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Journal of Mental Health Research in Intellectual Disabilities

ISSN: 1931-5864 (Print) 1931-5872 (Online) Journal homepage: http://www.tandfonline.com/loi/umid20

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**To cite this article:** Tracy Jirikowic, Maida Chen, Jennifer Nash, Beth Gendler & Heather Carmichael Olson (2016) Regulatory Behaviors and Stress Reactivity among Infants at High Risk for Fetal Alcohol Spectrum Disorders: An Exploratory Study, Journal of Mental Health Research in Intellectual Disabilities, 9:3, 171-188, DOI: 10.1080/19315864.2016.1183246

**To link to this article:** http://dx.doi.org/10.1080/19315864.2016.1183246

JOURNAL OF MENTAL HEALTH RESEARCH IN INTELLECTUAL DISABILITIES
2016, VOL. 9, NO. 3, 171–188
http://dx.doi.org/10.1080/19315864.2016.1183246

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# Regulatory Behaviors and Stress Reactivity among Infants at High Risk for Fetal Alcohol Spectrum Disorders: An Exploratory Study

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**ABSTRACT**

*Introduction:* This article examines regulatory behaviors and physiological stress reactivity among 6–15 month–old infants with moderate to heavy prenatal alcohol exposure (PAE), a group at very high risk for fetal alcohol spectrum disorders and self–regulation impairments, compared to low risk infants with no/low exposure. *Participants:* Eighteen infants and their biological mothers; nine infants [*M*= 10.7 (3.1) months] had moderate to heavy PAE and nine [*M*= 10.7 (2.9) months] had no/low exposure. *Methods:* Infant biobehavioral responses to the Still Face Paradigm (SFP), a standardized infant social stressor, were examined. Infant behaviors were compared across: 1) play; 2) still–face (stressor); and 3) reunion conditions, using the Infant and Caregiver Engagement Phases coding system. Salivary cortisol samples were taken at baseline, 15 and 30 minutes post SFP. Mothers completed the Infant–Toddler Symptom Questionnaire (ITSC) and Infant Behavior Questionnaire–Revised (IBQ–R). *Results:* Infants with PAE had significantly higher baseline salivary cortisol levels [0.26 mg/dl (.12 SD)] than controls [0.11 mg/dl (0.03 SD; $p \leq .05$]. Behaviorally, infants with PAE demonstrated significantly fewer social monitoring behaviors compared to controls. There were no significant group differences on caregiver-reported regulatory behaviors (ITSC) or temperament characteristics (IBQ–R). *Conclusion:* Biobehavioral findings revealed greater stress vulnerability and heightened risk for regulatory problems among infants with high–risk levels of PAE. Results need confirmation with a larger sample. These exploratory findings inform future studies designed to investigate early developmental problems among children with PAE and FASD, and support an overarching goal to improve early identification and early intervention strategies for a group of high–risk infants.

**KEYWORDS**

Behavior regulation; child development; fetal alcohol spectrum disorders; fetal alcohol syndrome; infants and young children; prenatal alcohol exposure; stress reactivity

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## Introduction

Prenatal exposure to alcohol (PAE) can increase risk for lifelong developmental and intellectual disabilities that fall under the nondiagnostic, umbrella term of fetal alcohol spectrum disorders (FASD; Warren et al., 2004). High rates of secondary disabilities and co-occurring behavior and mental health problems are significant among children and adults with FASD (Mattson, Crocker, & Nguyen, 2011; Streissguth et al., 2004). Early recognition of the effects of PAE (before age 6) is an important protective factor associated with increased odds of more positive outcomes later in life (Streissguth et al., 2004). Early intervention has the potential to attenuate damage from PAE by taking advantage of neuroplasticity in the first three years of life (Kodituwakku, 2010; Warren, Hewitt, & Thomas, 2011). Thus, timely identification and intervention may alter the downward spiral of problem behaviors and secondary disabilities so commonly described in this high-risk group of children (Olson & Montague, 2011).

Self-regulation impairments are a persistent finding in older children with FASD (Mattson et al., 2011), and are recognized as a central area of impairment in recently proposed *Diagnostic and Statistical Manual of Mental Disorders (DSM-V)* diagnostic criteria for neurodevelopmental disorder associated with prenatal alcohol exposure (ND-PAE) (American Psychiatric Association, 2013). Symptoms of poor behavioral regulation (e.g., irritability, negative affectivity, sleep difficulties) are common clinical concerns reported by caregivers of infants and young children with PAE or FASD (Olson, Jirikowic, Kartin, & Astley, 2007). Early regulatory difficulties among children with FASD or PAE also have been documented in developmental and sleep research (Brown, Olson, & Croninger, 2010; Chen, Olson, Picciano, Starr, & Owens, 2012; O'Connor, 2001; O'Connor, Kogan, & Findlay, 2002; Wengel, Hanlon-Dearman, & Fjeldsted, 2011).

Early regulatory functions involve the capacity to modulate positive and negative affective experiences and cope effectively with challenge or stress. These represent critical developmental tasks in the first years of life (Feldman, 2009). Regulatory development involves a complex interplay of infant behavior, physiological maturation and reactivity, and ecological influences (e.g., caregiver responses; Mayes, 2000). Disruptions in infant regulatory processes can be early indicators of central nervous system dysfunction or later neurobehavioral risk (Gunnar & Quevedo, 2007). Infants with regulatory problems are at greater risk for insecure attachment, poorer developmental outcomes, and later psychopathology (DeGangi, Breinbaur, Roosevelt, Porges, & Greenspan, 2000).

PAE has been associated with a disrupted physiological capacity to regulate arousal, respond and recover from stress, and develop adaptive self-regulatory behaviors. Among alcohol-exposed rodent and primate species, greater secretion

and slower recovery of stress hormones, an index of physiological dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, have been reported (Schneider, Moore, & Kraemer, 2004; Weinberg, 1989; Weinberg, Sliwowska, Lan, Hellemans, 2008). These physiological alterations also have been correlated with decrements in patterns of behavioral adaptation (Schneider et al., 2004), as well as vulnerability to anxiety, depression, and other mental health disorders (Hellemans, Sliwowska, Verma, & Weinberg, 2010).

In human studies, altered HPA-axis regulation, as measured by cortisol reactivity, has been reported among infants with PAE in response to physical stressors (e.g., blood draw, inoculation; Oberlander et al., 2010; Jacobson, Bihun, & Chiodo, 1999; Ramsay, Bendersky, & Lewis, 1996). Only one known study to date has examined infant responses to a social stressor. Haley, Handmaker, and Lowe (2006) reported an association between PAE and poorer physiological and behavioral adaptation among 5–7 month old infants ($n$ = 55) in response to the social-emotional challenge of the Still-Face Paradigm (SFP; Tronick, Als, Adamson, Wise, & Brazelton, 1978). Although this study lacked a comparison group, higher PAE levels were associated with increased cortisol reactivity and more negative affect. These alterations in the stress response system may persist across development. Keiver, Bertram, Orr, and Clarren (2015) reported that children 6 to 14 years old with high levels of PAE had higher afternoon and evening diurnal cortisol levels in comparison to children with low/no PAE, suggesting poorer day-to-day stress adaption in association with more PAE.

Regulatory development is especially significant during infancy and early childhood, and PAE appears to be associated with physiological and behavioral symptoms of dysregulation. Since marked developmental delays (e.g., motor, cognition, communication as identified by standardized developmental assessments) characterize only a small proportion of young children clinically affected by PAE, symptoms of dysregulation could be a more frequent early indicator of vulnerability, and might facilitate early identification and intervention (Olson et al., 2007). This important aim prompted the current study exploring regulatory functions among infants with high-risk levels of PAE.

We examined infant affective and physiological stress responses and caregiver-reported regulatory behaviors among infants at highest risk for FASD, based on carefully documented exposure levels, in comparison to a control group with no/low PAE. Hypotheses were that compared to low-risk controls, infants with moderate to heavy PAE would show more indicators of dysregulation and stress reactivity at a: (1) physiological level, as indexed by higher salivary cortisol reactivity in response to a social-emotional stressor; and (2) behavioral level, as measured by a higher frequency of observed negative affective behaviors in response to a social stressor. Secondary hypotheses were that, using standardized caregiver questionnaires, infants

with PAE would show more parent-reported symptoms of: (1) dysregulated behaviors during daily routines; and (2) challenging temperamental characteristics.

## Methods

### *Participants*

Eighteen 6–15 month old infants and their biological mothers participated. Nine infants had moderate to heavy PAE and nine infants had no/low PAE. All procedures were approved by a hospital institutional review board. Participants in the PAE group were recruited through local women's substance abuse treatment centers via posted flyers. Interested participants contacted the study investigators directly and were not recruited by treatment center personnel. Inclusion criteria for infants with PAE were: (1) male or female; (2) all races/ethnicities; (3) 6–15 months; and (4) moderate to heavy PAE as determined by a Frequency-Binge Aggregate Score in excess of the cutoff categorizing them with moderate-heavy alcohol usage in a daily or binge pattern (F-BAS binge score ≥ 4 or F-BAS daily score ≥ 24; see Table 1) (Barr & Streissguth, 2001). The F-BAS score is derived from a maternal self-report screening questionnaire that measures addiction severity and gestational alcohol use, either in the month prior to pregnancy recognition or during any part of known pregnancy (Barr & Streissguth, 2001). A positive F-BAS score has shown 100% sensitivity and 90% specificity for identifying mothers whose children were ultimately diagnosed with a condition in the category of FASD. In this study, women had to meet the F-BAS criteria for high-risk drinking with alcohol reported as the primary substance abused prior to and during pregnancy. However, participants were not excluded if other drug use was reported during pregnancy. Mothers also had to self-report treatment enrollment or that they were in substance abuse recovery to participate in the study to control for current substance abuse.

Participants in the control group were recruited through posted and web-based advertisements in the local community. Interested families contacted the study investigators directly. Inclusion criteria for control infants were: (1) male or female; (2) all races/ethnicities; (3) 6–15 months; and (4) maternal report of complete absence of PAE in a planned pregnancy, with either abstinence or ≤ 2 drinks per week prior to pregnancy recognition. Exclusion criteria for both groups were: (1) premature birth (< 37 weeks gestation); (2) any congenital abnormalities; (3) any acute or chronic medical conditions affecting cardiac, respiratory, or neuromuscular systems at enrollment, and/or (4) experiencing more than two different home placements

**Table 1.** Sociodemographic Characteristics of Infants and Caregivers.

| | PAE | | | Controls | | |
|---|---|---|---|---|---|---|
| Characteristic | M (*SD*) | Lo | Hi | M (*SD*) | Lo | Hi |
| Child age (months), M(SD) | 10.7 (3.1) | 7.3 | 15 | 10.7 (2.9) | 7.3 | 15 |
| Female (%) | 67% | | | 44% | | |
| Maternal age (years) M (SD) | 32.6 (6.7) | 21.3 | 39.5 | 33.2(4.3) | 27 | 39.8 |
| Annual Income ≤40K[1*] | 100% | | | 0 | | |
| Maternal Ed. ≥ HS diploma (%)[1*] | 11% | | | 89% | | |
| Tobacco use[1*] | 44% | | | 0 | | |
| Any illicit drug use (%)[1*] | 77.5% | | | 0 | | |
| Methamphetamines (%) | 55.6% | | | 0 | | |
| Marijuana (%) | 44.4% | | | 0 | | |
| Cocaine (%) | 22.2% | | | 0 | | |
| Heroin (%) | 22.2% | | | 0 | | |
| Other opiates | 11.1% | | | 0 | | |
| DLC Total M (SD)[2*] | 8.0 (5.6) | 3 | 22 | 1.9 (0.8) | 1 | 3 |
| CESD Total[2], M(SD) | 12.0 (5.7) | | | 10.3 (3.5) | | |
| F-BAS binge score, M(SD)[2*] | 34.7 (38.9) | 4 | 120 | .1 (.1) | 0 | 0.8 |
| F-BAS daily score, M(SD) | 4.9 (7.9) | 0 | 24 | 3.9 (6.2) | 0 | 20 |

*Note*. DLC = Difficult Life Circumstances; CESD =Center for Epidemiological Studies- Depression Screen; F-BAS = Frequency-Binge Aggregate Score. The F-BAS score was derived from maternal self-report on screening questions that asked about the monthly frequency and amount of alcohol consumed (i.e., 1-2, 3-4 and 5 or more drinks) for the month prior to and/or during the pregnancy. (1) The F-BAS binge score is a sum of the monthly frequency of ≥ 5 drinks plus 1/4 of the monthly frequency of 3-4 drinks per occasion. (2) The F-BAS daily score reflects the monthly frequency of daily or almost daily drinking of only 1-2 drinks per occasion, amounts that do not meet the binge criteria. Mothers in the moderate to heavy exposure group had to have F-BAS binge scores of ≥ 4 or F-BAS daily scores of ≥ 24 for this study. *p ≤ .01, two-tailed; [1] Fisher exact test; [2] independent t test.

since birth or living with their current caregiver for < 50% of their chronological age.

## *Procedures*

The study was conducted at a hospital-based pediatric clinical research center and was part of a larger study that included an overnight sleep study to examine sleep and nighttime regulatory behaviors. Sleep study procedures were noninvasive and a mother was given the option to withdraw from daytime study procedures if she felt her infant was not able to proceed due to poor overnight sleep quality. All mothers and children completing the overnight sleep study participated in the current investigation. After the infant was awake for an hour or more, fed, and settled, a research assistant conducted the SFP (Tronick et al., 1978) with the mother-infant dyad. The SFP is a well validated and robust standardized developmental paradigm used to study infant affect and stress regulation (Mesman, Van Ijzendoorn, & Bakermans-Kranenburg, 2009). Infants were seated in a child safety seat with a tray and the mother was seated 18 inches from the child for the three 2-minute SFP episodes involving: (1) normal mother-child play interactions; (2) still-face episode, where the mother maintains a neutral face and refrains from

interacting with her child; and (3) reunion episode, where the mother resumes normal interaction with the child. The social emotional challenge of the still face, which departs from the caregiver's usual pattern of interaction with the infant, has been reported to effectively elicit behavioral and physiologic stress responses in children with and without PAE (Haley et al., 2006; Mesman et al., 2009).

### Physiologic Measure: Neuroendocrine Activity

Salivary cortisol, an index of HPA-axis regulation, is a noninvasive physiological measure of stress reactivity that has been widely used and validated among typical and high-risk samples of infants and children (Gunnar & Donzella, 2002; Gunnar & White, 2001). Salivary cortisol samples were taken at (T1) baseline; (T2) 15 minutes post-SFP; and (T3) 30 minutes post-SFP. Stress reactivity was measured by calculating the difference between T1 and T2. The difference between T1 and T2 corresponds to the expected 15–20 minute delay between the release of cortisol post stressor and its presence in saliva. The 30 minute post-SFP sample was expected to gauge the beginning of recovery to baseline levels (Kirschbaum, Wust, Faig, & Hellhammer, 1992). Saliva samples were collected from the infant's mouth using a small sorbette held by the parent or examiner for one minute. All baseline samples were taken approximately 1 hour after the infant awakened, between 7:06 a.m. and 8:09 a.m., to control for potential diurnal variation in cortisol levels. The baseline samples were taken just prior to initiating the SFP procedure. Samples were transported to the hospital laboratory, spun down, and analyzed in duplicates using the Salimetrics immunoassay kit (Salimetrics, LLC, State College, PA).

### Behavioral Measures: Affective Behaviors

Infant affective responses during the SFP were recorded and a research assistant masked to group status coded the infant behaviors using the Infant and Caregiver Engagement Phases system (ICEP; Weinberg & Tronick, 1999). The ICEP is a well-established scoring system that codes the following mutually exclusive infant behaviors: social positive engagement; negative engagement; social monitoring; and object/environment engagement. Behaviors were coded in 1-second intervals for each 2-minute episode using INTERACT software (Mangold, 2010).

Interrater agreement for the ICEP was established before coding the study sample (> 90%). Reliability was estimated by calculating the number of agreements within episodes and dividing that figure by the sum of total observations. Interrater agreement was checked with the first author on four randomly selected participants (two per group; both raters masked to group status) and maintained at > 90%.

## Caregiver Questionnaires

Mothers completed questionnaires to assess daily infant regulatory behaviors and pivotal caregiver and postnatal ecological risk factors.

### Revised Infant Behavior Questionnaire Short Form (IBQ-R; Gartstein & Rothbart 2003)

The IBQ-R measures temperament and behavior in infants 3–12 months across three categories: (1) surgency (activity level and engagement with environment); (2) negative affectivity, and (3) orienting/regulation. Strong validity is reported for these three IBQ-R broad factors (Cronbach's alpha = .91 to .92). Interrater agreement between primary and secondary caregivers is reported as moderate ($r$ = . 30–.71).

### Infant-Toddler Symptom Checklist (ITSC; DeGangi 1995)

The ITSC assesses symptoms of dysregulation for infants 7–30 months of age in areas of self-regulation, attention, sleep, feeding, dressing, bathing and touch, movement, and attachment/emotional function. Good construct validity is reported (DeGangi, 1995) and the ITSC accurately differentiates typical infants from infants with regulatory disorder. Appropriate age-specific ITSC versions were used.

### Center for Epidemiological Studies-Depression Screen (CES-D; Radloff 1977)

The CES-D is a widely used 20-item self-rating scale that measures maternal depressive symptoms. Strong internal consistency reliability (Cronbach's alpha = .85–.90) and good concurrent and construct validity have been reported.

### Difficult Life Circumstances Scale (DLC; Barnard, 1989)

The DLC is a 28-item self-report questionnaire that measured the frequency of maternal/family social problems and stressful life events. The DLC has adequate test-retest reliability ($r$ = .40–.70), construct validity, and concurrent validity.

## Data Analysis

Descriptive statistics were used to summarize sociodemographic, child physiological, and caregiver-reported child behavioral variables. $T$-tests were used to compare groups on continuous variables; Fisher exact tests were used to compare categorical variables. Two-tailed significance levels were set at $\alpha = 0.05$. The frequencies of affective behaviors for each of the four ICEP coding categories (negative engagement, positive engagement, social monitoring, and object/environment engagement) were summed and the mean

percentage of time each respective behavior occurred within each 2-minute SFP episode were examined. ICEP codes of protest and withdrawn behaviors were collapsed under the category of negative engagement. The Mann-Whitney U Test was used to compare the frequency of affective behaviors between groups for each SFP episode. Affective data from one control infant was dropped due to technical recording errors; SFP behavioral data from nine infants with PAE and eight controls were used.

## Results

### Maternal and Child Characteristics

Sociodemographic data for infants and caregivers are reported in Table 1. As expected, mothers of infants with PAE reported significantly higher alcohol usage during pregnancy, which was primarily seen as a binge pattern of drinking (as opposed to daily use). There were small amounts of alcohol consumption reported for control mothers, which emerged in the F-BAS daily scores and largely reflected social drinking prior to pregnancy recognition. Caregivers of children with PAE reported significantly more life stressors on the DLC as well as other drug (77%) and tobacco (44%) use. Controls had higher household income and education levels compared to the group with PAE. Mean CES-D scores for maternal depression were comparable between groups.

### Neuroendocrine Measures

Salivary cortisol levels by group at baseline, 15 minutes and 30 minutes post-SFP episode are shown in Figure 1. Infants with PAE had significantly higher cortisol levels at baseline [0.26 mg/dl compared to controls [0.11 mg/dl (.03 *SD*) $p < .05$]. The infants with PAE also had a significant decrease in cortisol levels from T1 to T2 [1.1 mg/dl (.09 *SD*)] compared to controls [.08 mg/dl (.04 *SD*) $p < .05$].

### Affective Behaviors

Figures 2–4 illustrate the mean percentage of affective behaviors observed within each 2-minute SFP episode by group. Described first are findings from visual inspection of the affective behavior frequencies before, during, and after the stressor. Significant group differences are reported in the last section.

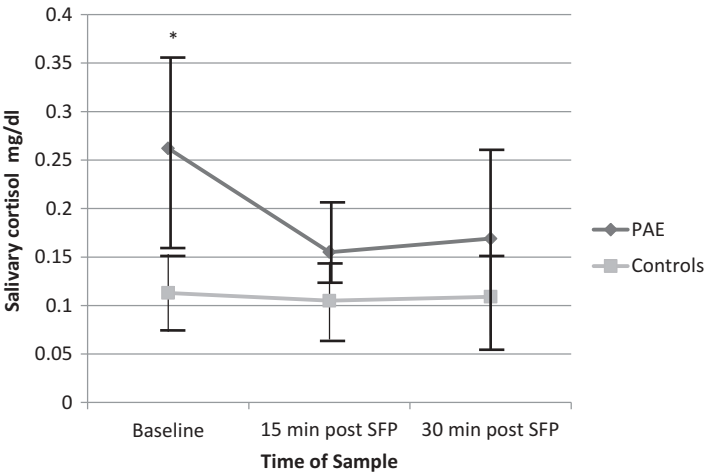


**Figure 1.** Salivary cortisol levels at baseline, 15 minutes post and 30 minutes post SFP stressor by group; * $p \leq .05$, two-tailed.

### *Play Episode*

Visual inspection showed that infants with PAE showed more negative affect and object/environment engagement than controls, who showed more social monitoring and positive engagement behaviors.

### *Still-Face Episode*

On visual inspection, both groups showed an increase in negative engagement and a decrease in positive engagement. The infants with PAE maintained a higher frequency of negative affective behavior with minimal change in object/environment engagement, while infants in the control group showed an increase in object/environment engagement behaviors.

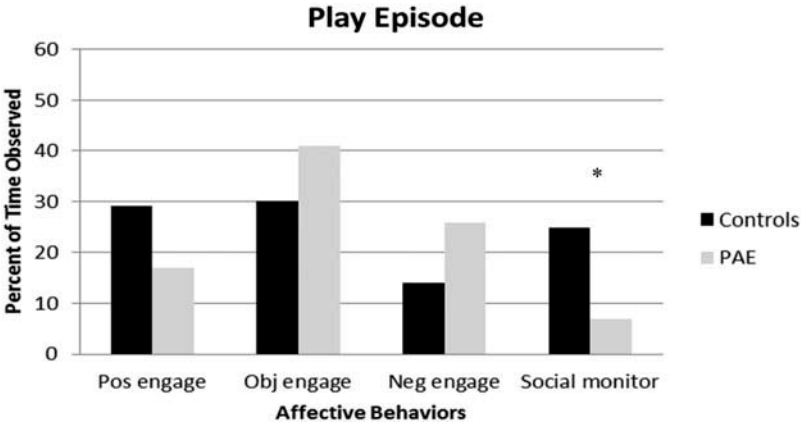


**Figure 2.** Percentage of Coded Affective Behaviors Observed During SFP Play Episode (PAE N=9; Controls N=8)* $p \leq .05$.

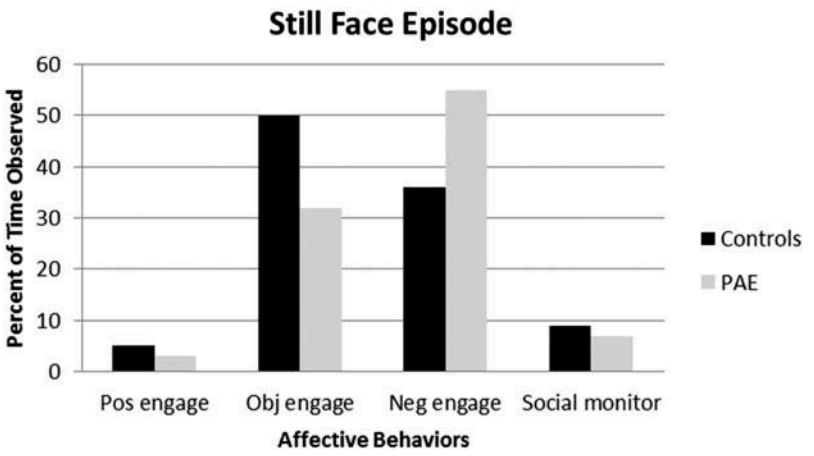


**Figure 3.** Percentage of Coded Affective Behaviors Observed During Still-face Episode (PAE N=9; Controls N=8).

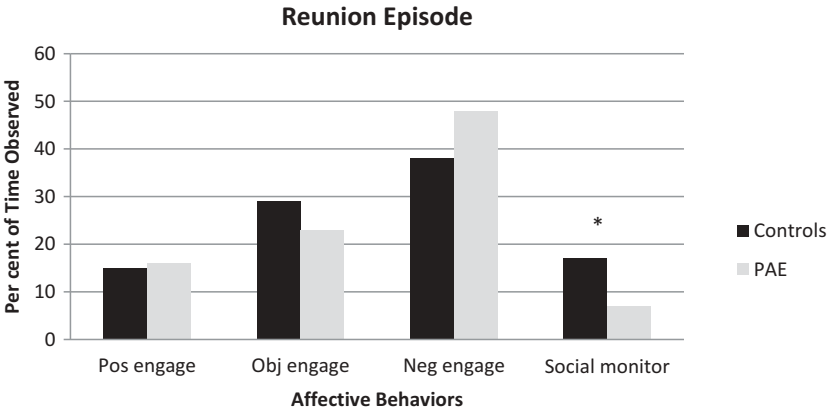


**Figure 4.** Percentage of Coded Affective Behaviors Observed During SFP Reunion Episode (PAE N=9; Controls N=8) * $p \leq .05$.

### *Reunion Episode*

Visual inspection revealed that both groups showed increased positive engagement and object/environment engagement, coupled by decreased negative affect. However, the still-face effect remained evident for both groups in that the predominant behavior was infant negative engagement.

### *Significant Group Differences*

Infants with PAE showed significantly fewer social monitoring behaviors than controls ($p < .05$) during both play and reunion episodes. The proportion of social monitoring behaviors for the infants in the high-risk PAE group stayed relatively consistent across each SFP phase.

**Table 2.** Descriptive Statistics: Caregiver Reported Regulatory Behaviors and Temperament Characteristics.

| | PAE | | | Control | | |
|---|---|---|---|---|---|---|
| Measure | M (*SD*) | Lo | Hi | M (*SD*) | Lo | Hi |
| ITSC Total[1] | 8.2 (8.5) | 0 | 26 | 4.9 (4.2) | 0 | 12 |
| IBQ-R Total[2] | 25.0 (4.6) | 19 | 31 | 23.9 (3.6) | 15 | 31 |
| Surgency | 5.1 (0.4) | 4 | 6 | 5.0 (0.6) | 4 | 6 |
| Negative affect | 3.8 (0.4) | 2 | 5 | 3.9 (0.6) | 3 | 4 |
| Orienting/regulation | 5.1 (0.4) | 4 | 6 | 5.1 (0.5) | 4 | 5 |

*Note*.[1] ITSC =Infant Toddler Symptom Checklist raw score; [2]IBQ-R = Infant Behavior Questionnaire-Revised raw score.

### *Caregiver-Reported Regulatory Behaviors*

Descriptive statistics and group comparisons of caregiver-rated regulatory behaviors are presented in Table 2. Group differences between maternal ratings of infant temperament and behavior on the IBQ-R, and symptoms of regulatory disorder on the ITSC, were not significant.

## Discussion

This small-sample exploratory study examined regulatory behaviors and stress reactivity among infants with moderate to heavy PAE (at high risk for FASD) in comparison to infants with no/low PAE. Regulatory behaviors and stress reactivity were measured with physiological, behavioral, and parent-reported outcomes. Physiologically, the infants with high-risk PAE had significantly higher baseline salivary cortisol levels compared to low-risk infants, although they did not show elevation in cortisol levels as an indicator of heightened reactivity after the stressor. From a behavioral perspective, an important but unexpected finding was that the infants with high-risk PAE showed significantly fewer social monitoring behaviors when observed during the stress paradigm, suggesting less orientation to or attunement to their caregiver's faces and social cues. There were no significant group differences on caregiver-reported measures of child temperamental characteristics or day-to-day regulatory symptoms. Results need confirmation with a larger sample, and statistical adjustment for the complex prenatal and postnatal factors often co-occurring with PAE is indicated. Nevertheless, current findings demonstrated that infants with moderate to heavy PAE had some bio-behavioral indicators, consistent with previous human and animal studies reporting physiological (HPA) dysregulation and early affective differences in young children, which place them at heightened risk for early regulatory problems.

### Neuroendocrine Outcomes and Implications

We hypothesized that infants with PAE would show greater cortisol reactivity in response to the stressor than the comparison group. Physiological differences were evident at baseline. Infants with moderate to heavy PAE displayed significantly higher cortisol levels than controls. Similarly, elevated pre-stressor (basal) cortisol levels have been described by Ramsay et al. (1996) and Jacobson et al. (1999) in 2- and 6-month-old, and 13-month-old, infants with PAE, respectively. Because increased distress and negative affect were observed through visual inspection of data during the SFP play episode before the social stressor, it is possible that pre-experimental conditions induced a stress response in the children with moderate to heavy PAE.

We did not find group differences in cortisol reactivity between baseline (T1) and post-stress (T2) levels. The lack of expected increase in cortisol reactivity from pre- to post-stressor could be explained by blunted HPA response. Hyporeactivity of the adrenocortical system has been reported in alcohol-exposed neonates and very young infants in response to pain stimuli (i.e., heel stick) (Oberlander et al., 2010; Ramsay et al., 1996). A vigorous cortisol response has been associated with more optimal functioning in children with typical development. However, blunted response patterns may serve an adaptive or compensatory purpose in response to early adversity (Oberlander et al., 2010).

Alternatively, the SFP may not have sufficiently provoked a physiological stress response, since neither group showed the expected increase in post-stressor cortisol levels (Gunnar, Talge, & Herrera, 2009). Our findings contrast with those of Haley et al. (2006), who used a younger cohort of infants and a modified SFP. The modified SFP had two still-face episodes, which provided a more intense stressor than the single still-face episode used in the current study. Procedures to elicit and measure acute physiological stress responses in infants and young children are widely used in research. However, outcomes are variable and reported to be sensitive to age and developmental factors (Gunnar et al., 2009). A larger two-group longitudinal study using more intense, developmentally adjusted stressors and carefully controlled pre-experimental conditions would be important considerations for future studies that aim to examine physiological regulation in very young children with high-risk PAE.

### Clinical Outcomes and Relevance to Caregiver-Child Interactions

Compared to controls, we expected that infants with PAE would be observed to show more frequent negative affect as a behavioral indicator of stress from the SFP. While visual inspection of data did reveal that negative affect predominated among children with PAE across all three SFP episodes

(play, still-face, reunion), differences were not statistically significant in this small sample. An intriguing finding in our study was that infants with PAE showed significantly fewer social monitoring behaviors during play and reunion episodes. Social monitoring behaviors were coded when the infant's attention was directed toward the caregiver, and when the infant was looking at the adult's face with a neutral or interested facial expression and neutral or positive vocalizations. These findings suggest that the infants with PAE were less attuned or oriented to caregiver faces or social cues during typical social interactions, which could be indicative of poorer maternal-child attunement and relationship quality in this vulnerable group.

Our findings are congruent with previous studies reporting associations between PAE and increased negative child affect and less-secure infant attachment (O'Connor, Sigman, & Brill, 1987; O'Connor et al., 2002). Increased emotional withdrawal, as measured by early social responsiveness behaviors (e.g., eye contact, facial expression, activity level) has also been reported in a cohort of heavily exposed South African infants. Notably, these early affective behaviors showing patterns of emotional withdrawal discriminated between children who later were diagnosed with FAS/PFAS. Infant withdrawal also predicted IQ at age 9 years, beyond social environmental factors such as maternal sensitivity and interaction quality (Molteno, Jacobson, Carter, Dodge, & Jacobson, 2014).

In contrast to our physiological and affective findings, no significant group differences were found on caregiver questionnaires. Temperament characteristics as measured by the IBQ-R, and symptoms of dysregulation as assessed by the ITSC, were similar between groups. The lack of statistically significant differences on these measures may be due to the small sample size, or reflect limitations of caregiver reported data, which can be subject to bias. Since measures relying on caregiver report are used often in the clinical evaluation of young children, study findings highlight the need to go beyond caregiver report and include objective, bio-behavioral measures of early regulatory development.

For young children with moderate to heavy PAE, our study results underscore the value of dyadic observations of child affective responses and maternal caregiving behaviors (coregulatory influences) to augment the usual clinical practice of administering standardized assessments plus parent questionnaires assessing behavior regulation. Mother-child interaction data from this study, reported elsewhere (Nash, 2013), revealed that mothers in both groups engaged in positive ways with their children. However, group differences in maternal behaviors reflecting sensitivity and responsiveness (e.g., acknowledging, parent supportive presence, and positive affect) were present. Mothers of infants with moderate to heavy PAE showed lower rates of these behaviors. Understanding the dynamics of coregulation, and pinpointing relational

challenges, could generate ideas of intervention tailored to this population. Maladaptive caregiving practices and interaction can undermine adaptive regulatory development, while responsive and sensitive interactions can enhance early coping, resiliency, and attachment security (Calkins & Hill, 2007; Treyvaud et al., 2009). Contemporary infant mental health and relationship-focused approaches that build on strengths and "coach" caregivers to use positive parenting strategies and interactive behaviors have shown promise with high-risk children and families, and are recommended for this clinical population (Olson & Montague, 2011).

Caregiver-child relationships are a central, developmentally meaningful context for understanding early regulatory processes and behavior (Calkins & Hill, 2007). However, examining infant stress reactivity within a relational context has inherent challenges. This is especially true for mothers with substance abuse histories. Mothers who reported high levels of alcohol use also reported poly-drug and tobacco use, more life stress, and lower levels of education and income. Cumulative risk is common within this population (Grant et al., 2011). Early adversity plus substance exposure has additive, negative effects on children's regulatory and developmental trajectories (Fisher, Kim, Bruce, & Pears, 2012). It is clear that the group of infants with moderate to heavy PAE had exposure levels that placed them at high risk for FASD, but they were also being raised in environments with potent socio-ecologic risk factors, which can render additional adverse effects on regulatory development.

These co-occurring social risk factors limit our ability to attribute current findings directly to the teratogenic effects of PAE. That would require a larger study in which covariate adjustment could be carried out. But, our study findings do illustrate the impact of cumulative risk on early development. A body of animal studies has clearly linked PAE to altered regulatory and neurobehavioral functions (Schneider, Moore, & Adkins, 2011), and primate models show that additional exposure to prenatal stress exacerbates the negative effects of PAE. Collectively, our data show very clearly that aspects of all factors involved in this interplay of regulatory development (Mayes, 2000)—infant affective responses, physiological reactivity, ecological factors—were different for heavily exposed infants compared to controls.

## Study Limitations

Current study findings should be considered exploratory and are limited by small sample size. Studies with larger sample size, stratified to account for co-occurring risk factors, could account for expected ecological confounders and dynamic influences on infant regulatory development. The dual purpose of the larger study and resources needed to conduct a comprehensive sleep

study also limited sample size for the current exploratory study of stress reactivity and infant regulatory capacity. Despite excellent interrater agreement for the ICEP, interrater reliability could have been obtained on additional children, which is another study limitation. Administration of a stress paradigm (SFP) after an overnight sleep study presented a potential confound for the current study. However, there were no statistically significant correlations between sleep and "daytime" bio-behavioral outcomes, even though findings revealed differences in physiological sleep quality among the infants with PAE compared to controls (Chen, Jirikowic, Gendler, & Olson, 2011).

## Conclusions

Findings of the current study revealed that infants with carefully documented levels of moderate to high levels of PAE showed different and less adaptive affective and physiological alterations in their response to stress when compared to a low-risk control group. However, because of co-occurring early adversity, it is difficult to draw firm conclusions about the relationship of PAE to group differences reported in this study. Results show that further study of behavior regulation, as a vulnerable developmental domain, is warranted. Methods and procedures used in this exploratory study were feasible, and ways to refine a larger study were identified. Neuroendocrine responses, social monitoring behaviors, and negative affectivity should be primary targets for further study. Early identification and intervention can capitalize on neuroplasticity and build critical protective factors (e.g., responsive caregiving) to buffer early adversity and help ameliorate early regulatory challenges. Therefore, it is vital to continue clinical and research attention to very early regulatory processes among children with PAE, who may be at high risk for the lifelong developmental disabilities of FASD.

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