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Effects of prenatal alcohol exposure on social competence: Asymmetry in play partner preference among heterogeneous triads of male and female rats

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Abstract

Social behavior deficits associated with prenatal alcohol exposure (PAE) are frequently described in terms of impaired social competence, which can be defined as the effectiveness in social interaction and the ability to employ social skills successfully within different interpersonal contexts. Play behavior – which peaks during adolescence – is critical for developing social competence, as well as for motor, cognitive, and emotional development. Studies of play behavior typically utilize protocols where animals interact in dyads. However, less is understood about how the social environment may shape PAE-related social behavior deficits, particularly in more complex social contexts. Here, we assess play partner preference utilizing a novel approach in which adolescent male and female animals interact within same-sex triads comprised of animals from mixed prenatal treatments to determine how play partner identity and social group composition interact to shape behavior. When triads included one PAE animal and two control animals (i.e., control animals had the option to play either with a fellow control or a PAE playmate), we observed play target asymmetry whereby controls preferentially played with fellow controls. Notably, these results were consistent for triads of both males and females, with subtle differences in frequency of initiations vs. reciprocations. We found no play target asymmetry, however, when triads included two PAE animals and one control animal or different configurations of control and pair-fed animals. Taken together, play target asymmetry resulting from ineffective social interactions, including a failure to engage with, respond to, and/or solicit play from control play partners appropriately, suggests that PAE negatively impacts the development of social competence.

Keywords

Adolescence; Play behavior; Prenatal alcohol exposure; Social behavior

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Conflict of Interest

All authors declare no conflict of interest.

Introduction

Fetal Alcohol Spectrum Disorder (FASD) refers to the spectrum of physical, neurobehavioral and physiological impairments resulting from prenatal alcohol exposure (PAE; the COMMITTEE ON SUBSTANCE ABUSE, 2015). Among the associated impairments across the entire spectrum, social behavior deficits are pervasive and have widespread implications for other domains such as executive function and emotional processing (Kully-Martens, Denys, Treit, Tamana, & Rasmussen, 2012; Streissguth, Barr, Kogan, & Bookstein, 1996). PAE-related social behavior deficits emerge early in development and become more pronounced as the individual approaches adolescence, when the transition to a more complex social environment may exacerbate existing social behavior impairments (Duquette, Stodel, Fullarton, & Hagglund, 2006; Streissguth et al., 1996; Thomas, Kelly, Mattson, & Riley, 1998). Social behavior deficits in individuals with FASD are frequently described in terms of impaired social competence, which can broadly be defined as the effectiveness in social interaction or the ability to employ social skills successfully within various interpersonal contexts (Dodge, 1986; Rose-Krasnor, 1997).

Development of social competence is an ongoing process that begins in infancy through interactions with the primary caregiver and involves the complex interaction of behavioral, cognitive, and emotional factors (Milligan, Sibalis, Morgan, & Phillips, 2017; Parent & Meaney, 2008; Raine et al., 2015). PAE has been shown to impair the development of social competence in the early postnatal period, as alcohol-exposed infants may show disorganized attachment to caregivers (O'Connor, Sigman, & Kasari, 1992; Platzman, Coles, Lynch, Bard, & Brown, 2001). As children with FASD transition into adolescence, impairments in social competence persist and generally worsen, as demonstrated by poor performance on behavioral assessments such as the Vineland Adaptive Behavior Scales, a parental rating of adaptive functioning in communication, daily living, and socialization domains (Whaley, O'Connor, & Gunderson, 2001). More specifically, children with FASD often struggle with reading social cues, engaging in socially appropriate interactions, and establishing reciprocal peer relationships (Bishop, Gahagan, & Lord, 2007; Stevens, Clairman, Nash, & Rovet, 2017).

Data from animal models of PAE have shown neurobehavioral deficits parallel to those observed in individuals with FASD (Driscoll, Streissguth, & Riley, 1990; Jänicke & Coper, 1993; Marquardt & Brigman, 2016; Sulik & Johnston, 1983; Weinberg, 1989; Weinberg & Bezio, 1987), including social behavior impairments (Hamilton et al., 2010; Hellemans et al., 2010; Kelly, Day, & Streissguth, 2000; Kelly, Goodlett, & Hannigan, 2009). In particular, disruptions in play behavior are a commonly reported deficit and provide an important behavioral context for investigating how PAE impacts social behavior development during adolescence (Charles Lawrence, Cale Bonner, Newsom, & Kelly, 2008; Lugo, Marino, Cronise, & Kelly, 2003; Meyer & Riley, 1986; Mooney & Varlinskaya, 2011; Royalty, 1990).

Play behavior is a characteristic form of social interaction observed in many mammalian species, particularly during the transition into adolescence when the expression of play behavior is highest (Meaney & Stewart, 1981; Spear, 2000; Trezza, Baarendse, &

Vanderschuren, 2010). As one of the first non-maternal social interactions in which young animals participate, play behavior is critical for developing social competence, as well as for motor, cognitive, and emotional development (Graham & Burghardt, 2010; Pellis, Burke, Kisko, & Euston, 2018). Evidence for the critical role of play behavior comes from studies employing social isolation, which demonstrate long-lasting neurobehavioral deficits into adulthood, including reduced social approach and/or interaction, increased aggression, and increased anxiety-like behavior (Cooke & Shukla, 2011; Sivi, Deron, & Kasten, 2011; Vanderschuren & Trezza, 2014). As in other forms of social behavior, play behavior is a dynamic collaboration, requiring each playmate to process salient cues and respond accordingly, so that the interaction can proceed in a coordinated fashion (Adolphs, 2003; Vanderschuren & Trezza, 2014). In this way, play is essentially dyadic, with playmates frequently switching between solicitor and responder across a social interaction such that each reciprocally influences the other. Moreover, play behavior involves not only the accurate perception of social stimuli and subsequent execution of an appropriate social response, but also the capacity to elicit appropriate behavioral reciprocity with a play partner in order to sustain play interactions (Thor & Holloway, 1984). Accordingly, investigations of play behavior offer important insight into the development of social competence.

In the dyadic protocols typically used in studies of play behavior, an experimental animal interacts either with a partner matched by treatment or with a naïve control animal (Himmler, Pellis, & Pellis, 2013; Thor & Holloway, 1984; Vanderschuren & Trezza, 2014). These paradigms offer useful information about how play may be altered by different experimental treatments or conditions, and provide important comparisons to increase understanding of social behavior (Himmler, Himmler, Pellis, & Pellis, 2016). For example, it was shown that the playfulness of one partner is influenced by the playfulness of the other partner, as high-playing rats exhibited decreased play behavior when paired with low playing playmates (Pellis & McKenna, 1992). Likewise, increasing play by systematically varying amount of social isolation results in increased play behavior by non-isolated playmates (Varlinskaya, Spear, & Spear, 1999).

While experiments utilizing dyadic protocols have significantly advanced the field, they do not allow for assessment of play in more complex social situations or for phenomena such as play partner preference. Our novel approach of utilizing triads of mixed-treatment composition allows us to assess not only play behavior, but also play partner preference. Specifically, we directly assessed play partner preference in adolescent male and female rats using same-sex triads of mixed-treatment composition to determine not only the impacts of PAE on social competence but also the impacts of the social environment on play behavior development.

Methods

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 ($21 \pm 1^\circ\text{C}$) and on a 12 h light-dark cycle (lights on at 0700 h) with *ad libitum* access to water and standard laboratory 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) Guide for the Care and Use of Laboratory Animals (2011), Canadian Council on Animal Care guidelines, and were approved by the University of British Columbia Animal Care Committee.

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 (C). Dams in the PAE group were offered *ad libitum* liquid ethanol diet (6.37% v/v) with 36% ethanol-derived calories (Weinberg-Keiver High Protein Experimental Diet #710324, Dyets Inc., Bethlehem, PA). Pair-fed dams were offered a liquid control diet (Weinberg-Keiver High Protein Control Diet #710109) 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). Control dams were offered *ad libitum* access to a pelleted form of the liquid control diet. 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. Diets are formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake (Lan et al., 2006). To determine 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 average of 95.85 ± 10.79 mg/dL (max BAL = 165.68 mg/dL; min BAL = 38.95 mg/dL). All animals had *ad libitum* access to water, and were provided with fresh diet daily within 1h 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 & 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, which occurred on G1, G7, G14, and G21. On the day of birth (postnatal day 1 – P1), litters were weighed and culled to 12 pups with equal numbers of males and females per litter when possible. Dams and pups were weighed on P1, P8, P15, P22, and after play behavior testing ($P30 \pm 1$ for females; $P37 \pm 1$ for males). No more than 2 males or females per litter were used for behavioral testing.

Play Behavior Triad Testing

To examine play behavior during adolescence there are two approaches: pubertal-dependent effects can be assessed by testing males and females at similar pubertal stages, which has the confound of testing at different ages; or pubertal-independent effects can be assessed by testing males and females at the same age, which has the confound of testing at different pubertal stages. In the present study we chose to assess pubertal-dependent effects and therefore tested males and females at different ages ($P30 \pm 1$ for females and $P37 \pm 1$ for

males). Habituation to the testing apparatus occurred over two consecutive days immediately prior to testing. Specifically, animals in same-sex littermate groups were habituated to the play arena (21.5" × 20" × 21.5"), which consisted of 3 mirrored side panels and a front panel made of clear Plexiglas and contained clean corn cob bedding. At the end of the second habituation period, pups were singly housed overnight in order to promote social play at testing (Himmler et al., 2013). The next day, animals were tested for 10 min in mixed-prenatal treatment triads, with four possible triad combinations: (1) two controls and a PAE (CCE), (2) two controls and a PF (CCP), (3) two PAEs and a control (EEC), (4) two PFs and a control (PPC). All triads consisted of same-sex, non-littermate animals that had never interacted.

Play behavior was filmed from the front of the clear enclosure and behavior scored later by trained observers blind to triad composition using a computer-assisted data acquisition system (Observer 5.0, Noldus, Netherlands). In each triad, the behavior of each of the two rats from the same treatment group was analyzed to determine the duration and frequency with which the experimental animals interacted with either the same (i.e. ingroup) or different (i.e. outgroup) treatment partners. The duration of social investigation (anogenital sniffing, body sniffing, allogroom) and play (rough/tumble play, boxing, follow/chase, evade, pinning) were measured; additionally, the frequency (over 10 min) of the play initiations (nape initiation, rump initiation), play reciprocation (half rotation, full rotation; i.e. turning halfway or completely over when initiated), and non-reciprocation (ignore, evade; i.e. either not responding when initiated or avoiding initiation) were quantified (Himmler et al., 2013; Hole, 1988).

Statistical Analyses

Data from female and male offspring were analyzed separately, as animals were assessed at different ages. Offspring weights were analyzed using a repeated measures ANOVA [prenatal treatment (between-subject factor) × day (within-subject factor)]. To examine whether animals from the same prenatal treatment showed social preferences within a given triad, the duration/frequency subjects spent investigating an animal from the same versus different prenatal treatment group was analyzed using paired t-tests. One-way ANOVAs were used to assess potential differences in play initiation frequency by outgroup animals across all triads. For all tests, the software package Statistica 13 (Statsoft, USA) was used. Data are presented as mean ± SEM. Significance was set at $p = 0.05$. Effect size measurements were made using partial eta squared and Cohen's d as appropriate.

Results

Effects of prenatal treatment on offspring weight

As expected, weights increased across development for both male and female offspring in all prenatal treatments [Table 1; within-factor effect of age (females: $F_{4,284}=5242.83$, $p<0.0001$, $\eta_p^2 = 0.99$; males: $F_{4,252}=5022.21$, $p<0.0001$, $\eta_p^2 = 0.99$]. In addition, female PF animals demonstrated slightly increased weight gain, which emerged at P22 and persisted until testing at P30 [main effect of prenatal treatment ($F_{2,71}=270.5$, $p=0.016$, $\eta_p^2 = 0.11$); interaction of prenatal treatment × age ($F_{8,284}=5242.83$, $p=0.011$, $\eta_p^2 = 0.07$)]. Specifically,

PF females weighed more than control females on P22, and more than both control and PAE females on P30. No other differences in weight were observed among males and females from the different prenatal treatment groups.

Triad composition unveils asymmetry in target of social interaction

Within each triad, assessment of behaviors directed to either the same or different prenatal treatment playmates revealed that female and male controls in CCE triads spent significantly less time playing with a PAE playmate than with a fellow control playmate [Figure 1b,c (female: $t_{17}=2.985$, $p=0.008$, $d=0.86$; male: $t_{15}=2.183$, $p=0.045$, $d=0.87$)]. Notably, play target asymmetry was exclusively observed in CCE triads, as analysis of EEC, CCP, and PPC triads revealed no differences in play duration between playmates from ingroup vs. outgroup prenatal treatments.

Play target asymmetry by control animals in CCE triads

Further assessment revealed differences in play initiation and play reciprocation in male and female CCE triads: male controls in CCE triads showed less initiation with a PAE versus a control playmate [Figure 2b ($t_{15}=2.248$, $p=0.04$, $d=0.79$)], whereas female controls in CCE triads showed less reciprocation with a PAE versus a control playmate [Figure 2c ($t_{17}=2.95$, $p=0.009$, $d=0.71$)]. Importantly, no differences in play initiations by the outgroup animals were observed across triads (Table 2). Again, differences in initiation/reciprocation frequencies between ingroup and outgroup playmates were exclusive to CCE triads, as EEC, CCP and PPC triads did not show play target asymmetries in initiation/reciprocation frequencies.

Discussion

Our novel approach utilizing triads of mixed-treatment composition provides insight on how play partner identity and social group composition interact to shape behavior, particularly in the context of PAE. Specifically, we found no play target asymmetry when the triad composition did not include a PAE animal (CCP or PPC), suggesting that control and PF animals do not discriminate between each other based on prenatal treatment, likely because both groups are socially competent. As well, we found no play target asymmetry within EEC triads, where PAE animals were given the choice between a fellow PAE playmate and a control playmate. However, when control animals had the option to play either with a fellow control or a PAE playmate (CCE), control animals showed play target asymmetry such that they biased their play behavior towards fellow control animals. This play bias suggests a deficit in social competence of PAE animals; that is, PAE-related social behavior deficits may not only serve to impede play with a control playmate but may also augment the relative attractiveness of a fellow control versus a PAE playmate. Notably, these results were consistent in CCE triads of both sexes, with subtle differences observed in the frequencies of initiations vs. reciprocations of male and female animals, respectively. Taken together, our data show that PAE animals are less effective at engaging and responding in playful interactions, which demonstrates negative effects of PAE on the development of social competence.

Triad composition unveils play target asymmetry in play behavior duration regardless of sex

That PAE alters play behavior – and social behavior in general – has long been established, though the exact mechanisms underlying these changes are still not completely understood (for excellent review, see Marquardt & Brigman, 2016). Methods utilizing dyadic play protocols in PAE rodent models have demonstrated that PAE may reverse the sex-typical expression of play behavior (Meyer & Riley, 1986), lead to overall increases in play behavior relative to control animals (Royalty, 1990), and disrupt processing of social cues important for play behavior (Charles Lawrence et al., 2008). Importantly, these PAE-related play behavior alterations may be the root of many of the long-lasting social behavior deficits observed in PAE animals, including altered patterns of social behavior (Donaldson et al., 2018), impaired social recognition memory (Holman, Ellis, Morgan, & Weinberg, 2018; Kelly, Leggett, & Cronise, 2009), and reduced social interaction (Hellemans, Sliwowska, Verma, & Weinberg, 2010).

In the present study, we observed play target asymmetry in both male and female CCE triads due to increased playfulness between control playmates (i.e., more time spent playing with a fellow control animal) and a subsequent attenuation in play duration with the PAE playmate. The observation that PAE animals are less desirable social targets for control playmates suggests that PAE animals have impaired social competence, as they are less effective at engaging and responding in playful interactions. Notably, control animals only showed biased play when given a choice between a fellow control playmate and a PAE playmate, and not when choosing between a fellow control and PF playmate (i.e. CCP triads). A similar lack of play asymmetry was observed when a PF animal was given a choice between a fellow PF playmate and a control playmate (i.e. PPC triads). This lack of play asymmetry in both CCP and PPC triads supports the specificity of the PAE insult on social competence. That is, neither control nor PF animals differentially bias play when playing in heterogeneous triads, suggesting that these animals are matched in social competence and thus do not differentiate each other by prenatal treatment. Importantly, PAE animals in EEC triads also did not show play target asymmetry; that is, they did not spend differential amounts of time in play with a control than with a fellow PAE playmate. Given that control animals show play asymmetry in CCE triads – as well as the lack of play asymmetry in EEC, CCP and PPC triads – our results suggest that PAE impairs social competence to solicit and engage a playmate, which subsequently may exaggerate the attractiveness of a fellow, socially competent control playmate.

Importantly, we did not observe differences in social investigation – a proxy measure for social interest – in any triad of either sex. These results suggest that social deficits following PAE appear to be driven by impaired social competence and not from an overall lack of social motivation. This finding is also supported by previous work from our lab utilizing the 2- or 3-chambered social interaction task, which demonstrated that adolescent PAE animals do not differ from their unexposed counterparts in social motivation (Holman et al., 2018). These results are also in line with the clinical literature, which reports that individuals with FASD exhibit typical and even enhanced levels of social motivation (Nanson, 1992), and have even been described as being inappropriately friendly (Kully-Martens et al., 2012).

Attenuated social motivation is generally associated with other neurodevelopmental conditions such as autism spectrum disorder, where deficits in social motivation are thought to be a core feature (Bishop et al., 2007; Chevallier, Kohls, Troiani, Brodtkin, & Schultz, 2012). Nevertheless, other studies of PAE effects on social behavior have reported reduced social motivation (Ignacio, Mooney, & Middleton, 2014; Mooney & Varlinskaya, 2011), though these studies differ from the present work in rat strain utilized, dose and pattern of PAE (e.g. single binge dose vs. chronic prenatal exposure), as well as the different behavioral paradigm utilized. Regardless, that control animals in CCE triads do not show differences in social investigation highlights the play-specific deficits resulting from PAE; control animals in CCE triads investigate PAE and fellow control playmates equally, and only show behavioral asymmetry with play likely resulting from impaired social competence of PAE animals.

Play target asymmetry related to differences in play initiation and reciprocation

Further assessment of play behavior allowed for a more detailed analysis of the nature of play target asymmetry, revealing that play target asymmetry in male and female CCE triads arose from differences in the frequency of initiations and reciprocations: male control animals preferentially initiated with fellow control playmates while female control animals preferentially reciprocated with control playmates. In other words, for male control animals in CCE triads, PAE animals did not appear to be attractive play targets and may instead have served to enhance the appeal of fellow control playmates. In female CCE triads, however, it seems PAE animals were not able to initiate play appropriately such that control animals were not persuaded to respond to their solicitations. We observed these differences exclusively within CCE triads and not in EEC, CCP, or PPC triads of either sex, again highlighting not only the specificity of PAE to impair social competence, but also revealing subtle differences in the patterns of play behavior for female and male control animals playing with PAE animals.

The literature has documented sex differences in play behavior expression, such that males typically show greater frequencies than females of rough-and-tumble play (Argue & McCarthy, 2015; Auger & Olesen, 2009; Meaney & Stewart, 1981). Interestingly, previous studies have employed dyadic play protocols within a chronic model of PAE, in which each experimental animal was tested across several sessions, but playmates were alternated between control, PF, and PAE animals (Meyer & Riley, 1986). This study found that PAE resulted in a reversal of sex-typical play expression, such that PAE males exhibited play levels similar to unexposed females, while play levels of PAE females were comparable to those of unexposed males. However, play interactions were collapsed across all play sessions thus precluding the investigation of differences in play associated with play partner identity. In view of these results, it is tempting to speculate that the play target asymmetry observed in CCE triads in the present study may be driven by sex-atypical expression of social play behavior by PAE animals. However, we cannot discount the potential effects of age at testing on play behavior asymmetry, as females and males were tested at different ages in an attempt to control for the differential timing of pubertal onset in males and females. As females were tested much closer to the developmental peak of play behavior observed in rats (Spear, 2000), it would be difficult to conclude whether the different

patterns of initiation/reciprocation observed between male and female CCE triads were due to sex differences or age differences. Moreover, careful inspection of the graphs indicates that the effects of PAE in both males and females – while not separately significantly different – show roughly similar patterns.

Possible mechanisms underlying impaired social competence following PAE

The neuropeptide oxytocin (OT) has been implicated in the regulation of various aspects of social behavior (Bredewold & Veenema, 2018; Dore, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2013), including social motivation (Lim & Young, 2006), social recognition (Bielsky and Young, 2004; Engelmann, Ludwig, & Landgraf, 1994; Ferguson, Young, & Insel, 2002; Veenema, Bredewold, & De Vries, 2012) and play behavior (Bredewold, Smith, Dumais, & Veenema, 2014). Though we did not assess the OT system in the current study, we have previously shown that with testing at a similar early adolescent age, PAE results in impairments to social recognition memory and alterations to OT receptor binding in the amygdala and prefrontal cortex (Holman et al., 2018), brain regions that show correlated activity during play behavior (van Kerkhof et al., 2014). Both the amygdala and prefrontal cortex are critically important for the typical expression of play behavior, as lesions to either region result in severe social behavior impairments, such as decreased play behavior in infant/adolescent rats (Bell, McCaffrey, Forgie, Kolb, & Pellis, 2009; Daenen, Wolterink, Gerrits, & Van Ree, 2002; M. Meaney, Dodge, & Beatty, 1981). Interestingly, research using prenatal valproic acid exposure – an animal model of autism spectrum disorders – has demonstrated reduced play behavior and OT receptor binding in the amygdala of exposed offspring (Bertelsen et al., 2017). Taken together, it is plausible that our observation of impaired social competence following PAE may be mediated, at least in part, by alterations to the OT system. Moreover, as impaired social behavior and alterations in OT binding in the amygdala have been observed in adult PAE rats, it appears that PAE has long-term consequences on social behavior development and limbic OT function (Kelly et al., 2009).

A possible alternative explanation for play target asymmetry in CCE triads is that PAE may alter the olfactory signature such that it could be antagonistic to control playmates. Indeed, stress-related odors emitted by conspecifics have been shown to impact on various aspects of rodent behavior (Abel & Bilitzke, 1990; Rottman & Snowdon, 1972). Of relevance to the current study, predator odor has previously been shown to suppress play behavior in rats, suggesting that particularly threatening olfactory cues in the peripheral environment can impact on play behavior expression (Siviy, Harrison, & McGregor, 2006). Besides environmental odor cues, conspecific odor cues have been shown to be important for mediating attachment, social recognition memory, and sexual behavior (Camats Perna & Engelmann, 2016). Nevertheless, rodent play seems most impacted by somatosensory and – based on more recent evidence in the literature – auditory cues in the form of ultrasonic vocalizations (Charles Lawrence et al., 2008; Wellmann, George, Brnouti, & Mooney, 2015). Indeed, striking early findings from the play literature demonstrated little to no effects of bulbectomy/anosmia on play behavior expression, suggesting that olfaction may not be as critical a sensory cue for play behavior (Beatty & Costello 1983; Thor & Holloway, 1982). While these data suggest that olfaction may not be a key factor for the typical expression of play behavior, they do not necessarily address whether olfactory cues

from PAE conspecifics can modify the expression of play behavior. However, that outgroup animals – and in particular controls in EEC/PPC triads – do not differentially initiate ingroup animals does not appear to support the alternative hypothesis of different conspecific olfactory cues driving play target asymmetry in CCE triads.

Implications

When viewed in the context of social behavior development, the ramifications of PAE-related impairments in social competence are noteworthy. Indeed, the present data demonstrate that, in addition to PAE-related alterations in social competence – presumably due to altered processing and integration of social cues in the brain (Charles Lawrence et al., 2008; Holman et al., 2018; Lugo et al., 2003) – PAE may levy a secondary insult by precluding age-appropriate opportunities for social experiences through the subsequent exclusion from play with competent playmates. Interestingly, an analogous situation has been described in the early neonatal period following PAE, when social interactions are restricted to the mother-pup dyad. Specifically, PAE pups show a diminished capacity to elicit retrieval by the dam as compared to unexposed pups, even when tested with a dam that never consumed alcohol (Ness & Franchina, 1990). Consequently, PAE serves as a primary insult to the pup's ability to elicit retrieval, which subsequently alters maternal responsiveness. Given the critical role of maternal behavior in shaping offspring development, this observed alteration in maternal responsiveness may further exacerbate PAE-related impairments to the development of social competence (Champagne, Diorio, Sharma, & Meaney, 2001).

Adolescence is a critical period of development encompassing significant maturational changes – including pubertal onset – that can have dramatic consequences for brain and behavioral development, including the development of social behavior, making this a unique period of increased vulnerability to social behavior dysfunction (Blakemore, 2008; Blakemore, Burnett, & Dahl, 2010; McCormick & Mathews, 2007; Sisk & Zehr, 2005). Indeed, studies utilizing a model of social instability stress during adolescence – in which animals are isolated and then re-housed with a novel cagemate each day from P30-P45 – have demonstrated a wide range of negative outcomes not only on social behavior, but also on cognitive and emotional neurobehavioral development (Green, Barnes, & McCormick, 2013; Hodges et al., 2017). Impaired social competence in PAE animals may serve as a form of social instability stress, which carries additional risk for suboptimal social, emotional, and cognitive development following PAE.

The clinical literature has described PAE-related impairments in social competence, reporting that children with FASD often struggle with reading social cues, socially appropriate interactions, and establishing reciprocal peer relationships (Bishop et al., 2007; Domeij et al., 2018; Stevens et al., 2017). Importantly, deficits in social competence increase the risk for encountering additional challenges with social behavior development, as well as development in motor, cognitive, and emotional domains (Welsh & Bierman, 2001). Consequently, it is not surprising that children with FASD also experience high rates of social rejection, bullying, and later social withdrawal that may contribute to difficulties in school, further social rejection, trouble with the law, and later mental health problems

(Carmichael Olson, Feldman, Streissguth, Sampson, & Bookstein, 1998; Streissguth et al., 1996). Notably, FASD-related impairments in social competence extend into adulthood, as affected individuals continue to exhibit deficits in social responsiveness and interpersonal relationship skills (Kelly et al., 2000; Kully-Martens et al., 2012), and consistently lag behind peers in social behavior function (Streissguth et al., 1991). The present data highlight the impact of the social environment in the context of impaired social competence, which has important implications for understanding the complete impact of PAE on social behavior development.

Results from the present study also raise important questions related to designing appropriate interventions for ameliorating PAE-related social behavior deficits. For example, several preclinical studies have investigated whether social enrichment can attenuate PAE-related social behavior deficits. In one study utilizing a moderate/low alcohol exposure paradigm, PAE and control rats were housed in mixed pairs from weaning until adulthood, when they were assessed using tests of social, motor, and cognitive behavior (Rodriguez et al., 2016). Not only did social enrichment (i.e. mixed housing) fail to ameliorate PAE-related social behavior deficits, it instead led to behavioral impairments in the control cagemates of PAE animals for all behavioral domains assessed. Conversely, another study of social enrichment performed in an acute binge model of PAE found that group housing a PAE rat with control animals positively impacted some but not all aspects of social behavior in a social interaction test. However, in addition to the different exposure paradigm, this study also utilized a slightly altered method of social enrichment, in which each PAE animal was housed with 2–3 control animals from weaning until adolescent behavioral testing (Middleton, Varlinskaya, & Mooney, 2012).

Though both earlier studies observed social behavior deficits following PAE, there are several caveats to understanding the contrasting results of social enrichment on PAE-related social behavior deficits. First, the two reference studies utilized different PAE paradigms, which induced unique deficits in social behavior; indeed, even within the same binge exposure paradigm, the timing of exposure can result in vastly different outcomes for social behavior (Mooney & Varlinskaya, 2011). Second, the social enrichment protocols between these two studies differed significantly in their duration, as the first utilized mixed housing of animal pairs over a ~13-week period while the other employed mixed housing of groups of animals for a relatively protracted period (~20 days) during adolescence. Finally, in the Middleton et al. study, PAE animals were compared to control animals housed with other control animals, and not to their control cagemates – as was done in the Rodriguez et al. study. Thus, while social enrichment using group housing with controls appears to improve PAE-related social behavior deficits, it is not clear if this positive effect is specific to the unique effects of G12 binge exposure and/or is limited to adolescent animals; moreover, the potential impact of mixed housing on control animals housed with a PAE rat is not addressed. Nevertheless, that control animals reared with PAE cagemates show aberrant social behavior would be predicted by our present results, and presumably arises due to impaired social competence of PAE animals during social development and subsequent asymmetry of social targeting by control animals.

Conclusions

Taken together, asymmetries in play behavior by control animals in male and female CCE triads, together with the lack of play target asymmetry in EEC, CCP, and PPC triads, suggests that PAE compromises social competence, which may in turn be exacerbated by reduced play opportunities with socially competent conspecifics. Though social behavior is dramatically different between the rat and human, the parallels between our results and findings from the clinical FASD literature are striking. Indeed, our results highlight the complexity of addressing social behavior impairments following PAE and indicate that behavioral interventions should consider the value of the social environment in promoting meaningful improvements to social behavior function in individuals prenatally exposed to alcohol.

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Figure 1.

Diagrams of each triad composition, color coded by prenatal treatment (row a). Duration of social investigation (rows b, d) and play behavior (rows c, e) for female and male animals.

Data are expressed as mean $\pm$ SEM. * indicates control animals in CCE triads spent significantly less time playing with a PAE playmate.

Figure 2.

Diagrams of each triad composition, color coded by prenatal treatment (row a). Frequency of play initiations (rows b, d) and play reciprocations (rows c, e) for female and male animals. Data are expressed as mean ± SEM. * indicates control animals in CCE triads showed significantly fewer initiations/reciprocations with a PAE playmate.

Table 1.

Developmental weights.

Developmental Data			
	Prenatal treatment group		
	Control	PF	PAE
Female			
P1	6.2 ± 0.1	6.5 ± 0.1	6.2 ± 0.1
P8	16.1 ± 0.3	16.7 ± 0.3	16.1 ± 0.2
P15	32.0 ± 0.5	34.6 ± 0.7	33.8 ± 0.5
P22	53.2 ± 0.8	57.4 ± 1.2 ^a	55.3 ± 0.9
P30	98.1 ± 1.8	104.4 ± 2.4 ^{ab}	99.3 ± 1.9
Male			
P1	6.8 ± 0.1	6.9 ± 0.1	6.4 ± 0.2
P8	17.4 ± 0.4	17.5 ± 0.4	16.9 ± 0.3
P15	33.8 ± 0.7	35.0 ± 0.7	34.3 ± 0.5
P22	56.0 ± 1.0	58.2 ± 1.0	56.8 ± 0.9
P37	176.0 ± 3.4	177.3 ± 4.0	171.3 ± 4.1

Data are expressed as mean ± SEM. Superscripts indicate a significant main effect of prenatal treatment

^aPFs are different from control or

^bPAE animals.

Table 2.

Mean frequency (#) of initiations by outgroup animals in each triad.

Mean frequency (#) of initiations by outgroup animals in each triad

	Triad			
	CCE	EEC	CCP	PPC
Female (n)	(18)	(26)	(12)	(22)
Initiations by outgroup animal (#)	17.4 ± 1.8	19.2 ± 1.5	20.7 ± 2.2	19.3 ± 1.6
Male (n)	(16)	(20)	(12)	(22)
Initiations by outgroup animal (#)	16.6 ± 2.7	16.6 ± 2.4	16.4 ± 3.1	22.3 ± 2.3

Data are expressed as mean ± SEM.