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Short communication

Environmental enrichment reverses increased addiction risk caused by prenatal ethanol exposure

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ABSTRACT

Prenatal ethanol exposure (PE) leads to multiple cognitive and behavioral deficits including increased drug addiction risk. Previous studies have shown that rearing environment plays a significant role in addiction propensity. In the present study, we investigated if environmental enrichment during development could be effective in lowering the PE-induced increase in addiction risk. To simulate heavy drinking during pregnancy in humans, pregnant Sprague-Dawley rats received ethanol (6 g/kg/day) or vehicle through intragastric gavage on gestation days 8-20. After weaning, the offspring were reared in either an enriched environment (EE) including neonatal handling and complex housing or an impoverished environment (IE) consisting of barren, single housing. Adult male offspring were then tested for locomotion, performance on the elevated plus maze, and amphetamine self-administration under a progressive ratio reinforcement schedule. Overall, EE rats, compared to IE rats, showed reduced locomotor activity in a novel environment and lower levels of anxiety, irrespective of prenatal treatments. Prenatal ethanol exposure increased amphetamine self-administration at both doses tested (0.02 and 0.05 mg/kg/infusion) and in each case EE, relative to IE, reversed this effect. These findings suggest that postnatal environmental complexity plays a determining role in addiction risk after PE.

1. Introduction

Prenatal ethanol exposure (PE) leads to a variety of physical, cognitive, and behavioral deficits, collectively referred to as fetal alcohol spectrum disorders (FASD). One of the deficits is increased risk of addiction to drugs of abuse (Alati et al., 2006; Barbier et al., 2009). Indeed, our previous work shows that PE leads to enhanced amphetamine conditioned place preference and self-administration in adult rats reared in the standard laboratory environment (Hausknecht et al., 2015; Hausknecht et al., 2017; Wang et al., 2017). These observations are associated with PE-induced changes in the function and plasticity of excitatory synapses onto dopaminergic (DA) neurons located in the ventral tegmental area, the origin of the mesolimbic/cortical DA system (i.e., the brain reward pathway). These changes are considered critical cellular mechanisms of addiction (Choong and Shen, 2004b; Hausknecht et al., 2015; Hausknecht et al., 2017; Xu and Shen, 2001).

So far, there are limited treatment options for PE-induced deficits (Murawski et al., 2015). Environmental intervention has been used to reduce impairments caused by PE (Gursky and Klintsova, 2017). Studies show that addictive behavior is influenced by rearing environments. Enriched environment (EE) can reduce addiction risk. For example, neonatal handling/short-term maternal separation and complex housing after weaning are two widely used approaches to decrease/protect against addictive behaviors later in life (Bardo et al., 2001; Moffett et al., 2007; Schwarz et al., 2011; Solinas et al., 2010). Furthermore, greater protective effects have been observed by combining these two approaches (Escorihuela et al., 1994; Fernández-Teruel et al., 2002; Pham et al., 1999). We have used the combined method previously in our laboratory to study sensory habituation (Wang et al., 2018). In the present study, this combined method was utilized to investigate if EE (neonatal handling followed by complex housing) could reverse PE-induced increase in addiction risk.

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2. Materials and methods

2.1. Animals, prenatal ethanol exposure, and rearing conditions

The methods used for breeding, prenatal ethanol exposure, and rearing have been reported before in detail (Choong and Shen, 2004a; Wang et al., 2018). Briefly, during gestation days (GDs) 8–20, pregnant Sprague-Dawley rats (Envigo, Indianapolis, IN, USA) were gavaged intragastrically twice (6 h apart) every weekday, each with 3 g/kg ethanol (15% w/v) or vehicle (22.5% w/v sucrose water, isocaloric to ethanol). Single daily treatment with 4 g/kg solution was given on weekends. The PE treatment mimicked heavy prenatal ethanol exposure in humans (Eckardt et al., 1998; Shen et al., 1999). To equate caloric intake, controls were pair-fed with PE rats on GDs 8-20. Additionally, thiamine (8 mg/kg, i.m.; twice/week) was administered in both PE and control rats to avoid vitamin B1 deficiency caused by ethanol administration or pair-feeding. Our PE treatment causes no major stress (Hausknecht et al., 2015).

Cross-fostering was conducted along with culling on Postnatal day (PD) 1. The PE litters were fostered by extra dams that received no treatment and gave birth 2-days earlier; the control litters were cross-fostered among themselves (switching pups between 2-litters). Each litter was culled to 10 pups with ≤ 8 males. Pups from each litter were randomly assigned to either an enriched (EE) or an impoverished postnatal rearing environment (IE). We did not assign pups from the same litter into different rearing groups because the neonatal handling procedure involved the whole litter. Only males were used in the tests to limit the scope of the study.

The EE and IE rats were reared differently during early postnatal development and after weaning. Specifically, the EE rats received neonatal handling and were reared in complex housing post-weaning. Neonatal handling consisted of a short (15 min) maternal separation and handling procedure daily between PDs 2 and 20. This procedure has been reported to enhance maternal care (Pryce et al., 2001). Rats were weaned on PD 21 and were group housed (10–15/cage) in large four-story wire cages (64 × 92 × 160 cm, Model: CG-71111, Drs. Forrest and Smith, Rhinelander, WI, USA) with 30 pet toys (pots, hideouts, ropes, wheels, etc.; Drs. Forrest and Smith). The toys were relocated or changed daily to create novelty. Control and PE rats were housed in different cages; littermates were always housed in the same cages.

The IE pups were left with their dams undisturbed by experimenters except for weekly cage change before weaning. From PD 21 onward, they were singly housed in small, hanging wire cages (17 × 24 × 20 cm) facing a wall and kept undisturbed. More details regarding the IE and EE conditions were described in Wang et al., 2018. Behavioral tests started when the rats were 8-weeks old, with ≤ 3 rats/litter used in each test.

2.2. Locomotor test and elevated plus maze (EPM) test

Locomotor activity was assessed in a novel environment as previously described (Gancarz et al., 2011). Eighty-nine rats (control, IE: n = 26 from 11 litters; PE, IE: n = 21/11 litters; control, EE: n = 21/9litters; PE, EE: n = 21/10litters) participated in this test (60 min). To assess rats' anxiety levels, an EPM test was conducted in a subset of the rats (70 in total; control, IE: n = 15/10 litters; PE, IE: n = 14/10 litters; control, EE: n = 20/9litters; PE, EE: n = 21/10litters). They underwent the 5-min EPM test after being habituated to the testing room for 15 min.

2.3. Amphetamine self-administration

Amphetamine self-administration experiments were conducted after one week of operant pre-training and another week of recovery from the jugular vein catheterization surgery (Hausknecht et al., 2017). All rats were singly housed in standard plastic cages after surgery, to protect the catheters. As such, the post-weaning EE or IE rearing lasted for 6-weeks (until they were 9-weeks old).

Rats were trained to self-administer amphetamine (d-amphetamine hemisulfate, Sigma-Aldrich, St. Louis, MO, USA) at 0.02 mg/kg/infusion for 3-hours daily under a fixed ratio (FR) 1 and then FR2 schedule. One priming infusion was dispensed at the beginning of each FR session, followed by response-contingent amphetamine infusions. If rats self-administered 9 infusions/session for 2 FR1 and 1 FR2 sessions within 8 days, they then underwent two rounds of amphetamine self-administration experiments; first at 0.02 mg/kg/infusion for 6 daily sessions and, followed by 10 days of abstinence; followed by 0.05 mg/kg/infusion for 6 more sessions. Both of these experiments were conducted under a progressive ratio (PR) schedule of reinforcement, as determined by $5e^{(0.25 \times \text{infusion number})}$-5 (Richardson and Roberts, 1996; Vezina et al., 2002) with no priming infusions. Similar procedures have repeatedly been used, as described in earlier reports (Hausknecht et al., 2015; Hausknecht et al., 2017; Vezina et al., 2002; Wang et al., 2017). Thirty-five (control, IE: n = 8/5 litters; PE, IE: n = 10/6 litters; control, EE: n = 9/5 litters; PE, EE: n = 8/6 litters) out of 44 rats satisfied the FR criterion to continue to the PR sessions. These rats were thus included in the statistical analyses.

2.4. Data analysis

Locomotor activity was measured by number of photobeam breaks, and the 1-h testing session was divided into twelve 5-min epochs. In the EPM test, the dependent variable (DV) was the percentage of time rats spent on the open arms. In the self-administration test, a number of amphetamine infusions obtained and responding time were the two DVs. Responding time referred to the duration between session initiation and the time point at which the last lever press was made, which indicates motivation for drug seeking (Richardson and Roberts, 1996). Data outside of 2 standard deviations of the mean in each group were identified as outliers (2.7% in total) and brought down/up to the next highest/lowest level (Aguinis et al., 2013). Analysis of variance (ANOVA) was conducted, using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), with the significance level set at $\alpha = .05$. Planned comparisons were used for pairwise comparisons after ANOVA to examine control vs. PE in the same rearing condition (IE or EE) or IE vs. EE with the same prenatal treatment (control or PE).

3. Results

3.1. Prenatal ethanol exposure did not produce major teratogenic effects

The litter size was 14.21 ± .54 in controls and 14.43 ± .43 in PE rats. The pup body weight on PD 1 was 6.54 ± 0.05 g in controls and 6.57 ± 0.04 g in PE rats. There was no significant group difference in litter size or pup bodyweight, suggesting that PE did not produce major teratogenic effects.

3.2. Enriched rearing reduced locomotor responding to novelty

Prenatal ethanol exposure had no effects on locomotor activity. However, the EE rearing condition, relative to IE, decreased locomotion in both control and PE rats (rearing condition × 5-min epoch interaction effect, $F_{11,983} = 7.95$, $p < .001$; litter effect: $F_{37,983} = 5.52$, $p < .001$, 3-way mixed ANOVA with litter as a nested variable, Fig. 1A).

3.3. Enriched rearing produced anxiolytic effects

Prenatal ethanol exposure did not affect the time staying/traveling on the open arms. The EE rearing condition, compared with IE, increased open arm time, indicating reduced anxiety levels in both