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Hypothalamic-pituitary-adrenal axis and behavioral dysfunction following early binge-like prenatal alcohol exposure in mice

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ABSTRACT

The range of defects that fall within fetal alcohol spectrum disorder (FASD) includes persistent behavioral problems, with anxiety and depression being two of the more commonly reported issues. Previous studies of rodent FASD models suggest that interference with hypothalamic-pituitary-adrenal (HPA) axis structure and/or function may be the basis for some of the prenatal alcohol (ethanol) exposure (PAE)-induced behavioral abnormalities. Included among the previous investigations are those illustrating that maternal alcohol treatment limited to very early stages of pregnancy (i.e., gestational day [GD]7 in mice; equivalent to the third week post-fertilization in humans) can cause structural abnormalities in areas such as the hypothalamus, pituitary gland, and other forebrain regions integral to controlling stress and behavioral responses. The current investigation was designed to further examine the sequelae of prenatal alcohol insult at this early time period, with particular attention to HPA axis-associated functional changes in adult mice. The results of this study reveal that GD7 PAE in mice causes HPA axis dysfunction, with males and females showing elevated corticosterone (CORT) and adrenocorticotropic hormone (ACTH) levels, respectively, following a 15-min restraint stress exposure. Males also showed elevated CORT levels following an acute alcohol injection of 2.0 g/kg, while females displayed blunted ACTH levels. Furthermore, analysis showed that anxiety-like behavior was decreased after GD7 PAE in female mice, but was increased in male mice. Collectively, the results of this study show that early gestational alcohol exposure in mice alters long-term HPA axis activity and behavior in a sexually dimorphic manner.

Introduction

Alcohol use during pregnancy is one of the leading known causes of birth defects (Riley, Infante, & Warren, 2011), and therefore, represents a major health concern. Current estimates on the prevalence of Fetal Alcohol Syndrome (FAS) in the United States' general population range from 0.2 to 7 per 1000 live births (May et al., 2009). FAS is characterized by clear morphological changes and functional deficits, and represents the extreme end of the spectrum that can result from prenatal alcohol exposure (PAE). A recent addition to DSM-5, Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE), encompasses many of the same functional deficits but can be diagnosed in the absence or presence of any physical abnormalities. Both fall within Fetal Alcohol Spectrum Disorder (FASD), an umbrella term that describes the full spectrum of deficits that can occur following PAE, which has a prevalence of 2–5% in the United States (May et al., 2009). Based on longitudinal studies, an alarming proportion of individuals who are exposed prenatally to alcohol develop a range of mental health disturbances, with several studies finding the prevalence to be 90% or greater (Rasmussen, Andrew, Zwaigenbaum, & Tough, 2008; Streissguth, Barr, Kogan, & Bookstein, 1996). Anxiety and depression, the more common of these problems, occur at higher rates than those found in the general population; rates of occurrence in prenatal alcohol-exposed

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individuals compared to the general population are 29% vs. 17% for anxiety (Barr et al., 2006; Somers, Goldner, Waraich, & Hsu, 2006) and 52% vs. 21% for depression (Cryan, Markou, & Lucki, 2002; Streissguth et al., 1996). The behavioral abnormalities in FASD are typically apparent in childhood (Fryer, McGee, Matt, Riley, & Mattson, 2007; O'Connor & Kasari, 2000; O'Connor et al., 2002; Steinhausen, Willms, Metzke, & Spohr, 2003) and often persist into adulthood (Barr et al., 2006; Famy, Streissguth, & Unis, 1998).

Of particular interest for the current study is the association of anxiety and depression with altered hypothalamic-pituitary-adrenal (HPA) axis activity (Burke, Davis, Otte, & Mohr, 2005; Nemeroff et al., 1984; Vreeburg et al., 2009; Wingenfeld & Wolf, 2011; Young, Abelson, & Cameron, 2004). Notably, the literature suggests that normalization of HPA axis dysregulation is a prerequisite for successful and persistent remission of these behavioral problems (Appelhof et al., 2006; Schüle, Baghai, Eser, & Rupprecht, 2009; Zobel et al., 2001). Overall, HPA axis dysfunction can be a risk factor for HPA axis-related pathologies such as depression, anxiety, and alcoholism, particularly when it has been induced by early life adversity (De Bellis, 2002; Gordon, 2002; Macrì, Spinelli, Adriani, Dee Higley, & Laviola, 2007). Direct clinical evidence of PAE-mediated alterations in the HPA axis has been reported with infants as young as 5 months showing increased cortisol responsiveness to stress (Haley, Handmaker, & Lowe, 2006; Jacobson, Bihun, & Chiodo, 1999). Thus, these HPA axis-associated consequences present early in life may contribute to the increase in later life mental health issues.

Not only do clinical studies show functional deficits, they also reveal structural deficiencies in the pituitary or hypothalamus after PAE. An investigation directly examining morphological consequences of PAE showed abnormal development of the hypothalamus of both fetuses and children (Peiffer, Majewski, Fischbach, Bierich, & Volk, 1979). While the exact period of alcohol exposure in the previous study is unknown, an anatomical report looking at a 2.5-month-old girl exposed to first-trimester maternal binge alcohol abuse showed absence of the posterior pituitary, an enlarged and bulbous hypothalamus, and pituitary hormone deficiency (Coulter, Leech, Schaefer, Scheithauer, & Brumback, 1993). To date, such clinical research has mainly focused on heavy alcohol exposure for extended periods of time, leaving it unclear whether exposures that are more limited can lead to HPA axis dysfunction along with increased anxiety or depression.

While human research cannot readily delineate critical alcohol exposure times or the pattern and amount of maternal alcohol consumption necessary for structural and functional teratogenesis, animal models have proven invaluable in this regard. As seen in clinical studies, animal research employing models with low to moderate alcohol exposure throughout gestation suggests that even low levels of PAE lead to HPA dysfunction, which in turn contributes to behavioral abnormalities. Exemplary are reports describing the impact of PAE on fetal programming of the HPA axis response to stressors (Hellemans, Sliwowska, Verma, & Weinberg, 2010; Kim, Turnbull, Lee, & Rivier, 1999; Ogilvie & Rivier, 1997; Weinberg, Sliwowska, Lan, & Hellemans, 2008; Weinberg, Taylor, & Gianoulakis, 1996). Both rodents (Kim, Giberson, Yu, Zoeller, & Weinberg, 1999; Lee, Imaki, Vale, & Rivier, 1990; Nelson et al., 1986; Taylor, Branch, Liu, & Kokka, 1982; Weinberg, 1992) and primates (Schneider, Moore, Kraemer, Roberts, & DeJesus, 2002) exposed prenatally to alcohol display enhanced HPA axis reactivity to multiple types of stressors, including morphine administration, restraint stress, footshock, cardiac puncture, and cold stress. Furthermore, an overall increase in depression-like symptoms as measured on the forced swim task (Carneiro et al., 2005; Hellemans, Verma, et al., 2010; Wilcoxon, Kuo, Disterhoft, & Redei, 2005) and anxiety-like behavior as measured on the elevated plus maze (Carneiro et al., 2005; Dursun, Jakubowska-Doğru, & Uzbay, 2006; Liang et al., 2014) was found in rats. These changes in anxiety-like behavior can be directly linked to changes in HPA axis activity, as evidenced by increased corticosterone (CORT) levels after testing on the elevated plus maze or open field in prenatal alcohol-exposed females, but not males (Hellemans, Verma, Yoon, Yu, & Weinberg, 2008; Osborn, Kim, Steiger, & Weinberg, 1998). Importantly, the above findings highlight a role for sex in influencing alcohol's teratogenic outcome and illustrate the importance of study designs that analyze both sexes.

The PAE animal models described above suggest consistent functional changes with alcohol exposure throughout gestation. However, acute, binge-like exposures have the advantage of targeting critical and precisely timed developmental events. This is especially important early in gestation, when developmental changes occur rapidly. Strongly supporting the potential of early, binge-like gestational insult to adversely affect the HPA axis are morphological studies showing that PAE limited to gestational day (GD)7 in mice (developmentally equivalent to the mid-third week post-fertilization in humans) can result in structural abnormalities of the pituitary gland as well as third ventricular enlargement, the latter of which may reflect hypothalamic deficiency (Godin et al., 2010; Lipinski et al., 2012).

The current study expands upon this previous anatomical work following an early gestational, binge-like exposure in mice to assess the functional deficits that occur at this period. The hypothesis tested was that early PAE would have lasting consequences on the sensitivity and activity of the HPA axis, that these would alter the expression of behaviors related to anxiety and depression, and that males and females would differ in both of these physiological and behavioral measurements. For this purpose, HPA axis activity was measured by CORT and adrenocorticotropic hormone (ACTH) levels following exposure to two differing stressors (restraint and alcohol injection), and affective behavior was measured in the elevated plus maze, light–dark chamber, and forced swim test.

Methods

Animal care and prenatal alcohol treatment

C57Bl/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed on a 12-h/12-h light/dark cycle (lights on at 7:00 AM) with *ad libitum* access to standard rodent chow (Isopro RMH 3000; Purina, St. Louis, MO) and water. Timed pregnancies were established by housing two female mice with one male mouse for a period of 1–2 h. GD0, 0 h, was defined as the beginning of the breeding period in which a copulation plug was detected. Pregnant dams were administered two intra-peritoneal (i.p.) injections of 25% (v/v) ethanol in a vehicle of lactated Ringer's solution (offspring defined as GD7 Alc) at a dosage of 2.9 g/kg, given 4 h apart starting on GD7, 0 h. Control mice were injected similarly with vehicle alone (offspring defined as GD7 Con) to control for the stress of injection. Blood alcohol concentrations resulting from this PAE procedure have been previously reported, with the peak reaching approximately 420 mg/dL 30 min after the second alcohol injection (Godin et al., 2010; Kotch & Sulik, 1992).

A total of 102 litters were used for all the experiments. An approximately equal proportion of GD7 Con and GD7 Alc litters were used for all experiments. Litters were culled to a maximum of 8 pups. There were no significant differences in litter size (average size approximately 6 pups/litter) or the number of pups that