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Prenatal alcohol exposure disrupts male adolescent social behavior and oxytocin receptor binding in rodents

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ABSTRACT

Social behavior deficits resulting from prenatal alcohol exposure (PAE) emerge early in life and become more pronounced across development. Maturational changes associated with adolescence, including pubertal onset, can have significant consequences for social behavior development, making adolescence a unique period of increased vulnerability to social behavior dysfunction. Unfortunately, little is known about the underlying neurobiology supporting PAE-related social behavior impairments, particularly in the context of adolescence, when the transition to a more complex social environment may exacerbate existing deficits in social behavior function. Here we perform a comprehensive evaluation of social behavior development in PAE animals during two different periods in adolescence using three separate but related tests of social behavior in increasingly complex social contexts: the social interaction test, the social recognition memory test (i.e. habituation-dishabituation test), and the social discrimination test. Additionally, we investigated the underlying neurobiology of the oxytocin (OT) and vasopressin (AVP) systems following PAE, given their well-documented role in mediating social behavior. Our results demonstrate that compared to controls, early adolescent PAE animals showed impairments on the social recognition memory test and increased OT receptor binding in limbic networks, while late adolescent PAE animals exhibited impairments on the social discrimination test and increased OTR binding in forebrain reward systems. Taken together, these data indicate that PAE impairs adolescent social behavior – especially with increasing complexity of the social context – and that impairments are associated with altered development of the OT but not the AVP system.

1. Introduction

Of the cognitive, physiological and behavioral impairments associated with prenatal alcohol exposure (PAE) documented in the clinical literature, lifelong social behavior deficits are particularly pervasive (Kelly et al., 2000; Thomas et al., 1998). PAE-related social behavior deficits emerge early in development and become more pronounced with age (Kully-Martens et al., 2012; Marquardt and Brigman, 2016). Animal models have demonstrated social behavior deficits parallel to those observed in humans with PAE. In neonatal rats, for example, PAE has been linked with disrupted attachment (Subramanian, 1992) and reduced ability to elicit retrieval by the mother (Ness and Franchina, 1990). Social deficits persist and often worsen during adolescence, with PAE rats showing disrupted play behavior and changes in social investigation (Lawrence et al., 2008; Mooney and Varlinskaya, 2011), which in themselves may have long-lasting effects on normal social development (Auger and Olesen, 2009). Indeed, social behavior deficits are present into adulthood, as PAE rats show altered social interactions and increased aggression with conspecifics (Hamilton et al., 2010; Hellemans et al., 2010; Royalty, 1990). Taken together, these data suggest that the transition to a more complex social environment, such as occurs during adolescence, which is characterized by expansions in social networks and increases in peer-directed social interaction, may exacerbate some of the social behavior impairments observed following PAE (Kully-Martens et al., 2012; Spear, 2000).

Adolescence is a critical developmental period in which significant neurobehavioral, cognitive and physiological changes occur, including the attainment of sexual maturity (i.e. puberty; McCormick and Mathews, 2010; Vetter-O'Hagen and Spear, 2012). Notably, many of the maturational changes that occur around pubertal onset can have significant consequences for social behavior development, such as steroid-dependent organization of neural circuits, making adolescence a unique period of increased vulnerability to social behavior dysfunction (Sisk and Zehr, 2005). In rats, play behavior peaks during adolescence

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(Spear, 2000), and most studies of adolescent social behavior following PAE have focused on observations of play, with relatively few assessing other aspects of social function such as social recognition memory or social discrimination (Marquardt and Brigman, 2016). Here, we expand this literature by performing a comprehensive evaluation of social behavior development in PAE animals during two different periods in adolescence, using a battery of social behavior tests.

Despite progress in characterizing social behavior deficits following PAE in humans and animal models, more research is needed to identify the underlying neurocircuitry. The neuropeptides oxytocin (OT) and vasopressin (AVP) have been implicated in the regulation of various aspects of social behavior (Bredewold and Veenema, 2018; Dore et al., 2013), including social motivation (Lim and Young, 2006), social recognition (Bielsky and Young, 2004; Engelmann et al., 1994; Ferguson et al., 2002; Veenema et al., 2012) and aggression (Ferris, 1992; Veenema et al., 2010). OT/AVP act by binding to their respective receptors, OTR or $V1_aR$. These receptors are widely distributed in the brain and correlate with sites of peptide release, particularly in brain areas implicated in social behavior function, including the amygdala, prefrontal cortex (PFC), bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAcc), and lateral septum (LS; Dumais et al., 2013; Neumann and van den Burg, 2013; Stoop et al., 2015). Despite their well-characterized role in regulating social behavior and other key functions, relatively few studies have assessed the OT/AVP systems within the context of PAE-related social behavior impairment. Kelly et al. demonstrated that PAE results in sexually dimorphic deficits in social recognition memory and reductions in OT-receptor binding in amygdala homogenates of adult rats (Kelly et al., 2009). In adult female mandarin voles, PAE resulted in reductions in OT fibers within the PVN and SON as measured by OT immunohistochemistry (He et al., 2012). Studies investigating the effects of PAE on the AVP system have generally done so within the context of stress regulation and have shown long-lasting alterations to the AVP system (Godino and Renard, 2018). Additionally, previous work from our laboratory indicates that PAE alters sensitivity of central AVP pathways to testosterone in adult males (Lan et al., 2006, 2009). These studies suggest an enduring effect of PAE on the OT and AVP systems; however, more work is needed to understand the role of the OT/AVP systems in mediating the deleterious effects of PAE on social behavior, particularly during the key developmental period of adolescence.

Here, we utilize a well-established rat model of PAE to investigate the neurobehavioral effects of PAE on adolescent social behavior development using three separate, but related tests of social behavior in increasingly complex social contexts: 1) the social interaction test, 2) the social recognition memory test (i.e. habituation-dishabituation test), and 3) the social discrimination test. To further address the specificity of the PAE insult to the social behavior domain and to confirm that deficits are not simply a downstream effect of impaired olfactory function, an additional set of animals was assessed using tests of olfaction and social odor discrimination (Wesson et al., 2010). Given the role of OT and AVP in regulating social behavior function, we assess the central OT/AVP systems, measuring hypothalamic mRNA expression of OT and AVP as well as receptor binding of the OTR and $V1_aR$ in key brain regions. Finally, as the OT/AVP systems have significant cross talk with the hypothalamic-pituitary-adrenal and -gonadal systems (Dore et al., 2013; Neumann and van den Burg, 2013), we also assess plasma levels of corticosterone and testosterone.

2. Methods

2.1. Animals and breeding

Male and female Sprague-Dawley rats were obtained from Charles River Laboratories (St. Constant, Canada). Rats were pair-housed by sex and maintained at a constant temperature (21 ± 1 °C) and on a 12 h light-dark cycle (lights on at 0700 h) with *ad libitum* access to water and standard lab chow (Harlan, Canada). After a 10-day acclimation period, male and female pairs were placed together for breeding. Vaginal smears were taken each morning, and the presence of sperm was used as an indicator of pregnancy (gestation day 1; G1). All experiments were performed in accordance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals, Canadian Council on Animal Care guidelines, and were approved by the University of British Columbia Animal Care Committee.

2.2. Prenatal alcohol exposure

On G1, females were single-housed and randomly assigned to one of three treatment groups: Prenatal Alcohol Exposure (PAE), Pair-Fed (PF), or *ad libitum*-fed Control. Dams in the PAE group ($n = 41$) were offered *ad libitum* liquid ethanol diet (6.37% v/v) with 36% ethanol-derived calories. The liquid ethanol diet was introduced gradually over the first 3 days with bottles containing: Day 1 - 66% control diet, 34% ethanol diet; day 2 - 34% control diet, 66% ethanol diet; day 3 - 100% ethanol diet. This diet is formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake (Lan et al., 2006). To determinate blood alcohol levels (BALs) of alcohol consuming dams, tail blood samples from a subset of dams ($n = 14$) were taken on GD15 during various times across the light/dark cycle. Serum was collected and stored at −20 °C until the time of assay. BALs were measured using Pointe Scientific Inc. Alcohol Reagent Set (Lincoln Park, MI, USA); the minimum detectable concentration of alcohol is 2 mg/dL. Alcohol-consuming dams showed an mean of 135.3 ± 50.8 mg/dL (max BAL = 215.1 mg/dL; min BAL = 52.08 mg/dL). For reference, most jurisdictions set 80–100 mg/dL as the legal limit of intoxication. Pair-fed dams ($n = 33$) were offered a liquid control diet with maltose-dextrin isocalorically substituted for ethanol, in an amount matched to the consumption of an alcohol-fed partner according to gestation day (g/kg body weight/day of gestation). *Ad libitum*-fed Control dams ($n = 39$) were offered *ad libitum* access to a pelleted form of the liquid control diet. Diets were prepared by Dyets Inc., Bethlehem, PA (Weinberg-Keiver High Protein Experimental Diet # 710324; Control Diet #710109; Weinberg/Keiver High Protein Pelleted Control Diet #710109). All animals had *ad libitum* access to water, and were provided with fresh diet daily within 1 h of lights off to prevent a shift in corticosterone circadian rhythms, which occurs in animals that are on a restricted feeding schedule, such as the pair-fed dams (Gallo and Weinberg, 1981).

Experimental diets were continued through G21; beginning on G22, all animals were offered *ad libitum* access to standard laboratory chow and water, which they received throughout lactation. Pregnant dams were left undisturbed except for cage changing and weighing (G1, G7, G14, and G21). On the day of birth (postnatal day 1 – PN1), litters were weighed and culled to 12 pups with an attempt to preserve an equal number of males and females per litter. Dams and pups were left undisturbed except for cage changing and weighing (PN1, PN8, PN15, P22). Subjects were male offspring housed in same-prenatal treatment, non-sibling pairs in standard rat cages (17″L × 10.5″W × 7.3″H, Allentown, Inc., Allentown, NJ) at P25. For behavioral testing, no > 1 male per litter was used at each age of testing. Behavioral testing occurred in early (P30–35) or late (P43–47) adolescence to account for potential effects of gonadal hormone changes across puberty. Unmanipulated juvenile male rats (P23–28), housed 2–4 per cage, were used as social stimuli for social motivation, recognition memory, and discrimination testing.

2.3. Experimental design

For all experiments, subjects were male offspring in either early (~P32) or late (~P45) adolescence. Behavioral testing was performed using three experimental cohorts to reduce repeated testing effects, as social experience has the potential to affect later function (Veenema,