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Moderate Prenatal Alcohol Exposure Impairs Performance by Adult Male Rats in an Object-Place Paired-Associate Task

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Abstract

Memory impairments, including spatial and object processing, are often observed in individuals with Fetal Alcohol Spectrum Disorders. The neurobiological basis of memory deficits after prenatal alcohol exposure (PAE) is often linked to structural and functional alterations in the medial temporal lobe, including the hippocampus. Recent evidence suggests that the medial temporal lobe plays a critical role in processing high-order sensory stimuli such as complex objects and their associated locations in space.

Methods: In the first experiment, we tested male rat offspring with moderate PAE in a medial temporal-dependent object-place paired-associate (OPPA) task. The OPPA task requires a conditional discrimination between an identical pair of objects presented at two spatial locations 180° opposite arms of a radial arm maze. Food reinforcement is contingent upon selecting the correct object of the pair for a given spatial location. Adult rats were given a total of 10 trials per day over 14 consecutive days of training. PAE male rats made significantly more errors than male saccharin (SACC) control rats during acquisition of the OPPA task. In Experiment 2, rats performed an object-discrimination task in which a pair of objects were presented in a single arm of the maze. Moderate PAE and SACC control rats exhibited comparable performance. The results suggest that moderate PAE rats can learn to discriminate objects, but are impaired when required to discriminate between objects on the basis of spatial location in the environment.

Keywords

medial temporal; entorhinal; perirhinal; object recognition; spatial behavior; fetal alcohol

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Author Contributions: LS and BJC designed the experiments. JW, SD, and DS provided the rat model. LS and JG contributed to the collection of the data. LS and JG analyzed the data with input from BJC and DH. LS and BJC wrote the manuscript. All authors reviewed the final manuscript.

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Introduction

Exposure to alcohol during prenatal development can lead to developmental disability including profound altered physical features, behavior, and neurological function [1–4]. Fetal Alcohol Spectrum Disorders (FASD) are a major public health concern worldwide and in the United States impacting approximately 2–5% of children. While a great deal of research has been done to understand the effects of high-dose prenatal alcohol exposure (PAE), there is increasing evidence that moderate PAE is much more common and can also have a long-lasting impact on cognition and behavior [5–8]. One of the most striking behavioral abnormalities after PAE are deficits in learning and memory which can have serious repercussions for scholastic performance. Learning impairments are particularly noticeable once children begin to engage in complex math and reading comprehension assignments [9]. Since moderate PAE is usually diagnosed later in childhood, there is a considerable need to establish behavioral assessments for early detection and subsequent intervention.

The neurobiological basis of learning and memory impairments after PAE is frequently linked to the integrity of the hippocampus and its subfields, which displays significant alterations in synaptic plasticity and structural changes after alcohol exposure prenatally [10–17]. While considerable attention has been directed toward the hippocampus, less is understood regarding how PAE affects other medial temporal structures (entorhinal, perirhinal, and parahippocampal cortices). A large body of theoretical and experimental work suggests a role for extra-hippocampal structures in processing high-order sensory stimuli, such as the recognition of complex objects and their corresponding spatial locations [18,19]. Studies on the cognitive functions of children with fetal alcohol syndrome are supportive of a possible deficit in sensory discriminations, with some reporting difficulties for memory of recently viewed objects and the spatial locations of objects [20], while others reporting impairments in visual perceptual memory [21]. Although some research using animal models demonstrate that relatively high doses of PAE can impair discrimination between objects and object locations in adult rats [24–26], these impairments were not seen by Kim et al [28]. Less is known regarding the impact of more moderate levels of PAE [5].

In Experiment 1, we examined whether moderate PAE (60 mg/dl peak BAC [26]) disrupts the performance of adult male rats (4 months of age) on an object-place paired-associate task (OPPA), which involves the pairing of objects and places using a bi-conditional association rule [27–30]. Briefly, rats are reinforced with food after selecting an object when it appears in a specified location, but not when it is presented in a second location (see Fig. 1A). A second object is reinforced only when it is encountered in the second location, but not in the other location. Therefore, subjects are required to select a specific object on the basis of where it is encountered in the environment and disambiguate this pairing from other object-place associates. Importantly, accurate task performance on the OPPA requires high-order sensory processing [30] and interactions within a distributed network of hippocampal and medial temporal structures, as indicated by recent human neuroimaging work [31] and lesion studies in rats [32–34]. In Experiment 2, we tested moderate PAE rats in a task requiring accurate discrimination between two objects based on their unique features, but independent of their spatial location in the environment (Fig. 2A). Here, we report that moderate PAE

impairs performance in the object-place paired-associate task, but fails to disrupt accurate object discrimination.

Materials and Methods

Subjects

Subjects included 16 male Long Evans rats which were obtained from the University of New Mexico Health Sciences Animal Resource Facility (breeding protocol can be seen below). Male rat offspring were selected as experimental subjects to allow for comparison with previous studies investigating the neurobiological mechanisms of object-place and object discrimination [28, 33, 36, 52]. Following weaning, all animals were pair-housed with animals given the same prenatal treatment (either alcohol or saccharine exposed) in standard plastic cages on a reverse 12-hour light: dark cycle at a room temperature of 22 ° C with food and water provided ad libitum throughout the pre-training and behavioral experiments. Animals were maintained in the reverse light-dark cycle throughout their lifetime because rats are more likely to move during their natural nocturnal cycle and facilitates training and testing. Rats were given 5 minutes of acclimation in the light before training began. All rat breeders and weaned offspring consumed a diet of Teklad global soy protein-free extruded food 2920. At 4 months of age, rat offspring were placed on a food restricted diet of 90% of their Ad libitum diet weight and given access to water ad libitum. Food restriction continued through Experiments 1 and 2. The Institutional Animal Care and Use Committee (IACUC) at the University of New Mexico central campus and/or Health Sciences Center approved all procedures for the studies reported here.

Breeding and Voluntary Ethanol Consumption During Gestation

Breeding procedures were conducted at the University of New Mexico Health Sciences Animal Resource Facility (ARF). Three to four-month-old rat breeders (Harlan Industries, Indianapolis, IN (currently Invigo) were single housed in standard plastic cages and placed on a 12-hour reverse light: dark cycle (lights on from 2100-0900 hours) and kept at 22 ° C with ad libitum food and water. Following a one-week acclimation period in the animal facility, the breeders were exposed to a voluntary ethanol drinking paradigm. Female rats were provided 0.066% (w/v) saccharin (SACC) in tap water from 10:00 to 14:00 hours (4 hours) each day. On Days 1-2, the saccharin water contained 0% ethanol, Days 3-4, saccharin water contained 2.5% ethanol (v/v). On Day 5 and subsequently, saccharin water contained 5% ethanol (v/v). The daily four-hour consumption of ethanol was monitored for at least two weeks and the mean daily ethanol consumption was determined for each female breeder. After two weeks of daily ethanol consumption, females that drank at levels less than one standard deviation of the entire group mean (~12-15% of all female breeders) were removed from the study [35]. The remaining females were then assigned to either a saccharin control or 5% ethanol drinking group. These breeding females were matched such that the mean pre-pregnancy ethanol consumption by each group (2.65 ± 0.09 mg / kg) was similar. As a result, dams of both groups experience equivalent preconceptual exposure to ethanol. Lastly, the female breeders were nulliparous and were not used in multiple rounds of breeding, while the male rats were experienced breeders.

Female rats were matched with a male breeder rat until pregnancy was verified, based on the presence of a vaginal plug. There was no ethanol consumption during breeding. Beginning on Day 1 of gestation, the rat dams were given access to saccharine (Sigma Life Sciences, St. Louis, Missouri) water containing either 0% (v/v) or 5% (v/v) ethanol (Koptec, King of Prussia, Pennsylvania) for four hours a day, from 10:00 to 14:00 hours. The volume of the 0% ethanol saccharine water provided to the control group was matched to the mean volume of the 5% ethanol saccharine water consumed by the ethanol group. During gestation and including the four-hour ethanol/saccharine drinking period, rats were provided with ad libitum water and rat chow (Teklad global soy protein-free extruded food 2920). Daily ethanol consumption was recorded for each rat dam. The mean maternal ethanol consumption throughout pregnancy was 2.14 ± 0.10 g/kg and did not vary significantly during each of the three weeks of gestation. In a separate set of rat dams, this level of ethanol consumption has been shown to produce a mean peak maternal serum ethanol concentration of 60.8 ± 5.8 mg/dl [36]. Daily maternal ethanol consumption ended at birth, and the litters were weighed and culled to 10 pups. As reported previously [26], the moderate exposure paradigm employed in these studies does not affect maternal weight gain, offspring birth weight or body weight at testing (data not shown). To minimize potential litter effects, only 1-2 animals were used from each litter. Thus, a total of 7 litters contributed to the PAE group and 7 litters to the SACC group.

Experiment 1: Object-Place Paired-Associate Task

Objects and Environment.—The maze was composed of two arms (each 40.1cm $\times$ 9.30cm) of an 8-arm radial-arm maze [30]. The two arms were separated by 180° and were fixed to a center stage (25cm in diameter). The end of each arm was a rectangular platform (20cm $\times$ 30cm), each containing three recessed food wells separated by transparent vertical Plexiglas dividers (each 5.1cm $\times$ 5.1cm). Transparent Plexiglas doors (20cm $\times$ 9.5cm) were present at the entrance of each arm from the center stage. A set of toy objects which are glued to a washer, were presented at the end of the two opposite arms and above two of the recessed food wells. The maze was placed in the center of a testing room that contained many extra-maze cues, including a sink, desk, chair, shelves, and wall posters [30,37]. Session recordings were conducted using a camera above the maze and fluorescent overhead lighting conditions. Digital videos were obtained for off-line analysis.

Pre-training.—Experimenters blind to condition handled rats for 5 days for approximately 5 minutes per day. To help increase motivation to forage, rats were placed on a food-restricted diet to a weight of 90% of ad libitum feeding diet. Rats were weighed daily prior to testing. The amount that was fed to the rats post-testing was determined by the weight of the animal as to make sure that they did not drop below the 90% of ad libitum feeding. After rats displayed signs of comfort with the experimenter (i.e. no defecation/urination), shape training began. On the first day of shape training, 5 pieces of food (1/2 of Froot Loop®, Kelloggs, Battlecreek, Michigan) were placed along each of the two arms and animals were allowed to freely explore and consume the food over a period of a maximum of 20 minutes. In subsequent training session, food was restricted to the recessed cups located at the end of each arm and rats were once again given up to a maximum of 20 minutes on the maze. Once rats retrieved 20 ½ Froot Loop® from the recessed food wells

(10 pieces per arm), metal washers (2.5cm in diameter) were placed above the recessed food wells and rats had to successfully push the metal washer to retrieve the ½ Froot Loop® for 20 trials (10 on each arm) over a maximum of 20 minutes. Shape training continued for 6 days. Rats were then trained for 20 trials per day. A trial consisted of the rat being placed in the center of the maze within the closed doors of the maze. One of the two arm doors were pseudo-randomly opened. The rat was then allowed to locomote to the end of the arm to the choice platform where it displaced the metal washer, retrieved the half Froot Loop® and was carefully guided back to the center to consume the food with the arm door closed. After a few days of washer displacement, the rats ran back to the center for food consumption with little experimenter guidance [38]. Once rats could displace the washer 20 times within a 20-minute time interval, they had completed pre-training and moved on to object-place-paired-associate training. All rats reached criterion within 6 days, there were no rats removed if they did not reach criterion.

Object-Place Paired-Associate Training.—Animals, 4 months of age, were trained in an object-place paired-associate task similar to [33 see also 31,36]. Washers were replaced with two distinctive objects (a toy superman and a Ping-Pong ball; see Fig. 1A). The toys were placed above the left and right recessed food wells on the choice platform and rats were required to displace one of the objects to uncover the food. If rats displaced the incorrect object, they were not allowed to change their choice but were gently guided back to the center of the maze. The positions of objects on the choice platform were pseudo-randomly selected across trials to prevent animals from learning a specific egocentric response to obtain the food reinforcement (see side bias described in Data Analysis). This required the animals to learn a rule which was associated with a particular object (superman or ball), and a particular place (1 of the 2 arms) in the maze. This rule is typically referred to as an object–place paired-association. The sequence of arm visits was pseudo-randomized with two different sequences that were alternated between days. Rats were trained to acquire the task for a total of 14 days. Each day consisted of a total of 10 trials with 5 trials on each arm. The maximum time allotted for each rat per day was 20 min. Based upon our previous findings that rats can use proximal cues to discriminate between maze locations [30], the maze surface and objects were cleaned between animals, and the maze was rotated 45° after each day of testing.

Experiment 2: Object-Discrimination Task

Two months after testing in the OPFA task, rats were trained to discriminate between two novel objects (black conical tube and red box; see Fig. 2A) to receive food reinforcement. One arm of the maze was used for the experiment [28,33]. As in the tasks above, rats were placed in the center of the maze, the experimenter opened the maze arm, and the rats were allowed to push one of the two objects to uncover the food reinforcement [30]. The same object was reinforced throughout the task. As in Experiment 1, the left-right position of the object was pseudo-randomly selected to prevent acquisition of a bias towards responding to a particular side of the choice platform (see side bias described in Data Analysis). If the incorrect object was chosen, the rat was not allowed to correct the choice but was guided back to the center without reinforcement. Each test day consisted of a total of 10 trials administered over 8 days.

Data Analysis

For Experiments 1 and 2, the performance of each animal was determined by calculating the percentage of correct trials per day (correct choices divided by total choices and multiplied by 100). During acquisition of the OPPA task, previous studies have reported that rats can express a bias for responding to a particular side of the choice platform or a bias for selecting a particular object [39,28,30]. To quantify these response biases, we created a side bias index similar to previous reports [39,28,30]. The side bias was calculated by taking the absolute value of the total number of left choices minus the total number of right choices and then dividing by the total number of trials. Similarly, an object bias index was calculated by taking the absolute value of the number of Object 1 choices minus the total number of Object 2 choices and then dividing by the total number of trials [28,30]. Latency-to-choice for each animal for each day was calculated. This was measured as the time it took for the rat to leave the center platform threshold and then make a choice of object (pushing the object off of the recessed cup). Group (PAE and SACC rats) means for all measures were analyzed using mixed-model repeated-measures analysis of variance (ANOVA) with between subject's factors of treatment. In addition, mean comparisons were conducted on data (percent correct, side bias, and object bias) from each test day of the OPPA (Experiment 1) using an independent samples t-test (one-tailed). Effect sizes for the ANOVA are reported using partial eta squared (η_p^2) and are reported for t-tests using Cohen's *d* (*d*). Data analysis was performed using IBM® SPSS Statistical software, v24.

Results

Experiment 1: Object-Place Paired-Associate Task

Animals were first tested on an OPPA task in which they were required to discriminate between a set of objects based upon their spatial position in the environment (Fig. 1A). Figure 1B plots the percentage of correct choices from PAE and SACC animals over 14 days of training. In general, animals in both groups improved their performance over days ($F(13,182) = 27.88, p < 0.001, \eta_p^2 = 0.637$). All animals improved in performance over the entire testing period. However, improvement in performance was greater for the SACC group. These observations were confirmed by a mixed-model repeated-measures ANOVA conducted on the percent correct across testing days. The ANOVA indicated a significant group-by-day interaction ($F(13,182) = 1.89, p = 0.034, \eta_p^2 = 0.043$), and a significant group effect ($F(1,14) = 5.46, p = 0.035, \eta_p^2 = 0.281$). Mean comparisons detected significantly lower measures of percent correct for PAE rats on days 9, 11, 12, 13, and 14 (Day 9: $p = 0.012, d = 1.27$; Day 11: $p = 0.04, d = 0.94$; Day 12: $p = 0.004, d = 1.54$; Day 13: $p = 0.024, d = 1.09$; Day 14: $p = 0.04, d = 0.94$). To assess variability in individual performance, we measured the number of testing days required to exceed >90% correct across two consecutive days. Overall, 7 out of 8 SACC rats reached >90% performance before 12 days of testing. In contrast, only 2 of the 8 PAE rats reached >90% performance at the same rate, with two PAE rats failing to reach this performance level by the 14th day of testing. Lastly, there was no significant group effect ($F(1,13) = 0.842, p = 0.376, \eta_p^2 = 0.061$) or group by day interaction

($F(13,169) = 0.731, p = 0.730, \eta_p^2 = 0.053$) found for measures of latency-to-choice (SACC: 10.90 ± 1.83 sec; PAE: 14.95 ± 4.60 sec). Taken together, PAE animals displayed impaired task acquisition relative to SACC rats in the OPPA task.

Figure 1C plots the side bias index for each group across the 14 days of testing in the OPPA. Both SACC and PAE animals showed similar use of this rule early in testing, but the side bias index decreased as a function of testing for both groups of animals. This observation is supported by a significant day effect as revealed by the mixed-model ANOVA ($F(df$ Greenhouse-Geisser adjusted: 5.15, 72.11) = 2.59, $p = 0.032, \eta_p^2 = 0.143$). However, on days 11 to 14, the average side bias index was greater for PAE rats compared to SACC animals (Fig. 1C). Although the ANOVA failed to detect a significant group effect ($F(1,14) = 1.51, p = 0.204, \eta_p^2 = 0.097$) or a group by day interaction ($F(df$ Greenhouse-Geisser adjusted: 5.15, 72.11) = 1.49, $p = 0.203, \eta_p^2 = 0.082$), mean comparisons between the two groups revealed significantly greater side bias measures for PAE rats on days 11, 13, and 14 (Day 11: $p = 0.005, d = 1.47$; Day 13: $p = 0.08, d = 0.75$; Day 14: $p = 0.07, d = 0.79$). A similar pattern of results was observed for measures of the object bias (Fig. 1D). An ANOVA detected a significant day effect ($F(3,182) = 3.95, p < 0.001, \eta_p^2 = 0.22$), but failed to indicate significant group ($F(1,14) = 0.414, p = 0.531, \eta_p^2 = 0.029$), or day by group interactions ($F(3,182) = 1.48, p = 0.129, \eta_p^2 = 0.095$). Nevertheless, mean comparisons revealed significantly greater object bias measures for the PAE group on days 13 and 14 (Day 13: $p = 0.03, d = 1.07$; Day 14: $p = 0.048, d = 0.90$).

Experiment 2: Object Discrimination Task

With the exception of one rat that died after Experiment 1, all animals were tested in an object-discrimination task which required a discrimination between two objects based on their unique sensory features (Fig. 2A). Because animals were tested in a single maze arm, they were no longer required to discriminate between the two objects based on spatial position. Figure 2B plots the percentage of correct choices by PAE and SACC groups across the eight days of testing. On average, rats in each group showed improvement in selecting the reinforced object across training. This observation was confirmed by a mixed-model repeated-measures ANOVA indicating a significant day effect ($F(df$ Greenhouse-Geisser adjusted: 3.41, 44.31) = 40.23, $p < 0.001, \eta_p^2 = 0.756$). Importantly, animals in both groups demonstrated similar performance across training as revealed by the absence of a significant group ($F(1,13) = 0.001, p = 0.981, \eta_p^2 = 0.000$), or group by day effect ($F(df$ Greenhouse-Geisser adjusted: 3.41, 44.31) = 1.00, $p = 0.407, \eta_p^2 = 0.072$). In sum, PAE animals displayed intact performance in the object-discrimination task.

Discussion

Given previous research indicating that deficits in object processing and object-location discrimination can appear in children with Fetal Alcohol Syndrome [20] or after a high dose

of PAE in animal models [22–24], we tested the hypothesis that a moderate dose of PAE would produce a similar pattern of impairments. In Experiment 1, male animals were trained to discriminate between two objects in two different locations. In moderate PAE rats, we observed slower learning compared to SACC rats. In addition, by the end of training, PAE animals failed to obtain similar performance on the conditional object-place association (Fig. 1B) and appeared more likely to adopt a simple rule of either entering a particular side of the reinforcement platform (Fig. 1C) or selecting a specific object (Fig. 1D). In Experiment 2, animals were trained to discriminate between two objects independent of spatial location in the environment. We found that both moderate PAE and SACC control rats were able to learn the object discrimination at similar competence (Fig. 2B). These results indicate that moderate PAE rats can learn to discriminate between objects, but not when they are conditionally associated with a spatial location.

Our findings of impaired OPPA performance after moderate PAE is consistent with previous work reporting deficits in object-place memory in children with Fetal Alcohol Syndrome [20]. However, research using animal models has been less consistent regarding the impact of PAE on sensory discriminations. For instance, recent work has shown that alcohol exposure targeting the developmental equivalent of the third-trimester in rats (postnatal days 4–9) does not impair object-location memory [40–42]. Terasaki & Schwarz [42] evaluated performance by adult rats exposed to alcohol for 6 prenatal days (prenatal days 10–16) and detected only subtle deficits in an object-location task. Because ethanol was administered throughout gestation in the present study, it is possible that a broad developmental range of PAE may be required to observe robust impairments in object-place memory. Although neurogenesis in hippocampal and extra-hippocampal regions is largely complete by prenatal day 19 [43,44], laminar development continues throughout the remainder of gestation and into the first postnatal week [45].

In the literature there are some conflicting findings to our results, but this could be attributed to the different behavioral paradigms of object-place memory being used. The OPPA task used in the present study requires that rats location in the maze during 14 daily training trials, discriminate between two maze arms, and utilize this spatial information to discriminate between the objects encountered in the maze arms [30,46,47]. Further, animals are required to inhibit other behaviors such as the preference to locomote to a particular side of the choice platform or a specific object [28,30,33]. In comparison, previous studies have largely evaluated object-place memory using a spontaneous object exploration task in which animals are required to explore objects after a change in their spatial configuration in the same environment [48], or during a single encounter of the same objects but in a different environmental context [40–42]. Thus, compared to previous studies, the OPPA task used in our studies may place a greater load on object-place discrimination and the requirement to inhibit inefficient behavioral strategies. This interpretation is consistent with the hypothesis that task complexity, or discrimination load, is an important variable in understanding the cognitive impact of PAE [23].

A second conclusion of the present study is that object discrimination was unimpaired in adult rats with moderate PAE. Because several previous studies using high dose animal models have reported impairments in object-discrimination [24–26 but see 27], our findings

suggest that moderate PAE is not sufficient to affect task performance. Nevertheless, some studies have reported doses within a comparable range to the present work, and administered only during one week of gestation, can significantly alter the structural features of the hippocampus and cortical regions involved in object discrimination [42]. Again, differences between the present study and previous work could be related to variability in testing procedures. For instance, prior studies have investigated object discrimination using spontaneous exploration paradigms, which can be influenced by differences in locomotor behavior rather than the capacity to discriminate, per se. In contrast, the present study used an object discrimination paradigm that was food motivated; thus, motivational variables differed between the present work and previous studies. Given that food-driven tasks can enhance synaptic engagement in reward circuitry [49], it is possible that similar changes to object processing circuitry may have obscured the impact of PAE in the present study. Lastly, our object discrimination procedures used objects with non-overlapping features (see Fig. 2A). Popovic et al [23] used objects with a similar shape (tin drinking cans) but with subtle differences in visual appearance (different can labels) and reported clear deficits in object discrimination task by PAE rats. Thus, it would be of considerable interest for future work to investigate discrimination performance in tasks that systematically vary the degree of stimulus overlap for objects and/or places [50].

A number of studies have indicated that performance in the OPPA task is dependent on a distributed network of medial temporal lobe, limbic, and neocortical structures [21, 22, 50, 48]. For instance, neurotoxic damage to the hippocampus [29], including select lesions of hippocampal CA3 or dentate gyrus [52,53], can produce impairments in object-place tasks. Lesions of the entorhinal and perirhinal cortices [32–34], and disconnecting lesions between the hippocampus and perirhinal cortex [33], can also produce severe deficits on object-place learning and memory. Neural ensemble recordings from behaving animals during OPPA performance has shown that while hippocampal CA1/CA3 neurons discriminate between object-place and item-place associates [54,55], the synchrony of neural firing between prefrontal-hippocampal [47] and entorhinal-hippocampal regions increases during associative learning [56]. Lastly, a recent study monitoring performance by human subjects using a combination of fMRI imaging and a virtual reality variant of the OPPA and revealed a broad network of hippocampal, temporal, and prefrontal engagement during task acquisition and retrieval [57].

Little is known regarding the impact of PAE on the functional connectivity between the hippocampus and medial temporal structures, however considerable evidence indicates that the moderate prenatal ethanol exposure model used here impairs long-term potentiation between the entorhinal perforant pathway and dentate gyrus [14,16,17,26,58]. Some studies have also reported disrupted long-term potentiation at CA1 and CA3 subregions (for review see [13]), and memory tasks thought to be dependent on the CA subregions are impaired after PAE [48]. With respect to the neocortical impact of PAE, studies have reported that PAE can alter pro-inflammatory and neurotrophic expression in the perirhinal cortex of adult rats [42], and reduce neuron growth and differentiation in the entorhinal cortex [59,60]. Given these observations, future studies should be aimed at understanding the relationship between OPPA performance and alterations to hippocampal and extra-hippocampal circuitry after moderate PAE.

To compare with previous studies investigating the neurobiological mechanisms of object-place and object discrimination [28, 33, 36, 52], the present study utilized male animals as experimental subjects. However, whether moderate PAE might have similar effects on discrimination performance by female rats should be investigated in future work. Some studies have been supportive of differential sex effects of PAE on hippocampal function. Electrophysiological studies have reported that reductions in hippocampal long-term potentiation is reliably produced in male PAE rats, and in some studies, is enhanced in female PAE rats [13]. In contrast, some behavioral studies have reported that PAE does not produce differential sex effects in hippocampal-dependent tasks. For instance, a recent study reported that male and female PAE rats were equally impaired in a temporal discrimination task [48]. In another recent experiment, there were no reported group differences between male and female PAE rats (or interactions) on acquisition of the Morris water task [61]. Finally, a previous study reported deficits by PAE female rats in fear conditioning, but males were not tested in this study [26].

In summary, the present study demonstrates that male adult rats exposed to moderate PAE are selectively impaired in an object-place task, suggesting that higher-order sensory discriminations are sensitive to developmental exposure to alcohol. Further experiments focused on characterizing the behavior of PAE rats in discriminations of varying complexity, and a characterization of cortical-hippocampal circuitry, will begin to clarify the underlying status of the medial temporal lobe after PAE and in FASD.

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Figure 1.

A.) Schematic illustration of the object-place paired-associate task. Rats entering Maze Arm 1 were required to push the ball to receive the food. Rats running to Maze Arm 2 were required to push the Superman in order to receive the food. B.) Mean (dark line) and SEM (shaded area around line) for the percentage of correct trial is plotted for SACC (blue) and PAE (red) groups across the 14 training days. Dashed line represents chance performance. Note that PAE rats performed a lower percentage of correct trials compared to SACC rats after the 7th day of training. Note: this data was significant, $F(13,182) = 1.89$, $p = 0.034$, $\eta_p^2 = 0.043$. C.) The mean and SEM is plotted for side bias index across 14 days of training. Note that both PAE and SACC animals showed a modest side bias early in training with greater side bias scores by PAE rats after the 11th day of training. D.) The mean and SEM is plotted for the object bias index across the 14 days of training. Note that the object bias scores are higher for the PAE group on day 13 and 14 of testing.

A **B** **Figure 2.**

A.) Schematic illustration of the object-discrimination task. The red square object was always correct regardless of the location of the box at the end of the arm. Note that only one maze arm was used during training; thus, accurate performance was not conditional on spatial location. B.) Mean and SEM for the percentage of correct trials is plotted for the 8 training days. Note that PAE and SACC performed comparably at discriminating between the two objects.