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Prenatal Alcohol Exposure Alters Biobehavioral Reactivity to Pain in Newborns

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Abstract

Objectives—To examine biobehavioral responses to an acute pain event in a Cape Town, South Africa, cohort consisting of 28 Cape Colored (mixed ancestry) newborns ($n = 14$) heavily exposed to alcohol during pregnancy (exposed), and born to abstainers ($n = 14$) or light (0.5 oz absolute alcohol / d) drinkers (controls).

Methods—Mothers were recruited during the third trimester of pregnancy. Newborn data were collected on postpartum day 3 in the maternity obstetrical unit where the infant had been delivered. Heavy prenatal alcohol exposure was defined as maternal consumption of at least 14 drinks / wk or at least 1 incident of binge drinking / mo. Acute stress-related biobehavioral markers [salivary cortisol, heart rate (HR), respiratory sinus arrhythmia (RSA), spectral measures of heart rate variability (HRV), and videotaped facial actions] were collected thrice during a heel lance blood collection (baseline, lance, and recovery). After a feeding and nap, newborns were administered an abbreviated Brazelton Neonatal Behavioral Assessment Scale.

Results—There were no between-group differences in maternal age, marital status, parity, gravidity, depression, anxiety, pregnancy smoking, maternal education, or infant gestational age at birth (all $ps > 0.15$). In both groups, HR increased with the heel lance and decreased during the postlance period. The alcohol-exposed group had lower mean HR than controls throughout, and showed no change in RSA over time. Cortisol levels showed no change over time in controls but decreased over time in exposed infants. Although facial action analyses revealed no group differences in response to the heel lance, behavioral responses assessed on the Brazelton Neonatal Scale showed less arousal in the exposed group.

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Conclusions—Both cardiac autonomic and hypothalamic–pituitary–adrenal stress reactivity measures suggest a blunted response to an acute noxious event in alcohol-exposed newborns. This is supported by results on the Brazelton Neonatal Scale indicating reduced behavioral arousal in the exposed group. To our knowledge, these data provide the first biobehavioral examination of early pain reactivity in alcohol-exposed newborns and have important implications for understanding neuro- / biobehavioral effects of prenatal alcohol exposure in the newborn period.

Keywords

Prenatal Alcohol Exposure; Neonatal Pain; Stress Reactivity; Cortisol; Brazelton Behavioral Assessment

The Developmental and neurobehavioral consequences of prenatal alcohol exposure have been the focus of substantial research since fetal alcohol syndrome (FAS) was first described over 30 years ago (Hoyme et al., 2005; Jones and Smith, 1973; Stratton et al., 1996). Beyond the well-documented alterations in cognitive capacity, convergent evidence from preclinical and human infant studies has linked prenatal alcohol exposure to alterations in attention, arousal regulatory capacities, and stress reactivity (Coles et al., 1997; Fifer et al., 2009; Haley et al., 2006; Olson et al. 1998). For example, performance on the Neonatal Behavioral Assessment Scale (Brazelton, 1984) reflected poorer attention and reduced arousal in alcohol-exposed than control infants in response to examiner manipulation during an assessment of neonatal reflexes and presentation of animate and inanimate stimuli (Jacobson et al., 1984; Smith et al., 1986; Streissguth et al., 1983). Lower heart rate variability (HRV) and blunted cardiac autonomic responses to a tilt table maneuver have recently been reported in alcohol-exposed neonates (Fifer et al., 2009). Prenatal alcohol exposure was also related to an increased time to cardiac deceleration in response to auditory and visual stimuli at 6 to 7 months of age (Kable and Coles, 2004), suggesting a deficit in the initiation of attention (orienting response), and thus impaired neurophysiological encoding of information. Interestingly, these infants were also rated as having higher arousal levels, suggesting a link between arousal level and attentional regulation.

Convergent evidence from preclinical and human studies also points to altered hypothalamic–pituitary–adrenal (HPA) axis reactivity following prenatal alcohol exposure, which may contribute to some of the neurobehavioral deficits that have been observed (Haley et al., 2006). For example, Jacobson and colleagues (1999) demonstrated that moderate to heavy drinking at conception and during pregnancy and higher scores on the Michigan Alcoholism Screening Test (MAST) were associated with higher basal and poststress (blood draw) cortisol concentrations in 13-month-old infants, after controlling for potential confounders including cocaine use and cigarette smoking during pregnancy. Similarly, infants exposed in utero to alcohol and / or cigarettes exhibited significantly increased basal cortisol levels at 2 months and a trend for increased basal levels at 6 months, and did not show the normal cortisol increase following inoculation stress shown by controls (Ramsay et al., 1996). Recently, Haley and colleagues (2006), using a modified “still face” procedure (a standardized paradigm to study emotion and stress regulation) in 5-to 7-month-old infants, found that greater prenatal alcohol exposure was associated with greater cortisol reactivity, negative affect, and elevated HR.

Consistent with the human data, findings from several groups using animal models have linked prenatal alcohol exposure to alterations in neuroendocrine activity and regulation. In rodent models, data indicate that early in life, prior to weaning, fetal alcohol-exposed offspring exhibit blunted HPA and β -endorphin responses to a wide range of stressors including ether, novelty, saline injection, and cold stress (Angelogianni and Gianoulakis, 1989; Taylor et al., 1986; Weinberg, 1989; Weinberg and Wong, 1986). In contrast, in adulthood, fetal alcohol-exposed offspring are typically hyperresponsive to stressors with increased levels of the stress hormones and delayed or deficient recovery to basal levels (Glavas et al., 2007; Kim and Druse, 1996; Lee et al., 1990; Osborn et al., 1996; Redei et al., 1993; Taylor et al., 1988; Weinberg et al., 2008). Similar findings were reported in a rhesus monkey model following the stress of maternal separation (Schneider et al., 2004). Timely, appropriate response and regulation of the HPA axis is a critical component of the body's stress response system. Thus, altered HPA development and regulation is thought to be a risk marker for cognitive deficits (Lupien et al., 1994), emotional disturbances (Smider et al., 2002), and psychopathology (Holsboer et al., 1995).

One common element that may provide a key link between prenatal exposure to early adverse conditions, such as drugs and alcohol, and altered stress reactivity is the role of the biogenic amine neurotransmitter serotonin (5-HT; Azmitia, 2001). Beyond its role as a neurotransmitter-regulating arousal, mood, and cardiovascular function, it plays a critical role as a neurotrophic signal directing ontogeny of the 5-HT system itself as well as other neurochemical systems in the developing brain (Azmitia, 2001). In animal models, prenatal alcohol exposure decreases concentrations of 5-HT or its metabolites during fetal life and in weanling animals (Druse et al., 1991; Rathbun and Druse, 1985) and alters 5-HT transmission (Zafar et al., 2000). In addition, prenatal alcohol exposure is associated with physiological and behavioral abnormalities consistent with altered 5-HT function, reflected by a lack of response inhibition (Fryer et al., 2007; Kodituwakku et al., 1995; Riley et al., 1979), increased anxiety and aggression (Jacobson et al., 2006; Olson et al., 1998), and decreased thermoregulatory ability (Hofmann et al., 2002). Moreover, altered hypothermic and HPA responses to 5-HT_{1A} receptor agonists suggest an altered interaction between the HPA axis and the 5-HT system (Hofmann et al., 2002; Sliwowska et al., 2008). Prenatal alcohol effects on the development and activity of the 5-HT system and / or on the normal developmental interactions between the 5-HT system and the HPA axis could influence the developing pain system and alter neonatal pain responses.

The cardiovascular system offers a unique insight into neurodevelopmental systems that link 5HT and early human experience, and has been widely used to study neonatal vulnerability (Porges, 1992). HR is a final common expression of multiple physiologic processes that reflect central nervous system (CNS) function, autonomic control mechanisms, metabolic activity, thoracic hemodynamics, and cardiac chemoreceptors and baroreceptors, as well as levels of arousal and levels of activity. As a reflection of these multiple processes, HR is a particularly well-suited measure for studies of pain reactivity in infancy, when biobehavioral distress is often indicated by nonspecific physiological and behavioral arousal. At a neuroanatomic level, there is convergent evidence demonstrating that systems which control cardiovascular function are also closely coupled to systems that modulate the perception of pain. Importantly, activation of vagal, cervical, thoracic, or abdominal afferents can both

facilitate and inhibit nociception (Randich and Maixner, 1984) and have been widely used to assess responses to painful stimuli.

Variability in HR is mediated primarily by changing levels of parasympathetic and sympathetic outflow from the CNS to the sino-atrial node at the heart. The HR power spectrum typically contains a peak at the respiratory frequency representing respiratory sinus arrhythmia (RSA) and peaks again at lower frequencies between 0.01 and 0.15 Hz. Studies using selective pharmacologic blockade of the cardiac sympathetic and parasympathetic receptors have shown that fluctuations in HR above 0.15 Hz are mediated exclusively by changes in parasympathetic activity, whereas lower frequency changes are mediated jointly by changes in parasympathetic and sympathetic efferent activity (Saul et al., 1991). Most of the work investigating autonomic modulation of HR has focused on variations in HR that occur with respiration—the RSA (i.e., 0.15 to 1.0 Hz)—and has led to the suggestion that infant HRV associated with the RSA (often termed vagal tone) may be a measure of individual differences in stress responsiveness (Fox and Porges, 1985; Porter et al., 1988).

Given these findings, it is conceivable that prenatal alcohol exposure—possibly via changes in central 5HT levels—influences critical 5-HT- and / or HPA-related functions during fetal ontogeny, which may subsequently be expressed as altered patterns of neonatal pain responses and / or HPA stress reactivity. To further our understanding of the antecedents of altered stress regulation in alcohol-exposed infants, we undertook the following study to examine relations between heavy prenatal alcohol exposure and biobehavioral responses to an acute noxious event (heel lance) in the first few days of life. We also examined whether such pain responses were associated with patterns of early neurobehavioral development.

METHODS

Sample

Our cohort was comprised of 28 infants—14 heavily exposed (designated “exposed”), 14 born to abstainers or 1–0.5 oz / d during pregnancy) drinkers (designated “controls”)—from the Cape Colored (mixed ancestry) population in Cape Town, South Africa, in which very heavy alcohol use during pregnancy (Croxford and Viljoen, 1999) and a high prevalence of FAS (May et al., 2000) have been documented. Women were recruited during the third trimester of pregnancy by a research nurse at a maternal obstetric unit (MOU), an outpatient clinic that provides antenatal care to a predominantly Cape Colored population and where most of the infants are delivered. This population, descendants of white Europeans, Malaysians, Khoi-San, and black Africans has historically comprised the large majority of workers in the wine-producing and fruit-growing region of the Western Cape. The heavy maternal drinking during pregnancy and subsequent high prevalence of FAS in this community (Croxford and Viljoen, 1999) is a consequence of poor psychosocial circumstances and the traditional *dop* system, in which farm laborers were paid, in part, with wine. Although the *dop* system has been outlawed since the 1920s, regular and heavy alcohol consumption persists in both the urban and rural Cape Colored communities (Carter et al., 2005; Jacobson et al., 2006, 2008).

Mothers were interviewed regarding their alcohol consumption during pregnancy, using an interview derived from the timeline follow-back approach to determine incidence and amount of drinking on a day-by-day basis (Jacobson et al., 2002; Sokol et al., 1985). Any woman averaging at least 1.0 oz absolute alcohol per day (AA / d), the equivalent of 2 standard drinks / d or 14 drinks / wk, or reporting at least 1 incident of binge drinking / mo (4 or more standard drinks / occasion), was invited to participate in the study. A control group of women who drank <0.5 oz AA / d (<7 drinks / wk) and did not binge drink was also recruited. Twelve of the 14 (85.7%) in this control group were abstainers. Women <18 years of age and those with diabetes, epilepsy, or cardiac problems requiring treatment were excluded. Religiously observant Muslim women were also excluded because their religious practices prohibit alcohol consumption, and they would, therefore, have been disproportionately represented in the abstaining alcohol group. Infant exclusionary criteria were congenital heart defects, major chromosomal anomalies, neural tube defects, multiple births, and seizures. Three women were invited but refused: 2 whose partner / husband refused to have them participate and 1 who was sent to the hospital for delivery.

Informed consent was obtained from each mother at recruitment. Approval for human research was obtained from the University of British Columbia Research Ethics Board, the Children's and Women's Health Centre of British Columbia (C&W) Research Review Committee, the Wayne State University School of Medicine Human Investigation Committee, and the University of Cape Town Research Ethics Committee of the Health Sciences Faculty.

Maternal Alcohol and Drug Ascertainment

In the timeline follow-back interview administered at recruitment, the mother was asked about her drinking on a day-by-day basis in a typical week during pregnancy, with recall linked to specific times of day and activities. Volume was recorded for each type of alcohol beverage consumed each day and converted to oz of AA using multipliers proposed by Bowman and colleagues (1975; liquor—0.4, beer—0.04, wine—0.2). Three summary measures were constructed—average oz AA / d, AA per drinking day (quantity per occasion), and proportion of drinking days (frequency). Women were also asked how often they drank 4 or more drinks / occasion (the equivalent of a binge) during pregnancy and were administered the alcohol module (NAWS) of the Alcohol Use Disorder and Associated Disability Interview Schedule—DIS-IV Version (AUDADIS-IV; Grant et al., 2001), which assesses whether the respondent has a history of alcohol abuse or dependence. All of the mothers were asked how many cigarettes they smoked per day and how many d / wk they used marijuana, methaqualone (mandrax), cocaine, methamphetamine (tik), or any other drugs during pregnancy. Mothers were also administered the Beck Depression Inventory (BDI; Beck et al., 1961), the Spielberger State and Trait Anxiety Scales (Spielberger, 1972), and the Depression module from the Structured Clinical Interview for DSM-IV (SCID; First et al., 1995) during an interview conducted by the research nurse.

Outcome Measures

Data from the 28 newborns were collected on postnatal day 3 at the same MOU where the mothers had been recruited and had delivered the infants. Neonatal pain reactivity and stress-

related biomarkers were assessed in response to a heel lance blood collection (phenylketonuria screen) during 3 phases: baseline, lance, and recovery. Each infant was held on his or her mother's lap during the procedure in an awake, alert, noncry state (Brazelton, 1984), and all the procedures were videotaped.

Cardiac Autonomic Responses

Analyses of short-term variations in HR were used to examine the cardiac autonomic responses to the acute noxious event as previously described (Oberlander et al., 1999, 2000, 2002). Continuous electrocardiographic (ECG) recordings (BioPac, Goleta, CA) yielded RR intervals from which HR and spectral power of HRV were tabulated to yield measures of low frequency power (LFP, 0.04 to 0.15 Hz), high frequency power (HFP, 0.15 to 0.8 Hz), and the low-to-high frequency power ratio (an indirect index of the sympathetic / vagal balance; Oberlander et al., 2000). In addition, from the interbeat interval (IBI), the RSA was obtained to reflect variations in HR that occur with respiration (i.e., 0.15 to 1.0 Hz) and used to quantify measures of parasympathetic cardiac modulation. HR signals were collected, sampled, and normalized, as described previously (Oberlander et al., 1999, 2000). HR epochs of 60 seconds were selected for analysis from a resting baseline period within 3 minutes before the lance, a lance period starting within 20 seconds following the lance, and a recovery period within 20 seconds following the end of handling by the blood-collecting technician. Epoch selection criteria were based on quantitative assessment of signal stability and the absence of gross movement artifact, as previously described (Oberlander et al., 1996, 2000).

Salivary Cortisol

Neonatal saliva was collected at 3 time points: immediately before the heel lance, 20 minutes after the heel lance, and 40 minutes after the heel lance. The infant was not fed until after the second (40 minutes) postheel lance cortisol collection. The feeding almost always put the infant to sleep. We then waited for the infant to wake up before administering the Neonatal Behavioral Assessment Scale (NBAS) testing, which typically occurred approximately 60 minutes after the heel lance. Maternal interviews took place before the second cortisol collection and / or while the baby was sleeping. Sometimes the maternal interview was conducted even before the test, while the mother was waiting for everything to be setup.

All saliva samples were collected by our research nurse with a dental roll (Sullivan Dental Products, St Laurent, Quebec) placed in the infant's mouth for 3 to 4 minutes—the first usually on arrival at the MOU and then 20 and 40 minutes following the lance. Saliva was then expressed into a vial and stored at -20°C . All the samples were shipped as a single batch to J. Weinberg at the University of British Columbia, where they were assayed using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit for quantitative determination of salivary cortisol (Salimetrics LLC, Philadelphia, PA). Intra- and interassay coefficients of variation were 2.92 and 3.41%, respectively.

Behavioral Data Acquisition

The infant's face was video recorded during the baseline, lance, and recovery periods, and facial activity was assessed using the Neonatal Facial Coding System (NFCS; Grunau and Craig, 1987). NFCS-trained coders, masked to the participant's prenatal exposure and all information about the infant, coded the presence or absence of a discrete set of precisely defined facial actions: brow bulge, eye squeeze, nasolabial furrow, open mouth, vertical mouth stretch, horizontal mouth stretch, and taut tongue. These 7 facial actions were selected because they have been associated with reactivity to nociceptive procedures in infants up to 18 months of age (Craig et al., 1993; Grunau and Craig, 1990; Grunau et al., 1998; Lilley et al., 1997). Facial actions during the last 20 seconds of baseline, the first 20 seconds immediately following the lance, and the first 20 seconds following last contact by the technician (recovery) were coded in random order, 0 (did not occur) or 1 (occurred) during 2-second segments for each 20-second phase. The videotapes were coded by 1 examiner reaching inter-rater reliability during training of greater than 0.85, using the conservative reliability formula described previously (Grunau and Craig, 1987).

Following the pain reactivity procedures, the infant was breast or bottle fed by the mother and fell asleep for about 1 hour. During this time, maternal interviewing was completed. An abbreviated Brazelton (1984) NBAS, a widely used tool for neonatal behavioral examination, was subsequently administered by C. Molteno, after the mother completed the background interviews. Sixteen of 27 NBAS behavioral scales and 6 of the 17 reflexes (hand grasp, automatic walking, asymmetrical tonic neck reflex, Moro, rooting, and sucking reflexes) were assessed. The behavioral scales included 4 orientation scales, general tone and motor maturity, consolability, activity, tremulousness, lability of skin color, self-quieting activity, quality of alert responsiveness, general irritability, robustness and endurance, regulatory capacity, and state regulation. In addition, passive muscular tone scoring from the scale of Dubowitz and colleagues (1970) was used to evaluate the upper limbs; recoil and scarf lower limbs; recoil and popliteal angle body axis; ventral incurvation and dorsal incurvation head control; and pull-to-sit, posterior and anterior. Two subscales particularly relevant to pain reactivity, self-quieting, and consolability, are 9-point scales assessing degree to which the newborn uses activities to quiet himself / herself in response to distress or responds to the examiner's efforts to bring the infant back to an alert and quiet state.

All newborn assessments, videotaped scoring, and salivary analyses were conducted by examiners who were blind with respect to prenatal alcohol exposure status and other background characteristics.

Data Reduction

The mean and standard error (SEM) of the HR and power spectra for each data segment were calculated. Cortisol levels were log-transformed to ensure a normal distribution. To examine the pattern of facial response across phase, the number of each of the 7 NFCS facial actions occurring during each phase was calculated within each of ten 2-second segments to yield 10 facial scores, each with a possible range of 0 to 7. In addition, the scores were summed for each event, with a possible range from 0 to 70. When the infant's face was out of view, missing data for that event was replaced with the value (0 or 1) immediately

preceding or following the missing cell, where possible, or the mean for that segment. Data was replaced for 3.5% of the cells.

Statistical Analysis

Univariate analysis was used to assess demographic group differences. The cardiac autonomic and facial action data were analyzed by a group (heavy parentally alcohol exposure vs. control) by phase (baseline, lance, recovery)-repeated measure of analysis of variance (ANOVA) to compare outcomes across study periods, controlling for appropriate covariates (birth weight, gender), where appropriate. Post hoc comparisons were conducted where appropriate, and a difference was considered statistically significant for $p < 0.05$. As a result of the small quantities of saliva produced by the newborn, cortisol levels were available for only 12 newborns at baseline, 17 at lance, and 16 at recovery, and data were available for only 7 infants for all 3 time periods. The cortisol data were, therefore, analyzed separately for each time point using nonparametric ranked sum tests. As the number of women who used methamphetamine (tik) was too small ($n = 3$) for this variable to be controlled statistically, analyses were conducted both with and without methamphetamine-exposed infants. Omission of the 3 methamphetamine-exposed infants did not alter any of the findings.

RESULTS

Demographic characteristics of the neonates and their mothers are summarized in Table 1. The heavy alcohol-consuming mothers did not differ from the controls in age at delivery, marital status, or education. Depression and anxiety scores were elevated in both groups, with 38.5% in the exposed and 33.3% of women in the control group reporting moderate to severe depression. None of the women used psychotropic or antidepressant medication during pregnancy, but 3 mothers (1 exposed and 2 controls) had scores on the BDI in the “severely depressed” category (range = 29 to 51) and had a positive diagnosis for major depression on the SCID. All 3 were referred to the University of Cape Town community psychiatric clinic.

All the infants were full-term (gestational age or GA = 37 weeks) with the exception of 1 exposed (36 weeks) and 2 controls (36 and 36.6 weeks, respectively). Five infants weighed <2,500 g: 4 exposed (1,900 to 2,340 g) and 1 control (2,460 g). As expected, prenatal alcohol exposure was associated with lower birth weight.

Alcohol use in the exposed and control groups is summarized in Table 2. Women in the exposed group drank as many as 7.8 drinks per occasion on 1 to 2 d / wk, whereas women in the control group reported drinking 1.0 drink / occasion on an average of <1 occasion during pregnancy ($M = 0.84$ occasions). Data from the NAWS indicated that 8 women in the exposed group had a history of alcoholism as contrasted to only 1 control mother, $\chi^2(1) = 7.67$, $p = 0.006$. The 8 alcohol-dependent or abusing women were current drinkers, whereas the control woman reported that she had stopped drinking 13 years ago. Although the number of cigarettes smoked per day did not differ between groups, cigarette use was more common among mothers in the exposed group, with 12 / 14 (85.7%) smoking as compared with only 6 / 14 (42.9%) in the control group, $\chi^2(1) = 5.60$, $p < 0.025$. None of the mothers

reported using cocaine, heroin, sedatives, or depressants. Only 1 reported ever using methaqualone (mandrax), 1 year prior to the pregnancy. Three mothers reported using methamphetamine (tik): 2 from the exposed group used it for 2 to 3 d / wk across pregnancy and 1 control mother used it daily for the first 25 weeks of pregnancy. Five mothers reported using marijuana: 1 mother in the alcohol-exposed and 1 in the control group used it for 1 d / wk; 2 in the exposed and 1 in the control group, 3 to 4 d / wk. Marijuana use did not differ between groups.

Physiologic Data

Owing to technical difficulties related to HR signal acquisition (i.e., lost ECG signals or background high frequency signal contamination from an air conditioner in the MOU), complete HR data records available for analysis were obtained from 12 of the 14 exposed and 9 of the 14 control newborns.

Mean HR—In both groups, HR increased in response to lance and decreased during recovery [Fig. 1; quadratic $F(1,22) = 49.2$, $p < 0.01$]. Exposed infants had lower mean HR than controls throughout the session, $F(1,19) = 5.88$, $p = 0.025$.

Respiratory Sinus Arrhythmia—Exposed infants showed no significant change in RSA over time [quadratic $F(2,11) = 0.437$, $p > 0.20$]. In contrast, among control neonates, RSA decreased in response to lance and increased during recovery, as expected [Fig. 2a; quadratic $F(2,8) = 4.817$, $p = 0.059$].

Power Spectral Estimates—Although the patterns of change for HFP appeared similar to those for RSA, the changes over time did not reach statistical significance for either the exposed [quadratic $F(1,8) = 0.003$, $p > 0.20$] or the control [Fig. 2b; quadratic $F(1,8) = 1.34$, $p < 0.20$] infants. Like RSA, LFP did not change over time for the exposed infants [quadratic $F(1,11) = 0.21$, $p > 0.20$], whereas LFP decreased significantly in response to the lance and increased significantly during recovery in the control neonates [Fig. 2c; quadratic $F(1,8) = 16.43$, $p < 0.01$]. Similarly, for the exposed infants, the LFP / HFP ratio did not change significantly across time [baseline to lance, $F(1,10) = 0.50$, $p > 0.20$; lance to recovery, $F(1,10) = F = 3.2$, $p > 0.1$], whereas for the control infants, the LFP / HFP ratio decreased from baseline to lance [Fig. 2d; $F(1,8) = 6.56$, $p < 0.05$] and increased during recovery [$F(1,8) = 7.68$, $p < 0.05$].

Cortisol Reactivity—Mean salivary cortisol did not change from basal to lance to recovery in the control infants ($p > 0.22$), but decreased significantly over time in the ethanol-exposed group [$F(1,4) = 16.0$; $p = 0.016$; Fig. 3]. In contrast, Pearson correlation analyses showed that there was no relationship between cortisol levels at any of the 3 time points and prenatal smoking or marijuana use (all $ps > 0.20$), or between cortisol and maternal depression ($p > 0.05$). Of note, the infant born to 1 of the 2 clinically depressed nonexposed mothers had the second highest basal cortisol levels. Unfortunately, basal cortisol levels were missing for the 2 other newborns born to the clinically depressed mothers.

Facial Action Pain Responses (NFCS)—Facial action pain-related responses increased in both groups in response to heel lance, and decreased during recovery [Fig. 4; $F(2,22) = 10.98$, $p < 0.001$]. Behavioral responses, reflected by facial action scores to the acute noxious event, did not differ significantly between the groups ($p > 0.10$).

Neurobehavioral Outcomes—There were no significant between-group differences on the NBAS, but newborns in the alcohol-exposed group tended to be more consolable and to self-quiet more readily than those in the control group, $M = 6.9$ versus 5.6 , $p = 0.06$, and 7.0 versus 5.5 , $p = 0.07$, respectively, data that are consistent with reduced responsivity to stress. Overall, consolability was related to lower basal levels of cortisol, $r = -0.60$, $p = 0.05$, and self-quieting to lower cortisol responses at 20 and 40 minutes after the lance, $r = -0.46$, $p = 0.07$, and $r = -0.59$, $p < 0.05$, respectively. Neurobehavioral outcomes were not related to HR or HRV responses to the noxious event.

DISCUSSION

This study is the first to document a relationship between prenatal alcohol exposure and biobehavioral responses to a noxious event in human newborns. A number of key findings emerged from this study. As expected, both alcohol-exposed and control infants responded to the heel lance. Importantly, although facial action scores were similar between exposed and control infants, physiological measures reflecting particular components of key stress reactivity systems were significantly blunted in exposed compared with control infants. Specifically, mean HR was lower in exposed than in control infants at all time points, whereas cortisol levels showed a decrease in exposed infants but no change in control infants following lance. Furthermore, control infants exhibited substantial parasympathetic withdrawal (reflected by reduced RSA) and significant changes in the sympathetic / vagal balance (reflected by a decreased LFP / HFP ratio at basal and lance and an increased ratio during recovery), whereas exposed infants showed no significant changes in these measures over time. Alcohol-exposed newborns exhibited more consolability and self-quieting responses on the Brazelton NBAS assessment, which were related, in turn, to reduced cortisol levels, suggesting reduced arousal and responsivity to stress. Together, these convergent biobehavioral findings may reflect altered stress reactivity, possibly resulting from fetal “programming” of key physiological systems by prenatal alcohol exposure, antecedent to adverse developmental, behavioral outcomes later in childhood.

The study of infant pain reactivity was chosen specifically as a probe of monoaminergically mediated processes that may be altered by prenatal alcohol exposure. Investigation of neonatal pain reactivity may assist us in understanding early antecedents of later behavioral disorders without many of the potentially confounding environmental factors that influence child behavior and long-term developmental outcome. Although it might be proposed that a “neonatal withdrawal” syndrome or acute alcohol toxicity may be present if the mother was inebriated at the time of delivery, close observation of the 28 infants in this study suggest that this is an unlikely explanation for our data. All the infants in this study appeared to be healthy full-term babies, and there was no indication of acute alcohol-related withdrawal during the pain study. It is conceivable that subtle neurochemical changes, particularly related to HPA axis function, may emerge over time, and thus it remains crucial to replicate

our findings with older infants (i.e., 6-month-olds) to rule out transient effects associated with a subtle late withdrawal phenomenon. Data regarding caffeine consumption, which could have also potentially influenced neonatal behavior, were not collected in this study. Previously, Jacobson and colleagues (1984) reported a relationship between increased alcohol and caffeine use during pregnancy. In that paper, caffeine exposure was related to greater arousal and irritability, whereas prenatal alcohol exposure was related to greater placidity, suggesting that caffeine might have been a suppressor variable that obscured the alcohol effect. In this study, even without controlling for caffeine exposure an alcohol effect was still evident.

Pain reactivity in this context may constitute a final common pathway that links prenatal exposure (i.e., alcohol), “programming” or resetting of neurotransmitter and autonomic systems, and arousal regulatory capacities that are predictive of subsequent childhood stress reactivity and developmental risk. In this sense, infant pain reactivity can be regarded as providing a “window” into the developing brain (Doussard-Roosevelt et al., 1997; Grunau et al., 2006; Oberlander et al., 2002) and reflecting or indexing developmental vulnerability. Interpreting our biobehavioral measures in this context provides a meaningful approach to the data. Previously, mean HR and HRV, and the variations that coincide with respiration (HFP or RSA), have been used as indices of developmental risk (Porges, 1992; Porges and Bohrer, 1990), neonatal capacity to respond to stress / pain (Oberlander and Saul, 2002; Oberlander et al., 2000), and sustained attention (Richards, 1987, 1994). Lower vagal tone in neonates at birth has been associated with greater CNS dysfunction and emotional reactivity (Fox, 1989; Gunnar et al., 1995; Stifter and Fox, 1990), and poorer developmental outcomes (Doussard-Roosevelt et al., 1997; Fox and Porges, 1985), and may be an indicator of overall health status (DiPietro and Porges, 1991).

To date, fewer studies of cardiac autonomic function have been performed in neonates prenatally exposed to alcohol. Recently, Fifer and colleagues (2009) reported lower HRV in active sleep in alcohol-exposed neonates, and a blunted HR response to a tilt table challenge. In this study, spectral analyses of short-term variations in HR were used to examine the cardiac autonomic responses. We found that alcohol-exposed neonates showed lower HR overall, as well as blunted RSA and LFP / HFP ratio in response to heel lance, an acute noxious event. The decreased or reduced change observed in the LFP and LFP / HFP ratio measures in response to the heel lance may reflect a reduced primary withdrawal of parasympathetic activity without a concomitant increase in sympathetic activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Why HFP did not change significantly in response to the lance is not clear. It is possible that the small sample size and increased variability in the HFP signal, which appeared to be owing to a high frequency background “noise” from an oscillating fan that could not be filtered, may have played a role. In this context, the RSA measure was considered a more precise reflection of parasympathetic responses. Interestingly, using animal models, Caul and colleagues (1983) and Hayne and colleagues (1992) reported no change in basal HR in response to novel olfactory stimuli in rats prenatally exposed to alcohol. In contrast, and consistent with our findings, Kelly and Richards (1997) observed that rats exposed to alcohol during the early postnatal period (human third trimester equivalent) showed a significant reduction in the typical developmentally based increase in

RSA (Kelly and Richards, 1997). Overall, our findings might suggest that prenatal alcohol “blunts” the parasympathetic cardiac autonomic stress response. Although our data support the suggestion that HRV might reflect the developing autonomic control of stress regulation, its use as an index of long-term developmental risk, such as vulnerability to sudden infant death syndrome (SIDS) (Harper et al., 2000), in fetal alcohol-exposed neonates and children remains to be studied.

As an index of parasympathetic activity, RSA has also been used as a measure of sustained attention control (Richards, 1987). Longer reaction times to visual stimuli (Jacobson et al., 1994) in the Visual Expectancy Paradigm (Haith et al., 1988), less efficient / slower speed of HR deceleration in response to auditory and visual stimuli, suggesting a deficit in the initiation of attention (i.e., orienting response; Kable and Coles, 2004), and less efficient / longer looking times (Jacobson et al., 1993) during 2 attention tasks have been reported in alcohol-exposed infants. Similarly, reduced sustained and focused attention (Coles et al., 1997) and reduced cognitive flexibility during childhood (Coles et al., 1997) have been reported. Interestingly, Kable and Coles (2004) proposed that their findings of deficits in orienting, indexed by less efficient HR deceleration to the stimuli, suggest an impairment in neurophysiological encoding of information, which could underlie attentional deficits observed in other studies (Kable and Coles, 2004). Whether a blunted neonatal RSA in response to an acute pain event reflects an early antecedent of a generalized impairment in arousal regulation remains to be determined.

NFCS pain-related facial action differences were not observed between exposure groups. However, Brazelton NBAS responses provided some evidence of an overall blunted behavioral response in alcohol-exposed newborns. Whereas the NFCS facial actions are relatively specific behavioral indicators of pain, the NBAS measures a greater range of arousal to noninvasive stimulation. As well, an acute noxious event may saturate the response system, whereas more subtle stimuli (such as utilized for the NBAS) likely recruit different regulatory systems. In this study, alcohol-exposed infants tended to be easier to console and to self-quiet more readily, consistent with previous reports relating maternal alcohol use with low arousal on the NBAS (Landesman-Dwyer et al., 1978), and with NBAS data demonstrating greater self-quieting and consolability and lower scores on peak of excitement and lability of state in alcohol-exposed infants (Streissguth et al., 1983). Similarly, Jacobson and colleagues (1984) reported that newborns exposed to even relatively low levels of alcohol prior to and during pregnancy exhibited less of the full range of awake to crying states on the NBAS. Moreover, Coles and colleagues (1987) found less optimal motor performance and autonomic regulation and more abnormal reflexes in a more heavily exposed sample (Coles et al., 1987). Consistent with these findings, Jirikowic and colleagues (2008) recently reported that early school-aged children with fetal alcohol spectrum disorder (FASD) were more likely to be rated as underresponsive or seeking sensation compared with typically developing children on sensory-processing measures, suggesting a similar long-term impact involving altered sensory regulation. Together, these studies suggest that blunted behavioral responses, particularly in relation to arousal to stimuli such as those measured by the NBAS exam, may be a consistent finding in the early neonatal period in infants exposed to a range of alcohol doses during pregnancy. The finding that arousal levels are typically increased in older infants and young children prenatally exposed to alcohol

speaks of the importance of examining and tightly controlling developmental stage in studies such as these.

The data on mean salivary cortisol levels are consistent with these findings of blunted behavioral and autonomic responsiveness. Contrary to our expectations, alcohol-exposed infants showed a decrease in salivary cortisol following heel lance, whereas control infants showed no change in cortisol levels over time. The cortisol decrease in the exposed group suggests a blunting of HPA activity. Consistent with previous findings (Jacobson et al., 1999), basal and poststress cortisol levels were not related to maternal smoking or marijuana use during pregnancy, suggesting that the alterations in cortisol levels were specific to prenatal alcohol exposure. Interestingly, basal cortisol levels showed a trend for a positive correlation with maternal depression, confirming findings in 2 previous studies (Field et al., 1988; Jacobson et al., 1999). However, because mothers in both the exposed and control groups reported similar levels of anxiety and depression during pregnancy and none were using antidepressant medication, the alcohol effects on infant cortisol cannot be attributed to prenatal maternal mood.

As noted, prenatal alcohol exposure is known to have long-lasting effects on HPA function, regulation, and adaptation to stressors. Although data from both human (Haley et al., 2006; Jacobson et al., 1999; Ramsay et al., 1996) and preclinical (Sliwowska et al., 2008; Weinberg et al., 2008; Zhang et al., 2005) studies show that in adulthood, alcohol-exposed offspring are typically hyperresponsive to stressors, during early neonatal life, fetal alcohol-exposed offspring typically show blunted or reduced HPA responses to stressors (Angelogianni and Gianoulakis, 1989; Taylor et al., 1986; Weinberg, 1989; Weinberg and Wong, 1986). Importantly, this dichotomy between early life hyporesponsiveness and later life hyperresponsiveness does not appear to be limited to the effects of alcohol. Hamilton and colleagues (2005) demonstrated that prenatal opiate exposure in a rodent model results in a decreased corticosterone response to both immunological and social stressors during the preweaning period, but heightened corticosterone responses to the same stressors in adulthood compared with those in controls (Hamilton et al., 2005). Interestingly, work by Grunau and colleagues (2007) have shown a similar phenomenon in preterm human infants. That is, basal salivary cortisol levels are lower in preterm compared with term infants at 3 months corrected age (CA), whereas at 8 and 18 months CA, infants born at extremely low gestational age (ELGA, 23 to 28 weeks) have significantly higher basal cortisol levels than both infants born at very low gestational age (VLGA, 29 to 32 weeks) and term born. Moreover, Oberlander and colleagues (2008) demonstrated that prenatal selective serotonin reuptake inhibitor (SSRI) antidepressant medication exposure is related to reduced evening cortisol levels in 3-month-old infants. Thus, our present findings demonstrating blunted HPA responsiveness in alcohol-exposed neonates are consistent with the findings of blunted autonomic and behavioral responses. Together, these data are consistent with a broad clinical and preclinical literature which suggests that blunting or down-regulation of behavioral and physiological responsiveness during the neonatal period may represent an adaptive or compensatory response to adverse early life experiences, including prenatal exposure to alcohol or drugs (de Kloet et al., 1988). An alternative, and not incompatible possibility, is that alcohol-exposed infants may “shut down” or withdraw in response to noxious events

encountered in the early neonatal period, perhaps because of increased sensitivity to environmental stimuli. These possibilities require further investigation.

Central 5-HT systems and the HPA axis interact closely and in a complex manner (Laplanche et al., 2002), and both systems are exquisitely sensitive to the effects of early adverse experience (Kofman, 2002; Weinstock, 2001). Early studies suggested that, in general, 5-HT stimulates the activity of the HPA axis, and that the 5-HT_{1A} receptor plays a major role in this effect (Chaouloff, 1993, 1995). Paradoxically, however, recent studies have shown that 5-HT may contribute to either facilitation or inhibition of basal and stress-induced glucocorticoid secretion, and that its action may be dependent on the specific nature of the stressor or the circuitry involved (Lowry, 2002). Circulating corticosteroids, however, exert an inhibitory effect on expression of hippocampal 5-HT_{1A} receptors (Chaouloff, 1993, 1995), and elevations of glucocorticoids may decrease hippocampal 5-HT_{1A} mRNA (Flugge et al., 1998; Joels et al., 2004), and attenuate hippocampal 5-HT and 5-HT_{1A} receptor-mediated responses (Joels et al., 2004).

Links between prenatal alcohol exposure and altered pain reactivity remain to be studied. However, effects of prenatal alcohol exposure could influence the emerging pain system via alterations to the developing 5-HT system and / or interactions between the 5-HT and the HPA systems. Preclinical studies provide strong evidence that prenatal alcohol exposure leads to altered serotonergic development and function (Sari and Zhou, 2004; Zhou et al., 2001). From early fetal life, 5-HT modulates pain responses in the developing brain and early pain pathways undergo considerable prenatal and postnatal organization. Blunted pain reactivity has been shown in developmentally “at-risk” infants and toddlers following prenatal exposure to cocaine (Jacobson et al., 1984; Magnano et al., 1992) and SSRI antidepressant medications (Oberlander et al., 2002, 2005), in preschool-aged children with autism (Nader et al., 2004), and in an animal model for Down syndrome (Martinez-Cue et al., 1999). In these conditions, it is possible that altered central 5-HT levels and function may be a common element that underlies blunted pain reactivity (see also Butkevich and Vershinina, 2003; Butkevich et al., 2003). It is conceivable that prenatal alcohol exposure changes central 5-HT levels during critical periods of brain development and thus influences critical 5-HT functions during ontogeny, which are subsequently expressed as altered 5HT-mediated stress / pain reactivity.

In summary, this study demonstrates, for the first time, reduced or blunted behavioral and physiological responses following an acute noxious event in neonates prenatally exposed to alcohol. Despite the limited sample size, the data appear to demonstrate convergence among different responses systems, as well as consistency with previous findings from both preclinical and human infant studies. Replication with larger samples is needed, as are studies examining neonatal pain reactivity as a potential biomarker of risk for later adverse neurodevelopmental outcomes.

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Fig. 1.

Heart rate (HR). Mean HR. Both groups showed increased HR in response to lance, which decreased during recovery [quadratic $F(1,22) = 49.2$, $p < 0.01$]. Exposed infants had lower mean HR throughout the session, $F(1,19) = 5.88$, $p = 0.025$.

Fig. 2.

Measures of heart rate variability. **(A)** Respiratory sinus arrhythmia (RSA). Among the control neonates, RSA decreased in response to lance and increased during recovery, as expected [quadratic $F(2,8) = 4.817$, $p = 0.059$]. In contrast, among exposed infants no significant change in RSA was observed over time [quadratic $F(2,11) = 0.437$, $p > 0.20$]. **(B)** High frequency power (HFP). No significant group differences were observed ($p > 0.05$). **(C)** Low frequency power (LFP). Among the control neonates, LFP decreased in response to lance and increased during recovery [quadratic $F(1,8) = 16.43$, $p < 0.01$]. Among exposed infants, no significant change in LFP was observed over time [quadratic $F(1,11) = 0.21$, $p > 0.20$]. **(D)** LFP / HFP ratio. In control neonates, LFP / HFP ratio decreased from baseline to lance [$F(1,8) = 6.56$, $p = 0.034$], but not among the exposed infants [$F(1,10) = 0.50$, $p > 0.20$]. In both groups LFP / HFP ratio increased during recovery, but as a result of substantial variance in L / H recovery in exposed neonates, only the controls increased with recovery [$F(1,8) = 7.68$, $p < 0.05$].

Fig. 3.

Mean salivary cortisol decreased significantly over time in the ethanol-exposed group [$F(1,4) = 16.0$; $p = 0.016$] but did not change significantly from basal to lance to recovery in the control infants ($p > 0.22$).

Fig. 4.
Facial action (Neonatal Facial Coding System) response to the acute noxious event.

Table 1**Maternal and Infant Characteristics**

	Exposed	Controls	
	<i>M</i> or % (SD)	<i>M</i> or % (SD)	<i>t</i> or χ^2
Maternal characteristics			
Age at delivery	28.8 (7.0)	28.0 (6.2)	0.29
Years of education	9.2 (1.6)	10.1 (2.2)	1.33
Marital status (% married)	14.3	21.4	0.24
Beck Depression score ^a	15.8 (13.4)	12.4 (10.5)	0.70
Trait anxiety ^b	40.1 (14.4)	37.2 (10.7)	0.56
State anxiety ^b	37.3 (11.1)	38.0 (14.3)	0.13
Parity	1.7 (1.4)	1.4 (1.1)	0.67
Gravidity	3.0 (1.5)	2.5 (1.0)	0.94
Infant characteristics			
Gestational age (days)	280.7 (13.5)	273.1 (11.0)	1.57
Birth weight (g) ^c	2,884.6 (600.2)	3,320.7 (444.5)	2.16 [*]
Length (cm)	47.8 (2.2)	49.3 (2.0)	1.85 ^{**}
Head circumference (cm)	33.9 (1.3)	34.8 (2.0)	1.53
Gender (% male)	28.6	64.3	3.59 ^{**}
Circumcised (%)	0.0	0.0	—

^{*} $p < 0.05$;

^{**} $p < 0.10$.

^aMissing for 1 exposed and 2 control mothers.

^bMissing for 2 exposed and 2 control mothers.

^cMissing for 1 exposed infant.

Table 2**Maternal Alcohol, Smoking, and Drug Use During Pregnancy**

	Exposed (<i>n</i> = 14)	Controls (<i>n</i> = 14)	
	<i>M</i> (SD)	<i>M</i> (SD)	<i>t</i>
At time of conception			
Absolute alcohol/d (oz)	1.0 (0.5)	0.1 (0.2)	6.52 ***
Absolute alcohol/drinking day (oz)	3.8 (1.8)	0.4 (1.4)	5.51 ***
Frequency (drinking days/wk)	2.0 (0.7)	0.1 (0.4)	8.19 ***
Across pregnancy			
Absolute alcohol/d (oz)	0.8 (0.5)	0.01 (0.01)	5.70 ***
Absolute alcohol/drinking day (oz)	3.9 (1.8)	0.5 (1.4)	5.62 ***
Frequency (drinking days/wk)	1.4 (0.9)	0.03 (0.1)	5.68 ***
Cigarettes/d	5.4 (5.0)	2.6 (3.7)	1.68
Marijuana use (d/mo)	2.5 (5.6)	1.6 (4.7)	0.49

 $p < 0.001$.