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Recognition memory is selectively impaired in adult rats exposed to binge-like doses of ethanol during early postnatal life

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ABSTRACT

Exposure to alcohol *in utero* can induce a variety of physical and mental impairments, collectively known as fetal alcohol spectrum disorders (FASD). This study explores the persistent cognitive consequences of ethanol administration in rat pups over postnatal days (PD) 4–9, modeling human third trimester consumption. Between PD65-70, ethanol-exposed (5E) and control rats were evaluated in two variants of recognition memory, the spontaneous novel object recognition (NOR) task, using 20 and 240 min sample-to-test delays, and the associative object-in-context (OIC) task, using a 20 min delay. No treatment group differences were observed in object exploration during the sample session for any task. In the 20 min NOR test session the 5E rats explored the novel object significantly less than controls, relative to the total time exploring both objects. Postnatal ethanol exposure is hypothesized to impede object memory consolidation in the perirhinal cortex of 5E rats, hindering their ability to discriminate between familiar and novel objects at short delays. The 5E rats performed as well or better than control rats in the 240 min NOR and the 20 min OIC tasks, indicating developmental ethanol exposure selectively impairs the retention and expression of recognition memories in young adult rats.

1. Introduction

Fetal alcohol spectrum disorders (FASD) represent a range of long-lasting somatic and cognitive impairments in children and adults exposed to alcohol in the womb (Mattson, Crocker, & Nguyen, 2011; Schaefer & Deere, 2011; Streissguth, 2007). Worldwide, FASD remains a major public health issue (Roozen et al., 2016) and one of the leading preventable causes of mental retardation (May et al., 2009; Senturias & Asamoah, 2014). Neuroimaging studies of the central nervous system (CNS) in individuals with FASD have shown decreases in white matter and overall brain volume (Archibald et al., 2001), including reductions in the hippocampus (HC) and prefrontal cortex (Bookstein, Sampson, Streissguth, & Connor, 2001; Spadoni, McGee, Fryer, & Riley, 2007). Binge drinking is particularly detrimental to fetal development (Maier & West, 2001) and, during the third trimester, induces persistent impairments in higher-order cognition (Brown et al., 1991; Korkman, Kettunen, & Autti-Ramo, 2003), including executive function, learning, and memory (Kodituwakku, Handmaker, Cutler, Weathersby, & Handmaker, 1995; Lee, Mattson, & Riley, 2004; Nanson & Hiscock, 1990).

Similar to humans, FASD model rodents demonstrate altered neurodevelopment following perinatal (pre- and/or postnatal) ethanol exposure (Driscoll, Streissguth, & Riley, 1990; Patten, Fontaine, & Christie, 2014). The extent of ethanol's neurotoxic effects is principally determined by the timing of exposure and the resulting peak blood alcohol concentration (BAC). Modeling FASD, our lab administers binge-like doses of ethanol to rat pups over postnatal days (PD) 4–9, a period comparable to the human third trimester (Bayer, Altman, Russo, & Zhang, 1993). The developing HC and medial prefrontal cortex (mPFC) are particularly vulnerable to postnatal ethanol, which induces cell death, aberrant cell migration and survival, and reductions in synaptic density and complexity (Gil-Mohapel, Boehme, Kainer, & Christie, 2010; Hamilton, Whitcher, & Klintsova, 2010; Livy, Miller, Maier, & West, 2003; Whitcher & Klintsova, 2008). Postnatal ethanol has also been dose-dependently linked to forebrain-dependent learning and memory deficits in juvenile and adult rodents, including spatial

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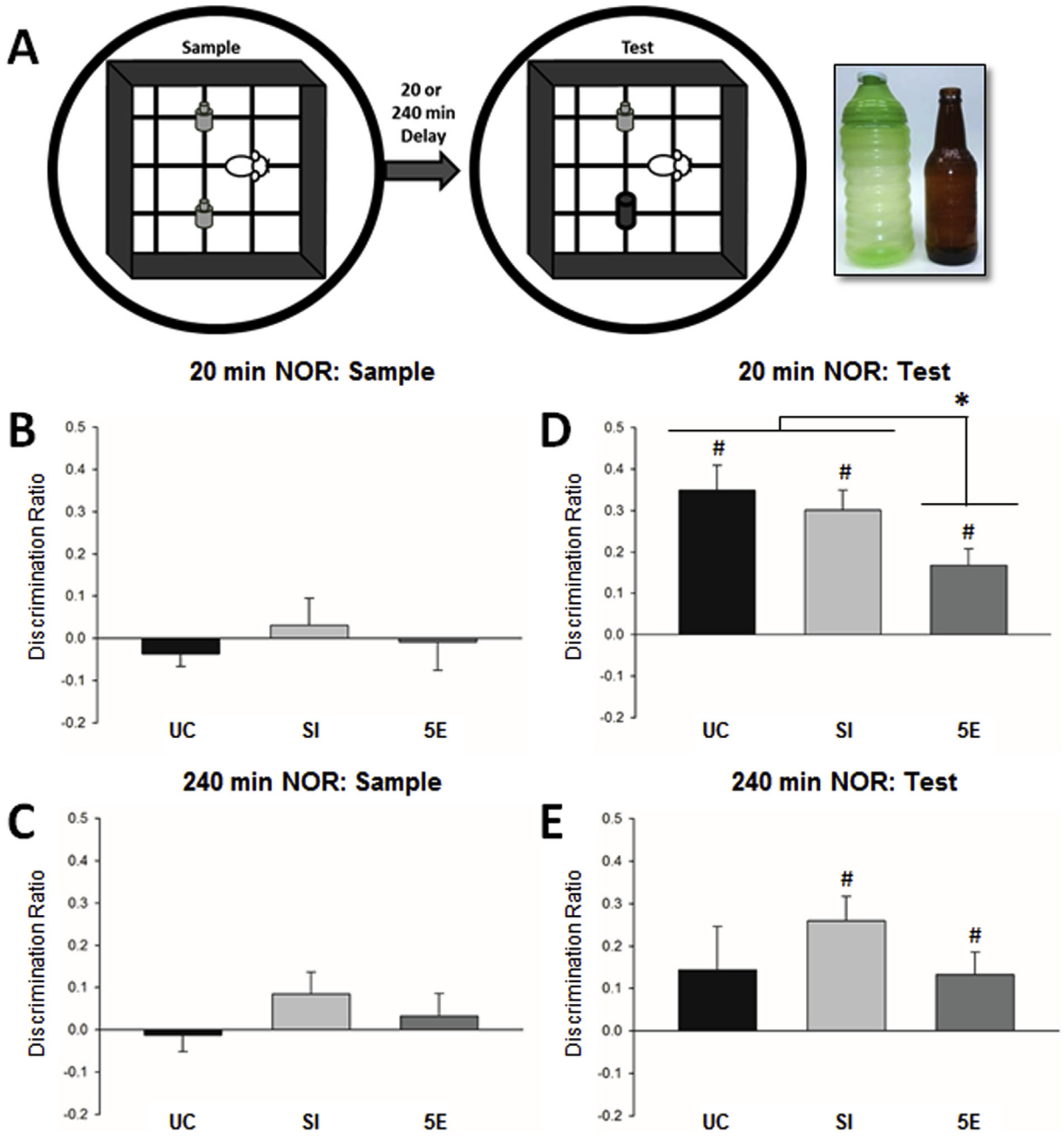

Fig. 1. Novel object recognition (NOR) memory task. (**A**) Subjects were exposed to context A, surrounded by a 180 cm high black curtain, containing one pair of identical objects (counter-balanced) during the sample session (5 min). Novel object recognition memory was tested 20 or 240 min later during the test session (3 min), with subjects exposed to one familiar and one novel object (counter-balanced across left-right position). The photograph shows the two objects used in the NOR and OIC (see Fig. 2) tasks. Discrimination ratios (mean ± SE) reflect the time exploring one object (as a proportion of total exploration). No treatment group differences were seen for the (**B**) 20 min or (**C**) 240 min NOR sample sessions. (**D**) Novel object exploration, with the 20 min delay, was significantly impaired in the 5E rats relative to UC and SI rats during the test session (asterisk). All treatment groups explored the novel object at rates greater than chance (pound sign). (**E**) Novel object exploration, with the 240 min delay, was not significantly affected by postnatal treatment during the test session. The SI and 5E, but not UC, treatment rats explored the novel object at rates greater than chance (pound sign).

learning (Goodlett & Peterson, 1995; Idrus, McGough, Riley, & Thomas, 2014), one-trial context fear conditioning (Goodfellow & Lindquist, 2014; Murawski & Stanton, 2010), and trace fear conditioning (DuPont, Coppola, Kaercher, & Lindquist, 2014; Goodfellow, Abdulla, & Lindquist, 2016; Hunt & Barnet, 2014).

In the FASD population, learning and memory deficits are selectively compromised rather than global in nature. For example, abnormal declarative (or explicit) memory but not procedural (or implicit) memory has been reported following prenatal alcohol exposure in both children and adults (Mattson et al., 2011; Olson, Feldman, Streissguth, Sampson, & Bookstein, 1998). One of the most widely studied forms of declarative memory is recognition memory (Manns, Hopkins, Reed, Kitchener, & Squire, 2003), which relies on a subject's sense of familiarity for previously encountered objects or environments and/or the recollection of specific details about the experience, such as what was encountered and where (Brown & Aggleton, 2001; Mandler, 1980). In rodents, attention and a preference for novelty leads to the incidental acquisition of

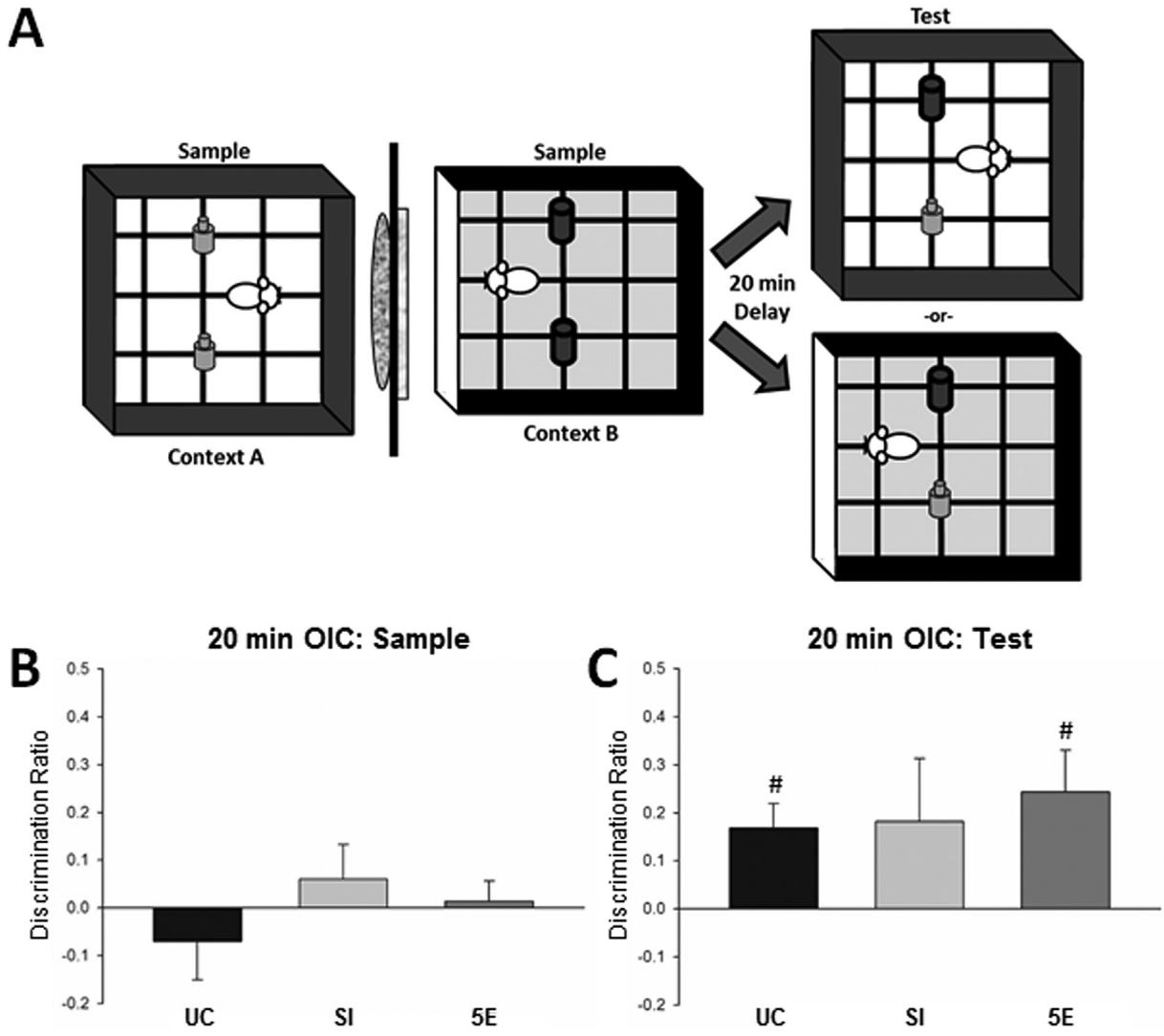

Fig. 2. Object-in-context (OIC) recognition memory task. (**A**) Subjects were exposed to contexts A and B (counter-balanced) containing distinct pairs of identical objects (counter-balanced) during each sample session (5 min), separated by 5 min. The room was divided in half by a 180 cm high black curtain with distinct pictures attached to each side. The testing session (3 min) commenced 20 min later. Subjects were again exposed to context A or B (counter-balanced) containing one matched-to-context object and one mismatched-to-context (counter-balanced across left-right position). Discrimination ratios (mean ± SE) reflect the time exploring one object (as a proportion of total exploration). (**B**) Results did not differ based on treatment group (collapsed across both contexts) during the sample session. (**C**) Mismatched object exploration did not significantly differ between treatment groups (collapsed across both contexts) during the test session. The UC and 5E, but not SI, treatment groups explored the mismatched object at rates greater than chance (pound sign).

recognition memories (Barker, Bird, Alexander, & Warburton, 2007), which obviates the need for extended training, external motivators, reward, or punishment (Ennaceur, 2010).

In the novel object recognition (NOR) task, subjects must spontaneously explore a pair of identical objects and, after a delay, distinguish between the now familiar object and a novel object (Ennaceur & Delacour, 1988). The "unconditioned preference" for the novel object is a behavioral indicator that a memory of the explored objects was acquired during the sample session and is accessible to retrieval at the time of testing (Ennaceur, 2010). Familiarity for previously encountered objects is principally mediated by the perirhinal (PR) cortex, a recipient of multi-modal input from all sensory cortices (Burwell, Witter, & Amaral, 1995; Kahn, Andrews-Hanna, Vincent, Snyder, & Buckner, 2008). PR damage produces significant deficits in object identity memory but not object location memory (Aggleton, Albasser, Aggleton, Poirier, & Pearce, 2010; Baxter & Murray, 2001; Bussey, Muir, & Aggleton, 1999), which relies, instead, on the HC (Barker & Warburton,

Table 1
Mean body weights (grams; mean ± SE) over development for each treatment group, collapsed across behavioral task. The 5E rats weighed significantly less than the UC and SI rats on PD6 to PD10 (*). The 5E rats also weighed significantly less than SI rats on PD15 (†). No significant treatment group differences were noted on PD21 or beyond.

Treatment	PD4	PDS	PD6*	PD7*	PD8*	PD9*	PD10*	PD15†	PD21	PD30	PD45	PD60
UC (n = 26)	9.0 ± 0.2	10.4 ± 0.2	12.1 ± 0.2	13.4 ± 0.2	15.3 ± 0.3	17.0 ± 0.3	18.6 ± 0.3	28.7 ± 0.6	44.6 ± 1.2	91.2 ± 1.4	181.0 ± 5.3	265.6 ± 10.7
SI (n = 27)	9.2 ± 0.3	10.7 ± 0.3	12.2 ± 0.3	13.8 ± 0.3	15.5 ± 0.3	17.1 ± 0.4	19.2 ± 0.5	30.0 ± 0.8	46.2 ± 1.4	92.3 ± 2.1	181.3 ± 5.8	263.8 ± 11.0
5E (n = 27)	9.2 ± 0.2	10.0 ± 0.2	11.1 ± 0.2	12.0 ± 0.3	13.4 ± 0.3	14.9 ± 0.3	16.5 ± 0.3	27.5 ± 0.6	44.1 ± 0.9	89.4 ± 1.7	182.6 ± 5.4	266.9 ± 10.4

2011; Brown & Banks, 2015). Relative to control subjects, visual recognition memory deficits have been reported following *in utero* alcohol exposure in humans and non-human primates (Burden et al., 2011; Clarren, Astley, Gunderson, & Spellman, 1992). Alternatively, rats exposed to ethanol during the postnatal period are unimpaired in NOR with a 5 min sample-to-test delay (Jablonski, Schreiber, Westbrook, Brennan, & Stanton, 2013), whereas prenatal exposure in mice induces significant NOR deficits with a 15 min delay (Summers, Henry, Rofe, & Coyle, 2008). In the experiments below, NOR test performance was assessed in ethanol-exposed and control rats 20 or 240 min after the sample (identical object exploration) session. The 20 min retention interval depends on short-term memory, whereas the 240 min interval necessitates an intermediate form of memory (Stough, Shobe, & Carew, 2006; Taglialatela, Hogan, Zhang, & Dineley, 2009) that relies on protein synthesis (Grimes et al., 2011). Long-term memory, at durations of ~24 h or longer, requires both protein synthesis and gene transcription (Alberini & Kandel, 2015; Amtul & Atta Ur, 2015).

To more fully investigate recognition memory capabilities following early-life ethanol exposure, a more challenging associative recognition memory task was also utilized using the same 20 min sample-to-test delay. The object-in-context (OIC) task requires the association of a pair identical objects and the environment (context) in which they were encountered (Dix & Aggleton, 1999; Spanswick & Dyck, 2012). Subjects are exposed to two distinct contexts, each containing a unique pair of identical objects. After a delay, subjects are placed into one context and presented with one matched-to-context object and one mismatched-to-context object. Preferential exploration of the mismatched object signifies intact associative (object/context) recognition memory. Lesion and pharmacological studies indicate the OIC task depends on a distributed neural circuit that includes, among other brain regions, the PR, HC and mPFC (Balderas et al., 2008; Langston & Wood, 2010; Martinez, Villar, Ballarini, & Viola, 2014; Norman & Eacott, 2005; Spanswick & Dyck, 2012).

The present study was designed to ascertain whether third trimester-equivalent ethanol administration affects the retention and expression of simple and/or associative recognition memory in adult rats. All three behavioral tasks (20 min NOR, 240 min NOR, and 20 min OIC) utilize the same simple materials and general procedures. The NOR task relies on the physical properties of explored objects and the subject's ability to encode and later discriminate between novel and familiar objects. These functions are not sufficient for the OIC task, in which the matched- and mismatched-to-context objects are equally familiar. Rather, subjects must encode and recollect specific object/context associative memories in order to detect the incongruent object at the time of testing. Test performance by FASD model rats is expected to inform our current understanding of the cognitive functions and underlying brain regions and neural circuits most vulnerable to postnatal ethanol administration.

2. Methods & materials

2.1. Breeding & housing

Long-Evans breeder rats were maintained on a 12 h light/dark cycle (lights on at 0600 h) in a temperature- and humidity-controlled, AAALAC-approved animal care facility at The Ohio State University, with *ad libitum* access to food and water. Breeding was initiated by pair-housing one male and one female rat for one week. Beginning 2 weeks later the female was checked each morning and evening for parturition. After birth, pups were housed with the dam through PD21, then same-sex housed with 2–3 littermates through PD60. Consistent with our prior studies (DuPont et al., 2014; Goodfellow et al., 2016), rats were singly-housed through the end of testing. All procedures were conducted in accordance with The Ohio State University's Institutional Animal Care and Use Committee (IACUC), and all necessary measures were taken to minimize pain and discomfort.

2.2. Postnatal treatment

On PD3, male and female rat pups from individual litters were paw-marked for identification and separated into 3 treatment groups: ethanol-exposed (5E), sham-intubated (SI) or unintubated control (UC). On PD4, the 5E pups experienced three intragastric intubations. The first consisted of 5 g/kg/day of ethanol in a milk solution (22.66% vol/vol), followed 2 and 4 h later by a milk-only solution in order to maintain subject body weight. From PD5-9, the last intubation was omitted, resulting in two intubations per day. The volume of ethanol-milk and milk-only solutions were calculated each day based on pup weight (0.0278 mL per gram). The SI pups underwent the same intubation procedure over PD4-9 but did not receive the ethanol-milk or milk solution. The UC rats were removed from the dam each day but were never intubated. All rats were weighed each morning across PD4-9, as well as PD10, 15, 21, 30, 45, and 60.

2.3. Blood alcohol concentration

Approximately 20 min before the second intubation on PD4, the tails of SI and 5E rats were clipped and blood collected (20 μL) in capillary tubes. Blood samples from SI rats were discarded, while the blood samples from 5E rats were transferred into micropipette tubes and centrifuged for 10 min at 8000 rpm. An Analox GL5 Analyzer (Analox Instruments, Lenenberg, MA) analyzed plasma samples and computed each subject's BAC, based on the oxidation of ethanol and the resulting rate of oxygen consumption from experimental samples, calibrated to known standards (100 and 300 mg/dl).

2.4. Experimental design

A total of 80 rats were used in the current study. The NOR task was performed in 56 rats based on the sample-to-test delay: 20 min (UC = 10, SI = 10, 5E = 10) or 240 min (UC = 9, SI = 9, 5E = 8). The 20 min OIC task was performed in 24 rats (UC = 8, SI = 8, 5E = 8). Behavioral training and testing was completed between PD65-70. All objects and contexts (i.e., open-field arenas) were wiped with 70% ethanol between each session to clean surfaces and eliminate possible odor cues. Behavior for the NOR and OIC tasks was recorded with a Panasonic HC-V720 high definition video camera, placed approximately 1 m above each open field arena.

For both tasks, object exploration was recorded during the sample session (5 min) and test session (3 min). Identical objects were presented during the sample session. The NOR test subjects were presented with one familiar object (a duplicate of the two identical objects used during the sample session, in order to control for odor or slight visual cues) and one novel object. The OIC test subjects were presented with one matched- and one mismatched-to-context object, both of which were duplicates of those used in the sample session. For all sessions, object exploration was defined as the rat having its nose $\leq$2 cm from the object; sitting or leaning on the object was not considered active exploration (Barker et al., 2007; Ennaceur & Delacour, 1988). The time spent exploring each object was rounded to the nearest second and recorded by blind raters. Two dependent measures were analyzed: total exploration time (for both objects) and the object discrimination ratio. The latter calculation [(object 1 − object 2)/(object 1 + object 2)] is

based on the methods of Ennaceur and Delacour (1988) and represents the proportion of time spent exploring one object relative to the total time spent exploring both objects. Chance performance is represented by a score of 0. Discrimination ratios were analyzed for treatment group differences in object exploration (sample session) and object memory or object/context memory (test session).

2.5. Novel object recognition task

The NOR sample and test sessions occurred in context A, an open field arena measuring 60 × 60 cm, with 40 cm high black translucent walls and a white floor with black gridlines spaced 15 cm apart (see Fig. 1A). Importantly, the availability of spatial and extra-maze sensory information during NOR is known to recruit the HC in addition to the PR (Barker & Warburton, 2011; Murray, Bussey, & Saksida, 2007). Context A was therefore encompassed 360° by a 180 cm high black curtain in order to block distal sensory information; object locations inside the arena were also held constant between sessions. Over 3 days, each subject was transported in its home cage and allowed to explore context A for 5 min, after which it was returned to the vivarium. During the sample and test sessions, objects were taped to the floor to ensure they could not be moved by the animal. As shown in Fig. 1A, the objects were a transparent green water bottle made of non-porous plastic (22 × 9 cm) and a semi-opaque, brown glass bottle (22 × 7 cm). The two objects were located 15 cm from the nearest arena wall and separated by 30 cm. For the sample session, each rat was placed near the center of the open field, facing away from the two identical objects (counter-balanced) and allowed to explore for 5 min. Rats were returned to the same context 20 or 240 min later. Using the same spatial locations within the arena, one familiar object and one novel object were presented, counter-balanced across the left-right position. The subject was again placed near the center of the open field, facing away from the objects and allowed to explore for 3 min.

2.6. Object-in-context recognition task

The OIC sample and test sessions occurred in context A, described above, and context B, measuring 56 × 60 cm, with 30 cm high white plastic walls and a gray floor with black gridlines. A thin (1/4″) black vinyl insert was wrapped around 3 walls (see Fig. 2A). Contexts A and B were housed in a single room with tables and equipment along three of four walls and partitioned by a 180 cm high 180 cm long black curtain. The two contexts were positioned in the center of their respective halves of the room. Large distinct pictures were hung from each side of the black curtain, adding to the unique extra-maze cues for each context. Over 2 days, each animal was exposed to context A and B (counter-balanced) for 10 min, separated by a 5 min interval. For the sample and test sessions, each rat was placed near the center of the open field, facing away from the objects, which were located 14 cm from the nearest wall and 28 cm apart. For the sample session, rats were sequentially exposed (in counter-balanced order) to contexts A and B for 5 min, separated by 5 min. Each context contained two green plastic water bottles or two brown glass bottles, as described above. Retention testing began 20 min later, with each rat again exposed to context A or B (3 min) containing one matched- and one mismatched-to-context object, counter-balanced for context order and across the left-right position.

2.7. Statistical analyses

BACs, body weights, and behavioral data were analyzed with single-sample t-tests and single-factor, multi-factor, or repeated measures analysis of variance (ANOVA) tests. Significant main effects or interactions were followed by Tukey's post hoc tests. A significant post hoc test implies $p < 0.05$. Notably, subject sex did not significantly affect the NOR or OIC results as an independent factor or in interaction with postnatal treatment—thus, the analyses below are based on treatment group differences only.

3. Results

3.1. Blood alcohol concentration

Blood alcohol concentrations (mean ± SE) were calculated for the 5E rats submitted to each of the three tasks: 20 min NOR (383.6 ± 14.1 mg/dl), 240 min NOR (370.0 ± 6.3 mg/dl), and 20 min OIC (391.2 ± 6.3 mg/dl). A single-factor (Task) ANOVA found no significant differences in BACs across behavioral tasks ($p = 0.37$).

3.2. Body weight

Body weights (mean ± SE) were calculated for each treatment group from PD4 to PD60 (see Table 1). Treatment group effects were initially examined across the intubation period (PD4-9) via a 3 (Treatment) x 6 (Day) repeated measures ANOVA. Results revealed a significant Treatment x Day interaction, $F(10, 385)$, 25.99, $p < 0.0001$. Single-factor (Treatment) ANOVAs performed on each postnatal day revealed significant main effects from PD6 to PD9. Post-hoc testing indicates the 5E rats weighed significantly less than the SI and UC rats. Treatment group effects were also analyzed on select days across development (PD10, 15, 21, 30, 45, and 60) though, due to substantial heterogeneity of variance, each day was analyzed separately via single-factor (Treatment) ANOVAs. Results indicate postnatal treatment significantly affected body weight on PD10 and PD15, with the 5E rats weighing significantly less than the SI and UC rats or SI rats only, respectively. No treatment group differences were found on or following PD21, indicating body weight was not a confounding variable in the NOR or OIC behavioral tasks.

3.3. Novel object recognition task

Total object exploration times during the NOR sample and test sessions are shown in Table 2. Single-factor (Treatment) ANOVAs revealed no significant differences for either session in rats trained with the 20 or 240 min delay (p-values shown in Table 2), signifying the 5E rats attended to and explored both objects as reliably as controls. Discrimination ratios for the sample session [(left object − right object)/(left object + right object)] and test session [(novel object − familiar object)/(novel object + familiar object)] were calculated for subjects in each treatment group. Single-sample t-tests were used to determine whether the discrimination ratio for any group exceeded chance performance. The sample session ratios (based on identical objects) did not significantly vary from 0 for any treatment group. Conversely, all treatment groups explored the novel object at rates significantly greater than chance with the 20 min delay (Fig. 1D): UC rats, $(9) = 5.82$, $p < 0.001$, SI rats, $t(9) = 6.28$, $p < 0.001$, and 5E rats, $t(9) = 4.25$, $p < 0.01$. For the 240 min delay, the SI rats, $t(8) = 4.50$, $p < 0.01$, and 5E rats, $t(8) = 2.35$, $p < 0.05$, also explored the novel object at rates significantly above chance (Fig. 1E). Thus, all treatment groups showed a significant preference for the novel object, with the exception of the 240 min UC rats ($p = 0.20$).

Next, differences in each group's discrimination ratio were analyzed based on single-factor (Treatment) ANOVAs. The ratios during the sample session (Fig. 1B–C) did not significantly differ

Table 2
Total object exploration times (sec; mean ± SE) in the novel object recognition (NOR) task as a function of treatment group and the sample-to-test delay. Times are based on exploration of two identical objects during the 5 min sample session and the familiar and novel objects during the 3 min test session. The p-values, based on single-factor (Treatment) ANOVAS, indicate all treatment groups explored the two objects for similar amounts of time during both sessions.

Treatment	Delay	Sample (s)	Test (s)
UC	20 min	70.5 ± 6.7	76.0 ± 5.3
SI		77.2 ± 6.7	88.5 ± 5.2
5E		70.9 ± 5.8	77.0 ± 11.7
		p = 0.80	*p = 0.48*
UC	240 min	83.3 ± 8.0	91.6 ± 8.6
SI		87.8 ± 6.3	89.2 ± 8.2
5E		70.9 ± 9.7	82.7 ± 8.1
		p = 0.33	*p = 0.73*

between treatment groups in the 20 min rats ($p = 0.69$) or 240 min rats ($p = 0.38$). A significant treatment group main effect was revealed for the 20 min NOR test (Fig. 1D), $F(2, 27) = 3.55$, $p < 0.05$. Post-hoc testing verified the 5E rats explored the novel object significantly less than the UC and SI rats ($p < 0.05$). No significant treatment group differences were seen for the 240 min NOR test ($p = 0.37$). Results indicate the 5E rats discriminated and explored the novel object at both delays, though the proportion of time exploring the novel object (relative to total exploration time) was significantly reduced in 5E rats during the 20 min test session only.

3.4. Object-in-context recognition task

Total object exploration times during the OIC sample and test sessions, in contexts A and B, are shown in Table 3. Single-factor (Treatment) ANOVAs based on each context found no significant treatment group differences during the sample or test sessions (*p*-values shown in Table 3), indicating all groups explored both objects at similar rates. Collapsing across treatment group, single-factor (Context) ANOVAs revealed no significant differences in total exploration times during the sample session ($p = 0.56$; rats exposed to contexts A and B) or the test session ($p = 0.60$; rats exposed to context A or B). Consequently, data from both contexts was averaged for the training discrimination ratio (same as above) and the test discrimination ratio [(mismatched object − matched object)/(mismatched object + matched object)]. Single-sample t-tests verified the sample session discrimination ratios (based on identical objects) did not significantly vary from 0 for any treatment group or context (Fig. 2B). For the test session, the UC rats, $t(7) = 3.31$, $p < 0.05$, and 5E rats, $t(7) = 2.78$, $p < 0.05$, but not SI rats ($p = 0.21$), explored the mismatched object at rates significantly greater than chance (Fig. 2C).

Next, differences in each group's discrimination ratio were analyzed based on single-factor (Treatment) ANOVAs. Results revealed no significant treatment group differences ($p = 0.39$) during the sample session (Fig. 2B). For testing, the actual sample-to-test delay varied based on which context (A or B) was presented first during the sample session. A new factor, context order (first or second) was added to the analysis. It had no significant effect alone or in interaction with postnatal treatment—thus, the test data was averaged across both contexts. Test session results revealed no significant treatment group differences ($p = 0.85$). Results indicate the 5E rats performed the associative OIC task comparably to controls, with all groups exploring the mismatched object at roughly equal rates (Fig. 2C).

4. Discussion

The main experimental finding of the current study is that recognition memory is selectively impaired, during the 20 min NOR test only (Fig. 1D), by third trimester-equivalent ethanol exposure, when assessed in young adult rats. Nevertheless, the significant discrimination ratio across all three tasks (20 min NOR, 240 min NOR, 20 min OIC) signifies object memories can be encoded and retrieved by 5E rats. The control rats also successfully discriminated the novel object with the 20 min delay but showed more variability than 5E rats in the remaining two tasks—i.e., the novel object discrimination ratio was significant in SI (but not UC) rats during the 240 min NOR test while the mismatched object discrimination ratio was significant in UC (but not SI) rats during the 20 min OIC test.

Following context habituation, each rat was allowed to explore two identical objects for 5 min in one context (NOR) or two (OIC). For the 5 min sample session, no treatment group differences were seen in any task for total object exploration time (Tables 2 and 3) or the proportion of time devoted to each individual object (Figs. 1B–C and 2B). After the appropriate delay, rats were then submitted to a 3 min retention test involving one familiar and one novel object (NOR) or one matched- and one mismatched-to-context object (OIC). Again, no treatment group differences were seen in any task for total object exploration time (Tables 2 and 3). Taken together, the attentional and motivational processes that drive exploratory behavior appear to be unaffected by postnatal ethanol exposure in adult 5E rats.

The ability to encode and store object identity information is highly dependent on the PR, which combines the component perceptual features of individual objects (Bartko, Winters, Cowell, Saksida, & Bussey, 2007; Warburton & Brown, 2015; Winters, Saksida, & Bussey, 2008). The resultant object identity memory is automatically retrieved when familiar objects are encountered, biasing exploration toward novel objects (Brown, Barker, Aggleton, & Warburton, 2012). Herein, novel object exploration with the 20 min delay was significantly reduced in 5E rats, relative to UC and SI rats (Fig. 1D). In two prior FASD rodent studies, NOR was unimpaired compared to controls with a 5 min delay in juvenile rats administered ethanol over PD7-9 (Jablonski et al., 2013), suggesting intact object memory encoding and retrieval, but impaired with a 15 min delay in adult mice exposed to ethanol on gestational day 8 (Summers et al., 2008). Combined with current results, the data suggests FASD model rodents can encode an object memory but

Table 3
Total object exploration times (sec; mean ± SE) in the object-in-context (OIC) task for contexts A and B as a function of treatment group. Times are based on exploration of two identical objects during the 5 min sample session (contexts A and B) and the matched- and mismatched-to-context objects during the 3 min test session (context A or B). The p-values, based on single-factor {Treatment) ANOVAS, indicate all treatment groups explored the two objects for similar amounts of time during both sessions in both contexts.

Treatment	Delay	Context A sample (s)	Context B sample (s)	Context A test (s)	Context B test (s)
UC	20 min	81.1 ± 6.9	71.6 ± 6.1	54.9 ± 8.5	58.5 ± 9.1
SI		62.5 ± 3.8	61.3 ± 4.8	47.5 ± 2.9	53.8 ± 3.4
5E		58.8 ± 3.7	61.3 ± 2.7	61.0 ± 7.7	59.4 ± 4.6
		p = 0.18	*p = 0.40*	*p = 0.41*	*p = 0.79*

may retrieve a weaker (i.e., less consolidated) memory at the start of the retention test, hindering their ability to discriminate and explore the novel object. Provisional support for this idea can be seen in the human literature—i.e., children whose mothers reported binge drinking during pregnancy performed a continuous recognition task comparably to controls, though event related potential (ERP) recordings showed reductions in the neurophysiological processes that underlie the consolidation and retrieval of visual recognition memories (Burden et al., 2011). While we cannot rule out retrieval deficits as a contributing factor to the significant 20 min NOR impairment in 5E rats, their novel/mismatched object discrimination ratios were significantly above chance for all three tasks (Figs. 1D–E and 2B), signifying object memories can be consistently retrieved. Thus, we hypothesize postnatal ethanol induces long-lasting PR dysfunction, such that the consolidation of short-term (20 min) object identify recognition memory is impeded or disrupted in adult 5E rats.

The HC plays a well-documented role in spontaneous NOR when the task incorporates spatial and/or contextual information (Chao, Huston, Nikolaus, & de Souza Silva, 2016; Warburton & Brown, 2010), which was the rationale for blocking distal extra-maze cues and holding the spatial relationship between each object and the arena walls constant (see Fig. 1A). The HC is also reportedly engaged in the consolidation of non-spatial object recognition memory (Cohen et al., 2013). Based on a review of 12 NOR studies that used permanent or temporary lesion techniques, Cohen and Stackman (2015) conclude the HC is necessary for object recognition memories with sample-to-test delays of 10 min or more. Consistent with this proposition, results from two prior drug studies—using muscimol to inactivate or anisomycin to block protein synthesis—suggest the HC is especially important for the consolidation of object recognition memory across intermediate retention intervals, up to 3–6 h following the sample session (Rossato et al., 2007; de Lima, Luft, Roesler, & Schroder, 2006). Hence, the PR can independently support NOR at short delays, while a second HC-dependent object memory is engaged in a delay-dependent manner as the retention interval is lengthened (Hammond, Tull, & Stackman, 2004). Considering the HC is required with sample-to-test delays of 10 min or more (Cohen & Stackman, 2015), the object memory formed by the HC could compensate for (putative) PR dysfunction in 5E rats submitted to the 20 or 240 min NOR test, though the latter intermediate-term (240 min) object memory is likely to benefit more from HC involvement. If correct, such an account could explain the counter-intuitive NOR results—i.e., the 5E rats were significantly impaired in the 20 min but not 240 min NOR test (Fig. 1D–E).

To our knowledge, this is the first study to examine associative recognition memory via the OIC paradigm in FASD model rodents. Subjects must form and recollect unique object/context associative memories in order to detect, at the time of testing, which object is incongruent with the surrounding context (Spanswick & Dyck, 2012). The 5E and UC rats explored the mismatched object at rates significantly greater than chance (Fig. 2C), suggesting the 5E rats are capable of forming and recollecting object/context memories, in spite of prior reports by our lab and others of significant deficits in a variant of context fear conditioning following postnatal ethanol exposure (e.g., Goodfellow & Lindquist, 2014; Murawski & Stanton, 2010). While the SI rats failed to explore the mismatched object beyond chance levels, the discrimination ratio did not significantly differ between the three treatment groups. Thus, the 5E rats were impaired in the spontaneous NOR task but not the more challenging OIC associative task using the same 20 min sample-to-test delay. By distributing task needs across the brain (Balderas et al., 2008; Langston & Wood, 2010; Martinez et al., 2014; Norman & Eacott, 2005; Spanswick & Dyck, 2012), the OIC paradigm might allow other neural regions or pathways to compensate for (putative) PR dysfunction in 5E rats, enabling efficient discrimination of the mismatched object.

In summary, recognition memory abilities in young adult rats were selectively impaired by binge-like postnatal ethanol exposure. The significant novel/mismatched object discrimination ratios indicate the 5E rats can successfully encode and retrieve an intermediate-term (240 min) object memory and a short-term (20 min) object/context associative memory. The same is true for the short-term (20 min) object memory, though the 5E rats' preference for the novel object was still significantly impaired relative to controls. We suggest the latter deficit is due to deficient consolidation of the object identity memory, yielding a weaker object memory on retrieval, which increases the difficulty of discriminating familiar from novel objects. While no studies, to our knowledge, have investigated the effects of early-life ethanol exposure on PR neurodevelopment, prior research in adolescent and adult rats indicates binge-like ethanol exposure does induce significant PR neurodegeneration and cell death (Crews, Braun, Hoplight, Switzer, & Knapp, 2000; Leasure & Nixon, 2010; Obernier, Bouldin, & Crews, 2002). Going forward, more research is required to delineate the precise consequences of third trimester-equivalent ethanol exposure on PR development, in relation to the encoding, consolidation, and retrieval of various types of object recognition memory at different ages over development.

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