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Moderate Prenatal Alcohol Exposure Impairs Visual-Spatial Discrimination in a Sex-Specific Manner: Effects of Testing Order and Difficulty on Learning Performance.

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Abstract

Background: Exposure to high levels of alcohol during development leads to alterations in neurogenesis and deficits in hippocampal-dependent learning. Evidence suggests that even more moderate alcohol consumption during pregnancy can have negative impacts on the cognitive function of offspring. Methods for assessing impairments differ greatly across species, complicating translation of preclinical findings into potential therapeutics. We have demonstrated the utility of a touchscreen operant measure for assessing hippocampal function in mice.

Methods: Here, we integrated a well-established ‘drinking-in-the-dark’ exposure model that produces reliable, but more moderate, levels of maternal intoxication with a trial unique, delayed nonmatching-to-location (TUNL) task to examine the effects of prenatal alcohol exposure (PAE) on hippocampal-sensitive behavior directly analogous to those used in clinical assessment. PAE and SAC offspring mice were trained to touch a single visual stimulus (“sample phase”) in one of 10 possible spatial locations (2×5 grid) in a touch-screen operant system. After a delay, animals were simultaneously presented with the original stimulus and a rewarded stimulus in a novel location (“choice phase”). PAE and saccharin (SAC) control mice were trained on a series of problems that systematically increased the difficulty by decreasing the separation between the sample and choice stimuli. Next, a separate cohort of PAE and SAC animals were given a brief training and then tested on a challenging variant where both the separation and delay varied with each trial.

Results: We found that PAE mice were generally able to perform at levels similar to SAC control mice at progressively more difficult separations. When tested on the most difficult unpredictable variant immediately, PAE showed a sex specific deficit with PAE females performing worse during long delays.

Conclusions: Taken together, these data demonstrate the utility of the TUNL task for examining PAE related alterations in hippocampal function and underline the need to examine sex-by-treatment interactions in these models.

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Keywords

prenatal alcohol exposure; visual-spatial discrimination; nonmatching-to-location; touchscreen

Introduction

While research indicates that no amount of alcohol is safe to drink during any point of pregnancy, reports indicate that nearly 10% of pregnant women continue to consume alcohol worldwide (Popova, Lange, Probst, Gmel, & Rehm, 2017). Exposure to alcohol during development, even at moderate levels, has been shown to disrupt spatial memory in both humans (Chiodo, Janisse, Delaney-Black, Sokol, & Hannigan, 2009; Dodge et al., 2020; Jirikowic, Olson, & Kartin, 2008) and rodents (Brady, Allan, & Caldwell, 2012a; Savage et al., 2010; Staples, Rosenberg, Allen, Porch, & Savage, 2013). The ability of animals to encode and then discriminate between distinct stimuli within an environment, which is heavily dependent on hippocampal functioning, is critically important for survival (Leutgeb & Leutgeb, 2007; MacDonald, Carrow, Place, & Eichenbaum, 2013; Sloan, Dobrossy, & Dunnett, 2006; White, 2004). Loss of hippocampal function has been linked to numerous neurodegenerative and neuropsychiatric disorders (Brady et al., 2012a; Mu & Gage, 2011; Small, Schobel, Buxton, Witter, & Barnes, 2011) and is a well-documented consequence of developmental alcohol exposure. The use of prenatal alcohol exposure (PAE) rodent models has become a widely used tool for studying these effects as studies in human patients have produced similar findings. In contrast to other developmental disorders that have complex genetic or epigenetic causes, the ability to reach blood alcohol content (BAC) congruent with those in humans means that behavioral alterations in PAE have clear determinants (Driscoll, Streissguth, & Riley, 1990). While studies have used a variety of timings, doses and testing approaches (for review see (Patten, Fontaine, & Christie, 2014) PAE models have provided strong insight on the impact of developmental alcohol exposure on visual spatial learning (Marquardt & Brigman, 2016). High levels of alcohol exposure across different exposure windows have reliably been shown to impair acquisition of spatial locations utilizing Morris Water Maze (MWM) paradigms (An & Zhang, 2013; Dursun, Jakubowska-Dogru, & Uzbay, 2006; Gianoulakis, 1990; Girard, Xing, Ward, & Wainwright, 2000; Goodlett & Johnson, 1997; Means, Russ, Medlin, & Gray, 1986; Wagner, Zhou, & Goodlett, 2014; Wozniak et al., 2004). While it is increasingly clear that even moderate exposures can have a long lasting impact on offspring, these approaches have had mixed results in terms of effects on spatial arena based learning in the MWM or Y-Maze task (Cullen, Burne, Lavidis, & Moritz, 2014; Hamilton et al., 2014; Sanchez Vega, Chong, & Burne, 2013; Zimmerberg, Sukel, & Stekler, 1991), which may be improved with early life supplementation (Thomas, Biane, O'Bryan, O'Neill, & Dominguez, 2007; Thomas, Idrus, Monk, & Dominguez, 2010; Waddell & Mooney, 2017). Additionally, moderate exposures in rats have been reported to produce both age-dependent effects in delay-dependent memory and alterations to stress sensitivity (Comeau, Lee, Anderson, & Weinberg, 2015; Nagahara & Handa, 1997).

In particular, studies using both limited-access drinking-in-the-dark and more traditional alcohol fed exposure models in rodents generally report no loss of spatial learning processes including rewarded Y maze learning and hidden platform location during MWM

performance (Allan, Goggin, & Caldwell, 2014; Hamilton et al., 2014; Riley, Lochry, Shapiro, & Baldwin, 1979; Wainwright et al., 1990). Regarding visual spatial discrimination specifically, moderate exposure has also been shown to spare the learning of distinct locations (4 arm separation) while impairing more similar locations (2 arm separation) on measures of delayed nonmatching-to-position (DNMTP) in the radial arm maze (Brady et al., 2012a). Interestingly, the contribution of hippocampal function to DNMTP tasks utilizing operant approaches (cued lever press) is not clear cut. While studies have shown that loss of hippocampal function impairs these behaviors (Aggleton, Keith, Rawlins, Hunt, & Sahgal, 1992; Dunnett, Wareham, & Torres, 1990; Hampson, Jarrard, & Deadwyler, 1999), others have shown that the hippocampus is not functionally necessary for successful performance (Sloan, Good, & Dunnett, 2006). While these tasks seem to have more translational potential, they are susceptible to mediating behaviors (positioning themselves on the side where the correct choice will appear) in animals (Chudasama & Muir, 1997; Herremans, Hijzen, Welborn, Olivier, & Slangen, 1996; Talpos, McTighe, Dias, Saksida, & Bussey, 2010) and are typically not sufficiently challenging for use with human subjects due to the manipulation of only one dimension (delay period presentations).

To address issues posed by traditional testing methods and increase translational utility, a touchscreen-based automated operant approach for both rats and mice has recently been established (Bussey et al., 2012; Oomen et al., 2013). These paradigms, called trial unique nonmatching-to-location (TUNL) tasks, have now been successfully adapted to assess spatial- and delay-dependent hippocampal memory in mouse models of hippocampal dysfunction (Josey & Brigman, 2015; Kenton, Castillo, Holmes, & Brigman, 2018; Kim et al., 2015; Oomen et al., 2013; Oomen et al., 2015). TUNL has been shown to be sensitive to loss of global dorsal hippocampal function, dentate gyrus-specific lesions and dorsal CA1 NMDAR subunit expression (Josey & Brigman, 2015; Kenton et al., 2018). However, to date the effects of prenatal alcohol exposure on these behaviors have not been explored.

Here, we tested the hypothesis that a moderate PAE mouse model, previously shown to impair DNMTP, but not Y-maze rewarded discrimination, would lead to deficits on TUNL performance as separation distances were decreased. We tested male and female PAE and SAC mice on an easy training variant of the task, and then systematically reduced the stimulus separation, while maintaining a constant 1 sec delay. Next, to examine training effects, we trained a second cohort on the easy training variant, and then tested them immediately on a challenging variant where both the separation and delay were variable.

Materials and Methods

All experimental procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

2.1 Drinking-in-the-Dark Model of Prenatal Alcohol Exposure.

Female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were used in a limited access PAE paradigm as described previously (Brady et al., 2012a; Brady et al., 2013; Marquardt, Sigdel, Caldwell, & Brigman, 2014). Starting 2 hours into their dark cycle, female mice

were given access to either 0.066% (w/v) saccharin or an EtOH solution (5% w/v for 4 days, then 10% w/v sweetened with 0.066% (w/v) saccharin for 4 hours per day (from 1000 to 1400 hrs; Figure 1). After 1 week of acclimation to the 10% EtOH or saccharin control solution, individual females were placed in a cage of a single male for 2 hours directly after the drinking period (~1400 hrs) for 5 days. Females continued to consume EtOH and saccharin solutions throughout the 5-day mating period. Pregnancy was determined by monitoring weight gain every 3 to 4 days. Access to EtOH was withdrawn beginning on postnatal day 0 using a step-down procedure over a 6-day period, during which time the EtOH concentration was reduced from 10% to 5% for 2 days, then to 2.5% for 2 days, and finally to 1% for the final 2 days. Offspring were weaned at ~23 days of age. The average EtOH consumption of the mothers of the offspring used in these studies was 7.17 ± 0.17 g EtOH/kg, in agreement with previous reports (Brady et al., 2012a). Although BACs were not evaluated in these animals, we have previously shown that this procedure produces BACs of 80–90 mg/dL at the end of the 4-hour drinking period and demonstrates no significant differences in pup development or maternal care (Brady et al., 2012a; Brady et al., 2013). Offspring from dams that were given either EtOH (PAE) or saccharin-sweetened water (SAC) were housed (2 per cage) in a temperature- and humidity-controlled vivarium under a reverse 12-hour light/dark cycle (lights off 0800 hrs).

Touchscreen apparatus

Behavioral testing was conducted in an operant chamber (21.6 × 17.8 × 12.7cm; model # ENV-307W, Med Associates, St. Albans, VT, USA) housed inside a sound- and light-attenuating box (Med Associates) as described (Brigman et al., 2013; Marquardt, Sigdel, & Brigman, 2017). A solid acrylic plate covered the grid floor to facilitate ambulation during testing. The chamber contained a house-light, tone generator, ultra-sensitive lever, a reward magazine and a dispenser for delivering 14 mg dustless pellets (#F05684, BioServ, Frenchtown, NJ, USA). Opposite the reward magazine was a touch-sensitive screen (Conclusive Solutions, Sawbridgeworth, UK). The screen was covered by a black acrylic plate allowing access to 2 rows of 5 touch areas (2.5 × 2.5 cm separated by 0.6 cm, located at a height of 1.6 cm from the floor). Presentation of stimuli and recording of mouse responses was controlled via the K-Limbic Software Package (Conclusive Solutions, Sawbridgeworth, U.K.).

Pretraining

To ensure motivation, all mice (6–7 weeks old) were first reduced and then maintained on a restricted diet to ensure 85% ad lib body weight. Mice were first acclimated to food reward in the home cage to prevent hyponeophagia. Pellets 10/mouse were provided in the home cage each day for 3 days. After reliably consuming pellets, mice were habituated to consuming reward in the operant chamber. Mice were allowed to freely explore the chamber with 10 pellets provided in the magazine. Mice were moved to a pre-training regimen if they consumed the 10 pellets within 30 min. In the first stage, mice were able to deliver a reward to the magazine via lever press of the ultra-sensitive lever adjacent to the magazine. Mice progressed to the next stage once they pressed and collected 30 rewards in less than 30 minutes. During touch training, a lever press now caused the appearance of a stimulus in one of the response apertures (white square 2.5 cm²; spatially pseudorandomized). Touches in

non-illuminated apertures had no response and the stimulus remained until a response occurred. Mice completing 30 trials (initiate, touch, reward collection) within 30 minutes moved on to the maximum separation, 1 second delay TUNL training task. All mice completed pretraining in approximately 14 days, and did not exhibit any group differences in pretraining performance.

Trial-unique nonmatching-to-location

After completing pre-training, PAE and SAC mice were tested on the touchscreen TUNL task as previously described (Josey & Brigman, 2015). During TUNL, a lever press initiated the onset of each trial phase. First, in the sample phase, the white square stimulus appeared in 1 of the 10 apertures until touched. Mice were encouraged to touch the sample by rewarding thirty-three percent of sample touches (Figure 2). Following a problem-specific inter-phase-interval (IPI) delay period, mice could initiate the choice phase via a second lever press. During the choice phase, both the original sample stimulus and a novel location were concurrently illuminated. A novel stimulus touch led to delivery of a food reward concomitant with illumination of the magazine light and a 1 sec pure-noise tone. Touching the sample phase stimulus led to a 15 sec house light-on timeout period before they could initiate a new trial. As in the sample phase, a touch in a non-illuminated window had no response. A correction procedure was utilized to aid TUNL training and extinguish position bias. Following an incorrect response during the choice phase, initiation led to an identical trial (sample and choice configurations) which repeated until a correct choice phase response was made. Following a correct choice phase response, there was a 5 sec inter-trial interval before the next trial could be initiated. Each daily testing session consisted of 60 first presentation trials or 2 hours. Throughout testing, all 10 response locations were utilized and touches at non-illuminated windows during any phase had no response.

One cohort of male and female controls PAE and SAC (n=8 per sex/trt, 1 mouse per sex per litter) were tested following our previous approach (Josey & Brigman, 2015). First, all mice were trained for 5 consecutive sessions with a maximum separation (3–4 spaces between stimuli) and a minimum IPI delay (1 second; Figure 2A). Mice were then tested for 5 consecutive sessions each on a decreased separation (2 spaces) followed by a minimum separation (1 space) and finally no separation (adjacent locations; Figure 2B). In order to test whether extensive training in PAE and SAC control mice improved performance, a second cohort of mice (n=8 per sex/trt, 1 mouse per litter) was first trained on the maximum (3–4 space) separation with a minimum (1 sec) delay for 5 consecutive sessions. Mice were then immediately tested for 5 consecutive sessions on the challenge problem that incorporated a variable IPI (1 sec/6 sec/12 sec) and variable separation (3–4 spaces/2 spaces/1 space; Figure 2C).

Statistical analysis

The following dependent measures were taken during each phase of TUNL testing: Accuracy (correct responses/first presentation trials attempted), Correction Trials, Sample Phase Stimulus Response (time from trial initiation to touchscreen response), Choice Phase Stimulus Response (time from trial initiation of choice phase to touchscreen response), and Reward Response (time from correct choice phase touch to reward retrieval). Main effects of

session, treatment, sex and delay/separation (during variable/variable testing) and interactions were compared for all measures using repeated-measures analysis of variance (ANOVA) followed by Fisher's PLSD post hoc tests.

Results

Systematic Reduction in Separation:

Analysis of training performance for cohort 1 on the easy training variant with maximum separation (3–4 spaces) and minimum delay (1 sec) found that all mice significantly improved across sessions (main effect of session: $F_{4,48}=26.16$; $p<0.01$ Figure 2A) but there was no significant effect of treatment ($F_{1,12}=0.26$; $p=0.61$) or sex ($F_{1,12}=0.29$; $p=0.59$) or interactions. Analysis of perseverative responding, as measured by correction trials (Session 1: PAE Male = 21.5 ± 4.44 ; SAC Male = 25.0 ± 4.01 ; PAE Female = 31.05 ± 11.9 ; SAC Female 31.8 ± 9.7 ; Session 5 PAE Male = 1.750 ± 8.5 ; SAC Male = 1.00 ± 0.8 ; PAE Female = 1.50 ± 0.6 ; SAC Female = 3.0 ± 0.7) similarly found that all mice significantly reduced perseveration as they learned the task demands (main effect of session: $F_{4,48}=28.94$; $p<0.01$), but there was no significant effect of treatment ($F_{1,28}=0.48$; $p=0.49$) or sex ($F_{1,28}=0.25$; $p=0.88$) and no interactions. Analysis of secondary response measures revealed no significant differences in average response latencies during the sample either by treatment or sex (main effect of trt: $F_{1,12}=0.82$; $p=0.37$; main effect of sex: $F_{1,12}=1.45$; $p=0.24$; no interactions; Figure 3A). Similarly, neither sex nor treatment significantly impacted response latencies during the choice phase (main effect of trt: $F_{1,12}=0.33$; $p=0.57$; main effect of sex: $F_{1,28}=0.05$; $p=0.82$). No significant differences between sex or treatment were seen in latency to retrieve reward after a correct response (main effect of trt: $F_{1,12}=0.02$; $p=0.90$; main effect of sex: $F_{1,12}=2.21$; $p=0.15$) with no interactions.

Analysis of medium (2 space) separation with a 1 sec. IPI revealed that while all animals improved performance over sessions, this did not reach significance (main effect of session: $F_{1,12}=2.51$; $p=0.05$; Figure 3B) and was not different between treatments or sex (main effect of trt: $F_{1,12}=0.85$; $p=0.38$; main effect of sex: $F_{1,12}=2.11$; $p=0.17$). During testing on the minimum (1 space) separation, all animals did not improve on performance (main effect of sessions: $F_{1,12}=1.72$; $p=0.16$, Figure 3C). Rather, there was a main effect of treatment ($F_{1,12}=5.44$; $p=0.04$) but not sex ($F_{1,12}=2.12$; $p=0.17$), with PAE mice initially having higher accuracies that declined across sessions while control improved, although the interaction was not significant (trt $\times$ session: $F_{4,48}=1.84$; $p=0.14$).

Accuracy on adjacent (0 space) separations did not significantly improve across training (main effect of session: $F_{4,48}=1.84$; $p=0.14$; 3D) and was not significantly affected by treatment ($F_{1,12}=0.777$; $p=0.40$). There was a non-significant reduction in performance by females (main effect of sex: $F_{1,12}=3.67$; $p=0.08$) with no interaction. For all separation problems there was no main effect of treatment, sex or interaction on latency to respond during the sample phase, choice phase, or to retrieve reward after a correct response.

Analysis of the average performance on each problem when separations were systematically reduced found that decreasing separation significantly reduced performance spaces (main effect of problem: $F_{4,48}=250.05$; $p=0.01$) that did not differ by sex or treatment (main effect

of trt: $F_{1,12}=0.18$; $p=0.67$; main effect of sex: $F_{1,12}=0.98$; $p=0.36$) with no interactions. Post hoc analysis found that there was a significant reduction in accuracy when separation was reduced from maximum (4–5 spaces) to medium (2 spaces) regardless of treatment or sex. In contrast, reducing separation from medium (2 spaces) to a minimum separation (1 space) with a 1 sec IPI, did not decrease performance. Similarly, decreasing from 2 spaces to 1 space, so that stimuli were adjacent (0 spaces), did not reduce performance further.

Variable Separation and Delay:

Performance on training with maximum separation (3–4 spaces) and minimum delay (1 sec) was similar to cohort 1, with a significant main effect of session ($F_{4,112}=2.50$; $p<0.01$) and no main effect of treatment ($F_{1,28}=1.74$; $p=0.20$) or sex ($F_{1,28}=0.002$; $p=0.96$) and no interaction (Figure 4A). Analysis of secondary response measures revealed no significant differences in average response latencies during either the sample either by treatment or sex (main effect of trt: $F_{1,28}=0.26$; $p=0.62$; main effect of sex: $F_{1,28}=3.08$; $p=0.09$; no interactions; Figure 4B). Similarly, neither sex nor treatment significantly impacted response latencies during the choice phase (main effect of trt: $F_{1,28}=0.61$; $p=0.44$; main effect of sex: $F_{1,28}=0.88$; $p=0.36$). No significant differences between sex in treatment were seen in latency to retrieve reward after a correct response (main effect of trt: $F_{1,28}=2.77$; $p=0.11$; main effect of sex: $F_{1,28}=1.89$; $p=0.18$) with no interactions.

When PAE and SAC mice were then immediately tested on the variable IPI and separation, analysis revealed a significant main effect of separation ($F_{2,168}=50.84$; $p<0.01$) but no main effect of delay ($F_{2,168}=0.32$; $p=0.73$) with no interaction ($F_{2,84}=0.70$; $p=0.51$). While there was no significant main effect of treatment ($F_{1,84}=0.09$; $p=0.76$) or sex ($F_{1,84}=0.04$; $p=0.84$) nor was there a sex $\times$ treatment $\times$ IPI interaction ($F_{1,84}=0.23$; $p=0.80$). However, there was a significant sex $\times$ treatment interaction ($F_{1,84}=4.615$; $p=0.03$, Figure 4C). Fisher's post-hoc tests revealed that female PAE mice had reduced accuracy at medium (2 spaces) separation at 1 sec IPI and at maximum (3–4 spaces) and medium (2 spaces) at a 12 sec IPI. Analysis of secondary response measures during the variable separation and delay problem revealed no significant differences in average response latencies (main effect of trt: $F_{1,28}=0.12$; $p=0.43$). Females, regardless of treatment, had a significantly slower sample reaction time (main effect of sex: $F_{1,28}=28.80$; $p<0.01$) with no interaction (Figure 4D). Similarly, treatment did not significantly impact response latencies during the choice phase (main effect of trt: $F_{1,28}=0.07$; $p=0.80$) while females were significantly slower to respond regardless of treatment (main effect of sex: $F_{1,28}=26.16$; $p<0.01$) with no interaction. There was a significant effect of treatment (main effect of trt: $F_{1,28}=5.34$; $p=0.02$), as well as sex (main effect of sex: $F_{1,28}=9.07$; $p<0.01$) and a significant interaction (trt $\times$ sex: $F_{1,28}=4.65$; $p=0.03$) whereby female PAE animals were significantly slower to retrieve rewards after correct responses versus other animals.

Discussion

Here, we found that a moderate 1st and 2nd trimester prenatal alcohol exposure model, previously shown to alter executive control, altered the ability to discriminate between visual-spatial stimuli on a mouse touchscreen TUNL task. In both of our studies, male and

female mice showed initial, non-significant impairments in performance of an easy training variant but demonstrated improved performance within 5 training sessions. Systematic testing that decreased separation in a fixed manner after 5 sessions showed that PAE had a significantly different pattern of responses only at minimum (1 space) separations, initially performing better than controls, but quickly falling off in performance as SAC improved. Surprisingly, on one of the hardest consistent problem, when novel stimuli were presented at a minimum separation (0 spaces) to sample stimuli, females PAE mice initially performed better than SAC females. Additionally, there was a non-significant reduction in accuracy of female animals at this separation. However, when tested on a variable separation and variable delay problem immediately after the easy training variant, a separate cohort of PAE mice showed a sex-specific deficit in performance, with female PAE, but not male, showing an impairment on minimum (1 space) separations during 1 sec IPI, and maximum (3–4 spaces) and minimum separations at long 12 sec IPI.

The effects of PAE on brain function and behavior are wide-ranging and convergent evidence from clinical and preclinical studies suggests that the hippocampus may be particularly sensitive to alcohol exposure during development (Berman & Hannigan, 2000). Clinical studies of confirmed exposure have consistently observed impairments in measures of hippocampal function including visual-spatial processing, spatial relations, and spatial motor behaviors regardless of diagnosis and across severity on the FASD spectrum (Mattson & Riley, 1998; Mattson, Riley, Gramling, Delis, & Jones, 1998; Meyer, 1998; Olson, Feldman, Streissguth, Sampson, & Bookstein, 1998; Uecker & Nadel, 1996, 1998). These results are consistent with studies examining adolescents with a range of FASD diagnoses on the Cambridge Neuropsychological Test Automated Battery (CANTAB) that found that even moderate exposures can impair spatial working memory even without the accompanying morphological changes seen in FAS (Green et al., 2009). While high dose exposure in rodent models has been shown to impair spatial learning and memory, models of more moderate PAE only impair these behaviors when the paradigms are altered to make them more stressful or challenging (Savage, Becher, de la Torre, & Sutherland, 2002; Westergren, Rydenhag, Bassen, Archer, & Conradi, 1996). Moderate exposures have been consistently shown to cause deficits in tasks that require pattern separation including contextual recall in delay fear conditioning, contextual discrimination, and maze-based delay nonmatch-to-place learning when conditions were sufficiently challenging (Allan, Chynoweth, Tyler, & Caldwell, 2003; Brady, Allan, & Caldwell, 2012b; Kajimoto, Allan, & Cunningham, 2013).

Visual-spatial discrimination behavior is thought to be mediated by a hippocampal network responsible for reducing overlap between input patterns from similar stimuli in order to avoid interference when memories are later retrieved. Current theories suggest that input from the medial and lateral entorhinal cortex (EC) is processed in the DG prior to establishing these distinct inputs. Recurrent networks in the CA3 then process the DG input in order to complete patterns from degraded stimuli or to distinguish distinct representations (Guzowski, Knierim, & Moser, 2004; Neunuebel & Knierim, 2014). Importantly, the CA3 is thought to mediate the fast acquisition and integration of novel sensory inputs directly via its ability to rapidly alter its firing rate to new environments and the presentation of new stimuli. Therefore, processing via rate encoding in of DG input in the CA3 supports the rapid integration of new spatial information that can be later interpreted in the CA1 in order

to remember and distinguish between distinct representations that have a high degree of similarity (Kesner, 2007; Kesner, Lee, & Gilbert, 2004; Leutgeb & Leutgeb, 2007). These processes are directly affected by alcohol during development with alterations in hippocampal circuit function, including changes in NMDAR subunit expression and loss of plasticity, reported even after moderate PAE (Brady et al., 2013; Sutherland, McDonald, & Savage, 1997; Varaschin, Akers, Rosenberg, Hamilton, & Savage, 2010).

The TUNL paradigm utilized in the current study was developed as a more translational analog of delayed non-matching-to-position (DNMTP) tasks that would also reduce the impact of mediating behaviors. While the current study did not directly measure hippocampal activity, TUNL paradigms require the generation of a distinct memory representation of a trial that is temporally and spatially similar to preceding trials (McClelland, 1995; Treves, 1994; Yassa & Stark, 2011). It is well established that the hippocampus plays an important role in these processes (Milner, Squire, & Kandel, 1998; Squire, Stark, & Clark, 2004), and we have shown that loss of dorsal hippocampal function, optimal neurogenesis, or NMDAR subunit expression is sufficient to impair accuracy on this paradigm (Carrica et al., 2019; Josey & Brigman, 2015; Kenton et al., 2018).

Surprisingly, in the current study, we found that our PAE model had only mild impacts on performance, as separations were systematically decreased in 5 session blocks. PAE mice only showed small, non-significant deficits with separations at adjacent (0 space) locations, primarily driven by decreases in PAE females. The pattern of response was also surprising as during both medium (2 spaces) and minimum (1 space) separations PAE mice initially showed superior accuracy, before quickly decreasing across sessions, even as SAC showed improvements. While this PAE model has previously been shown to be impaired on small separations on the DNMTP task, findings in mice with specific genetic knockouts are consistent with these results (Brady et al., 2012a). We previously showed that loss of GluN2B in the dorsal CA1 did not alter performance at minimal separations when they were tested as a long series of problems (Kenton et al., 2018). The current data suggest that, as in those animals, sufficient training across multiple problems is sufficient to overcome early training deficits in PAE mice. Additionally, PAE mice of both sexes are able to achieve levels above chance (~60%) although these improvements are not sustained across training. Indeed, the low performance demonstrated by both PAE and SAC mice may indicate a floor effect of the test, which may require further optimization of the systematic testing variant to determine the true difficulty of each task. Given the initial drops with decreased separation in performance to just above chance in both SAC and PAE mice tested systematically, future studies need to investigate whether problem order effects may lead to differences in performance in control mice, and whether these might lead to differences in PAE performance. Results in animals tested immediately on the variable separation and delay problem immediately after training support this hypothesis. These animals showed a robust sex-by-treatment interaction whereby female PAE animals had a specific impairment versus other animals.

Given the surprisingly small deficits seen after PAE on systemic testing, we tested a second cohort of animals on a challenging variable separation and variable delay problem after minimal training. In previous studies we found that mice, even with hippocampal lesions,

are insensitive to long delays in this task (Josey & Brigman, 2015). Importantly, increasing delay did impact performance when animals were tested on variable problems. Our current data show that when given variable challenges immediately after training, female PAE, but not male, were impaired on minimum (1 space) separations even with no delay and even maximum and minimum separations at long delays. These data are similar to previous findings from our laboratory utilizing a conditional knockout of the NDMA receptor subunit GluN2B in the dorsal CA1 of the hippocampus. As in the current study, those mice were able to perform even minimal separations as well as controls when given sufficient training via systematic testing, but were impaired when tested on the variable problem immediately (Kenton et al., 2019). Interestingly, when tested immediately on the challenging variant, all mice had slightly increased accuracy on medium (2 spaces) and minimum (1 space) separations than the cohort tested systematically. It is important to note that for systematic testing, the accuracy data are reported for each session, while in the challenging variant includes several different trial types per session (Fig 2B), and these data were therefore averaged across all sessions. This difference, combined with a smaller number of total trials of each type per session during variable testing may account for the improved performance seen at these smaller separations. In addition, in order to control for differences in total screen positions where stimuli can appear across separations, we have improved our protocol to only allow 20 trials per side and limit the total possibility of response options to match across sessions. Together, these data suggest that testing order and approach is an important variable in utilizing hippocampal-dependent touchscreen task. We hypothesize that training itself may act as an enrichment condition, and may stimulate neurogenesis during early, easier problems that then masks deficits on later challenging problems. Evidence that decreasing neurogenesis via the HIF-1 α mouse is sufficient to impair performance even at medium (2 space) separations lends further support to this interpretation (Carrica et al., 2018). Future studies are required to determine if systematic testing on the TUNL task is sufficient to stimulate increased neurogenesis in C57BL/6J mice, and how this is altered in PAE animals that have been shown to have differential responses to home-cage enrichment (Kajimoto et al., 2013).

We have previously seen sex-specific differences after PAE utilizing touchscreen approaches. We first showed that female mice had slower rates to acquire discrimination criterion versus males, although this was not different in PAE versus controls (Marquardt et al., 2014). More recently we found that while PAE spared attention on a touchscreen 5-choice Serial Reaction Time Task, PAE in general, and female PAE mice in particular, had significantly increased false alarm responses to non-target stimuli on the 5-Choice Continuous Performance Task (Olguin, Thompson, Young, & Brigman, 2020). Across these studies, female animals consistently had elevated latencies both to respond to stimuli, and to retrieve reward, although these measure did not always reach significance. Reports have shown that female rats work harder than males for appetitive rewards, such as sucrose (Sinclair, Hildebrandt, Culbert, Klump, & Sisk, 2017), however, in the current study the reward were non-sucrose based, which suggests females were slightly less motivated to work for unsweetened precision food rewards. Taken together, these data suggest that female animals may be less motivated by appetitive rewards in operant paradigms. Importantly, the current findings suggest that PAE may differentially effect hippocampal function in females

versus male animals. Additionally, while not directly measured in the current study, future work is necessary to examine the hippocampal activity of male PAE mice to determine if it is unaffected during task behavior, or if increased activation is required in the males to perform at the same levels as controls.

In conclusion, our current results show that moderate PAE impairs performance on a translational hippocampal-based visual-spatial discrimination task. Specifically, female PAE mice display decreased accuracy when trials are unpredictable, particularly when delay periods are long. Taken together, the data in this manuscript sets the stage for future work investigating the cellular and molecular mechanisms underlying deficits in translational hippocampal-dependent behavior.

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Fig 1.

PAE model A. Single-house female mice were acclimated to a sweetened alcohol solution (or sweetened control), bred, and given voluntary access to alcohol throughout gestation. Dams were weaned of solutions after parturition.

Fig. 2.

TUNL training, systematic and variable / variable testing. A. All mice were trained on a maximum separation, 1 sec delay (“easy”) variation of TUNL for 5 sessions. B. Mice on Systematic Testing were then tested on decreasing separations (medium, minimum, adjacent) with a consistent, 1 sec delay for 5 sessions each. C. Mice on Variable Separation, Variable Delay were given a challenging TUNL test with separation (maximum, medium, minimum, adjacent) and delay (1, 6, 12 sec) varied trial-by-trial.

Fig 3.

TUNL performance during systematic reduction in separation. A. Mice trained on maximum separation, 1 sec inter-phase delay show no treatment or sex related effects. B. When mice were moved to a harder (medium) separation, performance decreased, performance leveled out across treatment and sex. C. When stimuli were only one space apart (minimum separation), male mice showed no treatment differences, but female PAE mice were initially better than all other mice. Over time, female PAE mouse accuracy decreased while female SAC performance increased. D. Adjacent stimulus presentation initially impaired accuracy only in female PAE mice, but performance, although poor, normalized across treatments and sex. N= 8 per sex/trt. Data are average $\pm$ SEM.

Fig 4.

TUNL performance when separation and delay are unpredictable trial-by-trial. A. Mice across treatments and sex perform well on a maximum separation, 1 sec delay perform to the same degree. B. Mice show no differences in motor function or motivation during maximum separation, 1 sec delay training. C. When mice are immediately moved to a difficult TUNL variant wherein separations and inter-phase intervals are unpredictable, female PAE mice perform worse than SAC controls during the longest (12 sec) delay. D. Female mice of both treatments take longer to touch the screen during the both the sample and choice phases, while female PAE mice took the longest to collect rewards. N= 8 per sex/trt. Data are average $\pm$ SEM with individual data points (open circles). * = treatment $\times$ sex interaction $p < .05$. # = main effect of sex $p < .05$.