET tracks radiotracers carried by the blood to monitor glucose uptake in response to neuronal activity. However, because blood flow changes are sluggish, the temporal resolution is orders of magnitude slower for fMRI and PET (1 to 10 second) compared to MEG/EEG (approximately 1 ms) and limits the ability of these techniques to identify subtle deficits in cortical processing speed.

The goal of this study was to characterize auditory responses in preschool-aged children with FASD. Based on the results described above showing delays in auditory processing in infants and in children prenatally exposed to alcohol, we hypothesized that children with FASD would exhibit delayed auditory responses and/or reduced amplitude of these responses relative to typically developing children.

METHODS

Participants

We recruited parents/guardians of 19 healthy control (HC) children and 12 children identified as meeting criteria for FASD within the age range of 31 to 69 months. While the overall success rate was 96% for the MEG task, instrument error resulted in poor data in some children who succeeded in the task. Therefore, we describe only the good quality MEG data, collected from 15 HC and 10 children with FASD. This study was approved by the University of New Mexico Health Sciences Center Institutional Review Board and is in full compliance with the Declaration of Helsinki. The parent was fully informed of the study procedures prior to full participant recruitment and consented to the study prior to research procedures.

The children identified with FASD were recruited through the Fetal Alcohol Diagnostic Clinic at the Center for Development and Disability of the University of New Mexico Health Sciences Center. Children were diagnosed by a team of clinicians including a developmental pediatrician, clinical neuropsychologist, and a child clinical psychologist with extensive experience in the diagnosis and evaluation of children with FASD. Participants were classified as having fetal alcohol syndrome, partial fetal alcohol syndrome, or alcohol-related neurodevelopmental disorder (ARND) using the Institute of Medicine Criteria (Stratton et al., 1996). While all participants in the FASD group had confirmed prenatal alcohol exposure, 3 of them met criteria for fetal alcohol syndrome, 4 for partial fetal alcohol syndrome, and 3 for ARND. Maternal alcohol consumption was confirmed directly (maternal interview) or through the following: (i) multiple eyewitness reports of maternal drinking during pregnancy or (ii) legal records confirming alcohol consumption during pregnancy (e.g., DWI arrest). While none of the participants had hearing problems as determined by state-mandated newborn screening (otoacoustic emissions test) and follow-up screens based on any concerns with hearing, 3 of them had their vision corrected with glasses. Four of the children with FASD were taking medication unrelated to the FASD diagnosis (2 Albuterol, 1 Keppra for previous seizures, and 1 on Prevacid/Zyrtec).

The HC group was recruited from the community by word of mouth, through posted flyers/recruitment brochures or from previous studies, when the parents had agreed to be contacted for future studies. The HC group was screened using a brief questionnaire and did not have developmental or neurological disorders or prenatal exposure to alcohol or other substances.

MEG Data Collection

The children were scheduled for up to 2 MEG sessions and up to 3 MRI scan sessions, depending on success of each session. The MEG session was scheduled during the day at a time when the child was expected to be alert and well rested. The general MEG procedures are similar to those reported previously (Hansen et al., 2010; Stephen et al., 2002, 2006a). Bipolar electrocardiogram (ECG) and electrooculogram (EOG) recordings were obtained in conjunction with the MEG to allow offline artifact removal. The MEG head position indicator (HPI) coils were taped to a child-sized cloth cap

that could be form-fitted to each child's head. This reduced the need to place tape in the hair of the children and eased participant preparation. The cloth cap was taped to the available skin surfaces to ensure that the cap did not move relative to the child's head.

Head position indicator coils were registered relative to standard fiducial points (left and right preauricular and nasion) using the Polhemus 3D position tracking device. Additional head shape points were collected to facilitate MEG/MRI coregistration.

Magnetoencephalography data collection was performed using the Elekta Neuromag 306-channel whole-head biomagnetometer. Once preparation was complete, the child laid in a supine position on the Elekta/Neuromag MEG system bed. The MEG system is located within a 2-layer magnetically shielded room (Vacuumschmelze, Germany). The child's head position within the helmet was optimized, and padding was used to reduce the potential for major head movements. Care was taken to ensure that the placement of the cloth cap did not move relative to the child's head. During the sensory task, the children were allowed to watch a silent children's movie. This was projected onto a rear-projection screen that was positioned above the child to allow for viewing from the supine position. An investigator (and a parent, if needed) remained in the shielded room with the child during data collection to ensure that the child was comfortable and complied with the task. Everyone who remained in the shielded room during data collection was required to remove all metal and electronic devices to reduce magnetic noise. Metal-free clothes were provided as needed (hospital scrubs for adults/child-size t-shirt for children). The adult(s) sat quietly during the measurement and provided words of encouragement to the child, if needed. The participants were not sedated or restrained in any way.

Stimuli

The auditory stimuli were presented as part of a larger sensory paradigm. Because of the complexity of performing source analysis on child data, we report herein the results obtained for the frequent tones presented during a P300-style auditory task. The frequent, 1,000 Hz tones were presented at 72 dB measured at the helmet. Rare tones (1,200 Hz; 72 dB) were presented at a frequency of 16%. The duration of the stimuli was 50 ms (5 ms ramp at beginning and end), and the interstimulus interval was 1 ± 0.2 second. The tones were presented with speakers located in the room positioned to the left and right of the child. All auditory stimuli were presented binaurally and simultaneously from the left and right speakers. Approximately 600 trials were collected for the frequent tone condition. Intermixed with the auditory stimuli were tactile and simultaneous auditory/tactile conditions. The task took approximately 15 minutes to complete.

After the child was positioned within the helmet, the HPI coils were activated to determine the child's head position relative to the sensor array. The MEG data were collected using the Elekta Neuromag 306-channel biomagnetometer. The continuous HPI option was employed to monitor head movement during the scan. The Elekta software then corrects for head movement offline. Wehner and colleagues (2008) demonstrated that the head movement correction is effective in child studies.

MRI Data Collection

We obtained structural MRIs using a 3T Siemens Triotim MRI system to allow us to map the MEG activity onto the anatomical locations provided by the T1-weighted MRI sequence. The MRI and MEG data were collected during separate visits. Most children were requested to return for a night scan to allow MRI scanning to occur during sleep and minimize movement during the scan. Six children who had difficulty sleeping and were compliant during the MEG were successfully scanned while awake. These children were

allowed to watch a movie during the MRI scanning procedures. The room attendant and MRI technician monitored motion to re-run scan sequences as needed. The child was accompanied by an adult at all times, and a trained room attendant was positioned next to the child during the MRI scan. The child was provided with double ear protection (MRI headphones and ear inserts) to reduce the risk of hearing loss and waking the child. Sagittal T1-weighted anatomical images were obtained with a multi-echo 3D MPRAGE sequence [TR/TE/TI = 2,530/1.64, 3.5, 5.36, 7.22, 9.08/1,200 ms, flip angle = 7°, field of view (FOV) = 256 × 256 mm, matrix = 256 × 256, 1 mm thick slice, 192 slices, GRAPPA acceleration factor = 2].

Data Analysis

The MEG data were preprocessed using the temporal, signal space separation method available in the Elekta Maxfilter software (Taulu and Kajola, 2005) to correct head position and reduce noise from external sources. Data quality was assessed by viewing the continuous data offline. Heartbeat and auditory speaker artifact were projected out of the data using the Elekta signal space projection software. The stimuli were averaged by condition, and source analysis was performed for the frequent auditory condition (described below).

The MEG data were registered to each participant's MRI in most cases. In the event that we were not successful in obtaining an MRI, we used another child's MRI based on matched head size and age. This is common for source analysis in studies where MRIs were not obtained (Dalal et al., 2011). An automatic fitting routine in MRIVIEW was used to co-register the MEG and MRI data after identification of the fiducial points (right and left preauricular and nasion) on the MRI (Ranken et al., 2002). The MRI was then segmented and the cortical volume identified using automated algorithms in MRIVIEW. A pixilated set of points based on the cortical volume was identified to provide starting locations for the dipole fitting procedure. Local, sensor-fitted multiple spheres (Huang et al., 1999) were used as the head model for calculating the forward solution. We used the cortical-start spatio-temporal (CSST) analysis approach for performing the source modeling of the MEG data. This approach has a number of advantages. First, while the initial starting locations originate from the cortical volume, the locations during the search procedure are not constrained. Second, the procedure allows for multi-dipole spatio-temporal modeling of the data. This is preferred to fitting single dipoles because under-modeling can impact the source timing obtained from the model (Supek and Aine, 1993). Third, there are no *a priori* assumptions about where the source activity originates. The starting locations for the minimization procedure are chosen randomly from the entire cortical volume. This minimizes bias introduced by using *a priori* assumptions about source locations for a particular task. Fourth, the minimization procedure is performed thousands of times with different random starting locations for each fit. This helps to ensure that a global minimum is reached. When consistent locations arise across the thousands of fits and across participants using this approach, one can more confidently assert the accuracy of the source locations for the task.

The CSST source modeling approach has been employed by our group and others over the last 10 years (e.g., Stephen et al., 2003b, 2007, 2010; Susac et al., 2011), and the methods have been described in detail previously. Briefly, random starting locations are chosen from the pixilated set of points identified within the cortical volume. A preprocessing step helps simplify the number of parameters to be resolved by identifying dipolar activity using a MUSIC approach for a subset of the sources (for details see Ranken et al., 2002). This reduced complexity data set is submitted to a fully unconstrained Nelder-Mead minimization procedure. The Nelder-Mead procedure has been shown to reduce the likelihood that the minimization

procedure will be caught in local minima. Once this procedure is performed for the specified number of fits (2,500 to 5,000 fits depending on the number of dipoles being modeled), the best fits (based on a reduced chi-square fitting criterion and 90th percentile in the chi-square ranking or greater) are then subjected to a finer-grained Nelder-Mead minimization procedure. This 2-step process reduces the overall analysis time by focusing the fine-grain search on the best fits. Once this fine-grain search is complete, the best 10 solutions are displayed. As is necessary with dipole modeling, multiple dipole models are tested for each condition for each participant. The best dipole model is chosen based on the criteria identified by Supek and Aine (1993).

Once the best dipole model was identified for the frequent auditory condition, the sources common across subjects were identified. The timecourses obtained from the dipole model were compiled within source across subjects, and statistical analysis was performed on the latency and amplitude of the prominent peak(s) identified in the source timecourses. Timecourses for sources located in primary auditory cortex are reported in this manuscript (see Fig. 1).

Signal-to-noise ratio (SNR) was obtained from the averaged auditory waveforms for each participant. The reported signal-to-noise ratio is the maximum SNR across channels obtained by identifying the maximum signal in the response time window (20 to 350 ms) and dividing by the standard deviation of the baseline (-100 to 0 ms) noise for each channel.

Statistical Analysis

Differences in latencies and amplitudes were tested using repeated measures ANOVA, each with age as a covariate, peak, and hemisphere as the within-subjects factors and diagnosis as the between-subjects factor. A repeated measures ANOVA was performed to test whether the FASD diagnostic groups differed on the variables that were found significant in FASD versus control comparisons.

RESULTS

The demographic data for the participants are shown in Table 1. Although the FASD children appeared to be younger than the HC group on average, there was no significant difference in age between the groups. However, despite this lack of statistical significance, age was included in the statistical model because of the strong negative correlation ($r = -0.35$, $p = 0.08$) between age and auditory latencies at this young age. We were able to bring back 22/25 children for the MRI procedure. Of this group of 22, the MRI success rate was 92% for HC (12/13) and 78% for the FASD (7/9) children.

Based on head position tracking, we found that most children were able to maintain a consistent head position in the supine position. In particular, we consistently identified brief intervals of movement (position adjustments) with a close return to the original position. We did not identify any relationship between age or diagnosis and the child's ability to remain still during the task. Examples of head movement across the 15-minute data collection are presented in Fig. S1.

The SNR and the number of trials for the 2 groups were equivalent, indicating that the reported results are not because of the differences in data quality across the groups. The maximum SNR was 39 ± 4 (mean \pm SEM) for HC and 33 ± 4 for children with an FASD with no significant difference between groups, $t(22) = 1.0$, $p = 0.2$. The number of trials was statistically equivalent between groups, $t(22) = 0.33$, $p = 0.4$ with an average of 603 ± 32 trials for HC and 588 ± 30 trials for children with an FASD. In addition, we consistently identified 2 prominent peaks in the auditory response (M100 and M200) in the source timecourses across subjects and groups (see Fig. 2). Although it is well known that peak latencies decrease with increasing age (Paetau et al., 1995), the changes from 3 to 6 years of age are modest enough to allow one to reliably identify the same peak across the age range.

The $2 \times 2 \times 2$ RM-ANOVA for latency with peak and hemisphere as within-subjects factors and diagnosis as the between-subjects factor showed a significant between-subjects effect of diagnosis, with M100 and M200 latencies delayed for the FASD children relative to the HC (Table 2), $F(1, 19) = 8.1$, $p = 0.01$. As expected, there was a significant within-subjects' effect of peak latency between the M100 and M200 peaks (Table 2), $F(1, 19) = 53.4$, $p < 0.001$. There

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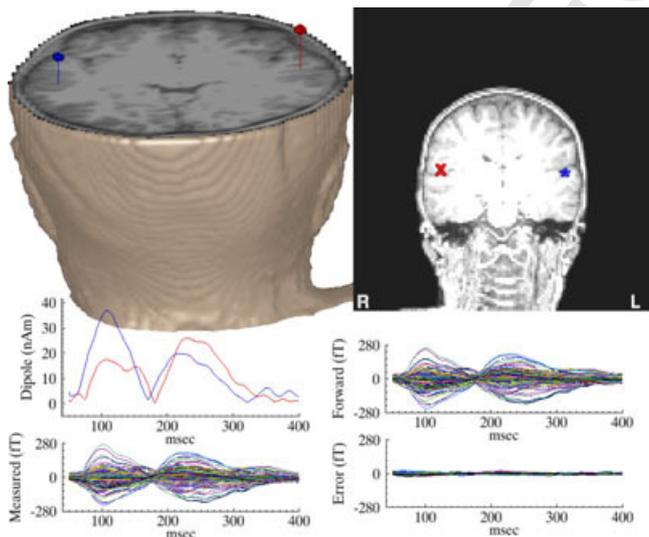


Fig. 1. Example results of the cortical-start spatio-temporal analysis for a 60-month-old HC child. The example source locations are shown in both the 3-dimensional view and the coronal slice (radiologic convention) showing good registration to left and right superior temporal gyrus (STG) consistent with primary/secondary auditory cortex. The timecourses ("dipole" plot) of the left and right STG sources are shown in blue and red, respectively. The original waveforms correspond to the "Measured" plot and the waveforms generated by the best model for this condition and subject correspond to the "Forward" plot. The "Error" plot is the difference between the "Measured" and "Forward" and shows that the model accounted for the visible response.

Table 1. Participant Demographics of Analyzed Group

	HC (N = 15)	FASD (N = 10)
Age	52 (11.4) months	48 (10.7) months
Gender	9 girls/6 boys (60% girls)	6 girls/4 boys (60% girls)
Race/ethnicity	13 caucasian (6 hispanic origin) 1 Asian/1 Native American	6 caucasian (3 hispanic origin) 3 Native American 1 mixed race
FASD subcategories		3—ARND/PAE, 4 pFAS, 3—FAS

ARND, alcohol-related neurodevelopmental disorder; FASD, fetal alcohol spectrum disorder; HC, healthy control.

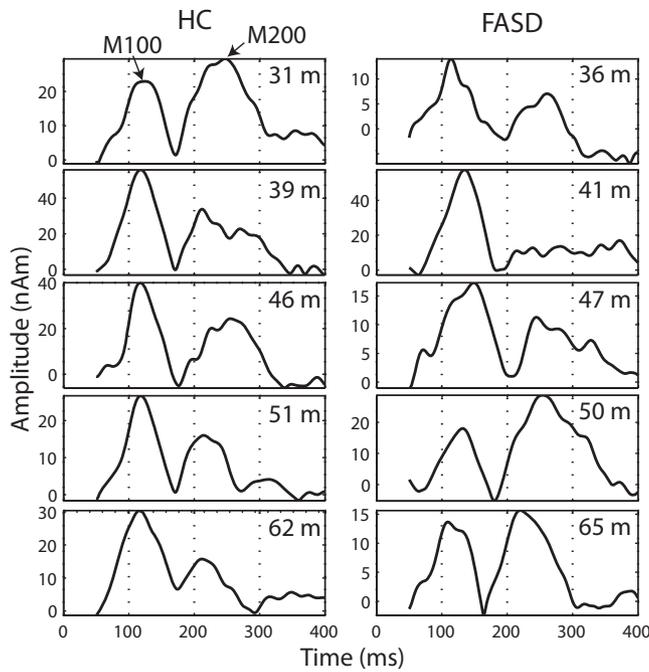


Fig. 2. Example superior temporal gyrus timecourses are shown across age (in months) for HC children and children with FASD. The M100 and M200 peaks are labeled (see arrows) in one example timecourse and are clearly visible across timecourses. The duration of the timecourses corresponds to the time window chosen for source analysis (50 to 400 ms).

Table 2. Mean (SEM) Latencies and Amplitudes for the M100 and M200 Peaks

	M100 latency	M200 latency	M100 amplitude	M200 amplitude
HC (N = 15)	114.4 (2.8)	234.9 (4.3)	35.1 (6.6)	29.9 (6.7)
FASD (N = 10)	125.0 (3.4)	247.8 (4.9)	30.1 (7.9)	17.5 (7.6)

FASD, fetal alcohol spectrum disorder; HC, healthy control.

were no significant interactions between peak, diagnosis, and hemisphere. The average timecourses from the right superior temporal gyrus (STG) source are shown in Fig. 3 (left STG showed a similar pattern). The individual M100 STG peak latencies are shown in Fig. 4. The RM-ANOVA comparing FASD subcategories showed no significant difference in latency and no trend of increasing latency with increasing disease severity (e.g., mean M100 latency ARND—124 ms, pFAS—129 ms, FAS—123 ms) based on subcategory, $F(2, 5) = 0.69, p = 0.54$. There were also no significant effects of medication on peak latencies within the FASD group ($p > 0.05$). There were no significant amplitude differences for either the M100 or M200 peak.

DISCUSSION

Consistent with our hypothesis, there was a significant main effect of diagnosis with auditory M100 and M200 latencies delayed in the FASD group relative to the HC group.

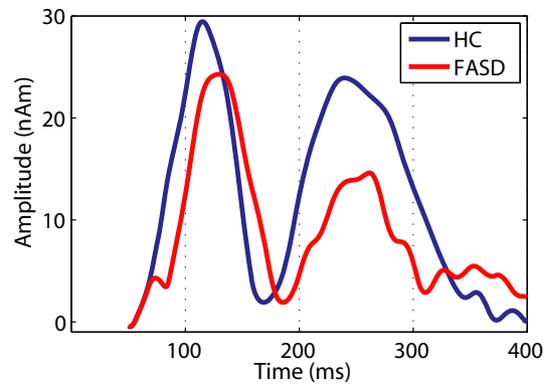


Fig. 3. The group-averaged timecourses for the right superior temporal gyrus source are shown to demonstrate the difference in latency observed for the M100 and M200 peaks. There were no significant differences in amplitude for the M100 or M200 peak. One can see a clear M100 and M200 peak for both groups as well as the delay in the peak latencies for the FASD relative to the HC children.

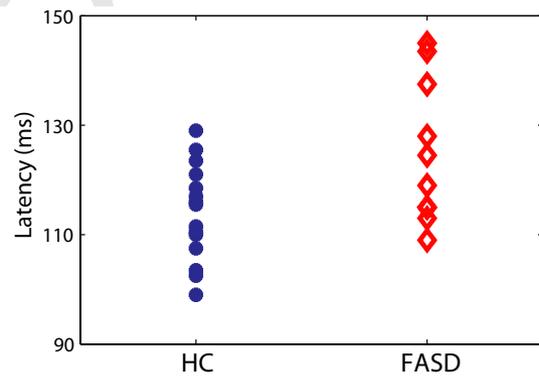


Fig. 4. The M100 latencies averaged across left and right superior temporal gyrus (STG) sources (no within-subjects effect of hemisphere). This plot denotes the difference in average STG latencies between HC and FASD children with an average 11 ms delay for the FASD group relative to HC.

However, no group differences in amplitude were observed. This study is consistent with animal literature reporting widespread auditory deficits with prenatal ethanol exposure. This study also extends the previous results in humans by performing source analysis to determine that this latency delay is present at the level of the auditory cortex and by identifying these latency changes in very young children across the FASD spectrum.

Our hypothesis of delayed auditory processing was largely based on previous animal studies, in which prenatal alcohol exposure was carefully controlled. The animal studies have identified alterations in auditory functioning in both the peripheral and central nervous system. For example, Church and colleagues (1987) demonstrated delays in all 4 brainstem auditory-evoked potential (BAEP) peak latencies across development in rats. In addition, they found the largest latency delays at the youngest ages (<17 days of age) with smaller differences in latency in rats 17 to 70 days of age (human age equivalent of approximately 18 months to

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6 year). Kaneko and colleagues (1993) also identified latency delays in auditory-evoked potentials measured in hippocampus in alcohol-exposed rats measured at 20 to 24 weeks of age. With a whole brainstem *in vitro* preparation, Pettigrew and Hutchinson (1984) identified poorly synchronized and delayed responses to auditory nerve stimulation in chick brainstem after a single exposure to alcohol early in incubation. The variability in responses in the alcohol-exposed chicks appeared similar to responses in younger control chicks, but the delays were larger in alcohol-exposed chicks; therefore, the central auditory processing deficits may be a result of both delayed and abnormal development following prenatal alcohol exposure. Histological studies on these brainstems confirmed atypical synaptic formation in the auditory brainstem nuclei. The results of Pettigrew and Subramanian (1985) suggest alcohol exposure causes a delay in Ca-dependent mechanisms in the brainstem, which impacts brainstem auditory response amplitudes at younger ages. Our results are consistent with these animal studies and suggest that the cortical latency delay likely encompasses both brainstem and higher-order cortical processing deficits. One important distinction is that the current results do not represent a simple propagation of delays originating in brainstem; latency delays in BAEPs in rats are approximately 1 ms compared to the approximately 10 ms cortical delays reported in the current study.

Three studies in infants/children have also identified atypical BAEP (Church et al., 1997; Pettigrew and Hutchinson, 1984; Rossig et al., 1994). The incidence of abnormal BAEPs ranged from 50 to 79% across these studies showing clear agreement with the previous animal studies. Pettigrew and Hutchinson specifically reported that the most common abnormality was difficult in identifying peak V of the human BAEP, consistent with synchronicity problems identified in the animal component of their study. However, the clinical report of "abnormal" BAEP reported in the remaining 2 studies does not distinguish whether the abnormality is based on the amplitude or latency criterion. Therefore, further comparisons are difficult.

To the best of our knowledge, only one previous study reported cortical auditory deficits in children with FAS (Kaneko et al., 1996). Using auditory-evoked potentials, Kaneko and colleagues (1996) contrasted auditory responses in 4- to 15-year-old children with FAS, Down syndrome, and HCs using an oddball paradigm. The primary finding was a delayed parietal P300 response in the FAS group relative to Down syndrome and HCs in response to the rare stimulus. A secondary finding was a significant group effect for N100 latency to the rare tone, with a trend for delayed latencies in the FAS relative to the Down syndrome and HC children. However, their post hoc tests did not reach significance for distinguishing the HC and FAS groups. At the same time, they did not identify any latency or amplitude differences to the frequent tones. The difference between the Kaneko study and the current results may be explained, in part, by the difference in age range across the 2 studies. The

lack of significance in latency in the Kaneko study may be related to the broader age range and the continued development of the auditory system across this age range (Gage et al., 2003). Based on their rat studies, Church (1987) suggested that sensory deficits normalize with increasing age (also suggested by Spohr and Steinhausen, 2008) such that sensory deficits are less pronounced with increasing age. In a follow-up study, Church and colleagues (2011) confirmed the normalization of the auditory brainstem response latencies in rats prenatally exposed to alcohol when comparing very young post-weaned rats with young adult rats. The Kaneko study did not include age as a covariate, despite the larger age range. Therefore, the difference between the 14- and 15-year-old children may have been small enough to reduce group latency differences below the level of significance. Furthermore, our study included children across the FASD spectrum. This perhaps unexpected consistency in the latency delay of the M100 with the previous report of children with FAS (where the delay was not found to be significant) is notable. Our results are consistent with the general pattern of early sensory deficits with prenatal alcohol exposure.

Hearing deficits in FAS children have been consistently reported (Church, 1987; Church and Gerkin, 1988; Church et al., 1997; Rossig et al., 1994). These deficits can include sensorineural hearing loss with an estimated incidence of up to 33% in FAS children compared to 2 to 3% of the general population, as well as conductive hearing loss because of recurrent ear infections which can have a prevalence of up to 92% in FAS children compared to <20% in the general population (for review see Church and Kaltenbach, 1997). It is well recognized that peripheral hearing loss directly impacts language acquisition (Church and Kaltenbach, 1997). Based on the nature of the current study (to investigate cortical differences in auditory perception), we specifically recruited children without hearing loss. Kaneko and colleagues (1996) also excluded children with hearing loss from their study. Despite normal hearing, there was still a significant central auditory delay without documented peripheral hearing deficits. Interestingly, Church and colleagues (1997) reported deficits in dichotic listening and word recognition in noise in 100% of the children tested despite only 50% of these children having abnormal BAEPs. Their conclusion was that BAEPs only identify some of the deficits related to language deficits in FAS children. Our current results of latency delays in the absence of peripheral hearing loss may represent an additional deficit in the auditory pathway that leads to the difficulties with language acquisition in FAS and FASD children. The lack of latency differences between diagnostic subtype is also notable and may suggest that source analysis with MEG may provide a more sensitive measure of delays in auditory processing.

Despite the clear latency delays identified in the FASD children relative to the HC children, the current study has some limitations. First, the sample size was small, requiring a replication of the current study for full generalizability of the results. It is possible that the small number of subjects in the

FASD subgroups led to a nonsignificant difference in auditory latency between the sub-categories. However, there was no trend for increased latency with increasing FASD severity (if one assumes the severity progression is from ARND, pFAS to FAS). Alternatively, the cortical auditory response latency may be related to atypical brain development at a later gestational age (GA) than the time window related to dysmorphia (8 to 13 weeks gestational age). Second, a more comprehensive developmental battery in FASD and HC children in future studies will allow one to better understand the broader cognitive consequences of these basic sensory deficits. Previous studies have confirmed language delays in FAS children with sensorineural hearing loss (Church and Kaltenbach, 1997). Third, a comprehensive hearing test for both HC and FASD children would provide additional evidence of the underlying deficits associated with prenatal alcohol exposure. Fourth, the latencies obtained from the primary auditory cortex may be the result of delays at multiple levels of auditory processing as the signal travels from the cochlea through the brainstem to auditory cortex. This summary latency may still provide a convenient marker, but without brainstem auditory potential measurements, the source (peripheral vs. central nervous system) cannot be determined. Finally, unlike animal studies, the human FASD group is not controlled for the amount or the pattern of alcohol exposure during pregnancy. Further prospective studies will be important for identifying if alcohol exposure at a particular gestational age is directly related to these delays in auditory processing.

Despite the above limitations, the current study is the first to document delayed auditory latencies in preschool-aged children with FASD. This MEG study extends the FASD literature to identify neurophysiological changes in young children across the FASD spectrum and better describes where and when auditory deficits related to prenatal alcohol exposure occur. The fact that the significant group difference was obtained with a small sample and that the effect was noted in less severely affected children suggests that delayed auditory signals may be a sensitive marker of prenatal alcohol exposure. This may prove to be a useful diagnostic tool, given the limited number of neurocognitive tests available for diagnosing young children with FASD who do not have physical dysmorphia. As young children show greater plasticity than adults, the opportunity for early interventions is critically important for prevention of secondary disabilities in FASD. Furthermore, Church and colleagues (2011) identified a resurgence of auditory deficits in middle age rats suggesting that sensory deficits at a young age may predict early age-related hearing decline consistent with the Barker hypothesis of aging. These results provide evidence that early identification of sensory deficits may be important for intervention purposes across the age-spectrum.

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