Received: 13 December 2021 | Accepted: 16 March 2022

DOI: 10.1111/acer.14813

ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

ORIGINAL ARTICLE

Sexually dimorphic organization of open field behavior following moderate prenatal alcohol exposure

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Funding information
National Institute on Alcohol Abuse and Alcoholism, Grant/Award Number: P50 AA022534, R01 AA029700 and T32 AA014127-15

Abstract

Background: Prenatal alcohol exposure (PAE) can produce deficits in a wide range of cognitive functions but is especially detrimental to behaviors requiring accurate spatial information processing. In open field environments, spatial behavior is organized such that animals establish "home bases" marked by long stops focused around one location. Progressions away from the home base are circuitous and slow, while progressions directed toward the home base are non-circuitous and fast. The impact of PAE on the organization of open field behavior has not been experimentally investigated.

Methods: In the present study, adult female and male rats with moderate PAE or saccharin exposure locomoted a circular high walled open field for 30 minutes under lighted conditions.

Results: The findings indicate that PAE and sex influence the organization of open field behavior. Consistent with previous literature, PAE rats exhibited greater locomotion in the open field. Novel findings from the current study indicate that PAE and sex also impact open field measures specific to spatial orientation. While all rats established a home base on the periphery of the open field, PAE rats, particularly males, exhibited significantly less clustered home base stopping with smaller changes in heading between stops. PAE also impaired progression measures specific to distance estimation, while sex alone impacted progression measures specific to direction estimation.

Conclusions: These findings support the conclusion that adult male rats have an increased susceptibility to the effects of PAE on the organization of open field behavior.

KEYWORDS
fetal alcohol spectrum disorders, home base, movement scaling, open field, spatial orientation

INTRODUCTION

Prenatal alcohol exposure (PAE) will impact ~3% to 4% of children in the United States (May et al., 2014). The resulting developmental neurotoxicity is associated with Fetal Alcohol Spectrum Disorders (FASD) which is a set of morphological, behavioral, and cognitive abnormalities in offspring after PAE. Spatial processing deficits are commonly observed in children with FASD and animal models of PAE (Hamilton et al., 2003; Sanchez et al., 2019). For example, children with FASD exhibit poor performance on a battery of neuropsychological assessments of spatial processing including the virtual Morris water task (Mattson et al., 2010). Sufficient alcohol exposure

later in postnatal development results in similar spatial processing deficits, as adolescent binge-drinkers exhibit poor performance on a manipulatory place learning task (Blankenship et al., 2016). Spatial processing deficits reflect functional changes in a variety of neural systems which can be observed even after moderate PAE (blood alcohol concentration: <100 mg/dl). Specifically, moderate PAE has been observed to produce changes in hippocampal function (Berman & Hannigan, 2000; Brady et al., 2012; Sanchez et al., 2019; Savage et al., 2010). For example, studies have shown that moderate PAE is associated with impairments in long-term potentiation (LTP) at dentate gyrus synapses (Sutherland et al., 1997), reduced $mGluR_5$ receptor function (Galindo et al., 2004), and alterations in dentate NMDA subunit composition (Brady et al., 2013). Additionally, recent work has observed that moderate PAE influences the firing characteristics of spatially tuned hippocampal cells, such that the prominence of firing in relation to environmental position is reduced (Harvey et al., 2020). PAE can also induce structural and functional changes in other neural systems involved in spatial behavior including the medial septum (Moore et al., 1997), anterior cingulate cortex (Moore et al., 1998), and orbitofrontal cortex (Licheri et al., 2021). These changes in hippocampal, cortical, and subcortical function likely contribute to observed impairments in spatial information processing.

Previous work investigating the effects of PAE on spatial information processing have frequently evaluated place learning in the Morris water task. While some studies have observed that PAE disrupts adult performance in the Morris water task (Hamilton et al., 2003; Savage et al., 2010), others have failed to observe an effect of PAE (Cronise et al., 2001; Cullen et al., 2014). At least two factors may be contributing to these conflicting results. First, PAE may result in a sexually dimorphic stress response. For example, while female rats exhibit a higher baseline corticosterone level and poor performance in the Morris water task compared to males (Beiko et al., 2004), the addition of PAE in male rats results in heightened baseline corticotropin releasing hormone concentrations (Gabriel et al., 2005) associated with an increased stress response relative to PAE female rats (Hellemans et al., 2010; Weinberg, 1992). Additionally, spatial orientation depends on the availability and familiarity of multiple sources of information (Maaswinkel & Whishaw, 1999). Performance in the Morris water task fails to dissociate the use of environmental and self-movement cues, and the absence of group effects may reflect one group adapting a compensatory spatial strategy to locate the hidden platform. Therefore, it is important to develop alternative behavior assessments of PAE that minimize stress and dissociate the impaired processing of different sources of information.

Spontaneously occurring behaviors have provided robust tools to investigate the neurobiological basis of spatial information processing. Movement in the open field is a behavior rodents will readily engage in without training or nutritional restriction, and may provide a less stressful testing environment compared to alternative spatial tasks (Harrison et al., 2009). Rodent open field behavior is highly organized and is typically focused around one location, or a home base (Eilam & Golani, 1989), where animals exhibit a high density of stops relative to other locations. Home base behavior has been observed across a range of invertebrate and vertebrate animals including humans (Frostig et al., 2020). Home base behavior provides refuge from environmental threats and helps maintain spatial orientation (Wallace et al., 2006). Movements around the home base are highly organized. Rodents typically make non-circuitous fast locomotor movements, termed progressions, directed toward the home base, and circuitous slow progressions directed away from the home base (Tchernichovski & Golani, 1995). Both environmental (Clark et al., 2005, 2006) and self-movement cues (e.g., vestibular, proprioceptive, and optic flow information; Banovetz et al., 2021; Wallace et al., 2006) have been implicated in organizing open field behavior around the home base. Specifically, rodents will use salient environmental landmarks to anchor home base position within the environment and will use self-movement cues as a continuous source of information to update a sense of position relative to the home base. Although rats may be trained to use odor cues to guide movement centered around the home base (Wallace et al., 2002), the removal of olfactory cues (Whishaw et al., 2001) or damage to the olfactory system (Hines & Whishaw, 2005) does not disrupt the establishment of a home base. In contrast, damage to structures implicated in maintaining spatial orientation have been observed to disrupt the organization of open field home base behavior. For example, genetic mouse models of vestibular pathology exhibit reduced home base stability, increased progression path circuity, and larger changes in heading between progressions (Donaldson et al., 2018). Additionally, rodents with hippocampal (Winter et al., 2013) and medial frontal cortical (Blankenship et al., 2016) lesions display more circuitous progressions toward the home base. The organization of movement in the open field provides a simple and efficient method to assess the effects of PAE on spatial information processing.

It is well established that open field behavior depends on neural circuits involved in spatial processing (Dudchenko & Wallace, 2018; Thompson et al., 2018); however, it remains to be determined whether moderate PAE influences the organization of open field behaviors (e.g., home base behavior, progressions, and stopping behaviors). Previous studies testing the impact of PAE (ranging ~60 to 130 mg/dl) on general open field locomotion have produced conflicting results with some showing heightened open field activity (Skorput & Yeh, 2016) and others reporting unaffected open field locomotion (Brady et al., 2012; Patten et al., 2016), with locomotor deficits more consistently observed at higher doses (Marquardt & Brigman, 2016). In addition, there are also conflicting findings regarding observed sexual dimorphisms in PAE effects on open-field behavior. Specifically, males exhibit an increase in anxiety-like behaviors (Bake et al., 2021; Lam et al., 2018), while other research suggests no sex differences (Cullen et al., 2013). Further, sex differences in spatial learning and memory have been reported after prenatal or postnatal alcohol exposure (Zimmerberg et al., 1991). Further work is needed to investigate whether PAE differentially influences female and male spatial orientation. In the current study, adult (4 months of age)

rats with and without moderate PAE (~60 mg/dl) were exposed to an open field under light conditions. Groups differences in the organization of open field behavior were consistent with sexual dimorphic effects of PAE on processing spatial information.

MATERIALS AND METHODS

Voluntary ethanol (EtOH) consumption paradigm procedures

The University of New Mexico Institutional Animal Care and Use Committees approved all procedures involving the use of live rats. LongEvans rats (Envigo Corporation) housed at 22°C were maintained on a reverse 12-h dark/12-h light schedule (lights on from 21:00 to 09:00 h) and were provided PMI Picolab 5LOD rodent chow (LabDiet Incorporated) and tap water ad libitum. The PAE and breeding procedures were the same as described previously (Davies et al., 2019). After 1 week of acclimation to the animal facility, all female breeders were single-housed and allowed to drink 5% EtOH in 0.066% saccharin in tap water for 4 h each day from 10:00 to 14:00 h. Daily 4 h EtOH consumption was monitored for at least 2 weeks and then the mean daily EtOH consumption was determined for each female. Females whose mean daily EtOH consumption was greater than one standard deviation below the group mean, typically about 12% to 15% of the entire group, were removed from the study. The remainder of the females were assigned to either a saccharin control or 5% EtOH drinking group and matched such that the mean pre-pregnancy EtOH consumption by each group was similar. Subsequently, females were placed with proven male breeders until pregnant, as indicated by the presence of a vaginal plug.

Beginning on Gestational Day 1 (GD1), rat dams were provided saccharin water containing either 0% or 5% EtOH for 4 h each day, from 10:00 to 14:00 h. The volume of saccharin water provided to the control group was matched to the mean volume of saccharin water consumed by the EtOH group. Daily 4 h EtOH consumption was recorded for each dam through GD21, after which EtOH consumption was discontinued. At birth, all litters were weighed and culled to 10 pups. Offspring were weaned at 24 days of age and transferred to the Department of Psychology Animal Research Facility where they were group-housed (two males or three females per cage). To minimize potential litter effects, one to two female or male rats used from each breeding pair were used in the behavioral studies.

Subjects

Subjects included 32 female (n = 16) and male (n = 16) Long Evans rats obtained from the University of New Mexico Health Sciences Animal Resource Facility (see breeding protocol above). The same breeding round produced all rats used in the study. Following weaning, all rats were pair-housed in standard plastic cages on a reverse 12 h light:dark cycle at a room temperature of 22°C with Picolab 5LOD diet and water provided ad libitum. Saccharin (SACC) rats (n = 16; counterbalanced for sex) and PAE rats (n = 16; counterbalanced for sex) were tested between post-natal day (PND) 135 and 137. The estrous cycle was not measured in female rats in the present study as previous work has failed to identify a significant influence of estrous on movement organization in similar open field tests (Köppen et al., 2015).

Apparatus

The open field was a walled (black) cylinder measuring 76.5 cm in diameter and 58 cm in height and a white floor. The apparatus was located approximately in the center of a well-lit testing room. Overhead ceiling lights provided illumination of the testing room and open field (135 Lux at bottom of open field). The room contained several objects that may have been visible to the rats as they locomoted the high walled cylinder, including a sink, poster, shelves, a computer bench, and a boom arm for the overhead camera. Ambient noise was kept to a minimum as the experimenter left the room after placing the rat in the open field. Open field behavior was recorded at 30 frames per second using a camera mounted above the center of the open field.

Procedure

All rats were tested during the dark portion of their light:dark cycle. Rats were transported directly from the colony room to the testing room and were placed in the center of the open field by the experimenter. The experimenter left the testing room, and the rat was then left alone in the open field for 30 min. Once the session was over, the rat was removed from the open field and returned to its holding cage. Between each session, the open field was wiped down with a cleaning solution to remove odor cues.

Behavioral analysis

Two minutes after the rat was placed on the open field, four consecutive 5-min samples of activity were captured for analysis. The 2-min delay was based on previous work demonstrating that rodents exhibit markers of organized open field behavior including home base establishment (e.g., circling, grooming, and rearing) within 2 min of exposure to a novel environment (Banovetz et al., 2021; Donaldson et al., 2018). Ethovision XT 13 (Noldus) was used to digitize body position in the open field at five frames per second. The resulting x- and y-coordinates were used to calculate moment-to-moment speeds. A rat's average speed for the session was used to segment movement into progressions and stops. Progressions were classified as periods of movement with speeds greater than the rat's average speed for at least two frames, whereas stops were classified as periods of behavior below the rat's average speed for at least two frames. Multiple

measures were then used to quantify general characteristics of movement, stop clustering, and progression topography.

General open field behavior

A variety of factors (e.g., locomotor patterns and emotion) have been observed to influence open field behavior (Denenberg, 1969). To assess general open field behavior, total distance traveled, and total stop time were calculated for the four samples. Total distance traveled was calculated by summing all progression distances during a sample. Total stop time was calculated by separately summing all stop times during each sample. These measures are descriptive of general movement in the open field.

Stopping behavior

Rodents organize stopping behavior around a specific established location in the environment termed the home base (Eilam & Golani, 1989). Each second of a stop was converted to an individual observation within an *x*- and *y*-cartesian coordinate system. These cartesian coordinates were then converted to a polar coordinate system (theta, *r*) with the center of the open field arena set as the origin. Transforming to a polar coordinate system allowed for an analysis of the distance and direction of stopping behavior relative to the center of the open field. The distance a stop was located from the center of open field was used to calculate the percent time spent on the outer 10% of the apparatus. Stop headings began at zero degrees and values increased counterclockwise. Circular statistics were used to quantify the stop clustering (Banovetz et al., 2021; Donaldson et al., 2018). First, the Rayleigh test was used to evaluate whether stop clustering significantly differed from random positions for the entire session. Next, the parameter of concentration (0.0 indicates directional headings of stops are uniformly distributed; 1.0 indicates directional heading of stops are concentrated in the same heading) was used to quantify the strength of stop clustering within each sample.

In general, most of the changes in heading between progressions in the open field occur during stops. Previous work has shown that manipulations which disrupt spatial orientation significantly increase the change in heading observed during a stop (Banovetz et al., 2021; Donaldson et al., 2018). The change in heading was calculated as the supplementary angle subtended by the progression peak speed location prior to the stop, the average stop location, and the subsequent progression peak speed location. Change in heading values range from 0° (no change in heading) to 180° (reversing heading). All changes in heading during stops were averaged for each sample.

Progression behavior

Previous work has demonstrated that rodents organize their movement into three modes of motion, or "gears" (Drai et al., 2000). Each rat's set of progressions (excluding progressions less than 20 cm, approximately two body lengths) were sorted into three classes based on length to investigate groups differences in topography and kinematic characteristics. First, path circuity of a progression was calculated by dividing the Euclidean distance by the distance traveled during the progression with values ranging from 0.0 (circuitous path) to 1.0 (direct path). Next, peak speed was recorded for each progression. Finally, observing that peak speed varied with progression class prompted an analysis of whether groups differed in the movement scaling strength, or correlation, between a rat's set of progression peak speeds and Euclidean distances.

Statistical analysis

Repeated measures ANOVAs were used to evaluate the main effects of group, sex, sample, progression class, and their interactions, with an alpha set at 0.05. The Greenhouse–Geisser correction was used in analyses where Mauchly's test indicated significant departure from the assumption of sphericity. Partial eta squared ($\eta^2 p$) values were reported for each main effect and interaction as a measure of effect size. The Holm post-hoc method was used to compare statistically significant differences. All statistical analyses used JASP 0.12.2.0 (University of Amsterdam) to calculate results.

RESULTS

Voluntary drinking paradigm measures

The rat dams that produced the moderate PAE offspring for these experiments consumed a mean of 2.08 ± 0.11 g/kg/day of EtOH throughout gestation (Table 1). This level of consumption, in a separate set of rat dams, produced a mean serum EtOH concentration of 42.0 + 3.0 mg/dl (Table 1) in blood samples collected 2 h after the introduction of the drinking tubes (Davies et al., submitted). Compared to the saccharin control group, this voluntary drinking paradigm did not affect maternal weight gain, litter size or offspring birth weight (Table 1).

General open field behavior

Rats alternated between stops and progressions (Figure 1) in the open field across the four 5-min samples. Topographic and kinematic profiles were plotted for a representative rat during one sample. Both female and male rats exhibited a significant decrease in distance traveled across samples (see Table S1, *Levene's test indicated unequal variance for S3 [$F = 5.983$, $p = 0.003$] and S4 [$F = 3.536$, $p = 0.027$]). Rats in the PAE group were observed to travel significantly longer distances compared to rats in the SACC group and female rats traveled significantly longer distances relative to males. No other significant main effects or interactions were observed in distance traveled.

TABLE 1 Effects of daily 4-h consumption of 5% EtOH on female rat dams and their offspring

	Saccharin Control	Prenatal Alcohol-exposed
Daily 4-h 5% EtOH consumption	NA	2.08 ± 0.11[a] (9)
Maternal serum EtOH concentration	NA	42 ± 3.0[b] (16)[c]
Maternal weight gain during pregnancy	103 ± 10[d] (9)	110 ± 9 (9)
Litter size	11.2 ± 0.8[e] (9)	10.0 ± 1.1 (9)
Offspring birth weight	7.33 ± 0.27[f] (9)	7.52 ± 0.29 (9)

Note: NA, Not applicable; (*n*), Group sample size.

[a]Mean ± S.E.M. grams EtOH consumed/kg body weight/day.

[b]Mean ± S.E.M. mg EtOH/dl serum, 2 h after introduction of the drinking tubes.

[c]Davies et al. Alcohol. Clin. Exp. Res., submitted.

[d]Mean ± S.E.M. grams increase in body weight from GD 1 through GD 21.

[e]Mean ± S.E.M. number of live births/L.

[f]Mean ± S.E.M. grams pup birth weight.

A significant increase in the time spent stopping was observed across the four samples (Table S1) in female and male rats. No other significant main effects or interactions were observed in the time spent stopping.

Stopping behavior

Rats spent most of their stop (Figure 2) time (mean: 90.8; 95% CI: 87.9 to 93.7) on the periphery of the apparatus. This tendency to stop close to the periphery significantly increased across samples; however, no other significant main effects or interactions were observed (see Table S2).

Application of the Rayleigh test to each rat's set of stop headings (Figure 3) for the session revealed that all rats in both groups exhibited directional clustering of stopping behavior that significantly differed from a random distribution. This is consistent with home base establishment; however, significant differences were observed in the strength of stop clustering within samples (Table S2). The SACC group exhibited significantly more concentrated stop clustering relative to the PAE group. This group effect was also observed to be influenced by the sex of the rat. Specifically, no group differences were observed between SACC female (mean: 0.57; 95% CI: 0.46 to 0.68) and PAE (mean: 0.58; 95% CI: 0.46 to 0.69) groups; however, SACC male (mean: 0.74; 95% CI: 0.62 to 0.85) and PAE (mean: 0.45; 95% CI: 0.34 to 0.56) groups significantly differed (i.e., a significant group × sex interaction; see Table S3). No other significant main effects or interactions were observed in the concentration of stop clustering.

Most of the changes in heading (Figure 4) along a path occur during stops. Both female and male rats exhibited a significant decrease in change in heading across samples (Table S2). In addition, differences in SACC and PAE groups depended on the sex of the rat. No group differences were observed between SACC females (mean: 87.4; 95% CI: 77.4 to 97.4) and PAE (mean: 97.2; 95% CI: 87.2 to 107.1) groups. In contrast, the SACC male (mean: 103.9; 95% CI: 93.9 to 113.8) group had significantly larger changes in heading relative to the PAE male (mean: 85.6; 95% CI: 75.6 to 95.5) group. No other significant main effects or interactions were observed in change in heading.

Progression behavior

Each rat's set of progressions (Figure 5) were sorted equally into long, medium, and short classes based on length. Increases in female and male rat progression class length was associated with following more circuitous progressions (Table S3). In addition, female rats (mean: 0.86; 95% CI: 0.84 to 0.88) followed significantly more circuitous paths relative to male rats (mean: 0.89; 95% CI: 0.87 to 0.91). Finally, sex differences varied as a function of progression class. Significant differences were observed between female (mean: 0.69 95% CI: 0.64 to 0.73) and male (mean: 0.77; 95% CI: 0.72 to 0.82) rats on long progression classes; however, no sex differences we observed on medium (*Levene's test indicated unequal variance in medium class average distance ratio [$F = 2.954$, $p = 0.050$]) or short progression classes. No other significant main effects or interactions were observed in progression path circuity.

Female and male rat peak speeds (Figure 6) were observed to vary across progression classes. Longer progression classes were associated with faster peak speeds (Table S3, *Levene's test indicated unequal variance in S4 [$F = 4.284$, $p = 0.013$]). In addition, female rats (mean: 16.7; 95% CI: 15.6 to 17.9) had significantly faster peak speeds relative to male rats (mean: 14.5; 95% CI: 13.4 to 15.6). No other significant main effects or interactions were observed in progression peak speed.

Rats scaled (Figure 7) their peak speed relative to the progression length. Female and male rat average movement scaling significantly increased in strength across the four samples (Table S3). The SACC group (mean: 0.92; 95% CI: 0.91 to 0.94) exhibited significantly stronger movement scaling relative to the PAE group (mean: 0.90; 95% CI: 0.89 to 0.91). SACC and PAE rats were only observed to differ throughout the first 10 min (Sample 1 and Sample 2). No other significant main effects or interactions were observed in progression movement scaling.

DISCUSSION

The current study presents the first examination of the effects of moderate PAE on the organization of open field behavior (e.g., home base behavior, stopping, and progressions) in female and male rats. Although all rats established a home base on the periphery of the open field, multiple differences were observed in the organization

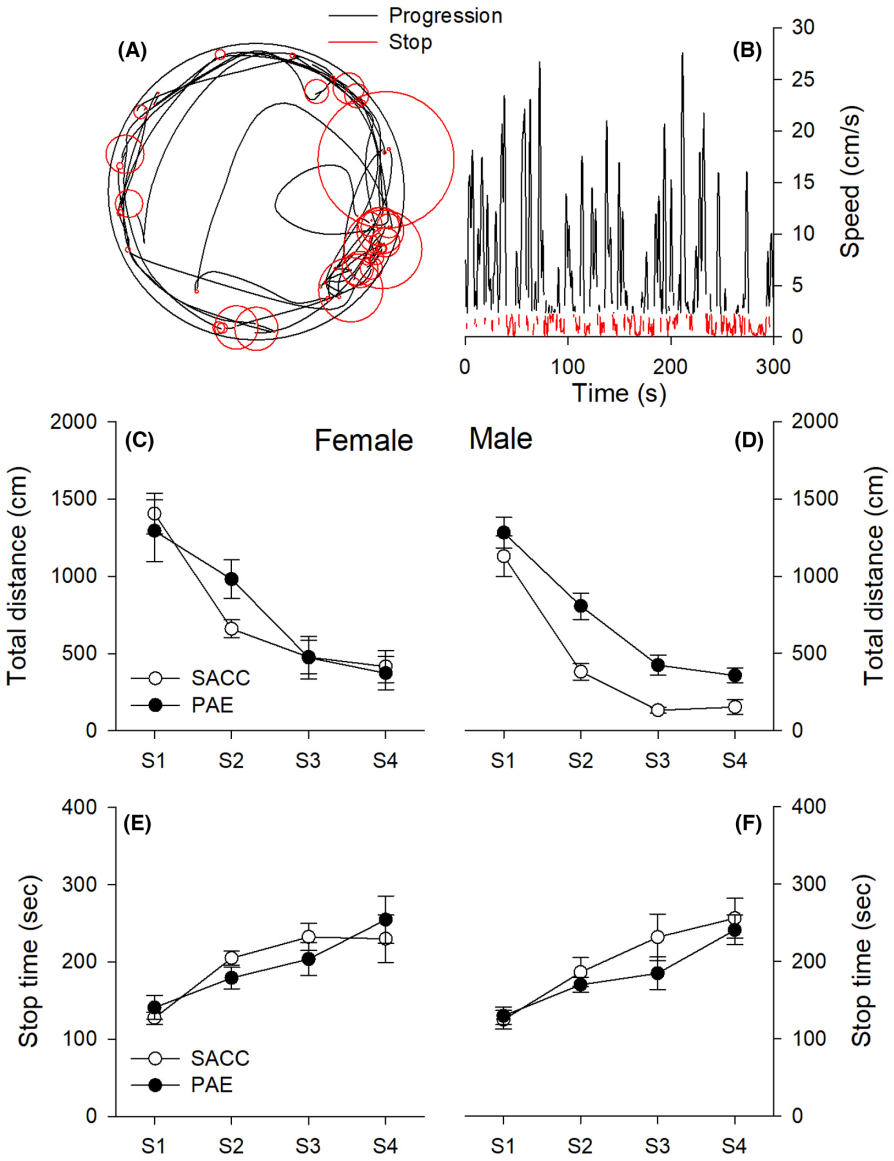

FIGURE 1 Topographic profiles of progressions (black) and stops (red) are plotted for a representative (selected to reflect the group mean) female control rat across one sample of open field behavior (A). The position and duration of stops are indicated by the diameter of the red circle, with longer stops associated with larger diameters. Kinematic profiles are plotted for progressions and stops from the same representative rat across one sample of open field behavior (B). The average total distance traveled, and total stop time are graphed for female (C and E) and male (D and F) across all four samples. PAE ($p = 0.038$) and female ($p = 0.019$) rats traveled significantly farther than SACC and male rats, respectively

of behavior around the home base. First, group differences were observed in general locomotor function. The PAE group traveled longer distances relative to the SACC group. Females also traveled longer distances relative to males. Next, several aspects of stopping behavior varied between groups. The PAE group had less concentrated stop clustering, with male PAE rats exhibiting significantly weaker stop clustering. In addition, male PAE rats had smaller changes in heading during stops. Finally, progression characteristics were observed to be significantly influenced by sex and PAE. Female rats exhibited progressions that were significantly more circuitous with faster peak speeds relative to males. In addition, the SACC group exhibited significantly stronger movement scaling relative to the PAE group. This set of observations suggests that male rats have an increased susceptibility to the effects of PAE in the organization of open field behavior. The following sections will consider the impaired information processing from PAE and potential mechanisms for the sexually dimorphic performance.

PAE and open field behavior

Although multiple studies have investigated the impact of PAE on general locomotor behavior in the open field (Marquardt & Brigman, 2016), this is the first study to examine the effects of moderate PAE

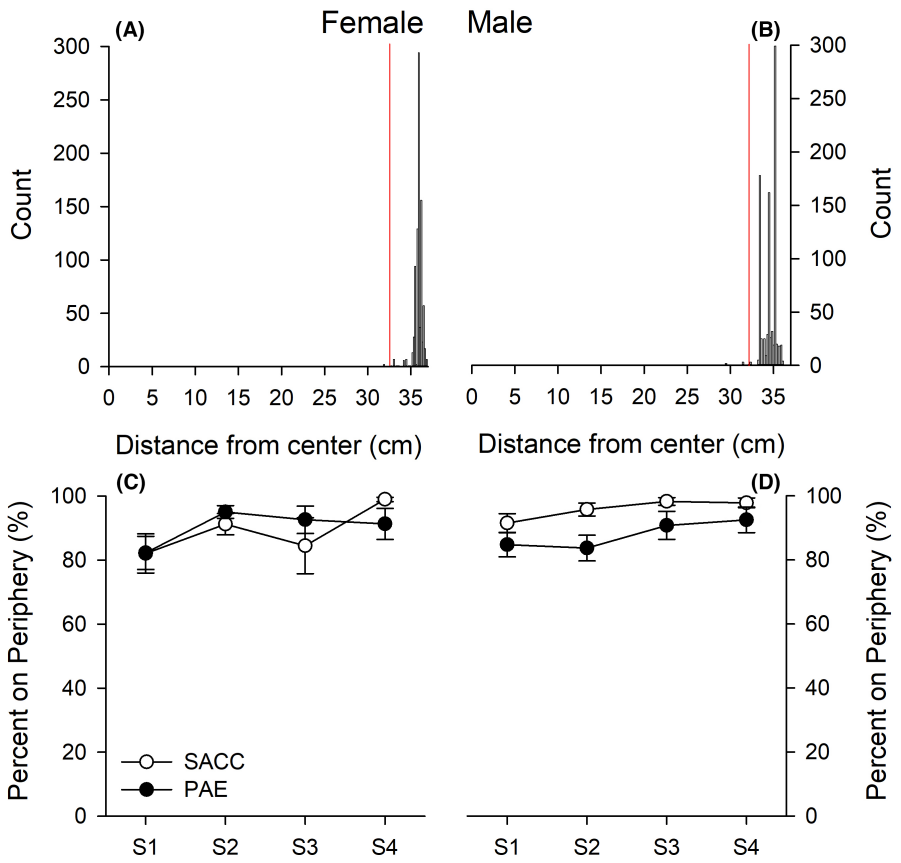

FIGURE 2 Frequency of stops relative to the distance from the center of the table is plotted for representative control female (A) and male (B) rats. The red vertical line represents the start of the table periphery. The average percent time spent on the periphery is plotted for female (C) and male (D) rats during each sample

on the organization of open field movement (home base behavior, stops, and progressions). Indeed, previous studies have largely focused on testing the impact of PAE on general measures of locomotor activity such as distance traveled, locomotor speed, or distance moved in specific regions of the apparatus. Using this approach, some studies have detected differences consistent with our findings. For instance, previous research has demonstrated that moderate PAE can result in open field "hyperactivity" expressed as increased distance traveled and increased locomotor speed measured over a 30 min session (Skorput & Yeh, 2016). It is possible these behavioral differences reflect moderate PAE-induced changes to hippocampal circuitry as hippocampal damage produces a similar pattern of hyperlocomotion in open field tests (reviewed in Clark et al. (2006) and Thompson et al. (2018)). It is important to point out that slightly different test designs such as including objects in the arena, conducting shorter duration tests, and time of testing may have contributed to the failure to detect locomotor differences after moderate PAE (Brady et al., 2012; Patten et al., 2016). For example, sexual dimorphisms in open field behavior have been observed under the light phase but not during the dark phase of the circadian cycle (Verma et al., 2010). Behavioral testing reported in the current study was conducted during the dark "active" cycle of rats' circadian cycle. Future work is needed to evaluate whether PAE sexually dimorphic effects persist in the open field when tested during the light phase of the rats' circadian cycle. Observing parallel effects of PAE across both phases would support a deficit in processing spatial information; however, observing behavior specific to the dark phase may support an emotional account of group differences. Specifically, moving animals from the dark phase of their cycle to a brightly lit testing room may serve as a stressor. In addition, studies have shown that circadian rhythms and sleep architecture can be impacted by developmental alcohol exposure (Wilson et al., 2016), and rapid changes in lighting may produce impairments subsequent behavior assessments. These possibilities warrant further investigation with careful consideration of variability in test design and environmental context (under light vs. dark conditions) which are known to influence the specific cues used in the expression of open field behaviors (discussed further below).

Spatial orientation in the open field

Rodents use multiple sources of information to organize movement in the open field. For example, previous work has shown that visual and tactile cues polarize the location of home base establishment in the open field (Clark et al., 2006; Hines & Whishaw, 2005). However, rodents have also been observed to establish home bases and organize movement under compete dark conditions and without tactile

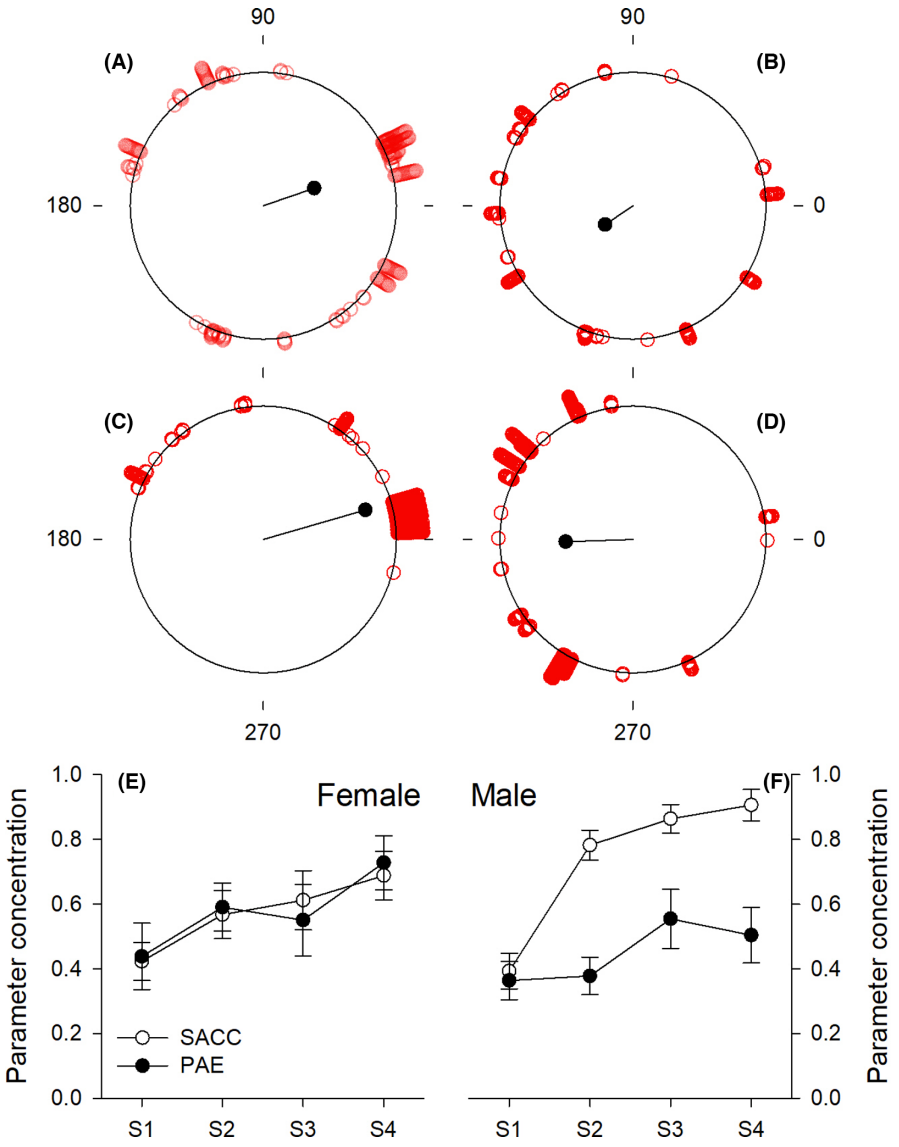

FIGURE 3 Stop clustering and circular statistics are plotted for representative SACC (A and C) and PAE (B and D) male rats for the first (A and B) and last (C and D) sample. Each red circle indicates a directional heading of one second of a stop on the open field. The within sample parameter of concentration is graphed across samples for female (E) and male (F) rats. SACC rats had significantly more concentrated stopping ($p = 0.016$) than PAE rats, and there was a significant group by sex interaction ($p = 0.013$)

cues (Osterlund Oltmanns et al., 2021). In addition, damage to systems involved in the generation of self-movement cues has been observed to disrupt home base establishment and the organization of movement in the open field under dark (and to a lesser extent, light) conditions (Banovetz et al., 2021). Considering that environmental and self-movement cues contribute to the organization of open field behavior, there are several factors contributing to the group differences in movement organization observed in the current study.

Moderate PAE may influence neural systems involved in processing self-movement cues. For example, the vestibular system might be developmentally sensitive to PAE. Genetic models of vestibular pathology have been observed to spare stop clustering in the open field behavior under light conditions (Donaldson et al., 2018). In contrast, the current study observed that PAE male rats exhibited weaker stop clustering across samples. These observations are consistent with PAE sparing vestibular function and possibly influencing higher level neural systems that process self-movement cues. Specifically, limbic system structures have been implicated in self-movement cue processing (Winter et al., 2013). In the current study, the PAE group exhibited weaker progression movement scaling relative to the SACC group. This observation has parallels to work demonstrating a role for limbic system structures in processing self-movement cues to estimate distance (Harvey et al., 2020; Winter et al., 2013). Therefore, it is possible that PAE impacted the development of limbic system structures and impaired distance estimation.

Disruptions in encoding the home base position relative to environmental cues may have also contributed to the group differences in the current study. For example, previous work has shown that a visual landmark will polarize rodent home base establishment (Clark et al., 2005, 2006; Hines & Whishaw, 2005; Thompson et al.,

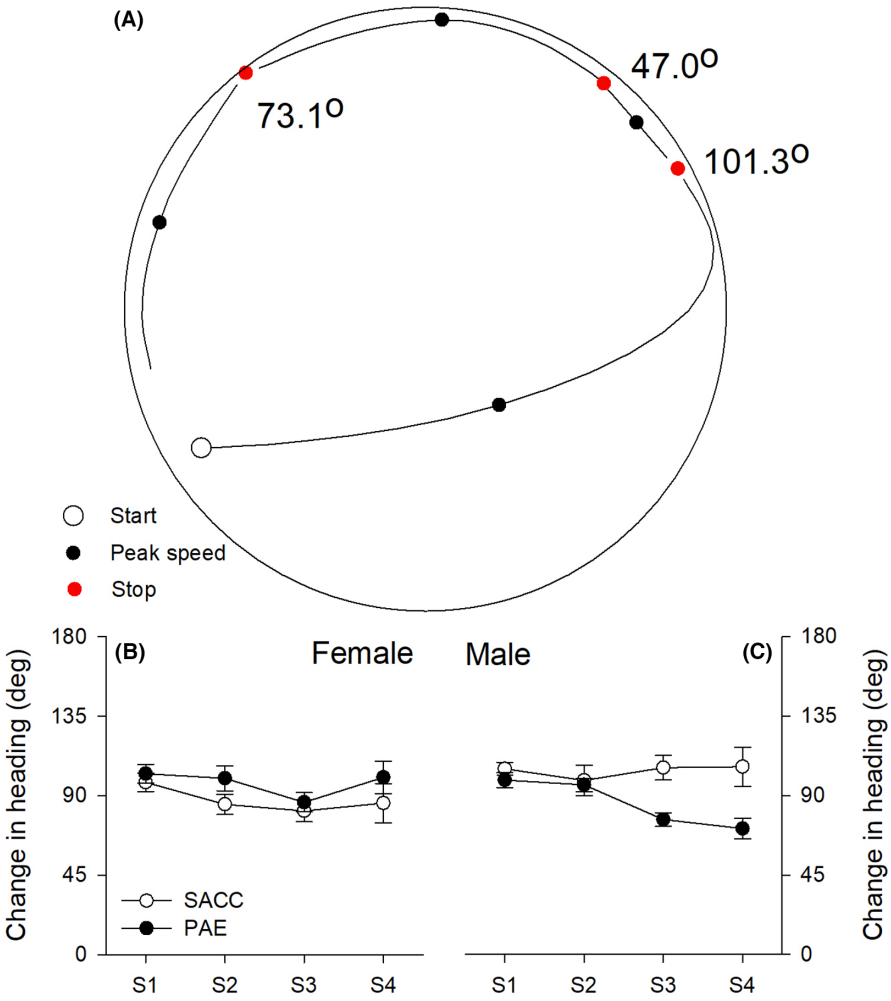

FIGURE 4 Topographic profile for three changes in heading are plotted for a representative control female rat (A). The start location of the path is indicated by the open circle, the black lines represent the progressions, the black circles represent the progression peak speed location, and the red circles indicate the stop location where the noted degree of change in heading was made. The average degree of change in heading across samples is graphed for female (B) and male (C) rats. There was a significant group by sex interaction ($p = 0.008$)

2018). Observing that open field behavior continues to be organized around the same position after the landmark has been removed is consistent with encoding the home base location within the environment. In addition, returns to a home base recalibrate head direction cell directional firing that drift while foraging for food (Valerio & Taube, 2012). It is possible that PAE may have attenuated encoding the position of the home base within the environment in the current study. This mnemonic deficit may have resulted in a home base that drifted over the session, consistent with the weaker stop clustering observed across samples in the PAE group. Future work should investigate the extent that PAE influences home base stability when access to environmental cues is varied across sessions.

Aside from environmental landmarks, intra-apparatus cues (tactile and geometric cues associated with walls) may play a critical role in the organizing open field behavior. For example, rats in a circular arena typically follow the walls and travel in circuitous paths compared to rats in a square arena (Yaski et al., 2011). Additionally, rodents tend to stop more frequently in the corners of a rectangular apparatus (Ben-Yehoshua et al., 2011). In the current study, the open field consisted of a walled circular table, and PAE rats displayed smaller changes in heading between stops compared to the SACC group. Typically, larger changes in heading are associated with genetic vestibular disorders (Donaldson et al., 2018) and acquired vestibular pathology in mice (Banovetz et al., 2021). However, most of the previous work on organization of open field behavior have not used a walled apparatus. Therefore, it is possible that PAE rats in the current study relied on the intra-apparatus cues to organize their behaviors, resulting in decreased changes in heading. Future work should consider the influence of intra-apparatus cues as it relates to the organization of open field behavior.

Sexual dimorphisms in PAE

Sex is a factor that may critically influence the effects of moderate PAE. While the majority of research has focused on male subjects (Patten et al., 2016), recent work including females demonstrates that PAE does have sexually dimorphic behavioral effects. Sex

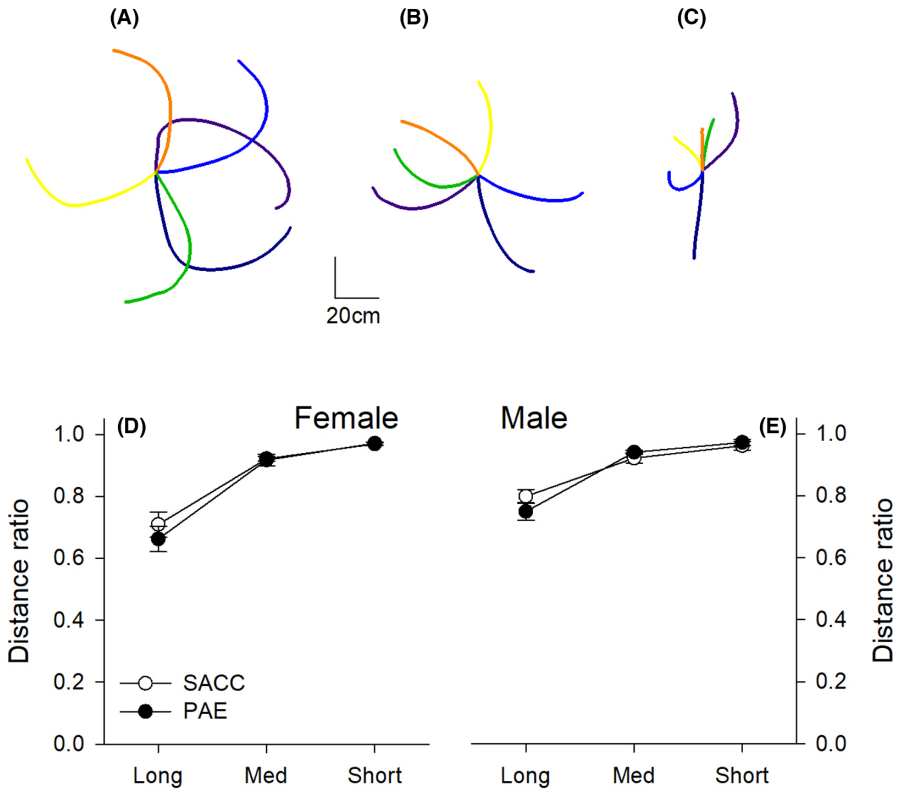

FIGURE 5 Start locations were normalized relative to the center of each plot for long (A), medium (B), and short (C) progressions from a representative control female rat. Each color represents a different progression. Average path circuity is plotted for each class of progressions for female (D) and male (E) rats. There was a significant length by sex interaction ($p = 0.020$)

differences in open field behavior as a measure for anxiety has been thoroughly studied following PAE. Typically, PAE male rodents exhibit greater distances traveled and increased center crosses in the open field (Lam et al., 2018; Rouzer et al., 2017). Sex differences in these anxiety-sensitive measures in previous work and the current study suggest males may be more sensitive to emotional processing following PAE (Cullen et al., 2013). Although the open field is commonly used to assess emotional characteristics, the organization of open field behavior can be used to detect sex-specific disruptions in spatial information processing commonly observed following PAE. For example, some studies report that PAE males have poorer working memory (Zimmerberg et al., 1991) and a greater disruption in recognition memory (Mooney & Varlinskaya, 2011) compared to PAE females. Further, a recent study reported that spatial perseveration errors (i.e., searching the previously reinforced location) during reversal learning in the Morris water task are more prominent in adult PAE male rats (Rodriguez et al., 2016). It is important to make note that moderate PAE can produce robust impairments in one-trial contextual fear conditioning in female offspring (Savage et al., 2010). Thus, although female rats are not entirely resilient to moderate PAE, the current study follows a similar general pattern finding evidence that PAE males are more susceptible to deficits in open field behavior. Why males seem to exhibit a greater vulnerability to the effects of PAE compared to females, however, is not well understood.

The hippocampus is vulnerable to the effects of PAE (Berman & Hannigan, 2000). Sexual dimorphisms in hippocampal development and cellular structures may contribute to these sex-specific effects of PAE. For example, females exhibit a faster maturation in density of the hippocampal cholinergic system compared to males (Darlington, 2010). Additionally, sexual dimorphisms have been observed in perforant path LTP (Maren, 1995). Males exhibiting larger magnitude LTP consistent with sex differences in glutamate neurotransmission. Further, females have a greater number of dendrites (Gould et al., 1990), and dendritic spines (Mendell et al., 2017) in the CA3 region of the hippocampus compared to males. Therefore, sexual dimorphisms in hippocampal neurotransmission and/or dendritic morphology may be implicated in the effects of PAE on learning and memory. Previous work has reported that hippocampal LTP is affected by PAE in a sex-specific manner, with greater deficits reported in PAE male rats (Sickmann et al., 2014). These differences were associated with an increase in glutamine synthetase in female rats. This difference may reflect a compensatory change in hippocampal function (Sickmann et al., 2014). Importantly, these effects are not dependent on the estrous cycle, as they are observed prior to ovulation. Therefore, it is possible that females have a higher baseline resilience to the effects of PAE compared to males due to a larger reserve of neuroplastic potential.

Female rodents show resilience to the effects of PAE on hippocampal neurogenesis. While PAE decreases cell proliferation in male

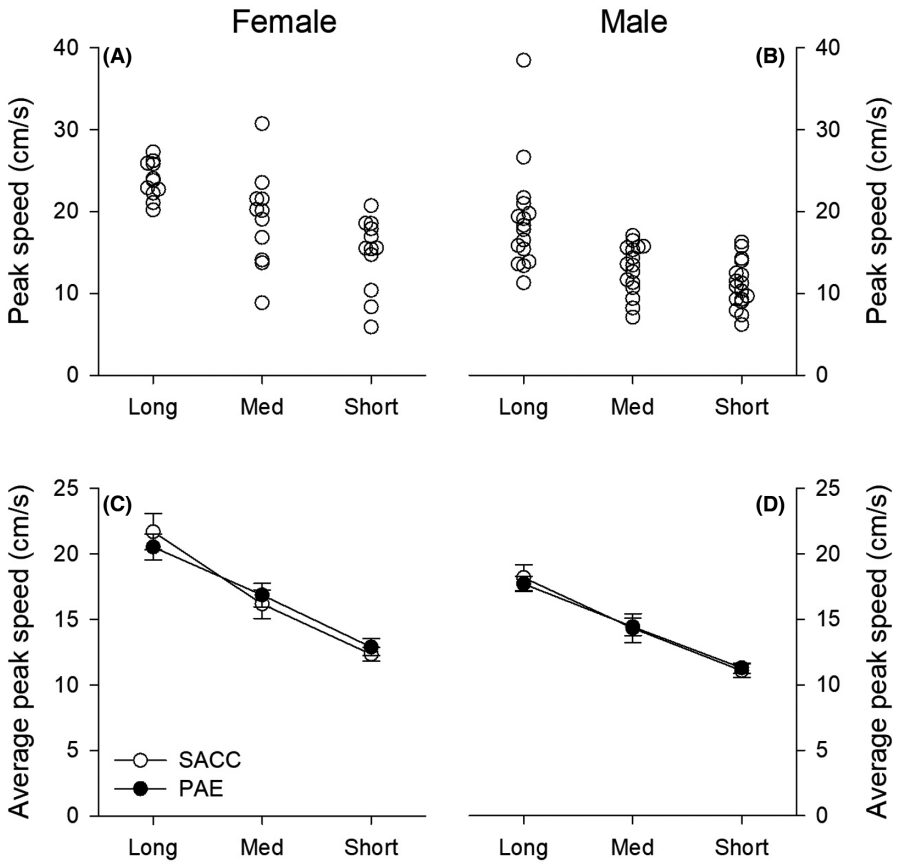

FIGURE 6 Distribution of progression peak speeds is plotted for representative control female (A) and male (B) rats across each progression class. Average peak speed is plotted for each class of progression for female (C) and male (D) rats. There was significant sex difference ($p = 0.007$) and length by sex interaction ($p = 0.041$)

rats (Sliwowska et al., 2010), female mice show an increase in cell proliferation (Choi et al., 2005). Stress may be a significant mechanism affecting this sexually dimorphic response in neurogenesis to PAE. In fact, the hypothalamic-pituitary–adrenal axis is especially susceptible to the effects of PAE (Zhang et al., 2005). PAE male rats exhibit higher baseline corticotropin releasing hormone concentrations (Gabriel et al., 2005), and have a higher stress response compared to PAE female rats (Hellemans et al., 2010; Weinberg, 1992). Stress has significant effects on neurogenesis. Specifically, stress reduces cell proliferation and survival in male rats, while females are largely spared from this effect (Falconer & Galea, 2003); however, the type and duration of stress is an important consideration. Nevertheless, it is important to consider the possibility that the increased male stress response from PAE may be a mediating factor in the neuroplastic sexual dimorphisms observed after PAE.

The mechanism underlying the effects of stress on neuroplasticity may be related to neuroinflammation. Aquaporin-4 (AQP4), a water channel that when disturbed causes cellular edema and neurotoxicity, is disrupted in binge drinking (Collins et al., 2014). Indeed, this edema may also be seen in offspring exposed to PAE. Hippocampal neuroinflammation is observed in PAE mice (Cantacorps et al., 2017) and may contribute to disruptions in neuroplasticity (Di Filippo et al., 2008). Interestingly, neuroinflammation may describe why PAE male animals exposed to stress have more deleterious effects on neuroplasticity compared to females. Stress is associated with persistent low-grade neuroinflammation in males (Liukkonen et al., 2011). Therefore, it is possible that neuroinflammation may be mediating both stress and neuroplasticity, both contributors to sexually dimorphic responses to PAE.

A final consideration is whether variability in levels of estradiol may have contributed to the observed differences in open field behavior. Although the current study did not track estrous during open field testing, wide variability was not observed in our female open field measures (home base behavior, stopping, and progressions) which would be expected if estrous had influence over the organization of these behaviors. A similar pattern has been described in a previous study investigating sex differences in open field behavior across lighted conditions (Osterlund Oltmanns et al., 2021). Further, previous work has failed to detect a significant influence of estrous on movement organization in a hippocampal-dependent food foraging task (Köppen et al., 2015). Lastly, estrous has been shown to have no effect on hippocampal place cell activity (Tropp et al., 2005), suggesting a limited effect of estrous on neural systems involved in spatial processing.

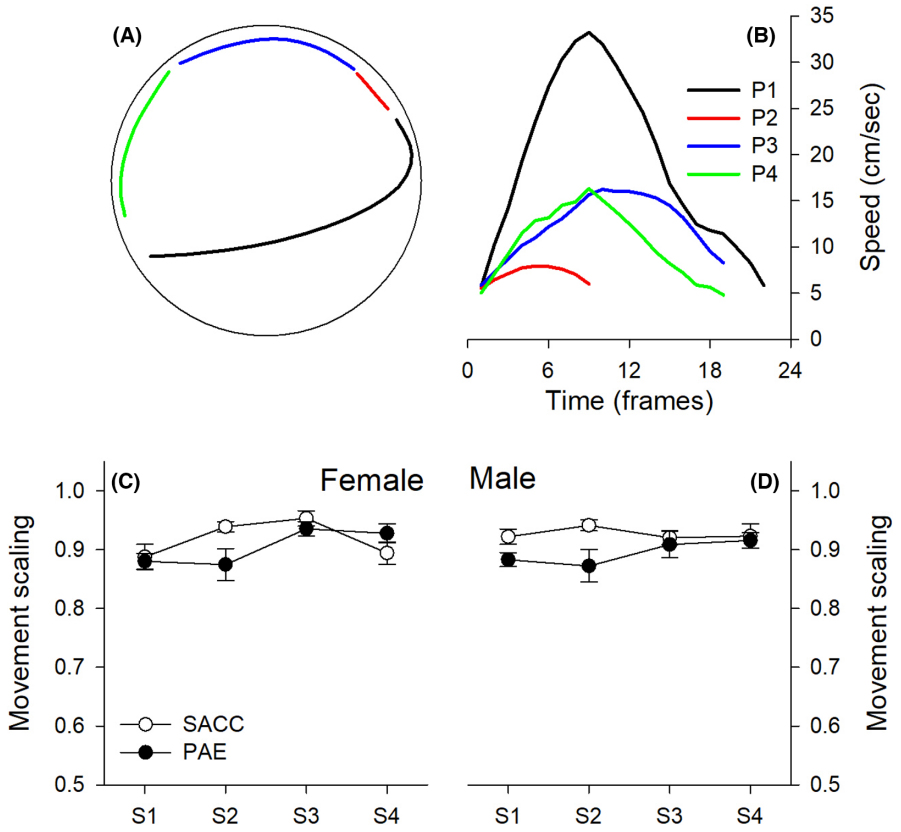

FIGURE 7 Topographic (A) and kinematic (B) profiles are plotted for a representative control female rat for a sequence of progressions that varied in length. Average movement scaling is graphed across samples for each group of female (C) and male (D) rats. SACC rats had significantly higher movement scaling ($p = 0.030$) compared to PAE rats

Clinical translatability of PAE assessments

Structures associated with spatial information processing such as the hippocampus are impacted by alcohol exposure across developmental stages (De Bellis et al., 2000). The behavioral effects of this exposure have been observed in work evaluating the effects of adolescent binge drinking on manipulatory scale spatial information processing (Blankenship et al., 2016). Specifically, adolescent binge drinker participants took more time and traveled longer distances to find a piece of Velcro hidden in a bead maze, analogous to what is observed in ambulatory scale variations of the Morris water task. This pattern of results suggests that adolescent binge drinking may influence the ability to recall previously searched locations, resulting in a less efficient search path.

The ability to recall where one has just traveled is essential for dead reckoning, a navigation strategy based on self-movement cue processing. Self-movement cues generated from one's own movement are a source of information animals can use to process spatial information such as distance and direction traveled. Animals use self-movement cues to guide movement across virtual (Wolbers et al., 2007), ambulatory (Wallace et al., 2006), and manipulatory (Wallace et al., 2010) scales of movement. For example, when restricted to using self-movement cues in a manipulatory Velcro search task, control but not binge drinker participants were able to return to their starting position with accurate distance and direction estimation (Blankenship et al., 2016). Recent work has demonstrated a critical role for the hippocampus in self-movement cue processing. For example, damage to the hippocampus precludes an animal from accurately returning to a starting position when restricted to using only self-movement cues (Gorny et al., 2002; Martin & Wallace, 2007; Martin et al., 2007; Wallace, Hines, & Whishaw, 2002; Wallace & Whishaw, 2003; Whishaw et al., 2001). Spatial tasks that restrict animals to using self-movement cues have consistently demonstrated the ability to detect hippocampal pathology.

A behavioral assessment of hippocampal function may provide a novel way to evaluate FASD in children. Currently, FASD is most commonly diagnosed based on characteristic facial features; however, the majority of children with FASD do not display these abnormalities (Mattson et al., 2010). An additional challenge in diagnosing FASD is that symptoms may not be apparent until later childhood, as the range in age for diagnosis is eight months to eight years of age. Given these challenges, it is estimated that less than 1% of children with FASD receive a clinical diagnosis (Burd & Popova, 2019), leading to missed opportunities for early protective interventions against the cognitive effects of FASD (O'Connor et al., 2006). Consequently, a main goal of preclinical research should focus on developing assessments that are sensitive in detecting PAE at an early age. Future work should investigate if a

manipulatory scale variation of the current spatial task and behavioral analysis is sensitive in detecting PAE. These tasks could provide a simple and inexpensive yet powerful tool for early detection of FASD in the clinical setting.

CONCLUSION

The current study evaluated the effects of moderate PAE on movement organization during open field behavior in adult female and male rats. PAE and female rats exhibited increased general open field behavior. PAE rats, particularly males, exhibited significantly less clustered home base establishment stopping and smaller changes in heading during stops. PAE and sex also impacted the organization of progressions in the open field. PAE rats exhibited weaker movement scaling, and female rats traveled more circuitous paths with faster peak speeds. This work provides evidence that moderate PAE influences spatial orientation in a sex-specific manner. Future work should dissociate the effects of PAE on environmental and self-movement cue processing in female and male rodents.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Danika Nelson, Dr. Nathaniel Pavlik, Ms. Ella Rappaport, Ms. Victoria Sugita and Dr. Kevin O'Hair for their outstanding animal care support for this project. This work was supported by the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Numbers P50 AA022534, T32 AA014127, and R01 AA029700.

CONFLICT OF INTEREST

The authors do not have any conflicts of interest for this article.

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How to cite this article: Osterlund Oltmanns, J.R., Schaeffer, E.A., Goncalves Garcia, M., Donaldson, T.N., Acosta, G., Sanchez, L.M., et al (2022) Sexually dimorphic organization of open field behavior following moderate prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, 46, 861–875. Available from: https://doi.org/10.1111/acer.14813