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Environmental enrichment may protect against neural and behavioural damage caused by withdrawal from chronic alcohol intake

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ABSTRACT

Exposure to stress and prolonged exposure to alcohol leads to neuronal damages in several brain regions, being the medial prefrontal cortex (mPFC) one of the most affected. These changes presumably reduce the ability of the organism to cope with these stimuli and may underlie a series of maladaptive behaviours among which include drug addiction and withdrawal. Drug-addicted individuals show a pattern of behavior similar to patients with lesions of the mPFC. This impairment in the decision-making could be one of the mechanisms responsible for the transition from the casual to compulsive drug use. The environmental enrichment (EE) has a protective effect on the neural and cognitive impairments induced by psychoactive drugs, including ethyl alcohol. The present study aims to determine the influence of withdrawal from intermittent long-term alcohol exposure on alcohol preference, emotional reactivity and neural aspects of early isolated or grouped reared rats kept under standard or complex environments and the influence of social isolation on these measures, as well. Our results point out new insights on this matter showing that the EE can attenuate the adverse effects of withdrawal and social isolation on rat's behavior. This effect is probably due to its protective action on the mPFC integrity, including the cingulate area 1 (Cg1), and the prelimbic (PrL) and infralimbic cortex (IL), what could account for the absence of changes in the emotional reactivity in EE alcohol withdrawal rats. We argue that morphological changes at these cortical levels can afford the emotional, cognitive and behavioural dysregulations verified following withdrawal from chronic alcohol intake.

1. Introduction

In humans, adverse life experiences are environmental factors implicated in the risk of alcohol use and abuse (Prescott and Kendler, 1999; Averna and Hesselbrock, 2001). In order to clarify the questions behind this phenomenon, laboratory animals have been used to model the influence of life adversities on the development of alcohol drinking behavior. For instance, the well-recognised influence of stress as a facilitator of drug self-administration (Dawson et al., 2005) was investigated in laboratory animals previously exposed to tail pinch (Piazza et al., 1990), aggression (Haney et al., 1995), and foot-shocks (Shaham and Stewart, 1994; Will et al., 1998). In addition, another valid tool to accomplish this task is the separation of animals from their peers during the early stages of development (Croft et al., 2005; Funk et al., 2005; Kosten et al., 2000; Toth and Neumann, 2013). In fact, early social isolation shares the ability of a chronic and unconditioned stressor that results in long-term behavioural changes, among them more sensitivity to the reinforcing effects of alcohol (Nunes Mamede Rosa et al., 2005; Spear, 2015; Butler et al., 2016).

Several studies have demonstrated that exposure to chronic stress alters the neuronal morphology and physiology of the prefrontal cortex (Musazzi et al., 2015; Radley et al., 2015, 2006a,b), an effect that was shown to be partially counteracted by environmental enrichment (Hellemans et al., 2004). Changes at this brain

Abbreviations: BAL, blood alcohol levels; BW, body weight; Cg1, Cingulate area 1; EE, enriched environment; EZ, elevated zero maze; GP, grouped; IL, infralimbic cortex; ISO, isolated; M2, secondary motor cortex; mPFC, medial prefrontal cortex; PrL, prelimbic cortex; SD, standard environment.

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level are supposed to play a role in the emergence of a series of maladaptive behaviours including excessive food intake, and drug dependence and withdrawal (Li and Sinha, 2008). Environmental enrichment has been shown to inhibit or reduce neuronal damage in many experimental models of neurological disease, including Huntington's chorea (van Dellen et al., 2000), Parkinson's disease (Faherty et al., 2005), Alzheimer's (Arendash et al., 2004), amyotrophic lateral sclerosis (Pang et al., 2006) and epilepsy (Young et al., 1999). Importantly, laboratory rats exposed to complex environments during the initial phase of development show significant increases in the dendritic arbors of medium spiny neurons of the mPFC and the nucleus accumbens (Kolb et al., 2003), brain regions mainly implicated in the efficacy of the reinforcing effects of drugs (Perry et al., 2011), and in cognition and the control of stress (Radley et al., 2006a).

Alcohol intake promotes a clear anxiolytic-like effect in rats previously submitted to social isolation (Pohorecky, 2008). Withdrawal from alcohol and other drugs of abuse, in turn, has the potential to elicit a myriad of symptoms, among them increased (rebound) anxiety (Breese et al., 2005). In this context, exposure to environmental enrichment has also been shown to reduce the vulnerability to drug use and abuse (Stairs and Bardo, 2009), as revealed by decreases in the reinforcing effects of alcohol (de Carvalho et al., 2010) and of analgesic opiate morphine (Xu et al., 2007). High levels of anxiety are also strictly correlated with the reinforcing effects of these drugs and the environment the individual experiences during his development seems to influence this vulnerability in a positive manner (Henniger et al., 2002; Will et al., 1998).

Taking the information above into account, the present study sought to determine the impact of environmental complexity on the levels of alcohol intake, following withdrawal from long-term intermittent alcohol exposure in rats. To evaluate the influence of environmental enrichment over the detrimental effects of chronic stress and the effects of chronic stress on withdrawal-induced anxiety, another group of rats will be isolated from their peers at weaning and maintained in this condition during the experiments. Blood alcohol levels (BAL) analysis will be performed to determine the levels of alcohol intoxication in rats randomly chosen from each of the experimental groups. Moreover, we try to ascertain the influence of the treatments on the cortical development taking into account the cortical size of the mPFC subareas (secondary motor cortex (M2), cingulate cortex area 1 (Cg1), prelimbic (PrL) and infralimbic (IL) cortices). Changes in emotionality induced by treatments will be assessed using the elevated zero-maze (EZM) apparatus. Based on the previous assumptions, we hypothesised that rats exposed to environmental enrichment, whether grouped or isolated, will show a small preference for alcohol following deprivation, mild emotional disturbances following withdrawal, and reduced neural changes (weakening of cortical development of the mPFC subareas), associated with chronic alcohol intake.

2. Materials and methods

2.1. Subjects and housing conditions

In this study, we used 96 new-borns male Wistar rats from the campus of Ribeirão Preto, University of São Paulo. The (weaned) animals were three weeks old, weighing 50 ± 10 g, at the beginning of the experiments. They were provided with food and water ad libitum and maintained in a colony in a temperature-controlled ($24 \pm 1^{\circ}$C) room, under a 12:12 h light–dark cycle (lights on at 7a.m.).

2.2. Ethical statement

We declare that the present study received formal approval (process 08.1.1547.53.3) from the Committee on Animal Research and Ethics (CEUA) of the University of São Paulo. Moreover, the experiments were conducted in compliance with the recommendations of the US National Institute of Health: Guide for the Care and Use of Laboratory Animals (NIH Publications, 8th Edition, 2010). The number of animals used was the minimum required to allow for the reliability of the results. In keeping with accepted practice, every effort was made to minimise animal stress and suffering.

2.3. Experimental apparatus and design for operant training

Water or alcohol oral intake training was conducted in two rat operant chambers. A liquid receptacle was located in the middle of the rear wall, 5 cm above the grid floor with two stainless steel response levers located on each side, 10 cm apart (S1 and S2). Two 200 ml pipettes located behind the wall, outside the chamber, and linked to each one of the two levers, allowed the experimenter to control the total volume of solution delivered. Five 2 h daily operant training sessions took place, with a 24-h interval between each session, during Mondays to Fridays where the animals were allowed to drink a solution of sweetened tap water as much as they wished (saccharin 0.2%, Synth, São Paulo, Brazil). These sessions took place during the week before the beginning of the treatments. The operant training used here has been described, with minor changes, previously (Ezequiel Leite and Nobre, 2012). Briefly, it consisted of the animals learning to press one of the two levers (S1 or S2), randomly active during the trials, to receive reinforcement. The fluid was supplied by a dipper fluid system in a volume of 50 μl after each lever press (5 s of delay until the lever became available again). A grille allowed unconsumed liquid to drain away; this was later subtracted from the total volume of the solution offered, in order to obtain the actual consumption. Once the animals had been trained in the operant response procedure, the second phase of the experiment, involving 70 days of the intermittent voluntary intake of water or alcohol solutions began.

2.4. Environmental enrichment

The animals were housed for 70 days, grouped (GP – 4 animals by cage) or isolated (ISO), in standard (SD – standard impoverished environment, $45 \times 35 \times 15$ cm), or large enriched Plexiglas-walled cages (EE – enriched environment, $65 \times 95 \times 50$ cm). SD boxes were provided with wood chipping bedding and food ad libitum. Enriched boxes were composed of a frame of stainless steel wire cast with two floors (30 cm height) connected by a stainless-steel ladder. Inside the boxes a running wheel, a rearrangeable set of PVC plastic tunnels and an acrylic maze were installed. A set of objects with different colours, sizes, shapes and textures were placed on the housing floor or at different heights. In addition, olfactory stimuli were offered one at a time in a random order, when the boxes were being cleaned. The olfactory stimuli used were lavender, sandalwood, rosemary, patchouli, lime, green tea and eucalyptus, provided by placing four drops of one of the essences in a clay pot. Fluids were available to the animals in the housing cage 24 h a day along with the treatments, except during the deprivation and testing periods, as noted elsewhere. The cages were cleaned three times a week, on Mondays, Wednesdays and Thursdays, at 8 a.m. in the morning. At this time, fluids were measured and replaced and all the stimuli (including the olfactory cues) were rearranged in the box. The location of the running wheel inside the box was changed. The form and location of the acrylic maze and the PVC plastic tunnels were changed. The other stimuli were frequently exchanged for new ones, and also placed in different locations in