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BRAIN REGIONS ASSOCIATED WITH THE REVERSAL OF COCAINE CONDITIONED PLACE PREFERENCE BY ENVIRONMENTAL ENRICHMENT

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Abstract—In addition to the known preventive effects of environmental enrichment (EE) on drug addiction, we have recently shown that EE can also have "curative" effects and eliminate addiction-related behaviors in mice and rats. In the present study, using Fos immunohistochemistry, we investigated brain regions involved in the elimination of cocaine conditioned place preference (CPP) produced by a 30-day exposure to EE. A first group of mice was conditioned to cocaine in the CPP apparatus, a second group that served as control received cocaine in a cage different from the CPP apparatus and a third control group received only saline injections. At the end of conditioning, we kept mice abstinent in the animal facility, housing them in standard environments (SE) or EE for 30 days and then we tested them for expression of CPP. SE, but not EE mice, conditioned to cocaine showed long-lasting preferences for the cocaine-paired compartment. Expression of CPP was paralleled by significant increases in the expression of Fos in the anterior cingulate cortex, the lateral caudate putamen, the shell of the nucleus accumbens, the dentate gyrus of the hippocampus, the basolateral and central nuclei of amygdala, the bed nucleus of the stria terminalis, and the ventral tegmental area. In contrast, EE mice showed levels of expression of FOS similar to control groups. These results demonstrate that EE can eliminate context-induced cocaine seeking and that this effect appears associated with a general reduction in the activation of several brain regions implicated in relapse.

Key words: enriched environment, reward, relapse, immediate early genes, stress, protracted abstinence.

Drug addiction is a brain disorder characterized by compulsive drug seeking and a high vulnerability to relapse, even after periods of prolonged abstinence (Mendelson and Mello, 1996; O'Brien, 2008). Environments are known to play a major role in determining differential vulnerability to addiction (Kreek et al., 2005; Piazza and Le Moal, 1998; Solinas et al., 2010). Early exposure to negative or positive environmental conditions, respectively increases or decreases the risk to develop addiction (Laviola et al., 2008; Piazza and Le Moal, 1998). In particular, in mice, exposure to environmental enrichment (EE) decreases the development of conditioned place preference (CPP) to cocaine, to heroin, and morphine, but not to methamphetamine (El Rawas et al., 2009; Solinas et al., 2009; Thiriet et al., 2010; Xu et al., 2007). These changes in the behavioral effects of drugs are associated with several neuroadaptive changes in the brain and in particular in the striatum, that may result in reduced reactivity to drugs (Bezard et al., 2003; Solinas et al., 2009, 2010; Thiriet et al., 2008).

In addition to these preventive effects, we have recently demonstrated that environmental conditions during abstinence, after that addiction is established, dramatically influence the expression of addiction-related behaviors (Solinas et al., 2008). In fact, behavioral sensitization is decreased in mice housed for a 30-day period in EE and is increased in mice housed for the same period in social isolation compared to mice that remain in standard environments (SE) (Solinas et al., 2008). In addition, expression of cocaine CPP as well as cocaine-induced reinstatement of extinguished CPP, are abolished in EE mice (Solinas et al., 2008). Finally, others and these "curative" effects of EE have been extended to cocaine relapse in self-administration procedures in rats (Chauvet et al., 2009; Thiel et al., 2009) and to food reinforcement (Grimm et al., 2008).

Importantly, in mice, we found that reduction in reinstatement induced by cocaine priming is associated with reduction of brain reactivity, as measured by Fos expression, in several brain areas involved in drug-induced relapse such as the shell and the core of the nucleus accumbens (NAC), the ventral tegmental area (VTA), the basolateral amygdala (BLA), and the infralimbic cortex (IL) (Solinas et al., 2008). On the other hand, in most cases in humans, cocaine administration follows rather than precedes drug-seeking behaviors (Epstein et al., 2006, 2009). Therefore, to better understand how EE can reduce the risks of relapse, it was central to investigate how the brain reacts to drug-associated stimuli in the absence of direct pharmacological stimulation.

In this study, we used functional neuroanatomy to highlight brain regions that might be implicated in the effects of EE on cocaine-seeking behavior. For this, a group of mice housed in SE was conditioned to cocaine (10 mg/kg, i.p.), a second group received cocaine in a cage different from the CPP apparatus and a third group received only saline injections. After conditioning sessions, we housed half of mice from each group in SE and the other half in EE and

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Abbreviations: AC, anterior cingulate cortex; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; CPP, conditioned place preference; dCPU, dorsal caudate putamen; DG, dentate gyrus; DMSO, dimethyl suphoxide; EE, enriched environments; ERK, extracellular regulated kinase; IL, infralimbic cortex; NAC, nucleus accumbens; OF, orbitofrontal cortex; PBS, phospate buffered saline; PrL, prelimbic cortex; SE, standard environments; VP, ventral pallidum; VTA, ventral tegmental area.

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we kept them abstinent for 30 days in the animal facility before testing the expression of CPP. 90 min after tests for expression of CPP, we obtained brains and we used immunohistochemistry to monitor Fos levels in several brain regions involved in relapse.

EXPERIMENTAL PROCEDURES

Animals

Seventy-two adult male C57/BL6 mice (Elevage Janvier, France), 10–12 weeks old, experimentally naïve at the start of the study, were housed in a temperature- and humidity-controlled room and maintained on a 12 h light/dark cycle with the lights on from 7:00 AM–7:00 PM and had *ad libitum* access to food and water. All experimentation was conducted during the light period. Experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) for the care of laboratory animals.

Environmental housing conditions

After arrival and during the development of CPP (see following sections), all mice were housed in groups of four in SE (housing cages, 25×20×15 cm^3). After being subjected to experimental manipulations, half of the mice were kept in standard environments, whereas the other half was switched to EE, which consisted of larger (60×38×20 cm^3) cages containing constantly a running wheel and a small house and four to five toys that were changed once per week with new toys of different shapes and colours.

Behavioral procedures

CPP experiments were performed in four identical boxes (IMETRONIC, Pessac, France). These cages are equipped with infrared sensors that allow determining mice position as well as measuring locomotor activity. We used an unbiased CPP procedure that consisted of three phases: pre-conditioning, conditioning, and test. For each manipulation, mice were brought to the experimental room 60 min before the start of the experiment to allow for habituation and to reduce stress. Pre-conditioning was performed on day 1. Mice were placed in the central corridor of the CPP apparatus with the doors closed. After 15 s, the doors were opened and mice were free to explore the entire apparatus for 30 min. The time spent in each compartment was recorded and was considered as a measure of spontaneous preference. Three groups of mice were used for conditioning: (1) the conditioned group received cocaine in one compartment of the CPP apparatus, saline in the other compartment and saline in another cage and in a different experimental room (neutral compartment); (2) the unconditioned group received saline in the two compartments of the CPP apparatus and cocaine in the neutral compartment; (3) the saline group received saline both in the CPP apparatus and in the neutral compartment (see Fig. 1A). Conditioning lasted 4 days and consisted of three daily sessions of 30 min per day. Morning and afternoon sessions were performed in the CPP apparatus and midday sessions in small opaque Plexiglas cages (20×10×12 cm^3) placed in a separate room. At the end of the conditioning phase, mice were housed in either SE or EE and kept in the animal facility for a 30-day period of withdrawal as we have previously described (Solinas et al., 2008). After this period, mice were tested for expression of CPP (Fig. 1B for general procedure). During test sessions, animals were placed in the central corridor for 15 s and then left free to choose a compartment for 30 min. The time spent in each compartment was measured and compared to the time spent in the same compartment during the pre-conditioning session. Preference scores, which served as a measure of drug seeking behavior, were calculated by subtracting the time in seconds spent during the pre-test from the time spent during the test day in the compartment paired to cocaine injections.

Molecular procedure

Tissue preparation. 90 min after behavioral testing, mice were deeply anesthetized using Avertin (500 mg/kg, i.p.) and intracardially perfused with 0.9% NaCl, followed by 4% paraformaldehyde (PFA) dissolved in 0.1 M PBS, pH 7.4. Brains were then removed and post-fixed in 4% PFA for 1 h and stored in 30% sucrose/4% PFA at 4 °C until sectioning. All serial brain sections (40 μm) were then cut using a freezing microtome (Leica RM2145, www.leica-microsystems.com). Sections were stored in cryo-protective solution (glycerol 20%, DMSO 2%, NaCl 0.9%, PB 0.1 M) at −20 °C until processed for immunolabeling.

Immunohistochemistry. Free-floating sections from mice in different groups were processed simultaneously for Fos protein expression. Sections were washed extensively in 0.1 M PBS (three times, 10 min), and incubated for 30 min in 0.3% hydrogen peroxidase. Then, they were washed again in 0.1 M PBS (three times, 10 min) and incubated for 2 h in 0.3% Triton X-100 in 0.1 M PBS containing 3% Bovine Serum Albumin (BSA, Sigma/Aldrich; www.sigmaaldrich.com). Subsequently, sections were then incubated for 48 h at 4 °C with an anti-c-Fos rabbit polyclonal primary antibody (1:10,000, Sigma) containing 0.1 M PBS, 0.3% Triton X-100, and 3% BSA. Sections were then washed in 0.1 M PBS (three times for 10 min each) and incubated for 2 h in 0.1 M PBS containing biotinylated goat anti-rabbit antibody IgG (1:600, Vector Laboratories; www.vectorlabs.com), 0.3% Triton X-100, and 3% BSA. Afterwards, the tissue was given additional washes in 0.1 M PBS (three times, 10 min) and incubated for 2 h in avidin-biotinylated horseradish peroxidase complex (ABC Elite kit, Vector Laboratories) diluted in 0.1 M PBS. Then, sections were washed twice in 0.1 M PBS, followed by a wash in 0.05 M Tris–buffer, pH 7.6 and they were incubated in 0.025% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma/Aldrich) containing 0.08% nickel ammonium sulfate (Sigma/Aldrich) and 0.01% hydrogen peroxidase (Sigma/Aldrich) for 3–5 min. The reaction was terminated by rinsing the tissue in 0.1 M PBS (two times, 10 min) and then 0.1 M Tris–buffer (10 min). Finally, sections were mounted onto gelatin-coated slides, dried, and dehydrated before coverslipping.

Immunoreactivity analysis. Fos immunoreactivity was examined using an Olympus opticals microscope set at 40× magnification and counted manually by an observer blind to treatment. Sections taken at +1.98 mm from bregma contained the prelimbic (PrL), infralimbic (IL), and orbitofrontal (OF) cortex; sections taken at +1.18 mm from bregma contained the anterior cingulate cortex (AC), the shell of the nucleus accumbens (NAC shell) and the core of the nucleus accumbens (NAC core), and dorsal caudate-putamen (dCPU); sections taken at −0.14 from bregma contained the bed nucleus of the stria terminalis (BNST) and the ventral pallidum (VP); sections taken at −1.7 mm from bregma contained the BLA, central amygdala (CeA), the dentate gyrus (DG) of the hippocampus; sections taken at −3.08 mm from the bregma contained the VTA (Fig. 2). The number of immunoreactive cells in each region were counted by hand from each hemisphere of three sections labeled for Fos for a total of six samples per animal. The sections were chosen such that the rostral-caudal extent of each region was sampled (340 μm) (Solinas et al., 2008). The area of each sample measured was 0.125 mm^2 regardless of the total size of the region investigated. This approach has been previously used to measure Fos expression following exposure to drugs or drug-related stimuli (Solinas et al., 2008; Thiel et al., 2010) and it has been found to produce good estimates of the activation of the entire region that is similar to those found adjusting the area sampled to the size of the region (for