Prenatal Alcohol Exposure Alters Neural Processing of Numerosity in Infancy

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# ****Abstract****

## Background

Research on and treatment of fetal alcohol spectrum disorders (FASD) are hampered by a lack of specificity in behavioral diagnostic criteria and a limited understanding of the neural substrates mediating the observed cognitive deficits. Previous studies have identified arithmetic as a particularly sensitive developmental endpoint in FASD. Behavioral and neuroimaging studies suggest that this impairment is mediated by a specific deficit in the core quantity system involving the ability to mentally represent and manipulate numbers. Using an event-related potentials (ERP) task, this study provides the first evidence—at the level of brain function—that an alcohol exposure-related deficit in quantity processing can be detected very early in development.

## Methods

Thirty-eight 6- to 9-month-old infants (19 prenatally alcohol exposed, 19 controls) were tested using a habituation-dishabituation paradigm. During each habituation trial, a specific quantity of stimuli varied in terms of location, total surface area, and color. ERPs were locked to the presentation of a novel quantity during the dishabituation trial.

## Results

Prenatal alcohol exposure was related to longer latencies and larger amplitudes of the dishabituation response, which resembled a “P300” response to novelty, and a smaller negative deflection in the right posterior parietal region, which is believed to reflect magnitude comparison. Results were not attributable to demographic or other exposure variables, including infant age or sex or maternal smoking or drug use during pregnancy.

## Conclusions

Our findings indicate an early fetal alcohol-related impairment in basic numerosity processing that may provide the basis for later difficulties in arithmetic. These findings advance an understanding of the pathophysiology of FASD and can contribute to the development of improved, targeted interventions for the specific number-processing deficits that characterize these disorders.

*Keywords:* prenatal alcohol exposure, fetal alcohol spectrum disorders, event-related potentials, numerical processing, infancy

# Introduction

Prenatal alcohol exposure is associated with a broad range of neurocognitive deficits in attention and memory (e.g., Coles et al. 1997; Mattson & Roebuck 2002; Lewis et al. 2015), eyeblink conditioning (Jacobson et al. 2008; S Jacobson et al. 2011), and cognitive processing speed (Streissguth et al. 1990; Jacobson et al. 1993, 1994; Coles et al. 2002; Burden et al. 2005), as well as lower IQ (Streissguth et al. 1990; Jacobson et al. 2004). Previous studies have identified arithmetic as a particularly sensitive developmental endpoint for fetal alcohol exposure (J Jacobson et al. 2011). Performance by alcohol-exposed children on arithmetic achievement tests is invariably more impaired than on reading or spelling tests (Kerns et al. 1997; Howell et al. 2005). Effects on arithmetic are dose-dependent and remain significant after statistical adjustment for IQ (Goldschmidt et al. 1996; J Jacobson et al. 2011).

Findings from behavioral and neuroimaging studies suggest that fetal alcohol-related impairment in arithmetic is mediated by a deficit in the core quantity system involving the ability to mentally represent and manipulate numbers (Santhanam et al. 2009; J Jacobson et al. 2011; S Jacobson et al. 2011). The intraparietal sulcus (IPS) is a brain region known to play a critical role in magnitude representation (Dehaene et al. 2003). Activation of the IPS during number processing is inversely related to the amount of alcohol ingested by a mother during pregnancy (Woods et al. 2015). Evidence that a deficit in quantity processing may already be evident in infancy was provided by pilot data from our Cape Town Longitudinal Cohort study, which showed poorer performance by alcohol-exposed infants on the Wynn (1992) looking-time infant numerosity paradigm (S Jacobson et al. 2011).

Studies have shown that children are able to discriminate and process quantities as early as infancy (e.g., Dehaene et al. 1998; Wynn 1992). This representation of numerosity appears to be innate and abstract as newborns can perceive the correspondence between numerical quantities across different modalities (e.g., visual and auditory; Izard et al. 2008). The first indications of infants’ abilities to discriminate between quantities were reported in the 1980s using the habituation/dishabituation paradigm (Antell & Keating 1983; Starkey & Cooper 1980). Infants were shown a series of displays with the same quantity of objects (e.g., “smiley faces”) that varied in color, location, and size. After 4 to 6 of these displays, there was a decrease in the looking time that infants devoted to the stimulus. Infant were then presented with a stimulus with a different number of objects, and dishabituation (i.e., recovery of looking time) indicated that they could recognize the difference in quantity.

Findings from habituation/dishabituation studies have provided strong converging evidence that infants can perceive the difference between numerosities, although their discrimination is heavily dependent on the ratio between the presented quantities (see review by Mou & van Marle, 2014). For example, 6-month-olds can discriminate auditory stimuli (Lipton & Spelke 2003) as well as visual quantities differing in 1:2 ratios (e.g., 4 vs. 2) but often fail to discriminate quantities with 2:3 ratios (e.g., 4 vs. 3; Xu & Spelke 2000).

Event-related potentials (ERP) studies with infants have provided additional supporting evidence for early basic numerical cognition (Berger et al. 2006; Berger 2011). ERPs are small deflections in electroencephalogram (EEG) recordings elicited by an external event (Luck 2005), which are derived by averaging the EEG activity coinciding with a specific event, such as the onset of a stimulus. Amplitude (μV) and latency (ms) of the components of the ERP wave form vary as a function of task demands and stimulus characteristics. The extant ERP literature using the habituation/dishabituation paradigm to study infant numerosity is sparse and varies in the methods used and the findings reported. Although Libertus and colleagues (Libertus & Brannon 2009, 2011) did not find an ERP signature for a numerosity change in 7-month-olds, Izard et al. (2009) found a middle central parietal (“P300-like”) effect at 3 months of age, and Hyde and Spelke (2011) reported a posterior-parietal P400 effect for changes in small numerosities (1, 2, or 3) at 6 to 7.5 months of age.

In older children and adults, these middle central and posterior parietal effects are clearly distinct. The middle-central parietal response to a novel numerosity (i.e., during dishabituation) is reflected primarily in the P300 component (Rushby et al. 2005), a strong positivity over the parieto-occipital scalp regions at about 300-600 ms poststimulus. The functional interpretation of the classic P300 is somewhat diverse, but it is generally characterized as an indicator of memory updating and stimulus categorization/discrimination in response to the appearance of a novel stimulus (e.g., Donchin & Coles 1988). P300 amplitude is expected to be larger for the dishabituation stimulus, compared to the habituated one. Importantly, P300 latency seems to reflect the duration of stimulus evaluation (Donchin & Coles 1988), with shorter latencies associated with better cognitive abilities (Polich & Martin 1992).

In contrast to the P300 response to novelty, a negative deflection in response to quantities of nonsymbolic stimuli (e.g., dots, smiley faces) at posterior parietal scalp electrodes (especially on the right side; Hyde et al. 2010) is believed to specifically reflect magnitude comparison processing in the IPS (Cantlon et al. 2006). We expected the timing of this response to be similar to the P2p distance effect seen in children and adults, that is, to occur about 200 ms after stimulus presentation (Ben-Shalom et al. 2012; Temple & Posner 1998). This posterior parietal effect has been demonstrated during magnitude comparison in infants in the study by Hyde and Spelke (2011). Using a different paradigm to examine differentiation between quantities in infants, Berger (2011) also found a negative deflection over the right posterior parietal region at around 200-400 ms poststimulus onset.

In this study, we used a numerosity habituation-dishabituation paradigm to determine whether prenatal alcohol exposure alters ERP response patterns that have been linked to processing numerical quantities in typically developing individuals and whether these alcohol-related numerical deficits can be detected as early as infancy. Two hypotheses were derived from the literature reviewed above: (1) alcohol-exposed and control infants would differ in the dishabituation response (i.e., detection of a novel numerosity) in the P300 component and (2) alcohol-exposed and control infants would differ in brain sensitivity to comparisons between quantities differing by larger ratios (i.e., > 2) at right posterior parietal scalp areas within the 200-400 ms time window.

# Materials and Methods

## Participants

This study was conducted in Cape Town, South Africa, where the prevalence of FASD in the Cape Coloured (mixed ancestry) community is among the highest in the world (13.6-20.9%) and where the incidence of fetal alcohol syndrome (FAS) has been estimated to be 18 to 141 times greater than in the U.S. (May et al. 2013). This population, composed of descendants of white European settlers, Malaysian slaves, Khoi-San aboriginals, and black Africans, has historically comprised the majority of workers in the wine-producing region of the Western Cape. The prevalence of FAS in this community is a consequence of very heavy maternal drinking during pregnancy (Croxford & Viljoen 1999), which, in turn, is due in part to poor psychosocial circumstances and the traditional *dop* system, whereby farm laborers were paid, in part, with wine. Although the *dop* system has been outlawed and despite numerous efforts to reduce pregnancy drinking, weekend binge drinking persists in a high proportion of women during pregnancy in rural and urban Cape Coloured communities (Jacobson et al. 2008; May et al. 2013).

Our sample consisted of 80 Cape Coloured infants (*M* age = 30.5 weeks) born to women recruited between 2011-2014 at their first visit (*M =* 19.1 weeks gestation, SD = 7.1) to one of two antenatal clinics serving an economically disadvantaged, predominantly Cape Coloured population (Jacobson et al. 2017). Age at assessment was adjusted for gestational age for 15 infants born prior to 37 weeks gestation. A research nurse interviewed each mother at her first antenatal visit using a 2-week timeline follow-back procedure (Jacobson et al. 2002) regarding her alcohol consumption both at time of recruitment and around conception. Volume was recorded for each type of beverage consumed each day and converted to ounces (oz) of absolute alcohol (AA; 1 oz AA ≈ 2 standard drinks). Any mother who reported drinking at least 14 standard drinks/week (≈ 1 oz AA/day) or engaging in binge drinking (≥ 4 drinks/occasion) was invited to participate in the study. Women who abstained or drank only minimally (< 0.1 oz AA/day) were recruited as controls. Exclusionary criteria included age < than 18 years of age, HIV infection, and those with diabetes, epilepsy, or cardiac problems requiring treatment. Infant exclusionary criteria included major chromosomal anomalies, neural tube defects, multiple births, and seizures.

Timeline follow-back interviews were re-administered by CDM, a developmental pediatrician, at 4 and 12 weeks after recruitment. Data from the three interviews were tabulated to provide continuous measures of alcohol exposure (oz AA/day; oz AA drinking/occasion; frequency of alcohol use [number of days/week]) at time of conception and during pregnancy. Mothers were also interviewed regarding their smoking (cigarettes/day) and illicit drug use (marijuana [“dagga”], methamphetamine [“tik”], cocaine, heroin, and methaqualone [“mandrax”]; days/month) during pregnancy.

Among the 80 infants who were tested, 10 (12.5%) were excluded because they were too fussy, 3 (3.75%) due to technical problems, and 29 (36.25%) who did not have adequate usable ERP data (as detailed below). These attrition rates are typical of those found in the infant ERP literature (e.g., DeBoer et al. 2007). Data were analyzed for the remaining 38 infants (19 alcohol-exposed, 19 controls). No significant differences were found between the infants who were included and those who were excluded from the final analyses for any of the background variables (all *p*s *>*.10).

## Procedure

Mothers and infants were transported to our research laboratory by our research nurse and driver for the ERP assessments and maternal interviews. Protection of human subjects approval was obtained by institutional review boards at Wayne State University and University of Cape Town Faculty of Health Sciences.

Prior to the task, the infant’s head circumference was measured and an appropriately sized 128-HydrocCel Geodesic Sensor Net (HCGSN) was placed on his/her head. During presentation of the stimuli, the infant was seated on the mother’s lap, 100 cm from the video monitor. The direction of the infant’s gaze was recorded by the experimenter during the session to ensure that data would be based only on trials in which the infant looked at the screen throughout the entire sequence of arrays (see below). The mother was instructed to avert her eyes from the monitor during the entire ERP trial and refrain from interacting with the infant during the assessment. The experimenter continuously verified that the mothers did not look at the stimuli or interact with the infant, and compliance was high. The session was discontinued if the infant became fussy or ceased paying attention to the stimuli presentations.

## Stimuli

The ERP task was a modified version of the habituation-dishabituation paradigm developed by Cantlon and colleagues (Cantlon et al. 2006) and Izard and colleagues (Izard et al. 2008). Consistent with the procedures in these studies, the length of the sequence was not based on a decrease in looking times. Instead, a quantity was presented repetitively for a varying number of times, presumably creating a habituation to this quantity, and ERP patterns created by this habituated quantity were compared to the ERP pattern created by the presentation of a novel quantity. The task was presented using E-Prime 2.0 software (released candidate 2.0.8.9; Psychological Software Tools Inc., Pittsburgh, PA).

Each infant was shown a maximum of 40 visual sequences of arrays, presented in random order. Before each sequence, a colorful rotating display accompanied by music was presented to elicit the infant’s attention, and sequences began only after the infant was focused on the screen. Each array was displayed for 1000 ms. To keep the infants engaged, each array presentation ended with the objects “bouncing” off the screen accompanied by a brief sound lasting 800 ms (as suggested by Hoehl & Wahl 2012). Array presentations were separated by an interstimulus interval of either 300, 400, or 500 ms.

Each sequence consisted of a series of five arrays, each one presenting the same number of objects (“smiley faces”), followed by an additional array consisting either of the same or a different quantity. In the *habituation* sequences (50% of all sequences), this last array contained the same quantity presented during the first part of the sequence, while in the *dishabituation* sequences, the last array contained a different quantity of objects (see Fig. 1). All quantities were within the subitizing range (quantities 1-4). Within the *dishabituation* condition, the ratio of change, that is, the ratio of the quantity displayed in the first part of the sequence (i.e., from the first to the next-to-last stimulus) and the quantity displayed in the last presentation, was either “> 2” (e.g., 1 vs. 3, 1 vs. 4) or “< 2” (e.g., 2 vs. 3, 3 vs. 4). Each pair was presented in both orders (e.g., both from 2 to 3 and from 3 to 2). To prevent the occurrence of the dishabituation array from appearing at a predictable time point in the sequence of arrays (e.g., always as the sixth array in the sequence), 40% were randomly inserted “sham” sequences of a different length (i.e., length of 4). The data from these “sham” sequences were not analyzed.

The color of the objects changed between sequences (yellow, blue, red, or green). Additional variables, such as location and total surface area, were controlled for and balanced between presentations. The location of each object was selected at random insofar as the objects did not overlap one another, locations were randomly drawn from a selected 200×200-pixel area at the center of the monitor screen to minimize eye movement, and mean total display area of all the objects was equal to the possible number of objects (approximately 6,000 pixels).

Insert Figure 1 about here

## EEG Recording and Preprocessing

Electroencephalographs (EEGs) were recorded using an EGI HydrocCel Geodesic Sensor Net and system (Electrical Geodesics, 2003); 128 electrodes were distributed on the scalp according to an adapted 10-20 method and were sampled at a rate of 250 Hz (Tucker 1993). Recording frequency band was constant at 0.1-100 Hz. The electrode impedance level was kept under 40 KΩ, which is an acceptable level for this system (Ferre et al. 2001). During EEG recording, all channels were referenced to the Cz channel (according to the 10-20 method). As noted above, a sequence was considered usable only if the infant looked at the screen during the entire presentation of the sequence. Only sequences that met this strict behavioral criterion were analyzed.

Continuous EEG data were filtered with a 40 Hz low-pass and then segmented into one long trial starting 200 ms before and ending 1200 ms after the stimulus presentation onset (stimulus-locked). The segmented data were inspected for artifacts (e.g., bad channel, blinks, and eye movement). Segments with more than 10 bad channels were excluded. In segments with 1-10 bad channels, the bad channel data were replaced with a spherical interpolation of the neighboring channel values.

Two separate sets of analyses were performed, as detailed below. Infants were included in an analysis only if they had at least one artifact-free trial per experimental condition. Sufficient data were available for both sets of analyses for 28 infants. An additional 10 infants had sufficient data for one of the two analyses (5 for the habituation-dishabituation analysis and 5 for the posterior parietal ratio analysis) providing a total *N* = 33 for each analysis. Mean number of good segments per experimental condition was 4 (SD= 2.4, range = 1-14). There were no between-group differences in the number of good segments for the exposed and control infants, and there were no significant correlations between number of good segments and any of the ERP measures (all *p*s > .15). In addition, supplementary analyses were conducted, which included only those infants who had at least 3 good trials in each condition to verify the findings seen in the larger sample.

Segments were averaged and re-referenced to the average of the channels across the scalp, after which a baseline-correction was conducted for 200 ms before the presentation onset. Baseline-corrected ERP data were averaged across subjects, resulting in an averaged segment ERP for each condition (grand average).

## ERP Measurements

Two separate segmentations and analyses were performed. The first set of analyses compared sequence type, that is, the *habituation* versus *dishabituation* conditions. These analyses used data from the 15 occipitoparietal-central electrodes between Pz and Oz of the 10-20 system that typically manifest the P300 effect (Lebel et al. 2008; see Fig. 2a). Mean amplitude and latency to peak amplitude for the final trial in each sequence were then extracted for each infant within the time-window of 300-1100 ms after stimulus onset, a time window typical for this effect (e.g., Hyde & Spelke 2011) that was verified by visual inspection of the grand averages plots. A measure of amplitude difference was extracted for each infant by subtracting mean amplitude for habituation from mean amplitude for dishabituation.

The *posterior parietal ratio* analyses compared mean amplitudes within the dishabituation trials for the sequences in which the ratio between the habituation and dishabituation stimuli differed by a ratio *<* 2 with those in which the ratio differed by *>*2. To examine the hypothesized negative right occipitoparietal response (Libertus et al. 2009), these analyses used data from 13 right parietal-located electrodes (below P4 in the 10-20 system; see Fig. 2b) and mean amplitudes were extracted within the time-window of 210-400 ms after stimulus onset for each subject for the dishabituation trials.

Insert Figure 2 about here

## Statistical Analyses

For the initial *habituation-dishabituation* analyses*,* mean amplitude was examined using repeated measures analysis of variance (ANOVA) with group (alcohol exposed vs. controls) as a between-subjects factor and condition (dishabituation vs. habituation) as a within-subjects measure. Independent samples *t*-tests were used to compare latency to peak amplitude for the dishabituation condition during the final trial in each sequence in the alcohol-exposed and control groups. In addition, Pearson *r* was used to examine the relation of the six continuous measures of prenatal alcohol exposure to these outcomes.

For the *posterior parietal* analyses, mean amplitude in the dishabituation trials was examined using repeated measures ANOVA with group (alcohol-exposed vs. controls) as a between-subjects factor and condition (ratio > 2 vs. < 2) as a within-subjects measure. In addition, Pearson *r* was used to examine the relation of the prenatal alcohol measures to three outcomes: mean amplitude in the dishabituation trials of the sequences with a ratio > 2, mean amplitude in the dishabituation trials of the sequences with a ratio < 2, and mean difference in amplitude between both the two conditions (ratio > 2 minus those with a ratio < 2).

Six control variables were considered: infants’ sex and age at ERP assessment and mothers’ years of education, smoking (cigarettes/day) and marijuana use (days/month) during pregnancy. Pearson *r* was used to examine the relation of each control variable to each ERP outcome measure. Any control variable that was even weakly related to a given outcome (*p* < .10) was considered a potential confounder of the effect of prenatal alcohol exposure on that outcome. Confounding was tested in multiple regression analyses in which the effect of prenatal alcohol on the ERP outcome was assessed after adjustment for potential confounders.

# Results

## Sample Characteristics

The sample characteristics are summarized in Table 1. Although the whole sample was poorly educated, control mothers completed about 2 more years of school than those in the heavy alcohol consuming group. Mothers of the exposed infants drank, on average, 9 drinks/occasion on 2-3 days/week. Four of the 19 exposed infants met criteria for FAS and 2 for PFAS. All the controls abstained from drinking during pregnancy, except for one control mother who abstained during the first trimester, when recruited, and later reported consuming 2 drinks twice/month. There were no between-group differences regarding maternal smoking or marijuana use during pregnancy or infant sex, birthweight, gestational age at birth, or age at ERP assessment.

Insert Table 1 about here.

## Habituation-Dishabituation Analysis

The expected dishabituation effect (main effect for condition) was observed at the occipitoparietal-central scalp electrodes for the sample as a whole, with larger mean amplitudes for the dishabituation condition, *M* = 10.1, SD= 34.3, than for the habituation condition, *M* = -15.7, SD= 56.4; *F*(1,31) = 4.99, *p*= .033. Although the alcohol group showed a larger dishabituation effect (mean amplitude difference = 42.9) than the control group (mean amplitude difference = 11.6; see Fig. 4), this interaction was not significant, *F*(1,31) = 1.64, *p* = .209, possibly due to large variance in the exposed group (SD= 93.1). Difference in amplitude between the habituation and dishabituation conditions was correlated with all six of the exposure measures (see Table 2).

Latency to peak amplitude for the dishabituation trials was significantly longer for the alcohol-exposed infants, *M* = 733.2, SD= 172.9, than for the controls, *M* = 591.3, SD= 93.8; *t*(31) = 2.99, *p* = .005 (Fig. 4), indicating a markedly slower neural response to the change in quantity. All six measures of exposure were associated with longer latencies. Because dishabituation latency was related to maternal education (Table 3), regressions were run relating the alcohol exposure measures to dishabituation latency controlling for maternal education. Four of the six alcohol exposure effects continued to be significant (standardized regression coefficients ranged from 0.34 to 0.43, *p*s *<* .05), whereas the remaining two (AA/occasion and frequency during pregnancy) fell just short of statistical significance (standardized regression coefficients of 0.33 and 0.34 respectively, *p*s *<* .07).

Insert Figures 3 & 4 and Tables 2 & 3 about here

## Posterior Parietal Analysis

Infants in the control group showed a larger negative deflection for the “> 2” condition (*M* = -8.6) compared to the “< 2” condition (*M* = 3.6), whereas infants in the alcohol-exposed group showed the opposite pattern, with the ratio “ > 2” trials having a smaller negative deflection (*M* = 16.0) than the “< 2” trials (*M* = -7.3; Fig. 5). A two-way ANOVA, with group (alcohol-exposed/control) as a between-subjects variable and condition (“< 2” vs. “> 2” ratios) as a repeated measure, indicated that this interaction was significant, *F*(1,31) = 6.67, *p* = .015. Pearson correlation analysis showed that prenatal alcohol exposure was associated with greater amplitude (i.e., less negative deflection) for the “> 2” ratio trials but was not related to amplitude for the “< 2” trials (Table 2). Neither of these outcome measures was related to any of the control variables (Table 3).

Insert Figure 5 about here

## Supplementary Analyses

Given the uniqueness of the sample, we included as many infants as possible in the main analyses described above. To examine whether the same effects are seen when more stringent inclusion criteria are used, we re-ran the analyses using data only from infants with at least 3 good trials in each experimental condition.

The habituation-dishabituation analyses for this subsample as a whole (*N* = 12, 8 controls, 4 alcohol-exposed) showed a main effect for larger amplitudes for the dishabituation condition compared to the habituation condition, *F*(1,10) = 6.71, *p* = .027. A marginally significant interaction between group and condition was also found, with the alcohol group showing a larger difference in amplitude between these two conditions compared to the control group, *F*(1,10)=3.87, *p* = .077. The difference in amplitude between these two conditions was still significantly correlated with all six measures of prenatal alcohol exposure (all *r*s > 0.71, *p*s < .01). Latency to peak amplitude for the dishabituation condition continued to be longer for the alcohol-exposed infants (*M* = 722.5 ms) compared to the controls (*M* = 585.8 ms), but this difference was not significant, *t*(10) = 1.48, *p* = .168. Correlations between these outcome measures and all six alcohol exposure measures continued to be of a medium effect size and similar to those found in the full sample (all *r*s > 0.31) but were no longer significant (all *p*s > .20), due to the smaller sample size.

When the posterior parietal analyses were repeated for the subsample meeting, the more stringent inclusion criteria (*N* = 16, 8 controls, 8 alcohol-exposed), the same interaction between group and change-ratio was found for amplitude, *F*(1,15) = 8.55, *p* = .01, as for the sample as a whole. Three of the six alcohol exposure measures were significantly correlated with the amplitudes of the > 2 ratio trials, *r*s > 0.49, *p*s < .043, and the correlations with the other three alcohol measures fell just short of significance, *r*s > 0.41, *ps* < .010). Additionally, amplitude differences between the > 2 and < 2 ratios were correlated with all six exposure measures, all *r*s > 0.47, *p*s = .05.

# Discussion

This study confirms previous ERP results indicating that 6-month-old infants can discriminate between small quantities (Berger 2011; Libertus et al. 2014) and that they can make this discrimination for quantities that differ by a ratio > 2 (Mou & van Marle, 2014). After being exposed to a specific quantity for five consecutive trials, the control infants in our study showed the expected dishabituation brain response. Although the infants with prenatal alcohol exposure showed a similar dishabituation response—indicating that they were also able to discriminate between these small quantities—the latencies and amplitudes of their ERP responses differed significantly from those seen in the control group.

Higher levels of prenatal alcohol exposure (measured in terms of maternal alcohol consumption during pregnancy) were associated with a longer latency of the P300 dishabituation response. P300 latency has been shown to be proportional to stimulus evaluation time and inversely related to cognitive ability (Kok 2001; Polich & Martin 1992). This finding thus suggests slower processing in the evaluation of the stimulus and discrimination of the novel quantity in infants prenatally exposed to alcohol. This interpretation is consistent with previous findings of slower information processing (Jacobson et al. 1993) and reaction time in alcohol-exposed infants (Jacobson et al. 1994) and slower number processing speed (Burden et al. 2005) and longer latencies of specific ERP components in alcohol-exposed children at school age (Burden et al. 2009). It is also consistent with DTI findings of poorer white matter integrity in alcohol-exposed children (Lebel et al. 2008; Wozniak et al. 2006; Spottiswoode et al. 2011; Fan et al. 2016), which may indicate myelination deficits that can affect processing speed, as well as alterations in white matter integrity in alcohol-exposed newborns from this cohort in our recent DTI-based tractography study (Taylor et al. 2015).

The present findings are also consistent with behavioral assessments reported in our Detroit Longitudinal Cohort during adolescence, which indicated that the fetal alcohol-related impairment in arithmetic is mediated primarily by a deficit in magnitude comparison and not by lower IQ or poorer executive function (J Jacobson et al. 2011). Magnitude comparison has been linked to activation in the anterior portion of the horizontal section of the IPS (Dehaene et al. 2003; Pinel et al. 2004). This region is activated by the representation of semantic information about magnitude, whether presented as Arabic numbers, sequences of words, or analogically (e.g., by numbers of dots; Naccache & Dehaene 2001). In a recent study, 50 school-aged heavily exposed and control children from the Cape Coloured community were administered two fMRI tasks: magnitude comparison (“Which of two numbers is closer to a third?”) and exact addition (Woods et al. 2015). Prenatal alcohol exposure was related to reduced activation of the right IPS on both tasks, providing direct evidence of reduced function in this region known to mediate magnitude comparison. A similar pattern was seen in an fMRI study using a subtraction task, in which young adults with fetal alcohol-related dysmorphia showed less activation in the inferior parietal and other math-related regions than the control subjects (Santhanam et al. 2009). Additionally, the Cape Town alcohol-exposed children exhibited more extensive and diffuse parietal and cerebellar activation during the magnitude comparison tasks, including activation in the left and right angular gyrus and posterior cingulate/precuneus (Meintjes et al. 2010). The larger amplitude difference between the habituation and dishabituation trials exhibited by the alcohol-exposed infants in the present study is consistent with the more extensive neural activation seen in the fMRI studies with older exposed children, suggesting a need to recruit more extensive neural resources in magnitude-comparison tasks at both ages.

The posterior parietal ratio analyses indicate that the number-processing deficit in the exposed infants involves not only a general slowing of processing and/or increased effort but is also related to discrimination of quantity. The control infants showed the expected ERP response in this region, that is, an occipitoparietal negative deflection for the quantity change that typically developing infants are able to detect (> 2; Izard et al. 2008), whereas the alcohol-exposed infants did not show this negative deflection. Moreover, in the analyses of the continuous measures of exposure, a larger quantity of alcohol consumed by the mother during pregnancy was correlated with a smaller amplitude in the negative deflection in response to the quantity change.

The exposure group differences in ERP response patterns are consistent with the behavioral findings from a pilot study with our Cape Town longitudinal cohort, which used a looking-time infant numerosity paradigm (S Jacobson et al. 2011). Wynn (1992) has shown that infants as young as 5 months of age can discriminate between correct displays (e.g., 1+1=2, 2-1=1) and erroneous displays (e.g., 1+1=1, 2-1=2) in simple arithmetic problems involving a small number of items. When the number of puppets shown to an infant does not agree with the number previously seen, typically developing infants look longer at the display than when the number agrees with their expectations (Wynn 1992). In our Cape Town pilot study, as predicted, nonexposed infants looked longer at puppet displays that were inappropriate, *t*(21) = 2.8, *p* < .01, that is, incongruent with their expectations. By contrast, the alcohol-exposed infants’ looking time was the same for both the expected and unexpected displays, *t*(17) = .03, n.s*.* (S Jacobson et al. 2011), indicating a failure to detect the numerical incongruity in the task. It is of interest that looking time difference *z*-scores (inappropriate minus appropriate) on the Wynn (1992) paradigm at 6.5 and 13 months of age predicted performance on the Number/Quantity and Digit Span subtests of the Junior South African Intelligence Scale (JSAIS; Madge et al. 1981) when these children were 5 years of age, but were unrelated to JSAIS vocabulary and fine motor function, indicating discriminant predictive validity (S Jacobson et al. 2011). These findings suggest that infant numerosity may provide an early, biobehavioral marker for impairment in number processing.

The present study extends the findings seen in the Wynn (1992) assessment, which are based solely on measurement of behavioral looking times. The behavioral measure does not provide information regarding which of the basic underlying mechanisms involved in this task—numerosity processing or error detection—is affected in the exposed infants. In addition, in this paradigm, an infant’s longer looking time at the incorrect solution depends not only on the processing of numerosity but also on the procedural understanding of simple “addition” and “subtraction.” In contrast, the ERP paradigm used in the present study specifically examines magnitude comparison and provides a direct assessment of the infant’s ability to discriminate between quantities that differ by a ratio > 2, including the accurate timing of this process. The present findings thus corroborate and strengthen the interpretation of the original Cape Town behavioral data, indicating a fetal alcohol-related deficit in infant numerosity.

Consistent with the extant literature, the 19 control infants clearly discriminated between numerosities differing by ratios > 2. This ability did not vary by age within the range that we tested. As in previous reports on nonsymbolic numerical processing in infants (Berger 2011), we also found an effect in the right parietal region. Effects in this region have also been seen in children and adults for discriminating small quantities within the subitizing range (Ansari et al. 2007). In adults, Libertus et al. (2007) have characterized this negative right lateralized early occipitoparietal ratio effect as an N1, which, as expected, was seen somewhat later in the infants in our study. Consistent with the infant literature, we did not find an effect on P2p, which is an ERP marker for quantity processing and comparison in children and adults (Temple & Posner 1998; Ben-Shalom et al. 2012), which apparently does not emerge until the preschool period.

There are some limitations to our study. As in previous infant ERP assessments, only a portion of the data could be used in the final analyses due to infant fussiness, fatigue, noncooperation, and the requirement that the infants clearly fixate on all the stimuli during a given trial. Nevertheless, this study generated data consistent with those seen in studies of older children with prenatal alcohol exposure and provided the first evidence at the level of brain function that a deficit in magnitude comparison can be seen in FASD as early as infancy. As in all correlational studies, the observed effects might be attributable to confounding from unmeasured control variables. However, our data show that these effects were not attributable to group differences in maternal education or exposure to smoking or marijuana during pregnancy or to infant sex, gestational age, birth weight, or age at testing.

In summary, our study demonstrates that ERP responses to changes in quantity are altered by prenatal alcohol exposure, providing important new evidence that the fetal alcohol-related impairment in number processing seen in children and adults is identifiable as early as infancy. Deficits were seen in both ERP components elicited by this task: (a) the positive occipitoparietal-central “P300-like” component that reflects memory updating and stimulus categorization/discrimination in response to the appearance of a novel stimulus in older children and adults and (b) the negative posterior-parietal component that has been linked specifically to magnitude comparison. These findings thus support the feasibility of detection of numerical processing deficits very early in life and suggest that it may be possible to develop early number processing interventions targeted for infants at risk.

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# Figure Legends

*Figure 1.* Example of a trial in the habituation-dishabituation task. In this example of a sequence ending in a dishabituation array, the quantity “2” was presented consecutively three times; subsequently, a dishabituation quantity of “3” was presented. (Not presented are the interstimulus intervals that occurred between array displays.)

*Figures 2 a & b.* Channel groups of the 128-channel HydrocCel Geodesic Sensor Net included in the different analyses: a) occipitoparietal-central group for the habituation-dishabituation analysis; b) right-parietal group for the posterior parietal analysis.

*Figure 3.* Topographic map of scalp voltages for the habituation condition (left panel) versus the dishabituation condition (right panel) for the sample as a whole, averaged across a time window of 550-650 ms. Parieto-occipital area of the effect is delineated by the yellow oval.

*Figure 4.* Latency and amplitude in the occiptoparietal-central area for a) control versus alcohol-exposed infants and b) habituation versus dishabituation trials. Note the between-group difference in the latency to peak amplitude for the dishabituation condition. The gray area marks the broad window of 300-1,100 ms poststimulus, in which latency differences were tested and in which correlations were examined between alcohol exposure and the amplitude measures.

*Figure 5.* Latency and amplitude in the posterior parietal region for a) control versus alcohol-exposed infants and b) ratio > 2 versus < 2. The gray area marks the time-window of 210-400 ms poststimulus.

Table 1

*Sample Characteristics (N = 38)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Control  *n* = 19 | Alcohol  exposed  *n* = 19 | *t* or *χ*2 | |
| Maternal Characteristics |  |  |  | 1 |
| Years of education | 11.1  (1.1) | 9.1  (1.3) | 4.69 | \*\* |
| Pregnancy drinking |  |  |  |  |
| At time of conception |  |  |  |  |
| oz absolute alcohol/day | 0.01  (0.04) | 1.9  (2.8) | 3.07 | \* |
| oz absolute alcohol/occasion | 0.1  (0.3) | 4.5  (4.1) | 4.74 | \*\* |
| Frequency days/week | 0.1  (0.2) | 2.3  (1.5) | 6.49 | \*\* |
| Across pregnancy |  |  |  |  |
| oz absolute alcohol/day | 0.004  (0.02) | 1.4  (2.2) | 2.75 | \*\* |
| oz absolute alcohol/occasion | 0.1  (0.3) | 4.4  (3.4) | 5.49 | \*\* |
| Frequency days/week | 0.02  (0.1) | 1.7  (1.3) | 5.48 | \*\* |
| Pregnancy smoking (cigarettes/day) | 3.4  (3.0) | 6.0  (4.5) | 1.84 |  |
| Pregnancy marijuana use (days/month) | 0.01  (0.03) | 1.2  (4.1) | 1.25 |  |
| Infant Characteristics |  |  |  |  |
| Sex (% male) | 57.9 | 57.9 | 0.00 |  |
| Gestational age at birth (weeks) | 39.3  (1.4) | 38.8  (2.1) | 0.85 |  |
| Birthweight (grams) | 3035.8  (596.9) | 2872.2  (388.3) | 1.05 |  |
| Age at ERP testing (months) | 6.9  (0.5) | 6.8  (0.4) | 0.30 |  |
| Note. Values are mean (SD).  \**p* < .01 \*\**p* < .001 | | | | |

Table 2

*Relation of Prenatal Alcohol Exposure to ERP Outcomes (N = 33)*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | At conception | | | | | |  | During pregnancy | | | | | |
|  | AA/day | | AA/occasion | | Frequency | |  | AA/day | | AA/occasion | | Frequency | |
| Dishabituation  peak response latency | 0.47 | \*\* | 0.44 | \* | 0.45 | \*\* |  | 0.41 | \* | 0.40 | \* | 0.41 | \* |
| Dishabituation-Habituation  amplitude difference | 0.41 | \* | 0.50 | \*\* | 0.35 | \* |  | 0.40 | \* | 0.46 | \*\* | 0.34 | \* |
| Ratio > 2  amplitude | 0.51 | \*\* | 0.42 | \* | 0.49 | \*\* |  | 0.50 | \*\* | 0.38 | \* | 0.56 | \*\*\* |
| Ratio < 2  amplitude | -0.03 |  | 0.05 |  | -0.08 |  |  | -0.02 |  | 0.02 |  | -0.04 |  |
| Ratio > 2 minus < 2  amplitude difference | 0.34 | † | 0.23 |  | 0.36 | \* |  | 0.33 | † | 0.22 |  | 0.38 | \* |
| *Note.* Values are Pearson *r*  †*p* < .10 \**p* < .05 \*\**p* < .01 \*\*\**p* < .001 | | | | | | | | | | | | | |

Table 3

*Correlation of Control Variables to ERP Outcomes (N = 33)*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Infant  sex | | Infant age  at testing | | Maternal  education | | Prenatal  marijuana | | Prenatal  smoking | |
| Dishabituation  peak response latency | 0.08 |  | -0.15 |  | -0.32 | † | 0.14 |  | 0.04 |  |
| Dishabituation-Habituation  amplitude difference | -0.05 |  | -0.14 |  | -0.18 |  | 0.22 |  | -0.24 |  |
| Ratio > 2  amplitude | -0.06 |  | 0.24 |  | -0.19 |  | -0.18 |  | 0.18 |  |
| Ratio < 2  amplitude | -0.09 |  | 0.15 |  | 0.27 |  | -0.23 |  | -0.01 |  |
| Ratio > 2 minus < 2  amplitude difference | 0.02 |  | 0.05 |  | -0.29 |  | -0.04 |  | 0.10 |  |
| *Note.* Values are Pearson *r*  †*p* < .10 | | | | | | | | | | |

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