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Environmental enrichment enhances conditioned place preference to ethanol via an oxytocinergic-dependent mechanism in male mice

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ABSTRACT

Environmental conditions, such as stress and environmental enrichment (EE), influence predisposition to alcohol use/abuse; however, the underlying mechanisms remain unknown. To assess the effect of environmental conditions on the initial rewarding effects of alcohol, we examined conditioned place-preference (CPP) to alcohol following exposure to EE in mice. Since social context is a major factor contributing to initial alcohol-drinking, we also assessed the impact of EE on the levels of the "social neuropeptide" oxytocin (OT) and its receptor, OTR. Finally, we assessed the effect of pharmacological manipulations of the oxytocinergic system on EE-induced alcohol CPP. While EE increased sociability and reduced anxiety-like behaviors, it caused a ~3.5-fold increase in alcohol reward compared to controls. EE triggered profound neuroadaptations of the oxytocinergic system; it increased hypothalamic OT levels and decreased OTR binding in the prefrontal cortex and olfactory nuclei of the brain. Repeated administration of the OT analogue carbetocin (6.4 mg/kg/day) mimicked the behavioral effects of EE on ethanol CPP and induced similar brain region-specific alterations of OTR binding as those observed following EE. Conversely, repeated administration of the OTR antagonist L,369−899 (5 mg/kg/day) during EE exposure, but not during the acquisition of alcohol CPP, reversed the pronounced EE-induced ethanol rewarding effect. These results demonstrate for the first time, a stimulatory effect of environmental enrichment exposure on alcohol reward via an oxytocinergic-dependent mechanism, which may predispose to alcohol abuse. This study offers a unique prospective on the neurobiological understanding of the initial stages of alcohol use/misuse driven by complex environmental-social interplay.

1. Introduction

Several factors contribute to the initiation of drug-taking, including environment, psychiatric conditions, stress and social factors. Chronic exposure to aversive stressors (foot shock, immobilization) enhances ethanol-induced conditioned-place preference (CPP) and alcohol consumption in rodents (Becker et al., 2011). In contrast, environmental enrichment (EE; model of "eustress") is known to protect stress-induced anxiety (Novaes et al., 2017) and prevent drug addiction progression (Solinas et al., 2010). Indeed, enriched environment prevents cocaine [(Solinas et al., 2008); but see (Green et al., 2010)], heroin (El Rawas et al., 2009) and morphine (Xu et al., 2007) CPP, diminishes amphetamine self-administration (Bardo et al., 2001) and abolishes sensitization to morphine (Xu et al., 2007) and nicotine (Green et al., 2003) in rodents. These effects might be associated with the ability of EE to prevent stress- and drug-induced hypothalamic-pituitary-adrenal (HPA) axis reactivity (Morley-Fletcher et al., 2003) and/or to modulate cognitive processes (van Praag et al., 2000).

Findings with respect to the effects of EE on alcohol-related behaviors are less clear. There is evidence showing that EE prevents alcohol-induced locomotor sensitization (Rueda et al., 2012), decreases alcohol self-administration in alcohol-preferring rats (Deehan et al., 2011), blunts alcohol consumption after stress (Marianno et al., 2017) and reduces ethanol CPP in spontaneously-

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hypertensive female rats (de Carvalho et al., 2010). Nevertheless, increased ethanol consumption has also been described in EE-raised rats (Rockman et al., 1989). Thus, the exact effects of EE on alcohol rewarding effects and the underlying mechanisms warrant further investigation.

Although the underlying neurobiology is not well understood, it is known that initiation of drug-taking is strongly influenced by several socio-environmental factors (York, 1999). Peer/social-drinking behavior has been linked with increased alcohol use among young adults, indicating social context as an important factor influencing alcohol-drinking behavior (Cruz et al., 2012; Osgood et al., 2013). In line with this, emerging evidence implicates the "social neuropeptide (Heinrichs and Domes, 2008)" oxytocin (OT), in the regulation of several addiction processes (Bahi, 2015; Georgiou et al., 2015a, 2015b, 2016a, 2016b; King et al., 2017; Zanos et al., 2014a, 2014b, 2015a). Whereas OT exerts protective effects in reducing addictive behaviors at later stages of the drug addiction cycle (i.e., chronic use, withdrawal, relapse; McGregor and Bowen, 2012; Zanos et al., 2017), this may not be the case during the initial drug exposure.

Conditioned-place preference is a model used to study the initial rewarding phase of the drug addiction cycle (Koob, 2009). It is worth noting that peripheral OT administration was reported to enhance the expression of morphine-induced CPP (Moaddab et al., 2015), but to reduce place preference for methamphetamine when it is administered directly into the nucleus accumbens core (Baracz et al., 2012). In addition, OT treatment not only increased nicotine intake, but it also alleviated nicotine aversion in rats (Lee et al., 2017), suggesting that elevated oxytocin levels may be a causative factor for the initial smoking behavior, which is also influenced by a variety of socio-environmental factors (Bellatorre et al., 2015; Greenlund et al., 1997).

Based on the aforementioned evidence, we hypothesized that exposure to enriched environmental conditions and/or repeated OT administration, which both exhibit pro-social effects, may augment alcohol reward. We thus examined the effects of EE and repeated carbetocin (OT analogue) administration on alcohol rewarding properties by assessing ethanol CPP in mice. To further shed light into possible interactions between enriched environmental conditions and alterations on the oxytocinergic system, we assessed the effects of EE on central levels of OT and its receptor (OTR) in the brain. Finally, to determine whether the impact of EE on ethanol CPP is mediated by an oxytocinergic mechanism, we assessed the effect of the selective OTR antagonist L,368−899 during EE exposure on alcohol CPP. Results from this study offer a unique prospective on the neurobiological understanding of the initial stages of alcohol use/misuse driven by a complex environmental-social interplay.

2. Materials and methods

2.1. Animals

126 male Swiss mice (PND: 70 at the beginning of the experiments; Biomedical Sciences Institute, University of São Paulo) were housed in groups of five/cage, with free access to food and water, in a temperature (21 ± 1 °C) and humidity-controlled environment, under a 12/12 h light/dark cycle (lights on: 07:00am). Procedures were approved by the Ethical Committee for Animal Use (CEUA) of the University of São Paulo, registered under protocol nº 132, page 110, book 02. All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

2.2. Drugs

Ethanol (20% v/v; Labsynth, Diadema, SP, Brazil) was diluted in 0.9% NaCl solution and administered intraperitoneally (i.p.) at a dose of 2 g/kg. Carbetocin (CBT; Sigma-Aldrich, St Louis, MO, USA) was diluted in 0.9% saline solution and administered i.p. at the dose of 6.4 mg/kg, based on previous studies (Georgiou et al., 2015b; Zanos et al., 2014a). CBT was chosen due to its greater stability and higher half-life time, compared to OT itself and due to the fact that it does not activate the vasopressin receptors (Passoni et al., 2016). L-368,899 (Tocris, Mississippi, USA), a blood-brain penetrant OTR antagonist was diluted in 0,9% saline and administered i.p. at the dose of 5 mg/kg based on previous studies (Lee et al., 2015).

2.3. Housing conditions

Animals were exposed to two different environmental conditions in this study (control and EE). Animals from the control (CT) group were housed in standard polypropylene cages (27.5 × 16.5 × 13 cm) throughout the experiment (see Supplementary Fig. 1).

2.3.1. Environmental enrichment (EE) protocol

EE mice were housed in large polycarbonate cages, (42 × 28 × 21.5 cm), where they were exposed to different stimuli, such as toys, tubes, ladders, houses and running wheels (objects were changed/moved three times a week), as previously described by Rueda et al. (2012). Depending on the experimental procedure, animals were maintained under enriched conditions for 31 days (see Supplementary Fig. 1 Ai, E) or 21 days (see Supplementary Fig. 1 Aii, B and C). EE is described as a combination of complex stimuli, including exposure to novelty, voluntary physical exercise and increased social interaction (van Praag et al., 2000). Previous attempts have been made to isolate these factors, including comparison between large spaces and exercise in the running wheel (Bernstein, 1973). Indeed, the variety of stimuli in a cage is more important than the large space in bigger cages to some behavioral responses (Whitaker et al., 2009). In our laboratory, we have observed that mice kept in EE cages did not show similar ethanol-induced behavioral sensitization as that seen in animals kept in large cages without the toys (data not shown). In Marianno et al. (2017), the control mice were kept in cages of the same size as the enriched mice and there were significant differences in ethanol intake between the two groups after stress.

2.4. Experimental procedures

The study was separated into 5 experiments with different cohorts of animals.

2.4.1. Experiment 1: behavioral characterization of mice exposed to EE

Mice undergoing EE or control housing conditions were introduced to their respective cage environment for a period of 31 days (Supplementary Fig. 1Ai). Throughout the duration of the study mice were exposed to a series of behavioural tests (Supplementary Fig. 1Ai).

2.4.1.1. Plasma corticosterone levels. In order to evaluate the effects of EE on the hypothalamic-pituitary-adrenal (HPA) axis activity, blood samples were collected from the caudal vein of mice in heparin tubes at the same time (9:00−11:00) on Day 1 and Day 21 of the experiment, to avoid circadian changes (Supplementary Fig. 1Ai). Blood samples were immediately spun at 2000 × g (4 °C)