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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.