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Environmental enrichment reduces cocaine neurotoxicity during cocaine-conditioned place preference in male rats

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ABSTRACT

Environmental enrichment (EE) has a neuroprotective role and prevents the development of cocaine addiction behavior in rats. Studies showing the role of EE in cocaine toxicity are nonexistent. We hypothesized that rats exposed to EE are protected from cocaine-induced changes in the redox profile and DNA damage after undergoing conditioned place preference (CPP). Ten male Wistar rats were placed in EE cages equipped with toys, a ladder and tunnels, and ten were provided clean, standard laboratory housing (non-EE). EE and non-EE rats were randomly allocated to the classical CPP cocaine vs. saline (COC/Saline) group, where cocaine (15 mg/kg; i.p.) was tested alternately with saline. Afterwards, intracellular reactive species and antioxidant enzymes were evaluated and the comet essay was performed in the prefrontal cortex and hippocampus of rats. As expected, EE rats spent less time in the cocaine-paired chamber, and as a new result, less cocaine-induced DNA damage was observed in the two brain structures. Altogether, our results demonstrate that EE decreases neurotoxicity in brain regions linked to cocaine addiction but does not extinguish it completely.

1. Introduction

It is known that individual factors such as biological and environmental characteristics can interact and exert positive or negative effects on drug abuse (Bozarth et al., 1989; Homberg et al., 2014). The environmental enrichment (EE) paradigm mimics some aspects of positive life experiences and has been used as a model for the study of positive stimuli in humans (Marmol et al., 2015). Evidence suggests that EE reduces the impact of psychiatric disorders (Nithianantharajah and Hannan, 2006) and facilitates recovery from brain injuries (Will et al., 2004). In the addiction context, the interaction between EE and drugs of abuse has received plenty of attention in recent years. Environmental enrichment facilitates abstinence in heroin-seeking rats (Peck et al., 2015) and directly alters the value of rewards and increases resistance to the effects of psychostimulants such as cocaine (Solinas et al., 2008) and amphetamines (Bardo et al., 1995, 2001). In conditioned place preference (CPP) in particular, exposure to environmental enrichment decreases the development of conditioning to cocaine (Solinas et al., 2009; Chauvet et al., 2011; Zakharova et al., 2009), heroin (El Rawas et al., 2009), and morphine (Xu et al., 2007).

Cocaine reinforcing properties are mediated primarily by the enhancement of extracellular dopamine (DA) levels via inhibition of the DA transporter (DAT) in several brain regions linked to the mesolimbic dopamine reward system (Rocha et al., 1998). In turn, the intense DA metabolism increases the generation of reactive oxygen species (Dietrich et al., 2005; Pereira et al., 2015). Under normal conditions, the reactive oxygen radicals produced during cerebral metabolism are scavenged by the brain's endogenous antioxidative defense mechanisms (Chan, 2001; Wang and Michaelis, 2010). However, during repeated cocaine consumption, these endogenous antioxidative defenses are likely to be perturbed, leading to persistent oxidative stress. The accumulation of oxidized proteins can lead to the loss of protein function, interference with the cell cycle, abnormal protein turnover, an imbalance in cellular redox potential, and eventually, cell death (Kannan and Jain, 2000; Briones et al., 2011). Compelling evidence exists that EE influences enhanced neuronal growth, restructuring and recovery following central nervous system injury (Briones et al., 2000; Saucier et al., 2010; Will et al., 2004; van Praag et al., 2000). However, little is

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known about whether EE housing has protective effects against cocaine-induced oxidative DNA damage. The aim of this study was to evaluate the effect of environmentally enriched rearing on rats exposed to the cocaine-CPP paradigm during adulthood through the evaluation of cocaine conditioning, ROS formation, antioxidant enzyme activity and DNA damage in the prefrontal cortex and hippocampus of these rats.

2. Methods

The protocols used in the present experiments were in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee of the UFCSPA (Ethics Committee #224/13).

2.1. Subjects

Twenty male Wistar rats were randomly assigned to a standard (non-EE) or environmentally enriched (EE) group on postnatal day (PND) 21 (weaning) and kept in these housing conditions until PND 50. They were group-housed in accordance with the vivarium guidelines under a 12 h light/dark cycle, with food and water available ad libitum. All experiments were conducted between 09:00 a.m. and 02:00 p.m. during the light phase of the cycle. All efforts were made to use only the number of animals necessary to produce reliable scientific data (Ethics Committee #224/13).

2.2. Housing conditions

The EE rats were housed 10 per cage in a large cage (70 × 60 × 80 cm) with 3 floors connected by a ladder, to force them to carry out physical activity to access the food and water provided on the third floor. Each cage had 6 toys of different shapes, a running wheel, a small plexiglass house and 2 cardboard tunnels. The objects were cleaned and rearranged 3 times per week. The non-EE rats were housed (2–3 per cage) in polycarbonate cages (40 × 33 × 18 cm) under standard conditions.

2.3. Solutions

Cocaine hydrochloride (COC) (Merck®, Germany) was dissolved in 250 mL sterile bags of 0.9% NaCl to a concentration of 15 mg/mL and stored at 4–8 °C. Before use in behavior tests, the solution was kept at room temperature (22 ± 2 °C).

2.4. Experimental procedures

2.4.1. Conditioned place preference

Six identical CPP equipment setups were used for all behavioral testing (Insight®, Brazil). The CPP equipment (40 × 60 × 38 cm) has three distinct chambers (unbiased apparatus): two larger conditioning chambers (40 × 23 × 38 cm) connected by a smaller neutral chamber (40 × 14 × 38 cm). The larger chambers were connected to the middle chamber by guillotine doors and had distinct visual (vertical or horizontal lines on the walls) and tactile (wire bars floor or wire mesh floor) characteristics. The middle chamber had gray walls and an aluminum plate on the floor. Photobeams located inside the boxes captured the horizontal movements of the animals, which were automatically monitored by software connected to the boxes (Pandolfo et al., 2009). Entrance into a chamber was defined as the animal having all four paws on the floor of the chamber. The apparatus was cleaned with ethanol solution (70%) and dried immediately to remove the odor of ethanol between trials. The experiments were conducted in a dimly lit room (red light).

The CPP protocol consisted of a schedule with 3 different phases: preconditioning (2 days), conditioning (8 days), and postconditioning (1 day). During preconditioning, rats were allowed to freely explore the three chambers for 15 min on the first day. Time spent in each of the three chambers was recorded on the second day to determine the least preferred side. A biased experimental design was used, in which cocaine was paired with the less preferred chamber in the preconditioning phase and saline was paired with the preferred side. A review of CPP procedures found that with the unbiased apparatus, significant place conditioning was obtained regardless of whether the drug was paired with the subject's initially preferred or nonpreferred side (biased or unbiased designs) (Tzschentke, 2007).

The second conditioning phase consisted of eight 30-min daily sessions. On uneven days, the animals were confined in the less preferred chamber, which had been identified in the preconditioning phase, immediately after receiving an intraperitoneal (i.p.) dose of cocaine (15 mg/kg); on even days, the animals received vehicle (saline 1 mL/kg; i.p.) and were confined in the opposite chamber. After the end of the sessions, the rats were placed back in their respective home cages (ST or EE). For the postconditioning phase (day 12), in a drug-free state, the animals were allowed an additional wash-out day and were individually placed in the neutral chamber. They had free access to all three chambers, and the time spent in each chamber was recorded for 15 min.

2.4.2. Sample collection and preparation

Immediately after the CPP paradigm, the animals were decapitated, and the prefrontal cortex (PFC) and hippocampus of each rat was dissected according to the Paxinos and Watson coordinates (Paxinos and Watson, 2007), quickly frozen in liquid nitrogen and stored at −80 °C.

2.4.3. Comet assay

The comet assay was performed for the evaluation of genotoxicity as described by Hartmann et al. (Hartmann et al., 2003). Briefly, the PFC and hippocampus were placed in separate microtubes with 400 μL of PBS cold solution with 20 mmol/L EDTA and 10% dimethylsulphoxide (DMSO) and were mixed with a vortex. The brain regions were allowed to settle and the supernatant containing single cells was collected. The isolated cells were counted in a Countess® (Invitrogen, by Life Technologies, Waltham, MA, USA) to determine cell concentration and survival by trypan blue exclusion assay. An aliquot of cell suspension (20 μL) was dissolved in 0.75% low-melting point agarose and immediately spread onto a glass microscope slide precoated with a layer of 1% normal melting point agarose. The slides were incubated in ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM, 1% Triton X-100 and 10% DMSO, pH 10.0). After lysis, the slides were washed three times for 24 h at 4 °C in enzyme buffer (40 mM Hepes, 100 mM KCl, 0.5 Mm Na2EDTA, 0.2 mg/mL BSA, pH 8.0), and incubated with FPG (30 min at 37 °C). The slides were then incubated with electrophoresis solution (300 mM NaOH and 1 mM EDTA, pH 13.0) for 20 min to unwind the DNA. Electrophoresis was conducted at 4 °C for 20 min at 0.94 V/cm. The slides were then neutralized with Tris buffer (0.4 M Tris, pH 7.5) and stained with silver. For the evaluation of DNA damage, 100 cells per slide were analyzed by optical microscopy. The cells were visually scored by measuring the DNA migration distance, and the amount of DNA in the tail was separated into five classes, from undamaged (0) to maximally damaged (4). The damage index (DI) value was calculated for each sample and ranged from 0 (no tail: 100 cells × 0) to 400 (with maximum migration: 100 cells × 4) (Burlinson et al., 2007).

2.4.4. Protein extraction

Preweighed tissues were homogenized in Tris-HCl buffer (pH 7.4) to determine brain protein levels for the measurement of dichlorofluorescein (DCFH-DA) and the activity of superoxide dismutase (SOD) and catalase (CAT). After 30 min, the tissues were subjected to 6 cycles of 30 s at ice cold temperature in the vortex mixer. Each solution was centrifuged for 10 min at 16,000 rpm to separate the tissue debris from the cell extracts. Protein concentration was determined by the Lowry