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Neural correlates of cerebellar-mediated timing during finger tapping in children with fetal alcohol spectrum disorders

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ABSTRACT

Objectives: Classical eyeblink conditioning (EBC), an elemental form of learning, is among the most sensitive indicators of fetal alcohol spectrum disorders. The cerebellum plays a key role in maintaining timed movements with millisecond accuracy required for EBC. Functional MRI (fMRI) was used to identify cerebellar regions that mediate timing in healthy controls and the degree to which these areas are also recruited in children with prenatal alcohol exposure.
Experimental design: fMRI data were acquired during an auditory rhythmic/non-rhythmic finger tapping task. We present results for 17 children with fetal alcohol syndrome (FAS) or partial FAS, 17 heavily exposed (HE) nonsyndromal children and 16 non- or minimally exposed controls.
Principal observations: Controls showed greater cerebellar blood oxygen level dependent (BOLD) activation in right crus I, vermis IV–VI, and right lobule VI during rhythmic than non-rhythmic finger tapping. The alcohol-exposed children showed smaller activation increases during rhythmic tapping in right crus I than the control children and the most severely affected children with either FAS or PFAS showed smaller increases in vermis IV–V. Higher levels of maternal alcohol intake per occasion during pregnancy were associated with reduced activation increases during rhythmic tapping in all four regions associated with rhythmic tapping in controls.
Conclusions: The four cerebellar areas activated by the controls more during rhythmic than non-rhythmic tapping have been implicated in the production of timed responses in several previous studies. These data provide evidence linking binge-like drinking during pregnancy to poorer function in cerebellar regions involved in timing and somatosensory processing needed for complex tasks requiring precise timing.

1. Introduction

Fetal alcohol spectrum disorders (FASD) are characterized by a broad range of physical and behavioral impairments, including poorer learning and memory (Burden et al., 2005; Jacobson et al., 1993; Mattson et al., 2011) and lower IQ (Jacobson et al., 2004; Mattson et al., 1997). Fetal alcohol syndrome (FAS), the most severe FASD, is characterized by a distinctive craniofacial dysmorphology, including a flat philtrum, thin upper lip and small palpebral fissures, smaller head circumference and growth retardation (Hoyme et al., 2005). A partial FAS (PFAS) diagnosis requires the presence of at least two of the facial features as well as either small head circumference, retarded growth, or neurobehavioral deficits and confirmation that the mother drank during pregnancy. Heavily exposed (HE) nonsyndromal children may also exhibit neurobehavioral and attention deficits but are more difficult to identify because they lack the characteristic facial features (Hoyme et al., 2005).

In the 5-year follow-up assessment of the Cape Town Longitudinal Cohort, we found a remarkably striking deficit in eyeblink conditioning performance in children with prenatal alcohol exposure (Jacobson et al., 2008), findings subsequently confirmed in a school-aged cohort (Jacobson et al., 2011a). None of the children in the longitudinal Cape Town sample with full FAS met criterion for delay conditioning at the end of three training sessions at 5 years, compared to 75% of the healthy controls. Children who blinked in anticipation of the air puff in at least 40% of the trials in a given session were considered to have met criterion for conditioning. Only 33.3% of the children with PFAS

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and 37.9% of the HE nonsyndromal children met criterion for conditioning. Eyeblink conditioning is a nonverbal elemental learning paradigm, in which a conditioned stimulus (CS), typically a pure tone, is presented 500 ms before a brief air puff to the eye (unconditioned stimulus (US)) that elicits a reflexive blink. After repeated pairings, the tone comes to elicit a conditioned eyeblink response just prior to the puff, as the subject is able to use the CS to anticipate the timing of the onset of the air puff. The cerebellar-brain stem neural pathways that mediate eyeblink conditioning have been studied extensively in animal models (Christian and Thompson, 2003; Lavond and Steinmetz, 1989).

Successful conditioning relies on a well-functioning cerebellar-mediated internal timing mechanism in order to produce responses with millisecond accuracy. Alcohol-related eyeblink conditioning deficits have also been demonstrated in rodents and sheep (Goodlett et al., 2000; Stanton and Goodlett, 1998) and in another human study (Coffin et al., 2005).

The cerebellum has been identified as playing a key role in producing and maintaining timed movements with millisecond accuracy (Ivry et al., 1988; Ivry and Keele, 1989; Spencer et al., 2003; Tesche and Karhu, 2000). Ivry and Keele (1989) used a paced/unpaced finger tapping task during which subjects were required to maintain a rhythm after a pacing metronome terminated to compare performance among patients with Parkinson's disease, cerebellar-, cortical- and peripheral neuropathy, and healthy controls. Patients with cerebellar lesions performed worst of all, with a 50% increase in the standard deviation (SD) of the inter-tapping interval (ITI) compared to controls. Subsequently, it was demonstrated that poor maintenance of rhythm in patients with lateral cerebellar lesions was attributable to deficits in the internal timing mechanism (Wing et al., 1984), whereas in patients with medial cerebellar lesions it was attributable to impaired motor response (Ivry et al., 1988). In a separate finger flexion/extension study, it was confirmed that cerebellar patients showed greater temporal variability during rhythmic discrete movements, but no timing deficits during continuous finger movement (Spencer et al., 2003).

Key areas identified as being involved in timed movements in adults using functional MRI (fMRI) include superior vermis and cerebellar lobules V/VI, all of which show greater activation during discrete finger flexion/extension compared to continuous movements (Spencer et al., 2007). Bengtsson et al. (2005) performed a conjunction analysis to localize brain regions involved in timing, independent of the effector used. Six tasks were performed by the subjects, including sequential bilateral finger tapping, bilateral isochronous finger tapping, and sequential and isochronous silent speech paced by auditory stimuli. fMRI results showed increased ipsilateral activation in vermis V/VI and lateral lobule VI during timed activity.

Neuroimaging studies have indicated that children often activate different or more extensive neural circuitry when performing simple tasks, compared with adults (Davis et al., 2009; Konrad et al., 2005; Meintjes et al., 2010). Similarly, children have been shown to activate more cerebellar regions than adults during unpaced rhythmic finger tapping, including right lobule VIIb and IX, bilateral crus II and vermis VI, VIIb, VIII and crus II (De Guio et al., 2012).

We were interested in examining whether the impaired eyeblink conditioning performance observed in children with FASD may, in part, be attributed to a deficit in the internal timing mechanism in these children and whether children prenatally exposed to alcohol recruit areas involved in the maintenance of timed responses with millisecond accuracy to the same extent as controls. We used fMRI in children prenatally exposed to alcohol and healthy non- or minimally-exposed controls during a finger tapping task, which interleaves blocks of rhythmic and non-rhythmic tapping in response to an auditory cue, to examine differences in cerebellar blood oxygen level dependent (BOLD) activations related to timing in these children. We hypothesized that significant differences in activation between rhythmic and non-rhythmic conditions will be seen between the children prenatally exposed to alcohol and the control children in areas involved in the maintenance of timed responses in control children.

2. Materials and methods

2.1. Participants

Pregnant women from the Cape Coloured (mixed ancestry) community in Cape Town, South Africa, were recruited between 1999 and 2002 at their first visit to an antenatal clinic (Jacobson et al., 2008). The incidence of FASD in this population is among the highest reported in the world (May et al., 2000, 2007).

The Cape Coloured population, comprised of descendants of white European settlers, Malaysian slaves, Khoi-San aboriginals, and black Africans, historically constituted the large majority of workers in the wine-producing region of the Western Cape. The high prevalence of FAS in this community is attributable to very heavy maternal drinking during pregnancy (Croxford and Viljoen, 1999; Jacobson et al., 2006; Jacobson et al., 2008), due to poor psychosocial circumstances and residual impact of the now-outlawed *dop* system, in which farm laborers were paid, in part, with wine.

All pregnant women who reported consuming at least 14 standard drinks/week or engaging in binge drinking (≥5 drinks/occasion) during pregnancy were invited to participate in the study. In addition, pregnant women who abstained or drank minimally during pregnancy were invited to participate as controls. Women younger than 18 years of age, as well as women with diabetes, epilepsy, or cardiac problems requiring treatment, and religiously observant Muslim women, whose religious practices prohibit alcohol consumption, were excluded from the study. Infant exclusionary criteria were major chromosomal anomalies, neural tube defects, multiple births, and seizures.

Maternal alcohol consumption was assessed using a timeline follow-back approach (Jacobson et al., 2002). At recruitment the mother was interviewed regarding the incidence and amount of her drinking on a day-by-day basis during a typical 2-week period at time of conception. She was also asked whether her drinking had changed since conception; if so, when the change occurred and how much she drank on a day-by-day basis during the preceding 2-week period. This procedure was repeated in mid-pregnancy and again at 1 month postpartum to provide information about drinking during the latter part of pregnancy. Volume was recorded for each type of beverage consumed each day, converted to absolute alcohol (AA) using multipliers proposed by Bowman et al. (1975), and averaged to provide three summary measures of alcohol consumption at conception and during pregnancy: average ounces of AA consumed/day, AA/drinking day (dose/occasion) and frequency (days/week). The number of cigarettes smoked on a daily basis, as well as the frequency of marijuana and other drug use were also recorded.

Each child was examined for growth and FAS dysmorphology by two U.S.-based expert dysmorphologists following the revised Institute of Medicine criteria (Hoyme et al., 2005) during a 6-day clinic in 2005 (Jacobson et al., 2008). Four children who did not attend the clinic (1 FAS, 2 HE and 1 control) were examined by a Cape Town-based dysmorphologist with expertise in FAS diagnosis. There was substantial agreement among the dysmorphologists on the assessment of all dysmorphic features, including the three principal fetal alcohol-related characteristics — philtrum and vermilion measured using the *Lip-Philtrum Guide* (Astley and Clarren, 2001) and palpebral fissure length (median $r = 0.78$). Each of the children was assigned to one of the following diagnostic groups at a case conference (conducted by HEH, LKR, SWJ, CDM, and JLJ): FAS, PFAS, nonsyndromal HE, or control.

The mother and child were transported to our University of Cape Town (UCT) Child Development Research Laboratory by a staff driver and research nurse for the IQ and eyeblink conditioning (EBC) assessments, which were administered by an MA-level neuropsychologist.

IQ data were collected from the children on the Wechsler Intelligence Scale for Children-IV (WISC-IV) at 10 years (Diwadkar et al., 2013; Jacobson et al., 2011b). In the 5-year follow-up of the children from our longitudinal cohort, we administered the *Junior South African Individual Scales* (JSAIS; Madge et al., 1981), which is available in Afrikaans and English and has been normed for South African children. 159 of those children were administered the *Wechsler Intelligence Scales for Children*, 4th ed. (WISC-IV) IQ test at 10 years. IQ scores from the *JSAIS* were strongly correlated with the WISC scores, $r = 0.73$, $p < 0.001$, confirming the validity of our translation of the WISC for use with this population (Jacobson et al., 2011a).

EBC assessments were administered using a commercially available human EBC system (Model #2325-0145-W, San Diego Instruments, San Diego, CA; see Jacobson et al., 2008, 2011a). Facing a monitor displaying a video, the child wore a light-weight headgear, which supported a flexible plastic tube that delivered an air puff to the right eye and a photodiode which measured eyelid closure. Two small 7-Ω speakers emitted an auditory CS, a 1-kHz, 80-dB tone. Each session consisted of five 10-trial blocks. Two 50-trial sessions were administered on the same day about 2 h apart with two more sessions on a second day within the same week. In delay EBC, the air puff was administered during the last 100 ms of the 750 ms tone. The trace conditioning procedure, which was administered 1.3–1.8 years after the delay task, was the same as in the delay task except that a 500-ms stimulus-free interval occurred between the offset of the 750-ms tone and the onset of the air puff. Eyeblinks executed within 350 ms prior to the air puff onset were considered CRs. EBC performance was assessed here in terms of percent conditioned responses during the third EBC session.

Mothers and children were transported on a separate day to the Cape Universities Brain Imaging Centre (CUBIC) for neuroimaging. 82 (10 FAS, 19 PFAS, 29 HE, 24 controls; 47 boys) right-handed children were scanned on the 3T Allegra (Siemens, Erlangen, Germany) MRI scanner at CUBIC between January 2009 and December 2011 (mean age ± standard deviation (SD) = 10.7 ± 0.6 years, age range 9.5–12.0). We acquired high-resolution structural images and functional MRI data during rhythmic and non-rhythmic finger tapping. All examiners were blind regarding prenatal alcohol exposure history and FASD diagnosis during the UCT and CUBIC assessments, except for a few severe cases.

2.2. Experimental tasks

The experimental tasks were programmed using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, USA) and were presented through a wave guide in-line with the bore of the magnet in the rear wall of the scanner room using a data projector and a rear projection screen mounted at the end of the magnet bore. Responses were recorded using a Lumitouch response system (Photon Control Inc., Burnaby, Canada). The child was able to talk to the examiner using an intercom that is built into the scanner and could stop the scan at any time by squeezing a ball held in his/her left hand. All children were accompanied into the scanner room by a research nurse/assistant who stayed with them throughout the scan.

All children practiced the task before the scan to ensure that they understood the instructions and could perform the task. Children also lay down in a mock scanner prior to the scan to listen to a recording of the scanner noises, which helped reduce anxiety.

The experimental task was designed to distinguish between brain regions activated during rhythmic tapping compared to non-rhythmic tapping. This task, which was adapted from the design (Fig. 1) used by Lutz et al. (2000), employed an auditory rather than a visual stimulus. Each block comprises two different active conditions (rhythmic and non-rhythmic finger tapping) interleaved with rest blocks. The children are instructed to press a button with their right index finger every time they hear a tone.

The first block is preceded by a rest block of 8 s, during which four dummy scans are acquired and an instruction to get ready is displayed. During the rhythmic blocks, tones are equally spaced (SD = 0 ms) with an inter-stimulus interval (ISI) of 736 ms. The non-rhythmic blocks comprise tones at irregular intervals (mean ISI = 736 ms, SD = 256 ms). Both the rhythmic- and non-rhythmic blocks last for 16 s and are interleaved with 10 s of rest between active blocks. Each set of blocks (rhythmic, rest, non-rhythmic, rest) is repeated four times. The principal performance measure is rhythmicity of tapping, determined by averaging for each condition the SDs of the inter-tap intervals (ITIs) within each block of that condition.

2.3. fMRI imaging protocol

High-resolution T1-weighted structural MR images were acquired using a 3D echo planar imaging (EPI) navigated (Tisdall et al., 2009) multi-echo MPRAGE (Van der Kouwe et al., 2008) sequence that had been optimized for morphometric analyses using FreeSurfer software. Imaging parameters were: FOV 256 × 256 mm^2; 128 sagittal slices, TR 2530 ms; TE 1.53/3.21/4.89/6.57 ms; TI 1100 ms; flip angle 7°; voxel size 1.3 × 1.0 × 1.3 mm^3. The 3D EPI navigator provided real-time motion tracking and correction, which served to substantially reduce the presence of any motion artifacts in structural imaging data, despite significant subject motion.

A T2*-weighted gradient echo, EPI sequence was used to acquire 114 functional volumes that are sensitive to BOLD contrast (TR 2000 ms, TE 30 ms, 34 interleaved slices, 3 mm slice thickness, gap 1.5 mm, FOV 200 × 200 mm^2, in-plane resolution 3.125 × 3.125 mm^2) while the children performed the task. Despite the low resolution of the fMRI data, this analysis succeeded in resolving the complex geometry of the cerebellum and its respective lobules.

All procedures were performed according to protocols that had been approved by the Institutional Review Board of Wayne State University and the Faculty of Health Sciences Human Research Ethics Committee at the University of Cape Town. All parents/guardians provided informed written consent, and all children provided oral assent.

2.4. fMRI analyses

To ensure that only data from blocks in which the child was fully engaged in the task were included in the fMRI data analysis, we applied performance criteria based on inspection of the distribution of the SDs of the ITIs in the rhythmic and non-rhythmic blocks. SDs displayed a bimodal distribution and the local minimum was used to select thresholds for each condition. In the rhythmic tapping condition, only blocks with SDs less than 150 ms, mean ITIs between 500 and 1000 ms, and 6 or fewer missed taps were included in the analyses. ITIs during the rhythmic blocks that exceeded 1200 ms were assumed to occur due to one or more missed taps, which occasionally occurred when a child did not press the button firmly enough. In such instances, for the purposes of computing SD, additional taps were inserted with an ITI as close to 736 ms as possible to ensure that missed taps were interpolated with the appropriate rhythm. Inserted taps were counted as "missed" in determining whether to include the block in the analysis. Non-rhythmic tapping blocks were included in the analysis only if their SDs were greater than 170 ms and if the difference between the number of tones presented and the number of button presses did not exceed 9. Blocks that did not meet inclusion criteria were labeled as bad blocks and treated as separate predictors in the general linear model (GLM). Only children who met behavioral performance criteria for two or more blocks in each condition were included in the analysis as only these children were considered to be fully engaged in the task.

fMRI data analyses were performed in Brain Voyager QX (Brain Innovation, Maastricht, The Netherlands). The first four dummy scans were excluded from all analyses. Pre-processing included motion correction relative to the first volume that was acquired during the

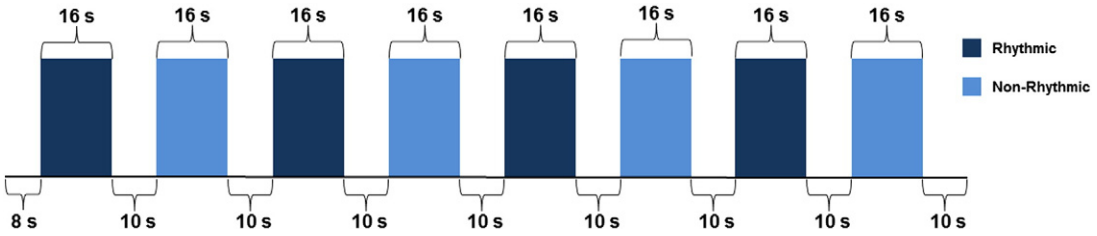

Fig. 1. Timing diagram of the rhythmic/non-rhythmic task.

functional scan, linear scan time correction, temporal filtering with a high pass filter of 3 cycles/point, and linear trend removal. Scans with motion exceeding 3 mm translation or 3° rotation within a functional run were excluded from all further analyses. Whole-brain group analyses were performed with a random effects analysis of variance using the general linear model with predictor time courses for the successful rhythmic and non-rhythmic tapping blocks convolved by the standard hemodynamic response function. The six motion correction parameters were *z*-transformed and added as predictors of no interest together with the predictors for the excluded (bad) rhythmic and non-rhythmic tapping blocks.

Beta maps were created for each subject for the contrast comparing BOLD activation during rhythmic and non-rhythmic finger tapping. The beta maps were exported into Analyze format for second level analyses using the spatially unbiased atlas template (SUIT) toolbox (Diedrichsen et al., 2009) in SPM5 (Statistical Parametric Mapping) to obtain more detailed information on activation patterns in the cerebellum. This atlas, which is based on the structural data of 20 healthy individuals, has been shown to significantly improve the alignment of individual fissures in the cerebellum when compared to normalization to the MNI whole-brain template (Diedrichsen et al., 2009).

Each subject's cerebellum was initially isolated in the structural images by calculating the probability of each voxel belonging to the cerebellum or brain-stem. The isolation maps were then used to transform each subject's cerebellum to the SUIT template in the subsequent step, which normalized the data. Manual correction was applied using MRICRON (Rorden and Brett, 2000) for each subject to eliminate contamination from the visual cortex. The functional data for the cerebella were then resliced according to the isolated and normalized structural data for each subject to render the data in the SUIT atlas space.

2.5. Statistical analyses

A one-sample *t*-test was used to identify clusters where percent signal change values comparing rhythmic and non-rhythmic tapping were significantly different from zero in the control children. Cluster size correction with a cluster defining threshold of 0.05 on the normalized group images was applied to reduce the risk of multiple comparisons and a minimum cluster size of 193 mm^3 was found to be statistically significant.

To determine whether normalizing the children's cerebella to an adult template would lead to excessively small effective regions of interest (ROIs), cerebellar volumes generated by FreeSurfer (version 5.1.0, http://surfer.nmr.mgh.harvard.edu) were compared to values reported in adult studies (Luft et al., 1999; Woodruff-Pak et al., 2001). Woodruff-Pak et al. (2001) calculated cerebellar volumes ranging from 122.73 to 142.37 ml in eight adults (age 21–35 years) and Luft et al. (1999) found a range of 99.86–170.6 ml in 48 adults (age 19.8–73.1 years). The children included in our functional study had an average cerebellar volume of 130.85 ± 13.03 ml (range 107.18–170.11 ml), which is within the limits of the aforementioned studies. The children from all diagnostic groups were included in this analysis, as previous studies have shown that children prenatally exposed to alcohol have reduced cerebellar volumes (Archibald et al., 2001; Mattson et al., 1994). It was, therefore, necessary to establish whether the volumes in these children were also comparable to the cerebellar volumes of adults. The overall effect of normalization to an adult template was, therefore, deemed negligible.

ROIs were defined with radius 3 mm, centered on the peak coordinates, in these regions. Due to the large cluster sizes in the vermal lobules, percent signal changes were extracted around the center of mass instead of the peak voxels in these two clusters. Mean percent signal change values were extracted in these ROIs for each child and exported to SPSS (version 20; IBM, New York, USA) to examine differences in activation in these regions as a function of diagnosis as well as associations with the extent of prenatal alcohol exposure.

Differences between diagnostic groups in each ROI were examined using analysis of variance. Eight control variables were considered as potential confounders: child's sex, age at assessment, postnatal lead exposure, IQ and cerebellar volume; maternal education, smoking (cigarettes/day) during pregnancy and age at delivery. Pearson correlations were used to examine the relations of the mean percent signal change values in the ROIs to each of the potential confounders. All control variables related to a given outcome at $p < 0.10$ were considered possible confounders. These variables were entered into an analysis of covariance (ANCOVA) to determine whether group differences in the ROIs remained significant after controlling for these measures.

Correlations between extent of prenatal alcohol exposure and activation were also examined in SPSS. Although the continuous measures of the control group were essentially all zero, the data for these children were included in the correlation analyses to avoid artificially truncating the range of exposure. Hierarchical multiple regression analyses were used to control for confounding. The alcohol measure was entered in the first step of each analysis for each outcome. All control variables related to the outcome at $p < 0.10$ were entered in the second step to determine if the effect of the continuous alcohol measure on activation patterns continued to be significant after statistical adjustment for potential confounders. Pearson correlations were used to examine the relation between BOLD activations in the ROIs and EBC performance.

3. Results

3.1. Sample characteristics

After applying exclusion criteria, we report data for 50 (30 male, 20 female) right-handed children (mean age 10.7 ± 0.6 year), including 7 children with full FAS, 10 with PFAS, 17 nonsyndromal HE children, and 16 non- or minimally-exposed controls. The data for 8 children were excluded due to excessive motion (1 PFAS, 2 HE, 5 controls), as were data from 24 children who did not meet performance criteria (3 FAS, 8 PFAS, 10 HE, 3 controls). Due to the smaller number of children with FAS, the FAS and PFAS groups were combined in the data analysis. Table 1 summarizes the demographic information for these children. The children in the HE group were slightly older than children in the other two groups. The low IQ scores of all of the children reflect the highly disadvantaged backgrounds and poor education of the children in this community; nevertheless, as expected, the lowest scores were seen in the FAS/PFAS group. Mothers reported that none of their children ever received medication for ADHD, and only four children had been given over-the-counter medications (e.g., aspirin for headache;

Table 1
Sample characteristics.

Demographic	FAS/PFAS	HE	Controls	F or χ^2
N	17	17	16	NA
Child's age at assessment (yr)[a]	10.5 ± 0.6	11.0 ± 0.7	10.6 ± 0.4	4.04*
Sex (M/F)	11/6	11/6	8/8	0.98
WISC-IV IQ[b]	67.2 ± 11.6	77.7 ± 8.9	73.8 ± 11.3	4.17*
Child's cerebellar volume (ml)[c]	124.4 ± 10.5	136.2 ± 12.3	130.9 ± 9.3	4.95*
Maternal age at delivery (yr)[d]	30.2 ± 7.8	24.8 ± 5.1	25.5 ± 3.2	4.37*
Maternal education (yr)[e,f]	7.4 ± 3.4	9.3 ± 2.4	10.4 ± 1.4	5.02*
Absolute alcohol consumed per day across pregnancy (oz.)	1.1 ± 0.9	0.4 ± 0.4	0.0 ± 0.0	16.16**
Absolute alcohol consumed per occasion across pregnancy (oz.)	4.1 ± 1.9	2.7 ± 1.6	0.1 ± 0.3	32.14**
Drinking days per week averaged across pregnancy	1.8 ± 1.0	0.9 ± 0.8	0.0 ± 0.0	22.51**
Cigarettes smoked per day during pregnancy	7.1 ± 5.2	6.3 ± 5.2	3.7 ± 6.8	0.56
Lead exposure (μg/dl)	11.6 ± 6.8	9.8 ± 3.0	8.8 ± 4.0	1.38

Values are means ± SD.
FAS = fetal alcohol syndrome, PFAS = partial FAS, HE = heavily exposed nonsyndromal.
[a] FAS/PFAS < HE ($p = 0.02$), HE > control ($p = 0.02$).
[b] FAS/PFAS < HE ($p = 0.01$), FAS/PFAS < control ($p = 0.09$).
[c] FAS/PFAS < HE ($p < 0.01$), FAS/PFAS < control ($p = 0.09$).
[d] FAS/PFAS > HE ($p = 0.01$), FAS/PFAS > control ($p = 0.03$).
[e] FAS/PFAS < HE ($p = 0.04$), FAS/PFAS < control ($p < 0.01$).
[f] Maternal education missing for mother of 1 child with FAS.
* $p < 0.05$.
** $p < 0.01$.

antihistamine for allergy). The mothers of the children in the FAS/PFAS group were older, as has been reported in previous studies (Jacobson, 1998, 2004; May, 1991), and had completed fewer years of formal education. Prenatal alcohol exposure was very high, averaging 8.2 standard drinks/occasion for the FAS/PFAS group and 5.4 for the nonsyndromal HE group across pregnancy. All but 1 (93.8%) of the 16 control mothers abstained from drinking during pregnancy, and that mother drank only 2 drinks on 3 occasions. No group differences were found for maternal smoking during pregnancy or lead exposure.

In accordance with previous findings (Archibald et al., 2001; Mattson et al., 1994), significant differences in cerebellar volumes were seen between the diagnostic groups and *post hoc* analyses showed that this result was driven by the significantly reduced cerebellar volume of the most heavily exposed children compared to both the HE and control groups.

3.2. Behavioral data

After exclusions, the groups did not differ on performance during rhythmic or non-rhythmic tapping (Table 2). Prior to exclusions, the only significant group differences were greater variability ($F = 4.05$, $p = 0.02$) in the rhythmic tapping blocks by the HE group compared to controls ($p < 0.01$) and increased number of missed taps ($F = 6.01$, $p < 0.01$) in the rhythmic tapping blocks by the HE group compared to the FAS/PFAS ($p = 0.02$) and control ($p < 0.01$) children. The groups did not differ in performance during non-rhythmic tapping. Since this study focuses on effects of prenatal alcohol exposure on functional activation, the behavioral results were used only to identify children who were able to perform adequately on the task, as evidenced from the absence of group differences in Table 2.

3.3. fMRI data

Four regions in the cerebellum showed greater activation during rhythmic tapping compared to non-rhythmic tapping in the control children (Table 3 and Fig. 2).

Table 4 summarizes mean percent signal change values in ROIs defined in these regions for each group. A significant group difference was detected in right crus I. *Post hoc* analyses showed that the activation in right crus I was significantly higher in control children than in both the FAS/PFAS ($p < 0.01$) and HE ($p = 0.01$) groups, with no difference between the FAS/PFAS and HE groups ($p > 0.20$). A group difference falling short of statistical significance ($F = 2.68$, $p = 0.08$) was seen in vermis IV–V, due to lower activation in the FAS/PFAS group compared with the controls (*post hoc* $p = 0.05$).

Pearson correlation analyses identified two potential confounding variables. Girls showed greater activations in right crus I ($r = 0.32$, $p < 0.05$), while maternal smoking during pregnancy was associated with lower activations in vermis IV–V ($r = -0.26$, $p < 0.10$). The group difference in right crus I remained significant ($F = 5.47$, $p = 0.01$) after adjustment for sex, and the effect on vermis IV–V was not reduced after adjustment for maternal smoking ($F = 2.63$, $p = 0.06$). None of the control variables were related to activations in vermis VI or right lobule VI.

Relations of extent of prenatal alcohol exposure to differences in activation between rhythmic and non-rhythmic finger tapping in the four cerebellar ROIs are summarized in Table 5. Greater prenatal alcohol exposure was associated with smaller differences in brain activation between rhythmic and non-rhythmic finger tapping in right crus I. The strongest association was with frequency of drinking across pregnancy (Fig. 3), a correlation that was also evident when the controls

Table 2
Behavioral performance by diagnostic group.

		FAS/PFAS	HE	CTL	F	p
	N	17	17	16	NA	NA
Rhythmic tapping	SD	91.12 ± 28.94	92.02 ± 43.31	81.56 ± 31.20	0.45	0.64
	Number of missed taps	2.32 ± 1.56	2.89 ± 1.67	2.16 ± 1.80	0.83	0.45
Non-rhythmic tapping	SD	351.60 ± 151.39	334.62 ± 131.65	328.83 ± 113.40	0.54	0.59
	Difference between taps and stimuli presented	2.72 ± 4.22	1.58 ± 3.24	1.81 ± 2.95	1.97	0.14

Values are means ± within-group standard deviations.
FAS = fetal alcohol syndrome, PFAS = partial FAS, HE = heavily exposed nonsyndromal.

Table 3
Cerebellar regions showing significantly greater activation during rhythmic finger tapping compared to non-rhythmic finger tapping in control children.

Brain region	MNI peak coordinates			*t*-statistic	Cluster size (mm^3)
	x	y	z		
Right crus I	50	−59	−36	5.18*	194
Vermis IV–V	2	−59	−2	4.40**	1151
Vermis VI	4	−79	−24	4.19**	796
Right lobule VI	12	−62	−17	3.55*	219

Minimum cluster size 193 mm^3.
Nomenclature as proposed by Schmahmann et al. (2000).
* P < 0.05.
** P < 0.01.

Table 4
Comparison by diagnostic group of differences in percent BOLD signal change between rhythmic and non-rhythmic finger tapping in four cerebellar ROIs that are activated more during rhythmic tapping than non-rhythmic tapping in control children.

Brain region	FAS/PFAS ($n = 17$)	HE ($n = 17$)	CTL ($n = 16$)	*F*	*p*
Right crus I[a]	−0.05 ± 0.39	0.03 ± 0.70	0.73 ± 0.98	5.59	0.01
Vermis IV–V[b,c]	0.10 ± 0.53	0.57 ± 0.65	0.60 ± 0.88	2.68	0.08
Vermis VI[c]	0.12 ± 0.43	0.22 ± 0.53	0.21 ± 0.26	0.28	0.76
Right lobule VI	0.12 ± 0.33	0.19 ± 0.51	0.29 ± 0.29	0.79	0.46

Values are means ± SD.
Nomenclature as proposed by Schmahmann et al. (2000).
FAS = fetal alcohol syndrome, PFAS = partial FAS, HE = heavily exposed nonsyndromal.
[a] FAS/PFAS < control ($p < 0.01$), HE < control ($p = 0.01$).
[b] FAS/PFAS < HE ($p = 0.06$), FAS/PFAS < control ($p = 0.05$).
[c] Percent signal change around center of mass.

were omitted from the analysis, $r = -0.42$, $p = 0.013$. Multiple regression analyses showed that the relation in right crus I remained significant after controlling for sex. In right lobule VI, greater absolute alcohol consumed per occasion, both around conception and across pregnancy, was associated with smaller differences in activation between rhythmic and non-rhythmic tapping (see Fig. 4), a correlation that was also seen when the controls were omitted from the analysis, $r = -0.43$, $p = 0.011$. Greater alcohol consumption per drinking occasion around conception and during pregnancy was also associated with lower percentage signal change in both vermal regions. Multiple regression analysis showed that the effect of drinking per occasion across pregnancy on activation in vermis IV–V continued to be significant after adjustment for maternal smoking.

At 9 years higher levels of delay and trace eyeblink conditioning (measured by % conditioned responses during session 3) were associated with lower levels of activation in right lobule VI in the control group (Table 6). This association was also strong, albeit not significant at age 5 years. There was a suggestion of a similar pattern for right crus 1. By contrast, there were no significant associations between activation of these regions and EBC performance for the exposed children.

4. Discussion

This study used fMRI to investigate differences in the neural circuitry involved in performing timed movements in children prenatally exposed to alcohol compared with healthy controls. The controls showed increased BOLD activations during rhythmic tapping compared to non-rhythmic tapping in four cerebellar regions that have been implicated in the production of timed movements in previous studies with adults (Gerwig et al., 2003; Grodd et al., 2001; Schlerf et al., 2006; Spencer et al., 2007). Our continuous measure of maternal alcohol intake per occasion during pregnancy was associated with reduced differences in activation between rhythmic and non-rhythmic tapping in all four regions. When the children were compared by diagnostic group, both the FAS/PFAS and nonsyndromal HE groups showed significantly less of an increase in brain activation during rhythmic tapping in right crus I compared with controls, while only the children with FAS or PFAS showed significantly smaller differences in activation between rhythmic and non-rhythmic tapping in vermis IV–V than the controls.

Vermis V and VI have been previously implicated in timing in a study in which these regions showed greater activation during discrete rhythmic finger extension/flexion than during continuous finger movements (Spencer et al., 2007). This finding, with the addition of the involvement of hemispheric lobule VI, was corroborated by the aforementioned study by Bengtsson et al. (2005). In a recent study of paced/unpaced finger tapping in children, both these regions also showed increased activation during unpaced tapping compared with rest (De Guio et al., 2012).

In a study of adults using a procedure very similar to our task, Lutz et al. (2000) also found differences in activation in vermis VI, as well as in the right cerebellar nuclei, when comparing rhythmic vs. non-rhythmic finger tapping. However, in contrast to the findings in the previous studies (Bengtsson et al., 2005; Spencer et al., 2007), as well as our own, Lutz et al. (2000) found more activity during non-rhythmic than rhythmic finger tapping in these regions. Schlerf et al. (2006) administered four timing conditions to adults in a rhythmic/non-rhythmic finger tapping task — one regular and three irregular that ranged from low to high ISI variability. Activation was generally higher in the anterior lobe and lateral lobule VI for the regular and most highly variable conditions, compared to the low and moderate variability conditions, indicating increased activation for processing both regular and highly irregular temporal patterns. When considered together, the Lutz et al. (2000) and Schlerf et al. (2006) studies suggest that the increased activation during the irregular tapping condition in vermis VI and lateral lobule VI may reflect greater effort to predict the timing of the onset of the next stimulus when the timing is irregular.

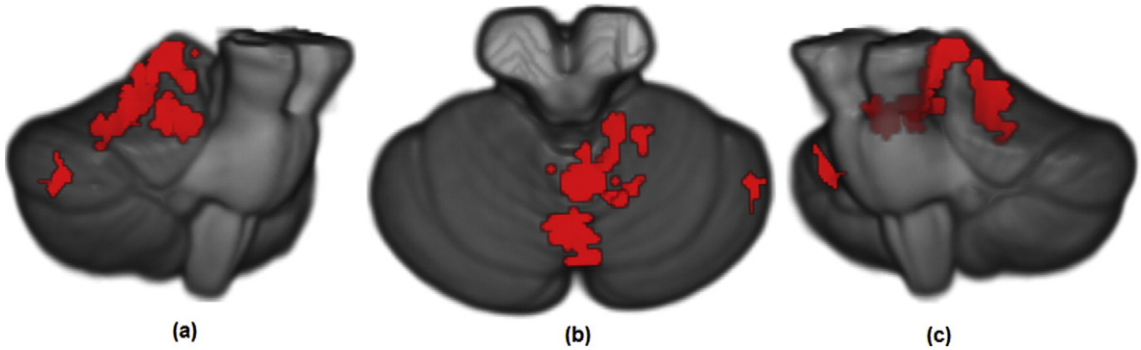

Fig. 2. (a) Right anterolateral, (b) superior coronal and (c) left anterolateral views of right crus I, vermi IV–VI and right lobule VI regions showing greater activation in control children during rhythmic tapping compared to non-rhythmic tapping. Functional data are shown in the Spatially Unbiased Cerebellar Atlas Template space (MNI coordinates).

Table 5
Relation of extent of prenatal alcohol exposure to BOLD activation in regions with significant differences in activation between rhythmic and non-rhythmic finger tapping in control children.

	Absolute alcohol consumed per day at conception		Absolute alcohol consumed per occasion around conception		Drinking days per week around time of conception		Absolute alcohol consumed per day across pregnancy		Absolute alcohol consumed per occasion averaged across pregnancy		Drinking days per week across pregnancy	
	r	β	*r*	β	*r*	β	*r*	β	*r*	β	*r*	β
Right crus I[a]	−0.32*	−0.28*	−0.18	−0.16	−0.40**	−0.35**	−0.36**	−0.32*	−0.30*	−0.28*	−0.46**	−0.41**
Vermis IV–V[b,c]	−0.23	−0.17	−0.31*	−0.26†	−0.23	−0.18	−0.20	−0.13	−0.35*	−0.28*	−0.18	−0.11
Vermis VI[c]	−0.04	−0.04	−0.29*	−0.29*	0.63	0.63	−0.04	−0.04	−0.25†	−0.25†	−0.02	−0.02
Right lobule VI	−0.25†	−0.25†	−0.42**	−0.42**	−0.09	−0.09	−0.26†	−0.26†	−0.37**	−0.37**	−0.15	−0.15

Nomenclature as proposed by Schmahmann et al. (2000).
r is the simple Pearson correlation between alcohol exposure and percent signal change values; β is the standardized regression coefficient after adjustment for the potential confounding variables.
[a] Controlling for sex in the multiple regression analysis.
[b] Controlling for maternal smoking.
[c] Percent signal change around center of mass.
† $p < 0.10$.
* $p < 0.05$.
** $p < 0.01$.

We did not see this increase during highly irregular tapping in the children in our study.

It is noteworthy that lateral lobule VI has been shown to be of major importance in eyeblink conditioning in numerous animal studies (Miller et al., 2003; Steinmetz, 2000; Yeo and Hesslow, 1998), as well as fMRI studies in humans (Dimitrova et al., 2002; Ramnani et al., 2000), including a recent study from our cohort (Cheng et al., 2014). In the present study we found that the differences in activation between rhythmic and non-rhythmic tapping in ipsilateral lobule VI were most strongly related to alcohol consumed per drinking occasion, an exposure measure that predicted lower activations in all four regions identified in the control group. This finding suggests that cerebellar timing is more sensitive to heavy episodic binge-like drinking than sustained moderate drinking around the time of conception and throughout pregnancy.

By contrast to vermis VI and lateral lobule VI, which have been most directly implicated in cerebellar-mediated timing, activations in vermis IV–V have been associated with the execution of intentional movements (Grodd et al., 2001) as well as somatosensory processing of motor response (Allen et al., 1997; Desmond et al., 1997; Nitschke et al., 1996). The greater response in vermis IV–V during rhythmic compared to non-rhythmic tapping by the control children in our study may be attributable to greater somatosensory demands in the rhythmic condition. Our finding that heavier maternal drinking during pregnancy is associated with lower activation in this region is consistent with a previous report that this region is smaller in alcohol-exposed children (Sowell et al., 1996). These data also suggest that the impaired eyeblink conditioning observed in children with FASD may involve both deficits in timing and impaired somatosensory function.

Although activation in ipsilateral crus I has not been implicated in timing during finger tapping tasks in either adults (Jueptner et al., 1995; Lutz et al., 2000) or children (De Guio et al., 2012), it has been shown to play a role during both reflexive eyeblinks (Dimitrova et al., 2002) and eyeblink conditioning (Cheng et al., 2014; Gerwig et al., 2003; Ramnani et al., 2000). This area corresponds to trigeminal projection areas and blink reflex control areas that have been identified in animal studies (Hesslow, 1994; Pellegrini and Evinger, 1997), suggesting a

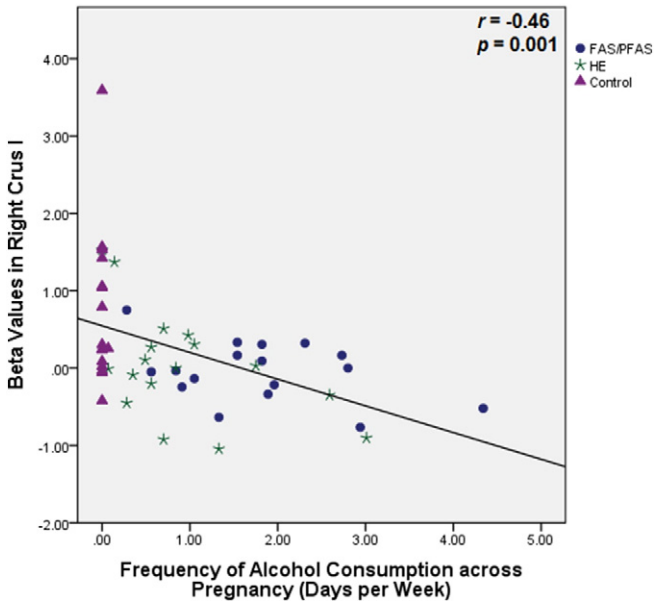

Fig. 3. Correlation of frequency of alcohol exposure across pregnancy with activation in right crus I.

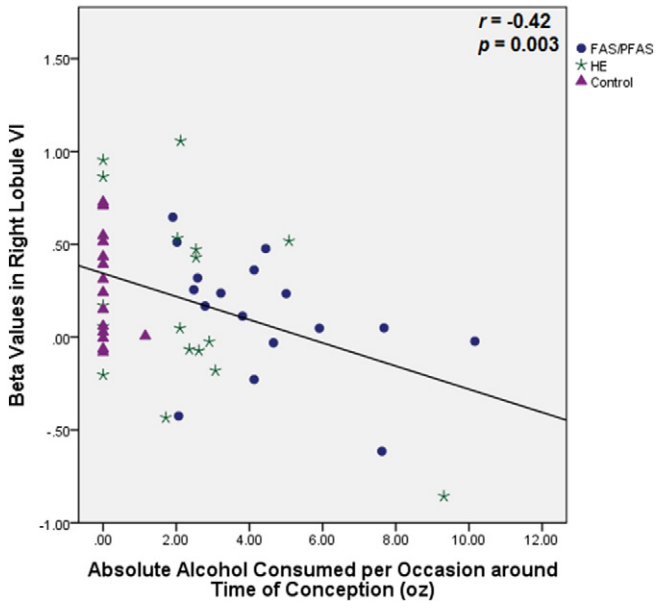

Fig. 4. Correlation of absolute alcohol consumed per occasion around time of conception and activation in right lobule VI.

Table 6
Relation of activation in regions of interest activated more during rhythmic than non-rhythmic finger tapping by control children to eyeblink conditioning performance in control children.

	Right crus I		Vermis IV−V		Vermis VI		Right lobule VI	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
% conditioned responses								
Delay − 5 yr ($n = 5$)	−0.79	0.12	−0.18	0.77	0.73	0.16	−0.77	0.13
Delay − 9 yr ($n = 14$)	−0.27	0.35	0.34	0.24	0.10	0.73	−0.59	0.03
Trace − 9 yr ($n = 11$)	−0.56	0.07	0.30	0.38	−0.24	0.49	−0.59	0.06

Values are Pearson *r*.

possible role in motor control and coordination for tasks that require millisecond accuracy.

This study was part of a larger study examining the neural bases of EBC (Jacobson et al., 2008, 2011a,b). We have previously reported that microstructural abnormalities in the cerebellar peduncles appear to partially mediate the effect of prenatal alcohol exposure on EBC performance (Fan et al., under review; Spottiswoode et al., 2011). In the present study we found a pattern of better delay and trace eyeblink conditioning performance associated with smaller increases in activation during rhythmic tapping compared to non-rhythmic tapping in right lobule VI and right crus 1 among the control children, which was not seen in children with prenatal alcohol exposure. Thus, these data suggest that the timing required for successful EBC performance may be mediated by other, less efficient brain regions in the alcohol-exposed groups.

5. Conclusions

Eyeblink conditioning is an elemental form of learning that is highly sensitive to prenatal alcohol exposure and requires precise millisecond timing. In this study, we used an fMRI finger tapping paradigm to examine effects of alcohol exposure on cerebellar timing with millisecond accuracy. Increased maternal alcohol intake per drinking occasion during pregnancy was associated with lower BOLD activation increases during rhythmic compared with non-rhythmic tapping in several cerebellar regions that have been implicated in millisecond timing in studies with adults. In addition, in comparisons by fetal alcohol diagnostic group, children in the FAS/PFAS group, which is particularly affected in eyeblink conditioning, showed lower activation particularly in vermis IV–V. This region has been implicated in the execution of intentional movements and somatosensory processing of motor response, suggesting that a deficit in those aspects of function may be more pronounced in children with FAS or PFAS. In summary, these data provide evidence linking binge-like drinking during pregnancy to poorer function in specific cerebellar regions involved in timing and somatosensory processing.

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The authors declare no competing financial interest.

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