

RESEARCH ARTICLE



Altered activity within the social behavior neural network in adolescent rats following prenatal alcohol exposure and/or early-life adversity

Parker J. Holman^{1,2} | Linda Ellis¹ | Amanda Chao¹ | Cecilia Mitchell¹ |
Charlis Raineke² | Joanne Weinberg¹

¹Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada

²Department of Psychology, Brock University, St. Catharines, Ontario, Canada

Correspondence

Parker J. Holman, Department of Psychology, Brock University, 1812 Sir Isaac Brock Way, St. Catharines, ON L2S 3A1, Canada.

Email: pholman@brocku.ca

Funding information

National Institute on Alcohol Abuse and Alcoholism, Grant/Award Number: F31 AA023151 and R01 AA022460; Canadian Foundation on Fetal Alcohol Research

Abstract

Background: Social behavior relies on the dynamic, complex, and coordinated activity of a highly conserved “social behavior neural network,” which includes the olfactory bulb (OB), piriform cortex (PCX), lateral septum, medial prefrontal cortex (mPFC), amygdala, paraventricular nucleus of the hypothalamus (PVN), and ventral hippocampus. Prenatal alcohol exposure (PAE) is known to disrupt social behavior development, leading to lifelong social functioning impairments. Individuals with PAE are at heightened risk of experiencing early-life adversity (ELA), which independently affects social behavior development; however, little is known about the combined effects of PAE and ELA on social behavior.

Methods: We previously demonstrated that PAE and ELA impact social recognition memory throughout adolescence; here, we combine animal models of PAE and ELA to gain insight into both independent and interactive effects of these insults on social behavior network neural activity in both early and late adolescent male and female rats. We measured neural activity (*c-fos* mRNA expression) across the network following social recognition memory testing.

Results: Our findings indicate that both PAE and ELA are associated with altered neural activity in regions supporting social recognition memory, notably the OB, PCX, mPFC, amygdala, and PVN. The direction of these effects and specific regions impacted vary by sex and age at assessment. Importantly, different brain areas exhibit distinct sensitivities to each type of insult, with PAE generally resulting in hypoactivity of the amygdala and ELA altering mPFC activity.

Conclusions: These data contribute to a more complete social neurobehavioral profile, accounting for both PAE and ELA, to support earlier diagnoses and interventions.

KEYWORDS

adolescence, *c-fos*, early-life adversity, neural activity, prenatal alcohol exposure, social behavior neural network

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). Alcohol, Clinical and Experimental Research published by Wiley Periodicals LLC on behalf of Research Society on Alcohol.

INTRODUCTION

Prenatal alcohol exposure (PAE) results in a wide range of neurobiological, behavioral, and physiological impairments (Marquardt & Brigman, 2016; Mattson et al., 2019; Wilson et al., 2024). Among the many PAE-related neurobehavioral impairments, social behavior deficits are especially pervasive (Kelly et al., 2000; Kully-Martens et al., 2012; Mooney & Varlinskaya, 2011; Rockhold et al., 2021); indeed, impaired social function is the second most commonly reported behavioral deficit after attention following PAE (Steinhausen & Spohr, 1998). PAE-related social behavior deficits emerge early in development and become more pronounced around adolescence (Duquette et al., 2006; Rockhold et al., 2021; Streissguth et al., 1996). Importantly, adolescence is a critical period of development encompassing significant maturational changes that can have dramatic consequences for social behavior, making it a unique period of increased vulnerability, especially in the context of PAE (Blakemore et al., 2010; Sisk & Zehr, 2005).

Individuals with PAE are at an increased risk of experiencing early-life adversity (ELA; Holman & Rainekei, 2023; O'Connor & Paley, 2006), which independently can produce long-term social behavior deficits (Conaway & Hansen, 1989; Rainekei et al., 2012), often making it difficult to separate the effects of PAE from those of ELA with regard to social behavior function. To address the unique and potentially interactive neurobehavioral effects of these two insults, we previously combined two animal models known to result in social behavior deficits: a well-established rat model of PAE (Helleman et al., 2010; Holman et al., 2018, 2019) and a naturalistic model of ELA (limited nest bedding from postnatal day [P]8–12; Rainekei et al., 2012, 2019; Rincón-Cortés & Sullivan, 2016) and assessed social recognition memory across adolescence (Holman et al., 2021). To evaluate social recognition, we used a behavioral assay that exploits the natural tendency of rodents to investigate a novel social stimulus more than a familiar social stimulus (Engelmann et al., 2011; Gabor et al., 2012). We found that PAE impaired social recognition memory in early adolescent males (P30) regardless of ELA. However, in early adolescent females, ELA impaired social recognition memory regardless of PAE (Holman et al., 2021). All social recognition memory impairments observed during early adolescence disappeared when animals were tested on a second day, suggesting that these PAE-related deficits in males and ELA-related deficits in females may be experience-dependent effects, likely representing delayed development. Another indication that the observed memory impairments in females may reflect delayed development is that neither PAE nor ELA affected social recognition memory function in late adolescence (P45). By contrast, while ELA impaired social recognition memory on the first test day for late adolescent males regardless of PAE, social recognition deficits persisted on the second test day only in animals exposed to both PAE and ELA, suggesting additive effects of PAE and ELA in late adolescent males (Holman et al., 2021).

Intact social recognition memory involves the coordinated activity of the highly conserved “social behavior neural network,” which is composed of interconnected brain regions, including the olfactory

bulb (OB) and associated olfactory cortices, such as the piriform cortex (PCX; Young, 2002), lateral septum (LS; Albers, 2012), medial prefrontal cortex (mPFC; Bell et al., 2009; Diamond, 2011), amygdala (Amaral, 2003; Katayama et al., 2009), hypothalamus (Ross & Young, 2009), and ventral hippocampus (Sun et al., 2020). Because of the intrinsic interconnectivity among these brain areas, altered neural activity within or among structures in this neurocircuitry could result in social behavior dysfunction. Despite our demonstration that PAE and ELA have unique and interactive sex- and age-specific effects on social recognition memory (Holman et al., 2021), the neural circuitry mediating such impairments has not been explored. To fill this gap, we used mRNA expression of the immediate early gene *c-fos*—a marker of neural activity—to evaluate the unique and/or interactive effect of PAE and ELA on the neural activity of the social behavior neural network in early (P30) and late (P45) adolescent male and female rats in response to the social recognition memory test.

MATERIALS AND 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 07:00 AM) 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) 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.

Prenatal alcohol exposure

On G1, females were single-housed and randomly assigned to one of two treatment groups: PAE or *ad libitum*-fed Control. PAE dams ($n=29$) were offered an *ad libitum* liquid ethanol diet (6.37% v/v) with 36% ethanol-derived calories (Figure 1). The liquid ethanol diet was introduced gradually over the first 3 days with bottles containing: Day 1—66% control diet and 34% ethanol diet; Day 2—34% control diet and 66% ethanol diet; Day 3—100% ethanol diet. This diet is formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake. To determine blood alcohol levels (BALs) of alcohol-consuming dams, tail blood samples from a subset of dams ($n=13$) were taken on G15 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 the Pointe Scientific Inc. Alcohol Reagent

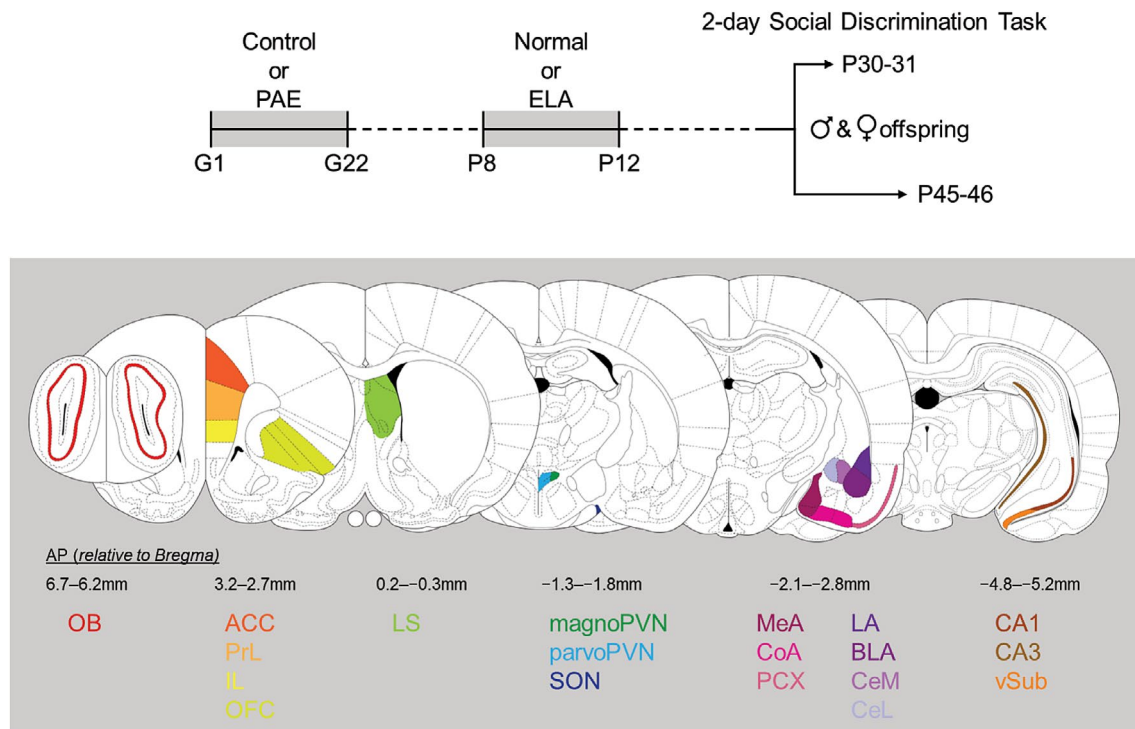


FIGURE 1 Experimental timeline and regions of interest. (Top) Schematic overview of the experimental design. Prenatal alcohol exposure (PAE) occurred throughout gestation (gestational days [G]1–22). Early-life adversity (ELA) was induced between postnatal days (P)8–12 using the low bedding model. Behavioral and neurobiological assessments were conducted during early (P30) and late (P45) adolescence. (Bottom) Representative coronal atlas sections illustrating the regions of interest (ROIs) analyzed for *c-fos* expression. Color-coded brain regions include the olfactory bulb (OB), anterior cingulate cortex (ACC), prelimbic cortex (PrL), infralimbic cortex (IL), lateral septum (LS), magno- and parvocellular divisions of the paraventricular nucleus (magnoPVN; parvoPVN), supraoptic nucleus (SON), amygdala nuclei: lateral (LA), basolateral (BLA), centro-lateral (CeL), centro-medial (CeM), medial (MeA), and cortical (CoA), ventral hippocampus: CA1, CA3, and ventral subiculum (vSub), shown with anterior–posterior (AP) coordinates relative to bregma (Paxinos & Watson, 2005).

Set (Lincoln Park, MI, USA); the minimum detectable concentration of alcohol is 2 mg/dL. Alcohol-consuming dams showed a mean of 176.9 ± 67.2 mg/dL (max BAL = 252.8 mg/dL; min BAL = 68.7 mg/dL). *Ad libitum*-fed Control dams ($n = 31$) 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. 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–P1), litters were weighed and culled to 12 pups with an attempt to preserve an equal number of males and females per litter.

Early-life adversity

ELA rearing condition: Within 2 h of lights off on P7, half the dams/litters from each prenatal treatment (PAE, $n = 16$, or *ad libitum*-fed control, $n = 15$) were transferred to clean cages with limited nesting/

bedding material that consisted of 300 mL of Beta Chip® bedding (Northeastern Products Corp, Warrensburg, NY; Figure 1). The animals remained in this limited bedding environment until the afternoon of P12 (within 2 h of lights off). Normal rearing condition: The remaining dams/litters (PAE, $n = 15$, or *ad libitum*-fed control, $n = 17$) were transferred to clean cages with abundant nesting/bedding material (3000 mL) within 2 h of lights off on P7 and remained in this environment until the afternoon of P12.

Experimental design

After weaning (P22), male and female offspring were housed by sex and prenatal treatment/rearing condition. At P25, nonsiblings were pair-housed by sex and prenatal treatment/rearing condition with no more than 1 male and/or female per litter used at each age of testing. Male and female offspring were tested on two consecutive days in a social discrimination task in either early (P30) or late (P45) adolescence to account for potential effects of gonadal hormone changes across puberty (Figure 1). Social discrimination behavioral data were published in Holman et al. (2021). Animals were decapitated 30 min after the end of the social discrimination test on the second test day; brains were collected, quickly frozen on dry ice, and stored at -80°C .

Brain slicing

Brains were sectioned coronally (20 μ m) using a cryostat (-16°C) and stored at -80°C until *c-fos* in situ hybridization (ISH) assays. Regions of interest for *c-fos* ISH assays included (see Figure 1) olfactory regions (olfactory bulb [OB] and piriform cortex [PCX]), lateral septum [LS], medial PFC (mPFC: anterior cingulate [ACC], prelimbic [PrL], infralimbic [IL], and orbitofrontal [OFC] cortices), amygdala (basal [BA], lateral [LA], medial [CeM] and lateral [CeL] divisions of the central amygdala [CeA], and medial [MeA] subnuclei), ventral hippocampus (vHPX: CA1, CA3, dentate gyrus [DG], and ventral subiculum [SubV]), and hypothalamus (paraventricular [PVN; magnocellular and parvocellular divisions]). Brain tissue from early and late adolescent animals was run simultaneously by brain region to be able to compare groups.

In situ hybridization

A ribonucleotide probe was used to measure *c-fos* and was prepared using a rat *c-fos* 2116-bp template provided by Dr. Victor Viau (Department Cellular and Physiological Sciences, The University of British Columbia, Canada). Probes were labeled with 35S-UTP (Amersham Biosciences, NJ, USA) using Polymerase T7 and the Promega Riboprobe System (Promega Corporation, Madison, WI, USA). All probes were purified using Micro Bio-Spin 30 Columns (Bio-Rad, CA, USA). One molar of DTT was added to prevent oxidation.

Sections were thawed (20 min) and went through prehybridization as follows: 1 $\times$ PBS twice for 10 min each, proteinase K (100 $\mu\text{g/L}$; at 37°C) for 9 min, 0.1 M triethanolamine-hydrochloride (TEA) for 10 min, 0.1 M TEA with 0.25% acetic anhydride for 10 min, 2 $\times$ SSC twice for 10 min each, dehydration by a graded series of ethanol, chloroform for 5 min, and finally 100% ethanol before being air dried. Hybridization buffer (75% formamide, 3 $\times$ SSC, 1 $\times$ Denhardt's solution, 200 $\mu\text{g/mL}$ yeast tRNA, 50 mM sodium phosphate buffer (pH 7.4), 10% dextran sulfate, and 10 mM DTT) was applied (1 $\times$ 10⁶ cpm/slide) and covered with HybriSlips (Sigma-Aldrich, ON, Canada). Sections were incubated overnight at 55°C in chambers humidified with 75% formamide. HybriSlips were removed and the slides were rinsed as follows: 2 $\times$ SSC twice for 20 min, 2 $\times$ SSC for 30 min, 50 $\mu\text{g/L}$ RNase A solution (at 37°C) for 60 min, 2 $\times$ SSC with 0.01 M DTT for 10 min, 1 $\times$ SSC for 10 min, 0.5 $\times$ SSC with 0.01 M DTT for 10 min, 0.1 $\times$ SSC with 0.01 M DTT (at 60°C) for 60 min, and 0.1 $\times$ SSC for 5 min. Sections were dehydrated by a graded series of ethanol and air dried overnight.

Kodak BioMax autoradiography film was exposed to hybridized slides of all brain regions except for the hypothalamus. Hypothalamus slides were dipped in Kodak NTB2 autoradiography emulsion (Eastman Kodak Co.) diluted 1:1 (Deionized H₂O) and exposed in desiccated, light-tight boxes at 4°C . Exposure times were as follows: 13 days for OB, 6 days for the mPFC and PCX, 25 days for LS, 91 days for the hypothalamus, and 38 days for vHPX. Slides were developed with Kodak D-19 developer at 14°C and fixed with Kodak fixer at 14°C , then counterstained with Cresyl Violet. Coverslips were mounted with Permount (Fisher Scientific Ltd., Nepean, ON, Canada).

Densitometric analysis

Autoradiographic films were scanned and analyzed with the Image J 1.50i software (National Institutes of Health, Bethesda, MD). The left and right OB (mitral cell layer), PCX, LS, mPFC, amygdala, and vHPX were traced freehand according to a stereotaxic rat brain atlas (Paxinos & Watson, 2005) in two sections per animal to determine mean gray density levels. Background was measured from the corpus callosum, and corrected gray levels were obtained by subtracting the background level from each of the four measurements. Left and right levels in each measured area were averaged together for analysis. For emulsion dipped slides (i.e., hypothalamus), ISH signals were visualized with a Q-imaging monochrome 12-bit camera attached to a Zeiss Axioskop 2 motorized plus microscope. Images were captured using Northern Elite 6.0v (Empix Imaging Inc., Mississauga, ON, Canada), and semi-quantitative densitometric analyses were performed using the Image J 1.50i software (National Institutes of Health, Bethesda, MD). The mean optical density of hybridization signal, corrected by background subtraction, was taken under dark-field illumination. Background signal was measured over the optic tract immediately adjacent to each side of the area of interest. The corrected gray levels from both sides of two sections of each region were averaged to obtain a mean corrected gray level of the four measurements for each animal.

Statistical analyses

All data are expressed as mean $\pm$ SEM. Outliers were identified and removed using the Robust regression and Outlier removal (ROUT) method with $Q=0.05$. *c-fos* mRNA expression were assessed by two-way ANOVAs, separate for sex and age, for the factors of prenatal treatment and rearing condition. Where appropriate, Fisher's LSD post hoc tests were used to test for differences among groups. Further analyses utilized planned comparisons to test the *a priori* hypotheses that: (1) PAE will alter neural activity responses (i.e., normally reared PAE animals will show differential neural activity as compared to normally reared control animals); and (2) ELA rearing will differentially alter neural activity responses (i.e., ELA control animals will show differential neural activity as compared to normally reared control animals). For all tests, the software packages Statistica 13 (Statsoft, USA) and GraphPad Prism 10.0 (USA) were used. Significance was set at $p \leq 0.05$.

RESULTS

c-fos mRNA expression in OB, PCX, and LS

In early adolescence, normally reared PAE females had increased *c-fos* mRNA expression in the OB relative to that in normally reared control females (Figure 2A; *a priori* analysis [$p=0.03$]). In late adolescence, control females exposed to ELA had increased *c-fos* mRNA expression in the OB compared with normally reared control females (Figure 2B). However, during late adolescence,

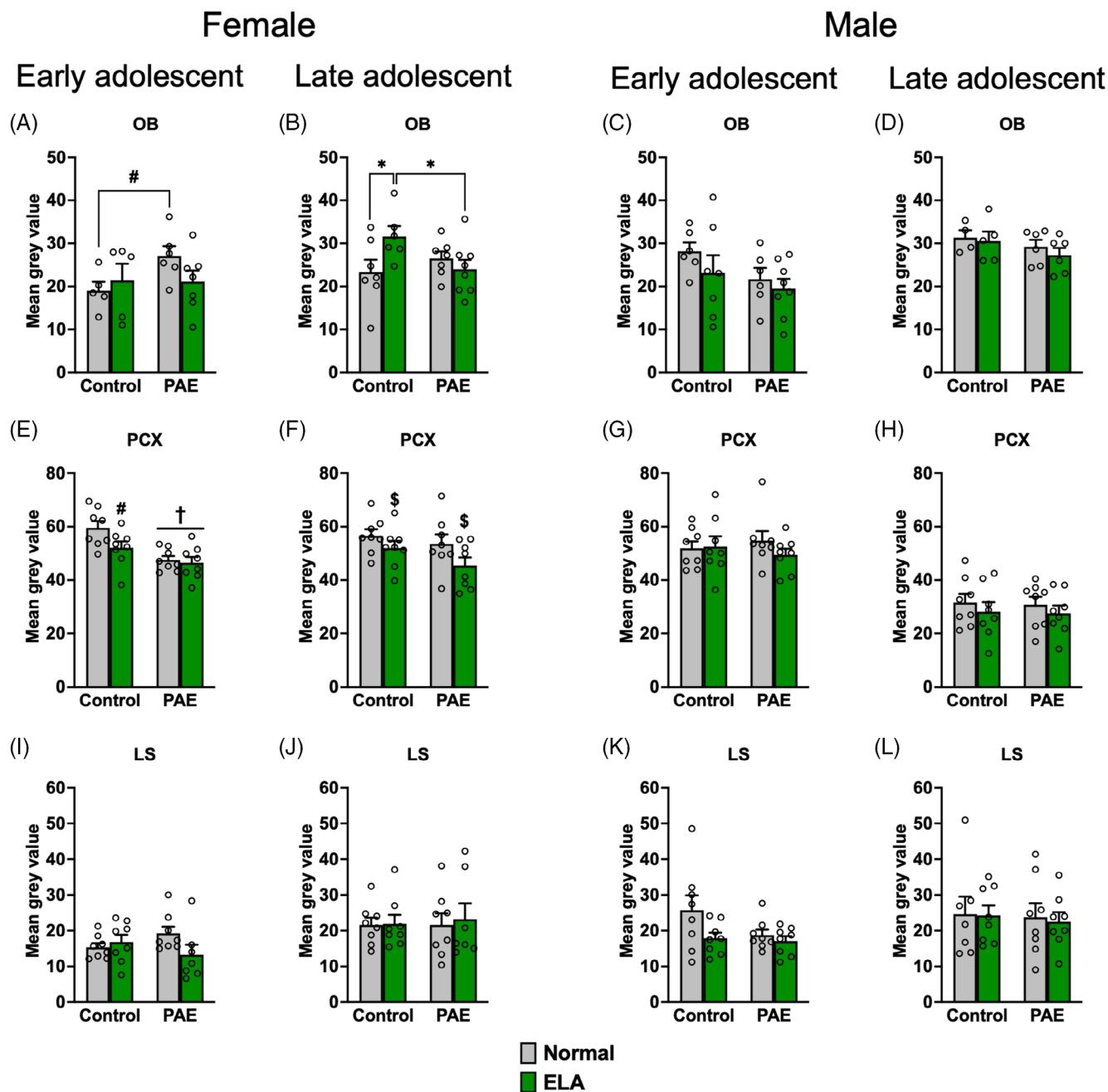


FIGURE 2 Olfactory bulb (OB), piriform cortex (PCX), and lateral septum (LS) *c-fos* expression following social recognition testing in male and female prenatal alcohol exposed (PAE) rats, with and without early-life adversity (ELA). Bars represent the mean $\pm$ SEM of the mean gray value of *c-fos* expression in the OB (A–D), PCX (E–H), and LS (I–L) ($n=4$ –8 for all groups). *Post hoc comparisons following a significant interaction between prenatal treatment and rearing condition; †PAE animals are significantly different from controls regardless of rearing condition; \$ELA animals are significantly different from normally reared animals regardless of prenatal treatment; for C, #PAE normally reared females are different from control normally reared females based on a *a priori* comparison; for G, #Control ELA females are different from control normally reared females based on a *a priori* comparison.

PAE females exposed to ELA had reduced *c-fos* mRNA expression in the OB relative to controls exposed to ELA (significant interaction between prenatal treatment and rearing condition [$F_{1,24}=5.46$, $p=0.03$]).

In the PCX, *c-fos* mRNA expression was reduced in PAE females relative to their control counterparts during early adolescence (Figure 2E). Moreover, ELA reduced PCX *c-fos* expression in both

PAE and control females relative to normally reared control females (significant main effect of prenatal treatment [$F_{1,28}=16.28$, $p<0.0005$]; *a priori* analysis comparing control normally reared and ELA animals [$p=0.05$]). In late adolescence, similar to what was seen in early adolescence, ELA reduced PCX *c-fos* expression relative to normally reared females regardless of prenatal treatment (Figure 2F; significant main effect of rearing condition [$F_{1,28}=4.66$, $p=0.04$]).

Neither PAE nor ELA affected *c-fos* mRNA expression in the LS of either early or late adolescent females and males (Figure 2I–L). Similarly, neither PAE nor ELA affected *c-fos* mRNA expression in the OB (Figure 2C,D) or PCX (Figure 2G,H) of both early and late adolescent males.

c-fos mRNA expression in mPFC

During early adolescence, ELA increased ACC *c-fos* expression relative to normally reared males regardless of prenatal treatment (Figure 3C; significant main effect of rearing condition [$F_{1,26}=6.15$, $p=0.02$]). A similar increase in *c-fos* expression was also observed in the IL of ELA control but not PAE males during early adolescence (Figure 3K, *a priori* analysis comparing control normally reared and ELA animals [$p=0.04$]). During late adolescence, ELA reduced IL *c-fos* expression relative to normally reared control females (Figure 3J, significant interaction between prenatal treatment and rearing condition [$F_{1,28}=4.21$, $p=0.05$]). Neither PAE nor ELA affected *c-fos* mRNA expression in the PrL (Figure 3E–H) or OFC (Figure 3M–P). Similarly, in females during early adolescence (Figure 3A,E,I,M) and males during late adolescence (Figure 3D,H,L,P), neither PAE nor ELA affected *c-fos* mRNA expression in any of the mPFC subareas analyzed.

c-fos mRNA expression in amygdala

During early adolescence, PAE females and males both showed reduced BL *c-fos* expression relative to controls regardless of rearing condition (Figure 4A,C; significant main effect of prenatal treatment for females [$F_{1,28}=4.37$, $p=0.05$] and males [$F_{1,28}=6.02$, $p=0.02$]). This PAE effect was only long-lasting in males, as PAE males still show reduced BL *c-fos* expression relative to controls regardless of rearing condition in late adolescence (Figure 4D; significant main effect of prenatal treatment [$F_{1,28}=5.17$, $p=0.03$]). In early adolescence, PAE also reduced MeA *c-fos* expression in females relative to controls, regardless of rearing condition (Figure 4I; significant main effect of prenatal treatment [$F_{1,28}=4.76$, $p=0.04$]). Neither PAE nor ELA affected *c-fos* mRNA expression in LA (Figure 4E–H), CeM (Figure 4M–P), nor CeL (Figure 4Q–T).

c-fos mRNA expression in hypothalamus

In early adolescent females, ELA reduced magnPVN *c-fos* expression relative to normally reared females regardless of prenatal treatment (Figure 5A; significant main effect of rearing condition [$F_{1,27}=4.70$, $p=0.04$]). In contrast, in early adolescent males, PAE reduced magnPVN *c-fos* mRNA expression relative to controls regardless of rearing condition (Figure 5C; significant main effect of prenatal treatment [$F_{1,26}=5.94$, $p=0.02$]). However, neither the ELA effect in females nor the PAE effect in males persisted into late

adolescence (Figure 5B,D). Finally, neither PAE nor ELA affected *c-fos* mRNA expression in parvoPVN (Figure 5E–H).

c-fos mRNA expression in vHPX

Neither PAE nor ELA affected *c-fos* mRNA expression in any of the vHPX subareas analyzed (Figure 6A–P).

DISCUSSION

Altered patterns of *c-fos* mRNA expression following social recognition memory testing revealed that overall, both PAE and ELA resulted in an attenuation of neural activity in specific nuclei within the social behavior neural network known to support social discrimination. Our results further suggest that the effects of PAE and ELA on *c-fos* expression are age-specific and sexually dimorphic. Moreover, brain regions supporting social recognition memory appear to be differentially sensitive to PAE and ELA, such that the amygdala is particularly vulnerable to PAE while the mPFC is more vulnerable to ELA.

The social behavior neural network comprises a complex and interconnected circuitry that is involved in multiple aspects of social behavior function, including social recognition memory formation and expression (Adolphs, 2003; Perna & Engelmann, 2017). As the dominant sensory system in rodents, the OB and PCX detect and provide the initial processing of socially relevant olfactory stimuli during social discrimination (Young, 2002). These olfactory areas project to limbic and cortical areas, such as the amygdala, vHPX, LS, and mPFC, which provide higher-order processing and integration that support social recognition memory and subsequent behavioral responses (Dias et al., 2016). The amygdala is a critical hub of the social behavior neural network, processing socioemotional stimuli to mediate social recognition memory (Adolphs, 2001; Perna & Engelmann, 2017; Zinn et al., 2016). Despite a more prominent role for the MeA, other nuclei, including the BL, LA, CeA, and CoA, are also implicated in social behavior (Insel & Shapiro, 1992; Katayama et al., 2009). The amygdala has reciprocal connections with the LS, a forebrain region associated with social motivation and essential for intact social recognition memory formation, particularly in males; indeed, silencing of the LS can block social recognition memory, and agonism or antagonism of the oxytocin (OT) and vasopressin (AVP) systems within the LS can enhance or impair, respectively, social recognition memory (Lukas et al., 2013; Mesic et al., 2015; Sheehan & Numan, 2000; Veenema et al., 2012). The amygdala also has reciprocal connections with ACC, IL, PrL, and OFC subdivisions of the mPFC (Kita & Kitai, 1990; McDonald, 1998; Swanson, 2003), a brain region generally associated with higher cognitive tasks (Diamond, 2011; Floresco et al., 1997) but also essential for social behavior (Adolphs, 2001). Involved in olfactory processing, the OFC is thought to encode information about odor identity and reward value to guide social and nonsocial behavioral responses (Bell et al., 2009; Rolls, 2000). For example, the OFC can shape behavioral

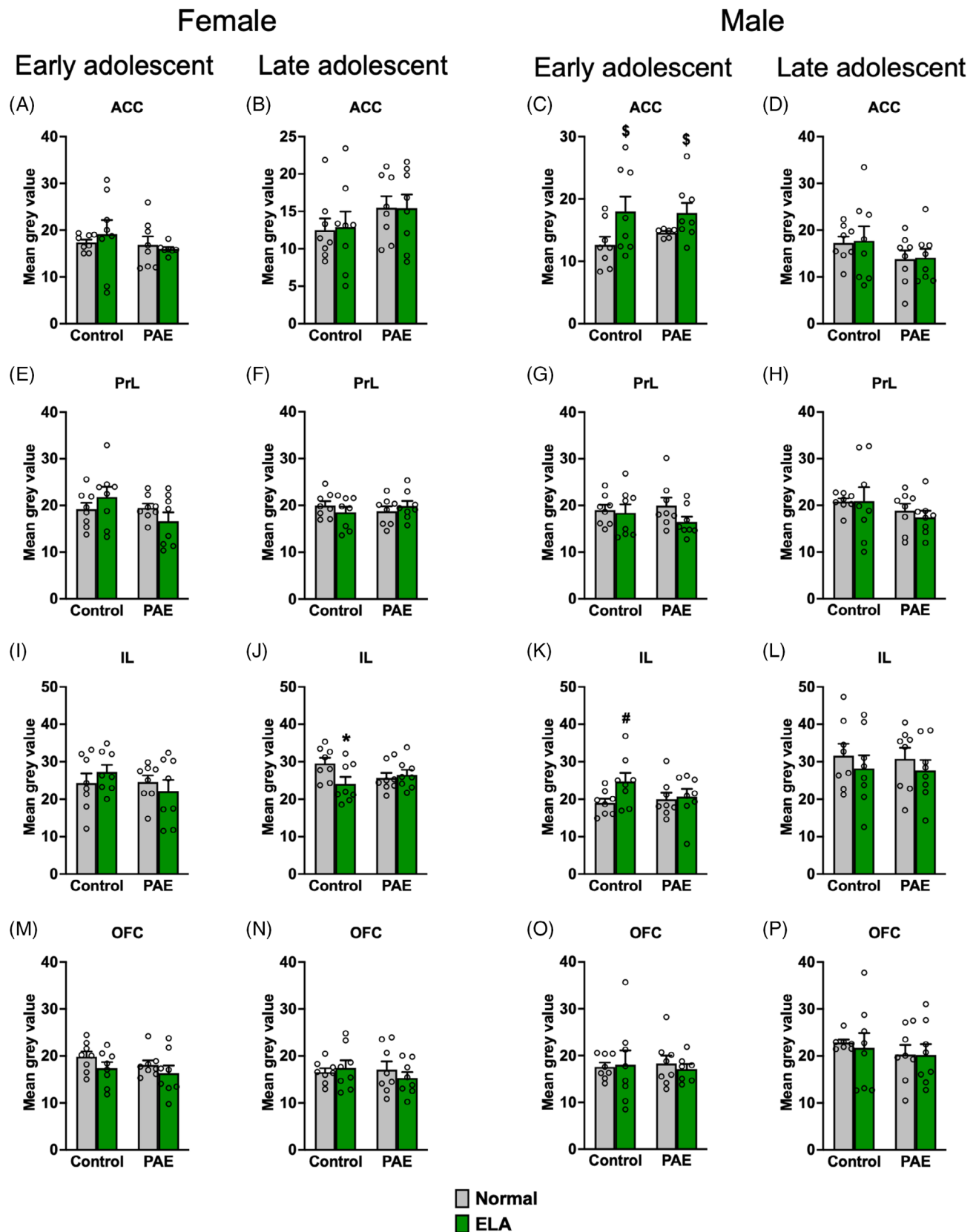


FIGURE 3 Medial prefrontal cortex *c-fos* expression following social recognition testing in male and female prenatal alcohol exposed (PAE) rats, with and without early-life adversity (ELA). Bars represent the mean $\pm$ SEM of the mean gray value of *c-fos* expression in the ACC (A–D), PrL (E–H), IL (I–L), and OFC (M–P) ($n=6-8$ for all groups). *Post hoc comparisons following a significant interaction between prenatal treatment and rearing condition; \$ELA animals are significantly different from normally reared animals regardless of prenatal treatment; #Control ELA males are different from control normally reared males based on *a priori* comparison.

responses based on play partner identity (Himmeler et al., 2018; Pellis et al., 2006); however, lesions of the OFC do not block adult social recognition memory (Rudebeck et al., 2007). Importantly, the ACC has been hypothesized to process the motivational states of social conspecifics during social interactions (Apps et al., 2016), and lesion studies have demonstrated an important role for the ACC in social recognition memory in rodents and nonhuman primates (Rudebeck et al., 2006, 2007), including responsiveness to social stimuli, such that rats with ACC lesions fail to habituate to repeated presentations of a social stimulus. The vHPX, particularly the CA1 subregion, has a critical role in social memory storage, particularly through its afferents to the nucleus accumbens involved in reward processing (Okuyama et al., 2016). Finally, all of the above regions are innervated by OT/AVP projections from the PVN, which influence their activity due to the high density of OT/AVP receptors in these regions (Althammer & Grinevich, 2018).

Our data suggest that adverse effects of PAE and ELA on social recognition memory are consistent with altered signaling within the social behavior neural network. Early adolescent PAE males exhibited attenuated *c-fos* expression of the BL and magnoPVN. Decreased activity of the magnoPVN following PAE suggests that altered OT/AVP signaling may contribute to the impaired social discrimination observed in this group. In late adolescent males, PAE reduced *c-fos* expression only in the BL, suggesting that PAE-related changes in neural activity observed in early adolescence appear to resolve across adolescent development except for those in the BL. This interpretation is supported by our finding that late adolescent PAE males exhibited social discrimination on both days of testing (Holman et al., 2021). Additionally, reduced function of the basolateral complex to mPFC projection has also been observed in a moderate PAE rodent model (Prybylski et al., 2023). Nevertheless, continued suppression of BL activity in early and late adolescent males does not preclude later social behavior dysfunction and/or poor performance on other tests of social behavior function, particularly with increasing complexity of the social environment (Helleman et al., 2010; Holman et al., 2018).

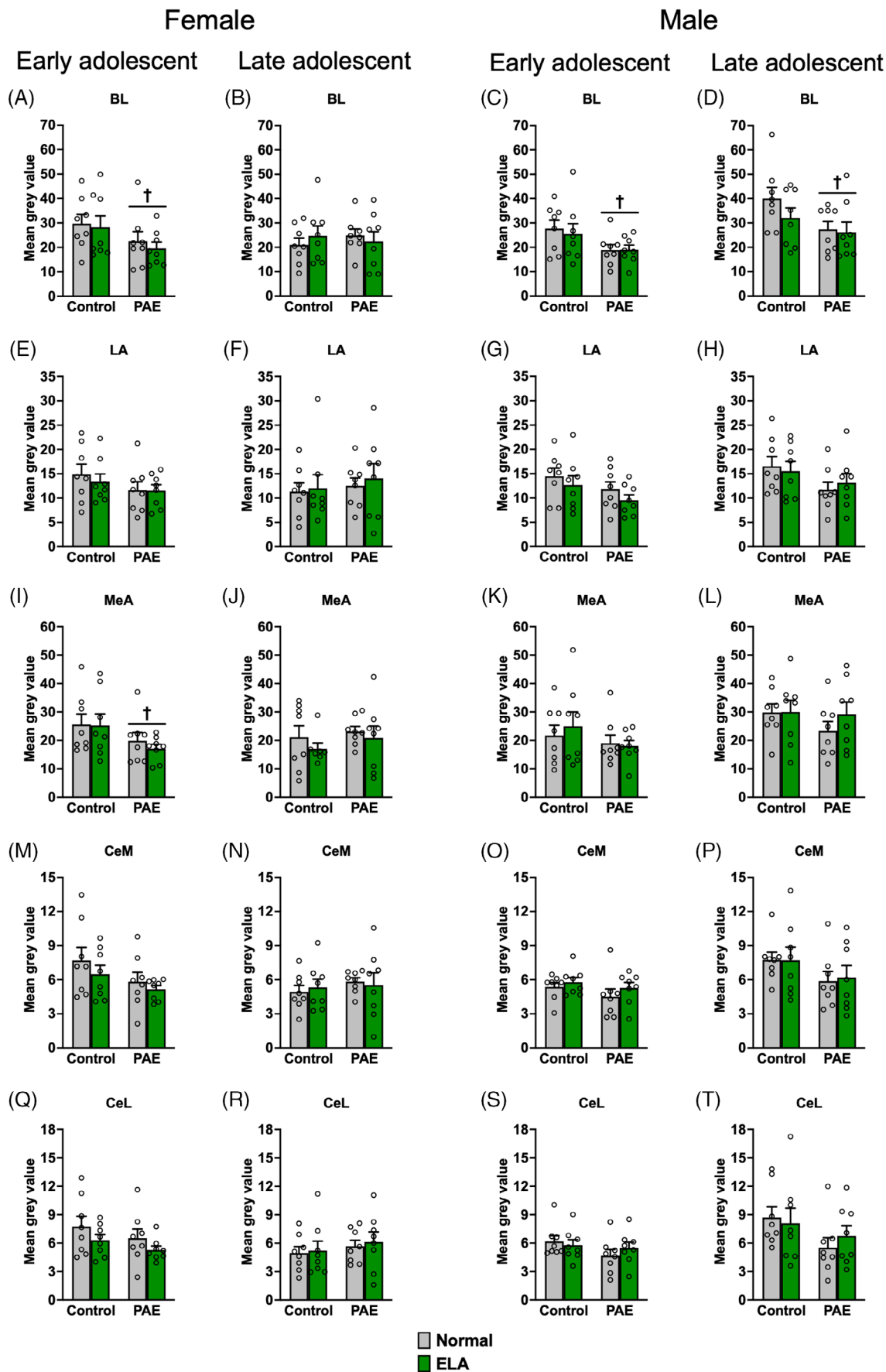
In males, ELA increased activity in the ACC and IL during early adolescence. ELA-related changes in mPFC circuitry/development are well documented in the clinical literature (Fan et al., 2014) as well as in preclinical models using low bedding (Fan et al., 2014; Rincón-Cortés & Sullivan, 2016) and maternal separation (Holland et al., 2014; Muhammad & Kolb, 2011). Our finding of increased ACC and IL activity differs from reports of decreased ACC and IL activity following social interaction in male and female adolescent rats exposed to ELA (Rincón-Cortés & Sullivan, 2016). An important difference between this earlier study and the present study, however, is their use of a social interaction test completed in a two-chamber

apparatus in which one chamber contained a social stimulus animal behind a wire mesh barrier to prevent physical interaction with the experimental animal. Social discrimination testing as performed in the present study is arguably a more complex social task and places stronger demands on the experimental rat to discriminate between novel and familiar social stimuli through physical interaction. Importantly, the ACC has been hypothesized to process the motivational states of social conspecifics during social interactions (Apps et al., 2016); in view of these data, it is possible that the increased ACC activity observed in ELA animals represents more effortful processing of social stimulus animals within the complex context of social discrimination testing. Finally—and somewhat surprisingly given the strong effect of ELA to impair social discrimination behavior—we did not detect any ELA-related alterations in *c-fos* expression among late adolescent males in any of the brain areas analyzed. Nevertheless, we cannot rule out altered function of these neural circuits in the absence of differential *c-fos* expression; indeed, despite the immense value of *c-fos* expression as a tool to interrogate changes in neural activation patterns, *c-fos* expression alone cannot account for the specific identity (e.g., glutamatergic and GABA-ergic) and/or connectivity of individual activated neurons. Thus, equivalent *c-fos* expression among groups does not necessarily indicate equivalent neural processing (Kovács, 2008). Moreover, ELA-related social discrimination deficits in late adolescent males may result from earlier developmental changes induced by altered circuit function or may be mediated by other brain regions not analyzed here.

Our data did not reveal any PAE-related changes in neural activity across the mPFC subareas analyzed, which contrasts with previous work using a more moderate PAE model that reported reduced *c-Fos* expression in the PFC of adult PAE animals following a social reunion task with a cage mate (Hamilton et al., 2010). Specifically, they demonstrated that PAE reduced *c-Fos* expression in the PrL of adult rats after social interaction, suggesting blunted PFC activation in social contexts. There are two major differences between the studies. First, the current study was conducted during adolescence, whereas the previous study examined adults, indicating that perhaps PAE-related effects on mPFC function may emerge later and require an incubation period. Nevertheless, we observed that PAE animals did not exhibit the typical ELA-related increase in IL *c-fos* expression, indicating that PAE alters the sensitivity of underlying mPFC circuitry to postnatal adversity. Second, the social behavior tasks employed differed: the current study used a more complex triadic social discrimination task (distinguishing between a familiar and a novel conspecific presented simultaneously), likely placing greater cognitive and mnemonic demands on the mPFC.

Despite the increased OB activity and reduced activity in the amygdala (BL and MeA) and PCX of PAE females during early

FIGURE 4 Amygdala *c-fos* expression following social recognition testing in male and female prenatal alcohol exposed (PAE) rats, with and without early-life adversity (ELA). Bars represent the mean $\pm$ SEM of the mean gray value of *c-fos* expression in the BL (A–D), LA (E–H), MeA (I–L), CeM (M–P), and CeL (Q–T) ($n = 7$ – 8 for all groups). [†]PAE animals are significantly different from controls regardless of rearing condition.



adolescence, we did not observe any PAE-related alteration in female social recognition memory (Holman et al., 2021). Previous work has shown that the encoding of social stimuli was impaired in adult PAE females only when the learning phase was reduced from 5 to 2 min (Kelly et al., 2009). Thus, the 5 min learning phase in the present study may have allowed females to overcome any behavioral impairments in the context of altered activity in olfactory/amygdala regions. In late adolescence, however, social discrimination (Holman et al., 2021) and *c-fos* expression in PAE females did not differ from controls in any of the brain areas analyzed. Alternatively, our observation of PAE-related alterations in olfactory/amygdala activation in females during early adolescence suggests that atypical function of these brain regions during development may contribute to later social processing impairments in adulthood despite equivalent overall activity within the social behavior neural network.

Like PAE, ELA also resulted in reduced *c-fos* expression in the PCX of early adolescent females, suggesting the possibility of impaired encoding of olfactory stimuli that could contribute to deficits in social recognition memory (Holman et al., 2021); related models of ELA have also shown altered activity of PCX (e.g., increased activity

of anterior PCX during olfactory learning during the sensitive period; Roth & Sullivan, 2005). The reduced *c-fos* expression in the magnoPVN of early adolescent females is interesting, particularly in view of previous research showing reduced OT release from the parvoPVN of females undergoing early handling (Todeschin et al., 2009). In late adolescent females, ELA increased neural activity in the OB, but decreased IL and PCX neural activity in the absence of social discrimination deficits. Our results of decreased IL activity replicate previous findings in late adolescent rats using the same ELA model and following social interaction testing, indicating a robust effect of ELA on mPFC function (Rincón-Cortés & Sullivan, 2016).

Collectively, we observed that PAE dampened BL amygdala activity in all but late adolescent females, regardless of rearing condition. Likewise, ELA potentiated ACC activity in early adolescent males, independent of prenatal treatment. Importantly, ELA also increased IL activity in control but not PAE males, suggesting that PAE alters circuit sensitivity to postnatal adversity. Our data support the hypothesis that PAE and ELA interact to alter the developmental trajectory of prefrontal-amygdala circuits, potentially by shifting the local balance of excitation and inhibition. Prior research supports this

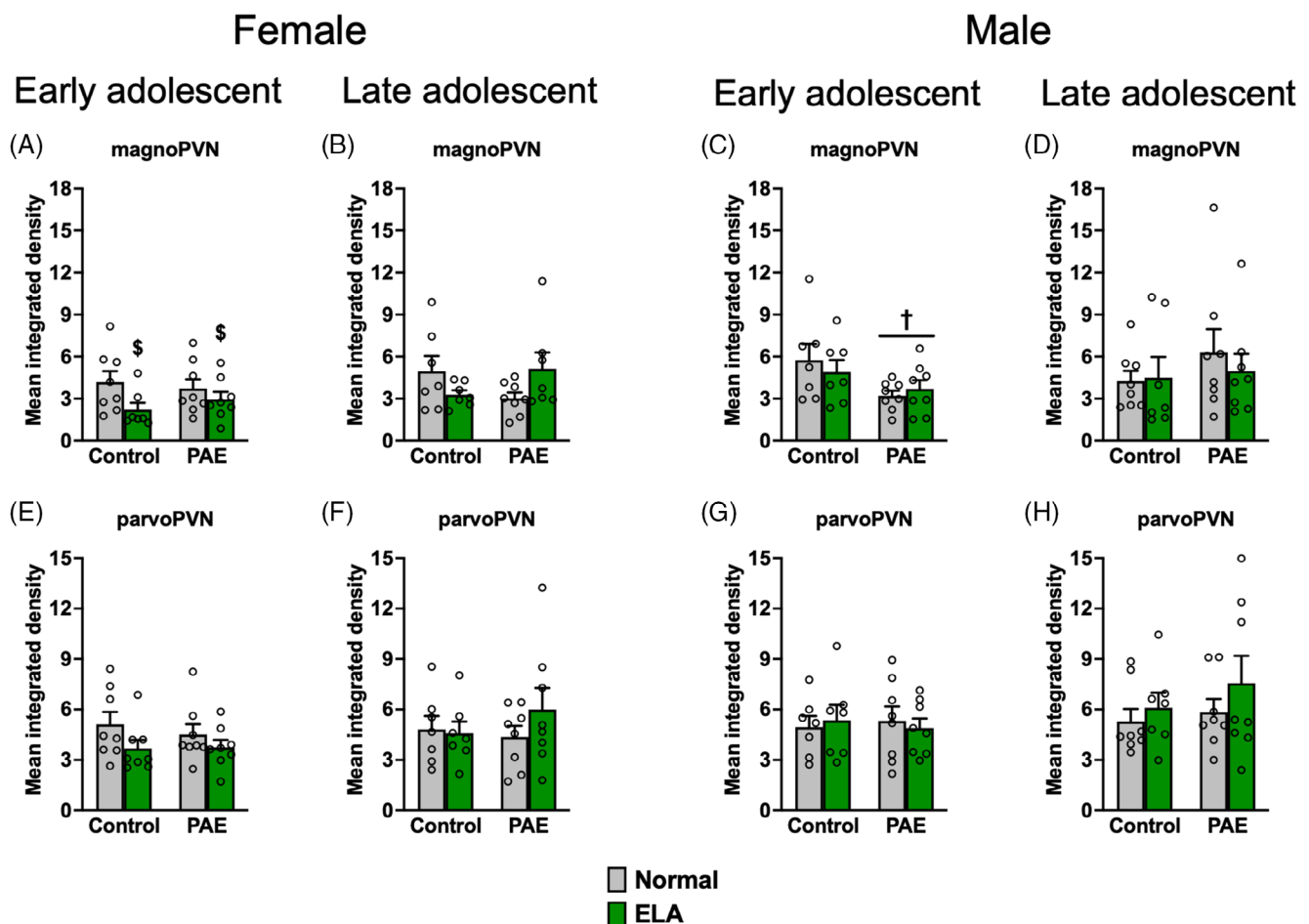


FIGURE 5 Paraventricular nucleus of the hypothalamus *c-fos* expression following social recognition testing in male and female prenatal alcohol exposed (PAE) rats, with and without early-life adversity (ELA). Bars represent the mean $\pm$ SEM of the mean gray value of *c-fos* expression in the magnoPVN (A–D) and parvoPVN (E–H) ($n = 7$ –8 for all groups). [†]PAE animals are significantly different from controls regardless of rearing condition; [‡]ELA animals are significantly different from normally reared animals regardless of prenatal treatment.

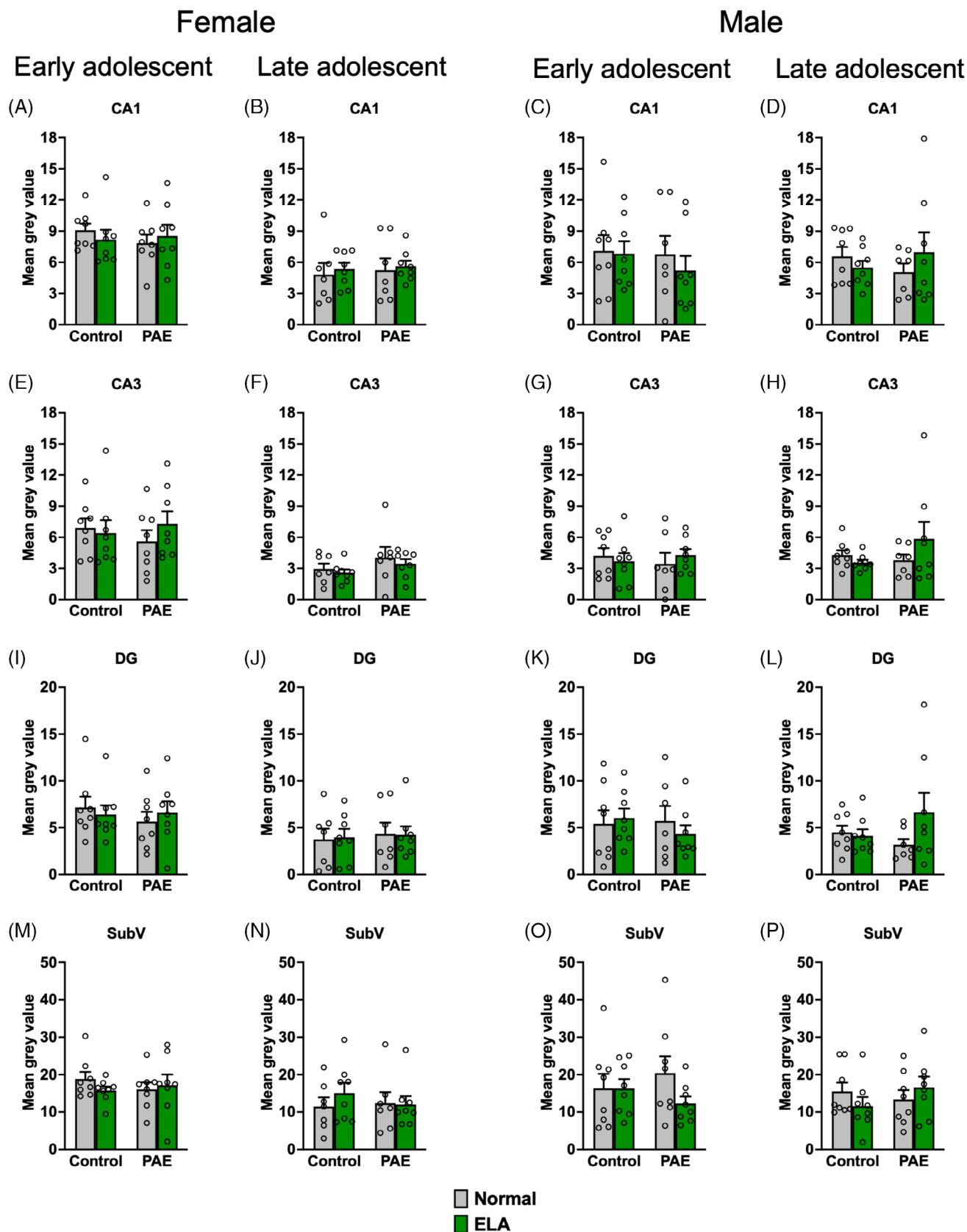


FIGURE 6 Ventral hippocampus *c-fos* expression following social recognition testing in male and female prenatal alcohol exposed (PAE) rats, with and without early-life adversity (ELA). Bars represent the mean $\pm$ SEM of the mean gray value of *c-fos* expression in the CA1 (A–D), CA3 (E–H), DG (I–L), and SubV (M–P) ($n=7-8$ for all groups).

interpretation. For example, Prybylski et al. (2023) showed that moderate PAE disrupts the basolateral complex–PrL circuitry, reducing the excitability of PrL pyramidal neurons via increased inhibitory interneuron activity, with notable sex-specific effects. These findings align with our observations of altered mPFC and BL amygdala activation and suggest that PAE-induced shifts in excitatory/inhibitory balance within key social-affective circuits may underlie long-term behavioral deficits. Similarly, research on ELA highlights the capacity of early environmental stressors to accelerate PFC-amygdala circuit maturation. ELA in humans is associated with earlier development of mPFC-amygdala connectivity and premature engagement of PFC top-down regulation of emotion (Tottenham, 2015). ELA is also associated with reduced adolescent social behavior and reduced c-Fos expression in the basolateral complex and mPFC (ACC, PrL, IL) in preclinical models (Rincón-Cortés & Sullivan, 2016). These findings suggest that early maltreatment perturbs typical circuit maturation and alters the functional balance between mPFC and amygdala regions, leading to distinct behavioral and neurobiological outcomes during adolescence.

Taken together, these studies suggest that both PAE and ELA disrupt normative trajectories of PFC-amygdala circuit development, potentially through alterations in excitatory and inhibitory balance and connectivity. Our results extend this literature by demonstrating that the combination of PAE and ELA produces sexually dimorphic effects on regional c-fos activation during adolescence—a sensitive period when social-affective circuits are undergoing refinement.

Importantly, there is an extensive literature on the importance of the social neuropeptide OT acting within the social behavior neural network for mediating social behavior function (Bielsky & Young, 2004). Our assessment of activity-dependent c-fos expression following social discrimination testing here reveals attenuated activity in many of the same brain regions where we identified increased OT receptor binding (Holman et al., 2021), including the mPFC and amygdala—which both receive OTergic axonal projections from the PVN—as well as the lateral septum (Johnson & Young, 2017).

CONCLUSION

In general, our results indicate that PAE and ELA target nodes of the social behavior neural network in unique ways, perhaps due to the differential vulnerability of these brain regions to early-life insults. Our neural activity data suggest that the effects of PAE and ELA on brain systems supporting social recognition memory are somewhat dissociable, such that the amygdala appears to be particularly sensitive to PAE while the mPFC seems to be more vulnerable to ELA; indeed, amygdala subnuclei were affected by PAE in all but late adolescent females, while ELA-related c-fos expression changes were more prominent in the mPFC. The relevance of the current research stems from the need for establishing a more specific social neurobehavioral profile that could support the development of strategies for earlier diagnoses and more targeted interventions following PAE and/or ELA.

AUTHOR CONTRIBUTIONS

PJH: Conceptualization; investigation; methodology; data curation; formal analysis; visualization; funding acquisition; writing—original draft; LE: Investigation; methodology; writing—review and editing; AC: Investigation; writing—review and editing; CM: Investigation; writing—review and editing; CR: Conceptualization; investigation; methodology; visualization; funding acquisition; writing—review and editing; JW: Conceptualization; funding acquisition; supervisor; writing—review and editing.

ACKNOWLEDGMENTS

We thank all members of the Weinberg laboratory for their assistance and expertise.

FUNDING INFORMATION

This work was supported by grants from the NIH/NIAAA R01 AA022460 to CR and JW, Canadian Foundation on Fetal Alcohol Research (CFFAR) grant to CR and JW, and NIH/NIAAA F31 AA023151 to PJH.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

ORCID

Parker J. Holman  <https://orcid.org/0000-0003-1631-9924>

Charlis Raineke  <https://orcid.org/0000-0003-1655-5935>

REFERENCES

- Adolphs, R. (2001) The neurobiology of social cognition. *Current Opinion in Neurobiology*, 11(2), 231–239.
- Adolphs, R. (2003) Cognitive neuroscience of human social behaviour. *Nature Reviews Neuroscience*, 4(3), 165–178.
- Albers, H.E. (2012) The regulation of social recognition, social communication and aggression: vasopressin in the social behavior neural network. *Hormones and Behavior*, 61(3), 283–292.
- Althammer, F. & Grinevich, V. (2018) Diversity of oxytocin neurons: beyond magno- and parvocellular cell types? *Journal of Neuroendocrinology*, 30(8), 1–10.
- Amaral, D.G. (2003) The amygdala, social behavior, and danger detection. *Annals of the New York Academy of Sciences*, 1000(1), 337–347.
- Apps, M.A.J., Rushworth, M.F.S. & Chang, S.W.C. (2016) The anterior cingulate gyrus and social cognition: tracking the motivation of others. *Neuron*, 90(4), 692–707.
- Bell, H.C., McCaffrey, D.R., Forgie, M.L., Kolb, B. & Pellis, S.M. (2009) The role of the medial prefrontal cortex in the play fighting of rats. *Behavioral Neuroscience*, 123(6), 1158–1168.
- Bielsky, I.F. & Young, L.J. (2004) Oxytocin, vasopressin, and social recognition in mammals. *Peptides*, 25(9), 1565–1574.
- Blakemore, S.-J., Burnett, S. & Dahl, R.E. (2010) The role of puberty in the developing adolescent brain. *Human Brain Mapping*, 31(6), 926–933.
- Conaway, L.P. & Hansen, D.J. (1989) Social behavior of physically abused and neglected children: a critical review. *Clinical Psychology Review*, 9(5), 627–652.

- Diamond, A. (2011) Biological and social influences on cognitive control processes dependent on prefrontal cortex. *Progress in Brain Research*, 189(1), 319–339.
- Dias, T.L., Golino, H.F., Oliveira, V.E.M., de Moraes, M.F.D. & Pereira, G.S. (2016) c-Fos expression predicts long-term social memory retrieval in mice. *Behavioural Brain Research*, 313, 260–271.
- Duquette, C., Stodel, E., Fullerton, S. & Hagglund, K. (2006) Persistence in high school: experiences of adolescents and young adults with fetal alcohol spectrum disorder. *Journal of Intellectual & Developmental Disability*, 31(4), 219–231.
- Engelmann, M., Hädicke, J. & Noack, J. (2011) Testing declarative memory in laboratory rats and mice using the nonconditioned social discrimination procedure. *Nature Protocols*, 6(8), 1152–1162.
- Fan, Y., Herrera-Melendez, A.L., Pestke, K., Feeser, M., Aust, S., Otte, C. et al. (2014) Early life stress modulates amygdala-prefrontal functional connectivity: implications for oxytocin effects. *Human Brain Mapping*, 35(10), 5328–5339.
- Floresco, S.B., Seamans, J.K. & Phillips, A.G. (1997) Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *Journal of Neuroscience*, 17(5), 1880–1890.
- Gabor, C.S., Phan, A., Clipperton-Allen, A.E., Kavaliers, M. & Choleris, E. (2012) Interplay of oxytocin, vasopressin, and sex hormones in the regulation of social recognition. *Behavioral Neuroscience*, 126(1), 97–109.
- Hamilton, D.A., Candelaria-Cook, F.T., Akers, K.G., Rice, J.P., Maes, L.I., Rosenberg, M. et al. (2010) Patterns of social-experience-related c-fos and arc expression in the frontal cortices of rats exposed to saccharin or moderate levels of ethanol during prenatal brain development. *Behavioural Brain Research*, 214(1), 66–74.
- Hellemans, K.G.C., Verma, P., Yoon, E., Yu, W.K., Young, A.H. & Weinberg, J. (2010) Prenatal alcohol exposure and chronic mild stress differentially alter depressive- and anxiety-like behaviors in male and female offspring. *Alcoholism: Clinical and Experimental Research*, 34(4), 633–645.
- Himmler, B.T., Mychasiuk, R., Nakahashi, A., Himmler, S.M., Pellis, S.M. & Kolb, B. (2018) Juvenile social experience and differential age-related changes in the dendritic morphologies of subareas of the prefrontal cortex in rats. *Synapse*, 72(4), 1–9.
- Holland, F.H., Ganguly, P., Potter, D.N., Chartoff, E.H. & Brenhouse, H.C. (2014) Early life stress disrupts social behavior and prefrontal cortex parvalbumin interneurons at an earlier time-point in females than in males. *Neuroscience Letters*, 566, 131–136.
- Holman, P.J., Baglot, S.L., Morgan, E. & Weinberg, J. (2019) Effects of prenatal alcohol exposure on social competence: asymmetry in play partner preference among heterogeneous triads of male and female rats. *Developmental Psychobiology*, 61(4), 513–524.
- Holman, P.J., Ellis, L., Morgan, E. & Weinberg, J. (2018) Prenatal alcohol exposure disrupts male adolescent social behavior and oxytocin receptor binding in rodents. *Hormones and Behavior*, 105(6), 115–127.
- Holman, P.J. & Raineke, C. (2023) Prenatal alcohol exposure and early life adversity: a translational perspective for dissecting compounding impacts. *Alcoholism: Clinical and Experimental Research*, 47, 2227–2230.
- Holman, P.J., Raineke, C., Chao, A., Grewal, R., Haghighat, S., Fung, C. et al. (2021) Altered social recognition memory and hypothalamic neuropeptide expression in adolescent male and female rats following prenatal alcohol exposure and/or early-life adversity. *Psychoneuroendocrinology*, 126, 105146.
- Insel, T.R. & Shapiro, L.E. (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences of the United States of America*, 89(13), 5981–5985.
- Johnson, Z.V. & Young, L.J. (2017) Oxytocin and vasopressin neural networks: implications for social behavioral diversity and translational neuroscience. *Neuroscience & Biobehavioral Reviews*, 76, 87–98.
- Katayama, T., Jodo, E., Suzuki, Y., Hoshino, K.-Y., Takeuchi, S. & Kayama, Y. (2009) Phencyclidine affects firing activity of basolateral amygdala neurons related to social behavior in rats. *Neuroscience*, 159(1), 335–343.
- Kelly, S.J., Day, N. & Streissguth, A.P. (2000) Effects of prenatal alcohol exposure on social behavior in humans and other species. *Neurotoxicology and Teratology*, 22(2), 143–149.
- Kelly, S.J., Goodlett, C.R. & Hannigan, J.H. (2009) Animal models of fetal alcohol spectrum disorders: impact of the social environment. *Developmental Disabilities Research Reviews*, 15(3), 200–208.
- Kita, H. & Kitai, S.T. (1990) Amygdaloid projections to the frontal cortex and the striatum in the rat. *Journal of Comparative Neurology*, 298(1), 40–49.
- Kovács, K.J. (2008) Measurement of immediate-early gene activation-c-fos and beyond. *Journal of Neuroendocrinology*, 20(6), 665–672.
- Kully-Martens, K., Denys, K., Treit, S., Tamana, S. & Rasmussen, C. (2012) A review of social skills deficits in individuals with fetal alcohol spectrum disorders and prenatal alcohol exposure: profiles, mechanisms, and interventions. *Alcoholism: Clinical and Experimental Research*, 36(4), 568–576.
- Lukas, M., Toth, I., Veenema, A.H. & Neumann, I.D. (2013) Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: male juvenile versus female adult conspecifics. *Psychoneuroendocrinology*, 38(6), 916–926.
- Marquardt, K. & Brigman, J.L. (2016) The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: insights from rodent models. *Alcohol*, 51, 1–15.
- Mattson, S.N., Bernes, G.A. & Doyle, L.R. (2019) Fetal alcohol spectrum disorders: a review of the neurobehavioral deficits associated with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, 43, 1046–1062.
- McDonald, A.J. (1998) Cortical pathways to the mammalian amygdala. *Progress in Neurobiology*, 55(3), 257–332.
- Mesic, I., Guzman, Y.F., Guede, A.L., Jovasevic, V., Corcoran, K.A., Leaderbrand, K. et al. (2015) Double dissociation of the roles of metabotropic glutamate receptor 5 and oxytocin receptor in discrete social behaviors. *Neuropsychopharmacology*, 40(10), 2337–2346.
- Mooney, S.M. & Varlinskaya, E.I. (2011) Acute prenatal exposure to ethanol and social behavior: effects of age, sex, and timing of exposure. *Behavioural Brain Research*, 216(1), 358–364.
- Muhammad, A. & Kolb, B. (2011) Maternal separation altered behavior and neuronal spine density without influencing amphetamine sensitization. *Behavioural Brain Research*, 223(1), 7–16.
- O'Connor, M.J. & Paley, B. (2006) The relationship of prenatal alcohol exposure and the postnatal environment to child depressive symptoms. *Journal of Pediatric Psychology*, 31(1), 50–64.
- Okuyama, T., Kitamura, T., Roy, D.S., Itohara, S. & Tonegawa, S. (2016) Ventral CA1 neurons store social memory. *Science*, 353(6307), 1536–1541.
- Paxinos, G. & Watson, C. (2005) *The rat brain in stereotaxic coordinates*, 5th edition, New York, NY: Academic Press.
- Pellis, S.M., Hastings, E., Shimizu, T., Kamitakahara, H., Komorowska, J., Forgie, M.L. et al. (2006) The effects of orbital frontal cortex damage on the modulation of defensive responses by rats in playful and nonplayful social contexts. *Behavioral Neuroscience*, 120(1), 72–84.
- Perna, J.C. & Engelmann, M. (2017) Recognizing others: rodent's social memories. In: Wöhr, M. & Krach, S. (Eds.) *Social behavior from rodents to humans: current topics in behavioral neurosciences*, vol. 30. Berlin: Springer Nature, pp. 25–45.
- Prybyls, K.R., Spodnick, M.B., Johnson, J.M., Varlinskaya, E.I. & Diaz, M.R. (2023) Moderate prenatal alcohol exposure produces sex-specific social impairments and attenuates prefrontal excitability and amygdala-cortex modulation of social behaviour. *Addiction Biology*, 28(1), e13252.

- Raineke, C., Cortés, M.R., Belnoue, L. & Sullivan, R.M. (2012) Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *Journal of Neuroscience*, 32(22), 7758–7765.
- Raineke, C., Opendak, M., Sarro, E., Showler, A., Bui, K., McEwen, B.S. et al. (2019) During infant maltreatment, stress targets hippocampus but stress with mother present targets amygdala and social behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 22821–22832.
- Rincón-Cortés, M. & Sullivan, R.M. (2016) Emergence of social behavior deficit, blunted corticolimbic activity and adult depression-like behavior in a rodent model of maternal maltreatment. *Translational Psychiatry*, 6(10), e930.
- Rockhold, M.N., Krueger, A.M., de Water, E., Lindgren, C.W., Sandness, K.E., Eckerle, J.K. et al. (2021) Executive and social functioning across development in children and adolescents with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, 45(2), 457–469.
- Rolls, E.T. (2000) The orbitofrontal cortex and reward. *Cerebral Cortex*, 10(3), 284–294.
- Ross, H.E. & Young, L.J. (2009) Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology*, 30(4), 534–547.
- Roth, T.L. & Sullivan, R.M. (2005) Memory of early maltreatment: neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. *Biological Psychiatry*, 57(8), 823–831.
- Rudebeck, P.H., Buckley, M., Walton, M. & Rushworth, M. (2006) A role for the macaque anterior cingulate gyrus in social valuation. *Science*, 313(5791), 1310–1312.
- Rudebeck, P.H., Walton, M.E., Millette, B.H.P., Shirley, E., Rushworth, M.F.S. & Bannerman, D.M. (2007) Distinct contributions of frontal areas to emotion and social behaviour in the rat. *European Journal of Neuroscience*, 26(8), 2315–2326.
- Sheehan, T. & Numan, M. (2000) The septal region and social behavior. In: Numan, R. (Ed.) *The behavioral neuroscience of the septal region*. Berlin: Springer Nature, pp. 175–209.
- Sisk, C.L. & Zehr, J.L. (2005) Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology*, 26(3–4), 163–174.
- Steinhausen, H.-C. & Spohr, H.-L. (1998) Long-term outcome of children with fetal alcohol syndrome: psychopathology, behavior, and intelligence. *Alcoholism: Clinical and Experimental Research*, 22(2), 334–338.
- Streissguth, A.P., Barr, H., Kogan, J. & Bookstein, F.L. (1996) Understanding the occurrence of secondary disabilities in clients with fetal alcohol syndrome (FAS) and fetal alcohol effects (FES): final report. In: *Final report to the Centers for Disease Control and Prevention (CDC)*. Seattle, WA: University of Washington Publication Services.
- Sun, Q., Li, X., Li, A., Zhang, J., Ding, Z., Gong, H. et al. (2020) Ventral hippocampal-prefrontal interaction affects social behavior via parvalbumin positive neurons in the medial prefrontal cortex. *iScience*, 23(3), 100894.
- Swanson, L.L.W. (2003) The amygdala and its place in the cerebral hemisphere. *Annals of the New York Academy of Sciences*, 985, 174–184.
- Todeschin, A.S., Winkelmann-Duarte, E.C., Jacob, M.H., Aranda, B.C., Jacobs, S., Fernandes, M.C. et al. (2009) Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Hormones and Behavior*, 56(1), 93–100.
- Tottenham, N. (2015) Social scaffolding of human amygdala-mPFC circuit development. *Social Neuroscience*, 10(5), 489–499.
- Veenema, A.H., Bredewold, R. & De Vries, G.J. (2012) Vasopressin regulates social recognition in juvenile and adult rats of both sexes, but in sex- and age-specific ways. *Hormones and Behavior*, 61(1), 50–56.
- Wilson, D.A., Sullivan, R.M., Smiley, J.F., Saito, M. & Raineke, C. (2024) Developmental alcohol exposure is exhausting: sleep and the enduring consequences of alcohol exposure during development. *Neuroscience & Biobehavioral Reviews*, 158, 105567.
- Young, L.J. (2002) The neurobiology of social recognition, approach, and avoidance. *Biological Psychiatry*, 51(1), 18–26.
- Zinn, C.G., Clairis, N., Cavalcante, L.E.S., Furini, C.R.G., Myskiw, J.C. & Izquierdo, I. (2016) Major neurotransmitter systems in dorsal hippocampus and basolateral amygdala control social recognition memory. *Proceedings of the National Academy of Sciences of the United States of America*, 113(33), E4914–E4919.

How to cite this article: Holman, P.J., Ellis, L., Chao, A., Mitchell, C., Raineke, C. & Weinberg, J. (2025) Altered activity within the social behavior neural network in adolescent rats following prenatal alcohol exposure and/or early-life adversity. *Alcohol: Clinical and Experimental Research*, 00, 1–14. Available from: <https://doi.org/10.1111/acer.70082>