

Cardiac Orienting Responses Differentiate the Impact of Prenatal Alcohol Exposure in Ukrainian Toddlers

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Background: Prenatal alcohol exposure (PAE) has been found to impact neurophysiological encoding of environmental events negatively in the first year of life but has not been evaluated in older infants or toddlers. Cardiac orienting responses (ORs) collected during a habituation/dishabituation learning paradigm were obtained from 12- to 18-month-olds to assess the impact of PAE beyond the first year of life.

Methods: Participants included women and their toddlers who differed in PAE histories and enrolled in a randomized clinical trial of multivitamin/mineral usage during pregnancy. Those who were randomly assigned to the no intervention group were used for this analysis. The habituation/dishabituation paradigm consisted of 10 habituation and 5 dishabituation trials. Baseline heart rate (HR) was collected for 30 seconds prior to stimulus onset, and responses to the stimuli were assessed by sampling HR for 12 seconds poststimulus onset.

Results: The speed of the OR in response to auditory stimuli in the dishabituation condition was found to be altered as a function of maternal alcohol use around conception. For visual stimuli, positive histories of PAE were predictive of the magnitude but not the speed of the response on habituation and dishabituation trials. A history of binge drinking was associated with reduced magnitude of the OR response on visual encoding trials, and level of alcohol exposure at the time of conception was predictive of the magnitude of the response on visual dishabituation trials.

Conclusions: Cardiac ORs collected in the toddler period were sensitive to the effects of PAE. The magnitude of the OR was more sensitive to the impact of PAE than in previous research with younger infants, and this may be a function of brain maturation. Additional research assessing the predictive utility of using ORs in making decisions about individual risk was recommended.

Key Words: Prenatal Alcohol, Cardiac Orienting, Toddlers.

ALTHOUGH MUCH IS known about the neurodevelopmental consequences of prenatal alcohol exposure (PAE; Riley et al., 2011), early identification of individuals who are neurodevelopmentally impaired as a function of their PAE histories remains challenging as a result of limitations in standardized tests of infant and toddler neurocognitive functioning (Bornstein and Krasnegor, 1989; Matheny, 1989). Greater differentiation of PAE effects has been found

in experimental tasks that assess infant information processing skills and basic learning mechanism, suggesting that the impact of PAE on the early developing brain is detectable as long as the measurement tool is appropriately sensitive to the neurocognitive damage caused by PAE (Jacobson, 1998; Jacobson et al., 1992, 1993, 1994, 2008). The assessment of infant neurophysiological encoding of environmental events using cardiac orienting responses (ORs) has been found to be a promising alternative to standardized developmental tests as ORs obtained from both humans (Kable and Coles, 2004) and animal models (Hunt and Phillips, 2004; Morasch and Hunt, 2009) have been sensitive to the impact of PAE.

ORs enable the heart to gate oxygen to the central nervous system and away from the periphery to allow for higher level information processing and learning about environmental events (Sokolov, 2002). They occur as a function of stimulation from the thalamus to the heart via the 10th cranial nerve and are characterized by a specific pattern of heart rate (HR) deceleration (Graham and Jackson, 1970a) in the presence of novel or interesting stimuli. ORs can be elicited within the first few months of life in all mammals (Sokolov et al., 2002). The trough of the OR reflects the degree of neurophysiological encoding and sustained interest to the stimuli and is characterized by a sustained deceleration in HR (Lansink et al., 2000; Richards,

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1995). In humans, ORs collected in the first 6 months of life have been found in response to the onset of a variety of stimuli (Berg et al., 1971; Brown et al., 1977; Lewis et al., 1966) and to be predictive of later neurodevelopmental status (O'Connor, 1980; O'Connor et al., 1984). Relatively little, however, is known about the predictive validity of ORs collected later in life, but cardiac vagal control, a measure of HR variability, has been linked to executive functioning (EF) skills in adults (Kimhy et al., 2013).

Using animal models, electrical stimulation of the medial prefrontal cortex can elicit an OR (Powell et al., 1994), suggesting that ORs can provide an early index of the efficiency of prefrontal cortical functioning involved in the neural circuitry that gates energy resources between basic attention and arousal systems needed to effectively process information (Ruff and Rothbart, 1996). Evidence suggests that the prefrontal cortex (Fryer et al., 2007; Kfir et al., 2009; O'Hare et al., 2009; Olegard et al., 1979; Sowell et al., 2007; Warren et al., 2004) and the connectivity of the prefrontal cortex to other brain regions (Wozniak et al., 2013) are adversely impacted by PAE and may be the neural substrate from which the deficits in EF skills arise. Thus, ORs may provide an early estimate of EF skill impairments associated with PAE that could be used to identify alcohol-affected individuals in need of early intervention services.

Using a sample of toddlers who were identified based on their mother's prenatal alcohol consumption during pregnancy and enrollment in a randomized clinical trial of micronutrient supplementation (Coles et al., 2015; Kable et al., 2015), the impact of PAE on ORs obtained in the second year of life was assessed. The study was carried out in Ukraine where the prevalence of alcohol use among women has been reported to be high (Bakhireva et al., 2011; Chambers et al., 2014) to determine whether the impact of PAE on neurophysiological encoding persisted beyond the first year of life. We hypothesized that toddlers with a history of PAE would have less change in HR in response to environmental stimulation and a slower response speed relative to toddlers without a PAE history. The amount of PAE was also anticipated to be related to the magnitude of the effects.

MATERIALS AND METHODS

Data used in this study are from a subset of participants who were enrolled in a multisite clinical trial conducted as part of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD), an international consortium of basic science and clinical investigations funded by the National Institute of Alcohol Abuse and Alcoholism (NIAAA). The study protocol was approved by institutional review boards at the Lviv National Medical University in Ukraine and the University of California San Diego, La Jolla, California. Participants were recruited from a diagnostic medical center located in Rivne, Ukraine (Rivne Regional Medical Diagnostic Center), which is a referral center for routine prenatal ultrasound and other diagnostic services for pregnant women, during their first prenatal care visit. Informed written consent was provided by all participants.

Recruitment and Procedures

Women ($N = 372$) were recruited between April of 2008 and August of 2012 for a clinical trial study (Chambers et al., 2014). Based on a screener interviewer conducted by a clinical nurse, women who reported at least weekly binge drinking episodes (5+ drinks), at least 5 episodes of 3 to 4 standard drinks, or at least 10 episodes of 1 to 2 standard drinks either in the month around conception or the most recent month of pregnancy, were invited to participate in the study and provided with information on the risks of alcohol consumption during pregnancy. The next woman seen at the center who reported no binge episodes, minimal (<2 drinks on 1 occasion) or no alcohol in the month around conception, and no continued drinking in pregnancy was asked to participate to provide a comparison group of toddlers with minimal or no PAE. Participants were then assigned to 1 of 3 intervention groups: (i) standard of care (recommendation to take prenatal vitamins), (ii) multivitamin/mineral supplementation, or (iii) multivitamin/mineral supplementation plus choline (750 mg) supplementation. Only those assigned to the standard of care condition, which was to recommend using prenatal vitamins without providing them directly to the participants, were used for the current analysis to avoid potential effects associated with the treatment conditions that were observed in the 6-month ORs (Kable et al., 2015). All participants were advised to avoid alcohol in pregnancy and provided a standard brochure with information on the risks of alcohol in pregnancy. The nurse interviewer also offered information about resources in the community.

Using structured questionnaires, information regarding the mothers' demographic characteristics, lifestyle, and substance use in pregnancy, including maternal alcohol and tobacco consumption and paternal alcohol use, was obtained. Mothers were interviewed at enrollment, in the third trimester at approximately 32 weeks of gestation, and at postpartum. A timeline follow-back procedure was used to assess day-by-day alcohol quantity and type consumed in the week around conception and in the 2 weeks prior to enrollment. Absolute ounces of alcohol per day (AA/day) and per drinking day (AA/drinking day) were computed from the mother's report of the amount and frequency of alcohol intake for each of the 2 time points to assess average and episodic or binge drinking behaviors (≥ 5 drinks on 1 occasion). Standard drink size was considered 1.5 oz of hard liquor, 4 oz of wine, and 12 oz of beer. Quantities reported for each type of alcohol were recorded in number of standard drinks for that type of alcohol on that day. Total absolute ounces of alcohol was computed for each type of alcohol on each day by multiplying the number of standard drinks by the ounces per standard drink size for that type of alcohol and that sum was multiplied by the alcohol content for that type of alcohol. The absolute ounces of alcohol per drink type were summed per day in the timeline follow-back period and summed across the period. Absolute ounces per day at the time of enrollment was computed by dividing the total absolute ounces by 14 days. Absolute ounces per drinking day at the time of enrollment was computed by dividing the total absolute ounces by the number of days with >0 alcohol reported of the 14 possible days. The same procedure was used for the week around conception except that a 7-day period was used.

Women enrolled in both groups were asked in the third trimester if their drinking pattern had changed from what they reported at the enrollment interview. Although many women reduced or discontinued their drinking after enrollment, we did not identify any women in the control group who reported starting to drink after enrollment.

Second-Year Follow-Up Assessment Procedures

Mothers and their toddlers ($n = 222$) were seen when the child was between 12 and 18 months of age at the diagnostic center for medical and developmental follow-up evaluations. Of these, 68

mothers had been assigned to the no intervention group at the time of enrollment during pregnancy and had children for whom cardiac data were available for this analysis to evaluate differences in ORs associated with a history of PAE.

Toddler Measures. Toddlers were placed into an age-appropriate child seat, and their mothers were allowed to observe the testing but were instructed to be nonresponsive to their toddler. The neurophysiologist was blinded to group status. A fixed trial habituation/dishabituation paradigm, consisting of 10 habituation trials followed by 5 dishabituation trials, was used to assess neurophysiological encoding and dishabituation of both visual and auditory stimuli. Stimulus presentation software available from the James Long Company (Adirondack Park, NY) was used to present the digitized stimuli for a total of 12 seconds followed by an interstimulus interval of 12 seconds. The auditory stimuli consisted of a standard stimulus of alternating 400 and 1,000 hz pure tones presented contiguously for 2 seconds each with a 5 msec controlled linear rise and fall time for each tone during the habituation trials and a novel stimulus consisting of alternating 700 and 1,000 hz pure tones presented in a similar format during the dishabituation trials. The visual stimuli consisted of chromatic Caucasian faces of a baby (habituation stimulus) and a woman (dishabituation stimulus).

Cardiac responses to the stimuli were monitored continuously using an electrocardiogram (EKG) amplifier connected to a data acquisition computer that was triggered by the stimulus presentation software. HR was averaged for 30 seconds prior to initial stimulus onset for each stimulus type to be used as a baseline and then 12 seconds after the onset of each stimulus. The latency of the OR response was computed by determining the poststimulus second when the HR reached more than 2 bpm of deceleration from the average baseline level of HR. For toddlers that did not have an OR response on a given trial, the maximum second of 9 was assigned to the trial. Nine seconds was selected as the peak deceleration in HR usually occurs between 7 and 9 seconds poststimulus onset. Decelerations after this interval are more likely to be random fluctuations in HR rather than a true OR as most are disengaging from the stimulus after this interval rather than initiating the response. Toddlers' arousal level was rated for each trial using a scale ranging from 1 to 7 (Als et al., 1977), and those who were in either state 1—Deep Sleep or state 7—Vigorous Crying were excluded from analysis. The first 3 trials of the habituation and dishabituation trials were used for analysis as significant diminution of the OR response typically occurs by the fourth trial (Kable and Coles, 2004; O'Connor, 1980). The stimuli and procedures were consistent with those used in data collection at 6 months with this cohort (Kable et al., 2015).

Data Analysis

Maternal interview and neurophysiological data were collected and entered on site in Ukraine and transmitted to the University of California San Diego, La Jolla, California and Emory University, Atlanta, Georgia. To assess group differences in family and child characteristics, analyses of variance were performed on continuous measures and chi-squares were performed on categorical measures.

Movement artifacts and poor-quality cardiac traces resulted in some attrition. On the habituation trials, data were available on 64 participants for the auditory task and 68 for the visual task. Additional data were lost due to fatigue or attrition within the tasks, resulting in 59 participants for the auditory and 62 for the visual dishabituation tasks. Latency in seconds for each of the first 3 habituation and dishabituation trials was analyzed using a repeated-measures analysis of covariance with trial as the repeated measure and PAE history (yes vs. no) as a between subjects factors for each of the 4 conditions assessed in the paradigm (stimuli [auditory or visual] by learning condition [habituation or dishabituation]). The

magnitude of the HR for each of the 12 seconds poststimulus was then aggregated across the first 3 trials for each stimulus condition (auditory or visual) and learning condition (habituation or dishabituation) for analyses. Repeated-measures analyses of covariance were then performed for each of the conditions with PAE group status (yes vs. no) as the between subjects factor and time, as measured by seconds poststimulus onset (1 to 12), as the repeated subjects factor in the model. Indices of the magnitude of prenatal alcohol consumption (the presence of binge drinking and AA/day in the month around conception and in pregnancy during the 2 weeks prior to the enrollment interview) were also entered in the model. Covariates in the models were the toddler's adjusted gestational age at the time of the assessment as a result of maturational effects of the OR, the child's baseline level of HR prior to the stimulus presentation to control for individual differences that might impact changes in HR in response to the procedures (Manning and DuBois, 1962), and other preexisting group differences.

RESULTS

Group Characteristics of Sample

Family and toddler characteristics by group status are presented in Table 1, including mean group differences and statistics to assess group differences. No significant group differences were found in maternal and paternal age, marital status, and social class as rated by the Hollingshead scale (Hollingshead, 2011). There were also no group differences in gestational age at enrollment. In addition to reporting greater levels of alcohol consumption and greater proportions of drinking days around the time of conception and in pregnancy during the 2 weeks prior to the enrollment interview and a greater likelihood of being a binge drinker, mothers in the PAE group were more likely to be smoking cigarettes at enrollment relative to the comparison group. Mothers in the PAE group also had less education and fewer previous children than did mothers who were in the comparison group. Toddlers with a history of PAE did not differ in gestational age at delivery or age at the posttest assessment, birthweight, birth length, or head circumference from those without a history of PAE. There were also no differences in the gender distribution between the groups.

Neurophysiological Outcomes

Baseline Heart Rate. Toddlers with a history of PAE had higher baseline levels of HR on both the auditory (133.11 [16.24]) and visual (132.29 [11.83]) trials than did the toddlers without a history of PAE (auditory: 127.73 [10.79]; visual: 127.68 [13.15]) but the differences were not significant, auditory: $F(1, 66) = 2.48, p < 0.123$; visual: $F(1, 67) = 2.35, p < 0.130$.

Latency of the OR Response. After controlling for child's age at assessment, the child's baseline HR, maternal education level, parity, and cigarettes per day at enrollment, on the auditory dishabituation trials, a significant trial by AA/day the month around conception effect was found, $F(2, 90) = 3.57, p < 0.032$, partial $\eta^2 = 0.074$, but parameter

Table 1. Sample Characteristics by Prenatal Alcohol Use History

Measure	Prenatal alcohol	Prenatal alcohol	Statistic and <i>p</i> -value
	No <i>n</i> = 32	Yes <i>n</i> = 36	
Maternal age (years)	26.9 (5.3)	25.3 (5.0)	ns
Paternal age (years)	28.4 (5.7)	29.9 (6.2)	ns
Child's gender (% male)	56.3%	42.9%	ns
Marital status (% with partner)	96.9%	88.8%	ns
Parity (number of previous children, SD)	1.0 (1.7)	0.3 (0.7)	$F(1, 65) = 4.35, p < 0.04$
Maternal education (years, SD)	14.3 (1.5)	13.3 (2.0)	$F(1, 65) = 5.78, p < 0.02$
Social class ^a	37.9 (10.0)	36.5 (11.2)	ns
Gestational age at recruitment (weeks, SD)	19.9 (6.1)	20.8 (5.4)	ns
Gestational age at birth (weeks, SD)	39.7 (1.1)	39.1 (2.4)	ns
Adjusted age at assessment (months, SD)	14.1 (2.0)	13.7 (2.7)	ns
Birthweight (grams, SD)	3266.9 (473.7)	3176.3 (589.6)	ns
Birth length (centimeters, SD)	50.8 (2.5)	50.8 (3.5)	ns
Birth head circumference (centimeters, SD)	33.9 (1.4)	33.8 (1.8)	ns
Proportion of drinking days the month around conception	0.00 (0.0)	0.347 (0.22)	$F(1, 65) = 77.75, p < 0.000$
AA/day mean (absolute ounces, SD) the month around conception	0.00 (0.0)	0.51 (0.27)	$F(1, 65) = 115.4, p < 0.000$
AA/drinking day (absolute ounces, SD) the month around conception	0.00 (0.0)	1.7 (0.98)	$F(1, 65) = 93.6, p < 0.000$
Proportion of drinking days the month in pregnancy	0.00 (0.0)	0.041 (0.11)	$F(1, 65) = 4.18, p < 0.045$
AA/day mean (absolute ounces, SD) in pregnancy	0.00 (0.0)	0.02 (0.05)	$F(1, 65) = 7.16, p < 0.009$
AA/drinking day (absolute ounces, SD) in pregnancy	0.00 (0.0)	0.22 (0.49)	$F(1, 65) = 6.77, p < 0.011$
Percentage reporting binge drinking (% any)	0%	74.3%	$\chi = 38.0, p < 0.000$
Current cigarette smoker at enrollment (%)	9.7	20.0	$\chi = 20.6, p < 0.000$

^aHollingshead scale (Hollingshead, 2011) includes measures of educational attainment and occupation for mother and father.

estimates were nonsignificant for any of the trials. There were no group differences in latency of the OR on the auditory habituation, visual habituation, or visual dishabituation trials.

Magnitude of the OR Response. After controlling for the covariates in the analysis of the auditory habituation trials, only trends were found for main effects on binge drinking during pregnancy, $F(1, 50) = 3.33, p < 0.074$, partial $H\eta^2 = 0.062$, and PAE group status, $F(1, 50) = 3.04, p < 0.087$, partial $H\eta^2 = 0.057$. For magnitude of the OR response, there were no group differences in the auditory dishabituation condition.

On visual habituation trials, main effects were found for maternal binge drinking, $F(1, 53) = 5.48, p < 0.023$, partial $H\eta^2 = 0.094$, and PAE group status, $F(1, 53) = 8.40, p < 0.005$, partial $H\eta^2 = 0.137$; No PAE: 123.5 vs. PAE: 135.2, and trends were found for interaction effects with time for AA/day in pregnancy in the 2 weeks prior to the enrollment interview, $F(11, 583) = 1.74, p < 0.062$, partial $H\eta^2 = 0.032$, and PAE group status, $F(11, 583) = 1.62, p < 0.088$, partial $H\eta^2 = 0.030$. Parameter estimates indicated that binge drinking and PAE group status were associated with overall higher HR in the OR.

On the visual dishabituation trials, main effects were found for AA/day, $F(1, 47) = 9.53, p < 0.003$, partial $H\eta^2 = 0.169$, around the time of conception and PAE group status, $F(1, 47) = 6.07, p < 0.017$, partial $H\eta^2 = 0.114$; No PAE: 124.0 vs. PAE: 137.0. In addition, a significant binge by time, $F(11, 517) = 1.80, p < 0.051$, partial $H\eta^2 = 0.037$, and an AA/day the month around conception by time effect was found, $F(11, 90) = 2.71, p < 0.002$, partial $H\eta^2 = 0.054$. Parameter

estimates were not significant for the binge by time effect but were for AA/day the month around conception on seconds 2 to 6 and 8 to 12 with higher levels of alcohol use associated with higher HR at each of these points.

Table 2 contains the effect sizes of the 4 indices of maternal alcohol use as measured by partial eta squared relative to their relationship with each of the indices of the cardiac ORs. For speed of the OR, the effect sizes of the interactions with trial are presented and for the magnitude of the OR, interactions with time (second interval) are presented. Figure 1 depicts the average change in HR aggregated over the first 3 trials for auditory (Fig. 1A) and visual (Fig. 1B) stimuli by seconds poststimulus for the habituation and dishabituation conditions.

DISCUSSION

Fetal alcohol spectrum disorders encompass a range of neurodevelopmental disabilities (Riley et al., 2011) and early identification of these individuals may provide a greater window for maximizing the neuroplasticity associated with the developing brain (Fox et al., 2010). Unfortunately, traditional tests of infant and toddler functioning have not been found to be useful in identifying the full spectrum of alcohol-affected individuals (Kable et al., 2015) and in general are known for not being effective in predicting long-term intellectual development (Bornstein and Krasnegor, 1989). ORs provide an early index of the efficiency of prefrontal cortical activity (Powell et al., 1994), which is associated with later EF skills known to be adversely affected in children with a history of PAE (Fuglestad et al., 2015; Kodituwakku et al., 2001; Vaurio et al., 2008). Although ORs collected in early

Table 2. Partial Eta-Squared Values for Each of the Parameters of Maternal Alcohol Use in Predicting Indices of the Cardiac Orienting Response

Measure	Alcohol group (yes vs. no)	Binge drinking	AA/day around conception	AA/day in pregnancy
Auditory habituation speed	Main: 0.033 Interaction: 0.029	Main: 0.026 Interaction: 0.007	Main: 0.002 Interaction: 0.008	Main: 0.013 Interaction: 0.028
Auditory dishabituation speed	Main: 0.006 Interaction: 0.021	Main: 0.007 Interaction: 0.013	Main: 0.014 Interaction: 0.074*	Main: 0.001 Interaction: 0.043
Visual habituation speed	Main: 0.011 Interaction: 0.001	Main: 0.006 Interaction: 0.012	Main: 0.003 Interaction: 0.002	Main: 0.000 Interaction: 0.020
Visual dishabituation speed	Main: 0.013 Interaction: 0.001	Main: 0.041 Interaction: 0.038	Main: 0.0210 Interaction: 0.006	Main: 0.028 Interaction: 0.010
Auditory habituation magnitude	Main: 0.057^T Interaction: 0.005	Main: 0.062^T Interaction: 0.011	Main: 0.009 Interaction: 0.004	Main: 0.000 Interaction: 0.004
Auditory dishabituation magnitude	Main: 0.010 Interaction: 0.008	Main: 0.019 Interaction: 0.008	Main: 0.006 Interaction: 0.005	Main: 0.000 Interaction: 0.0170
Visual habituation magnitude	Main: 0.137** Interaction: 0.030^T	Main: 0.094* Interaction: 0.028	Main: 0.043 Interaction: 0.015	Main: 0.000 Interaction: 0.032^T
Visual dishabituation magnitude	Main: 0.114* Interaction: 0.031	Main: 0.000 Interaction: 0.037*	Main: 0.169** Interaction: 0.054**	Main: 0.001 Interaction: 0.009

Trends and significant effects are bolded and marked as follows: ^T $p < 0.10$, * $p < 0.05$, and ** $p < 0.01$.

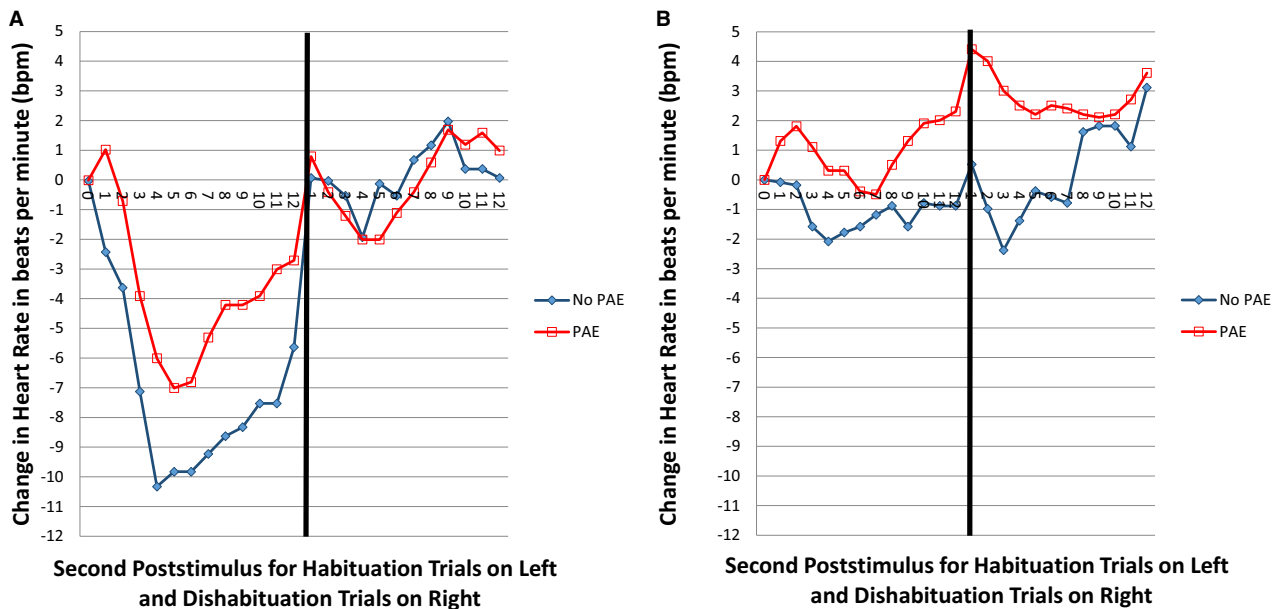


Fig. 1. (A) Cardiac OR to auditory stimuli. (B) Cardiac OR to visual stimuli.

infancy have been found altered as a function of PAE (Kable and Coles, 2004), little is known about the sensitivity of this measure with older infants or toddlers. To address this problem, the impact of PAE on ORs collected in a neurophysiological learning paradigm was explored using a sample of Ukrainian toddlers recruited to participate in a clinical trial of the efficacy of multivitamin/mineral supplements in preventing negative outcomes in alcohol-exposed pregnancies. Using participants assigned to the no intervention group, the results suggested that ORs collected in the toddler period continued to be sensitive to the effects of PAE.

Although the speed of the OR response was most vulnerable to the effects of PAE at 6 months (Kable and Coles,

2004), only maternal drinking levels around conception resulted in slower ORs during the dishabituation trials of the auditory stimuli and there was even variability in this relationship across trials. The lack of other significant relationships between indices of PAE and speed of the OR response in this sample suggests that caution should be used in interpreting this finding as it may be due to chance alone.

For the visual stimuli, prenatal alcohol variables were not predictive of the speed of the OR but were predictive of the magnitude of the response. An overall reduction in the magnitude of the OR was associated with reported binge drinking during pregnancy and a positive PAE group status on the visual habituation trials, and for the visual dishabituation

trials, maternal drinking levels during the month around conception and PAE group status were related to a reduction in the magnitude of the OR. Significant interaction effects were also found for binge drinking during pregnancy and estimates of maternal alcohol consumption around conception across time on the magnitude of the ORs collected in the visual dishabituation condition. Although individual estimates of these effects at each second for binge drinking were not significant, estimates of maternal alcohol consumption around the time of conception resulted in differential effects in the initial response, seconds 2 to 6, and in the later phase of the response, seconds 8 to 12. A reduction in HR in the later phase of the OR response suggests disruption to the toddler's capacity to maintain sustained interest in the stimuli. As indicated by earlier research that simultaneously collected HR and coded behavioral responses as a function of stimulus onset (Richards, 1995), these results suggest that levels of alcohol consumption around the time of pregnancy disrupted the toddler's ability to sustain attention to the stimulus.

Examination of Fig. 1B suggests that the reduced magnitude of the HR during the sustained attention period of the ORs in the toddlers with a history of PAE may be mediated by an initial increase in HR in response to the visual stimuli. This acceleratory response, often referred to as tachycardia, is often seen in very young infants and newborns (Graham and Jackson, 1970b; Jackson et al., 1971; Porges et al., 1973) and those with neurodevelopmental problems (Roberts et al., 2013; Rose et al., 1980) in response to stimulation, suggesting that the pattern is indicative of a less mature or damaged neural system.

Although the latency of the response has been effective in differentiating the impact of PAE (Kable and Coles, 2004) and sensitive enough to assess the impact of interventions to ameliorate the impact of PAE (Kable et al., 2015) in infants <1 year of age, the results of this study suggested that the magnitude of the OR was more sensitive to PAE group status and estimates of the quantity of PAE in a sample of toddlers. The sample size used in this study was smaller than that of previous studies using this methodology in alcohol-exposed infants (Kable and Coles, 2004; Kable et al., 2015), which may have limited the power available to detect significant effects on the speed outcome variables. Examination of the estimates of the effect sizes of the obtained mean differences, however, suggests that sample size alone cannot explain the differences in these studies, and speed of the OR at 12 months is not as differentiated by the impact of PAE as was previously found in younger infants (Kable and Coles, 2004).

Changes in brain maturation (Bell, 1998), particularly myelination of the prefrontal cortical areas of the brain, over the course of the first 2 years of life may contribute to the differential sensitivity of the indices of the cardiac OR. These brain maturation changes correspond to developmental maturational processes allowing for effortful control over the regulation of attention and capacity to sustain mental effort

(Ruff and Rothbart, 1996). Exploring the impact of different PAE histories in older children may be useful in future research with ORs to clarify the impact of brain maturation on these responses and their sensitivity in capturing PAE effects over the lifespan of an individual with FASD. Additional research is also needed on the predictive validity of ORs obtained at 12 months to clarify whether parameters of the OR in the toddler period are as predictive of long-term cognitive functioning as are those obtained from 6-month-olds (O'Connor et al., 1984).

Reports of level of alcohol consumption around the time of enrollment were not predictive of characteristics of the OR despite alcohol group status, a binge drinking history, and estimates of level of alcohol consumption around conception being related to parameters of the OR responses during the learning paradigm. Although one conclusion that can be drawn from this is that early prenatal alcohol consumption has a stronger impact on the brain development of areas involved in the OR response relative to later alcohol consumption in pregnancy, this may not be accurate as a result of the low rate of continued drinking during pregnancy reported by mothers in this sample. Both the level of alcohol consumption and the proportion of drinking days decreased from the estimate obtained around the time of conception to the estimate obtained around the time of enrollment. Higher thresholds of exposure in this period may be needed to produce significant effects. Inaccuracies in reporting of levels of alcohol consumption at the time of enrollment may have also obscured relationships as mothers are often uncomfortable with acknowledging alcohol consumption during pregnancy.

In the assessment of neurophysiological encoding in 6-month-olds in the overall study from which this toddler sample is drawn, responses to the visual stimuli were found to be more sensitive to micronutrient supplementation than were the auditory stimuli (Kable et al., 2015). Specific characteristics of the stimulus presentation in the Ukrainian facility were discussed as one potential bias for interpreting the differential responsiveness to the visual stimuli in this paradigm in that the testing room had more ambient light allowing for greater visual distractions relative to the original use of the stimuli in alcohol-affected infants (Kable and Coles, 2004) where no differences were found relative to stimulus modality. In this sample of toddlers from this same cohort, responses to both the auditory and visual stimuli were impacted by PAE but the impact to the responses to the visual stimuli appeared more robust in that the effect sizes were larger.

Alterations of prefrontal cortical functioning have been posited as underlying the disruption to complex thinking, psychosocial functioning, and interpersonal relations commonly seen among individuals with an FASD (McGee and Riley, 2006; Schonfeld et al., 2006, 2009; Thomas et al., 1998). ORs provide a relatively quick assessment of the efficiency of prefrontal cortical activity and can be applied readily in diverse cultural settings with little modifications of the

stimuli used to elicit the responses, making them an ideal tool for assessing neurodevelopmental functioning for comparisons of group differences and may ultimately be important for early identification of individuals who would benefit from intervention.

The transition from studies comparing group differences of the impact of PAE to making individual assessments of risk after PAE, however, requires additional research. Establishing parameters of the OR that can be used to identify individuals who are impaired or not has not been performed, including information on the predictive utility of the indices of the OR in making this distinction. Historical indices of ORs (Kable and Coles, 2004; Kable et al., 2015; O'Connor, 1980; O'Connor et al., 1984), including this analysis, have used point estimates of a complex phasic pattern of HR change over time to assess individual differences in information processing skills, which may not provide the most optimal estimates of individual risk (Carnahan et al., 2003). Future research should explore alternative methods of operationalizing the characteristics of the OR as advancements in statistical analysis modeling procedures, such as machine learning (Carnahan et al., 2003), may be better able to capture individual differences in the phasic pattern of HR needed to provide the most optimal estimates of prefrontal cortical functioning needed for identifying individual risk status.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflict of interests.

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