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Research report

NMDAR dependent intracellular responses associated with cocaine conditioned place preference behavior

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HIGHLIGHTS

- Cocaine-CPP is blocked by NMDAR antagonism during conditioning.
- NMDAR antagonism reduces NAc pERK and pCREB and CPu ΔFosB and SIRT2 levels.
- NAc pERK levels during cocaine-CPP expression are positively correlated to CPP scores.

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ABSTRACT

The aim of this study was to investigate the intracellular responses associated with the acquisition and expression of cocaine-context associations. ERK (extracellular regulated kinase), CREB (cAMP responsive element binding protein), FosB and ΔFosB proteins were of particular interest due to their involvement in cocaine reward and in synaptic plasticity underlying learning and memory. We used the conditioned place preference (CPP) paradigm, which employs a Pavlovian conditioning procedure to establish an association between a drug-paired environment and the drug's rewarding effects, to study the role of these signaling pathways in cocaine-context associations. *N*-methyl-D-aspartate receptor (NMDAR) antagonism prior to cocaine administration during conditioning blocked the acquisition of cocaine CPP and reduced Nucleus Accumbens (NAc) phosphorylated-ERK (pERK) and phosphorylated CREB (pCREB) levels following the CPP test (drug-free). We also show that cocaine-induced increases in Caudate Putamen (CPu) FosB and ΔFosB levels are decreased after MK-801 pre-treatment during conditioning. In addition, our results provide evidence for the involvement of striatal SIRT (Silent Information Regulator of Transcription) proteins in cocaine-CPP. These results will aid in the advancement of general knowledge about the molecular formation and retrieval of cocaine-associated memories that can be used in the future when designing treatments for cocaine addiction that target both prevention and relapse.

1. Introduction

Learned cocaine-environment associations play a major role in cocaine addiction and relapse. Cocaine increases synaptic concentrations of dopamine (DA) and glutamate, leading to the activation of molecular signaling cascades that cause functional changes in protein and gene expression and behavior [1–3]. The dorsal and ventral striatum, the caudate-putamen (CPu[1]) and nucleus accumbens (NAc) respectively, have been implicated in the regulation of habitual and reward-associated responses associated with addiction [4]. These brain regions undergo cocaine-induced neuroplastic changes in intracellular signaling similar to those underlying long-term memory processes [5–7]. For example, ERK, CREB and Fos proteins are signaling molecules that have been implicated in memory processes [5,7–12].

Activation of DA receptors (DARs) is required for the glutamatergic induction of LTP at synapses in the striatum [13]. The interaction between D_1 DAR and the NR1 subunit of the NMDA

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receptor (NMDAR) forms a complex at striatal synapses and serves to maintain and strengthen synaptic activity in response to changes in synaptic DA concentrations [14]. Downstream, ERK, a signaling molecule of the MAPK signal transduction family, is phosphorylated after acute cocaine administration [8,11,12,15,16]. The ERK pathway is an important regulator of phosphorylation of the transcription factor CREB and subsequent transcription [17] and much evidence supports a critical role of neuroadaptations produced by CREB and ERK signaling cascades in regulating synaptic plasticity through the alteration of gene activation [18–20]. Inhibition of ERK activation (pharmacologically or genetically) blocks cocaine CPP, indicating that the ERK pathway may be an essential requirement for the development of cocaine-associated memories [5,11,21–24].

Downstream of ERK and CREB signaling, SIRT1 and 2 (Silent Information Regulator of Transcription) are class III histone deacetylases (HDACs), recently implicated in the epigenetic changes underlying plasticity mechanisms including those associated with drug abuse [25]. Specifically, SIRTs are enzymes that play a role in the modification chromatin structure, which leads to long-term epigenetic changes in gene transcription and expression [26,27]. Recent evidence suggests that an increase in SIRT1/2 subtypes enhances the rewarding effects of cocaine [25] and mediates drug-induced neuroplasticity. NAc SIRT 1 and 2 protein levels increase following cocaine administration [28] and Resveratrol (a SIRT1 and 2 agonist) increases the rewarding effects of cocaine [26]. Local inhibition of SIRT1 and SIRT2 in the NAc decreases cocaine reward exemplified by attenuated CPP [28]. Inhibition of SIRT1 has been found to decrease ERK phosphorylation and resveratrol increases ERK phosphorylation suggesting that SIRTs may play a role in regulating ERK activity [29]. SIRT activity may also regulate CREB phosphorylation and ΔFosB overexpression after cocaine exposure [28,30,31].

Given the extensive role of NMDARs in memory formation and cocaine CPP [32–35] we aimed to investigate changes in NMDAR-dependent intracellular signaling cascades associated with cocaine-context associations. Specifically, we used the non-competitive NMDAR antagonist MK-801 to block the acquisition of a cocaine-environment association using a CPP model. We hypothesized that striatal ERK phosphorylation induced by cocaine is dependent on glutamate signaling through NMDARs and that MK-801 administration prior to cocaine administration would block CPP acquisition and subsequent ERK phosphorylation during the CPP expression test. We also expected to see similar NMDAR/ERK-dependent changes in pCREB, FosB, and SIRT1/2 protein levels.

2. Materials and methods

2.1. Animals

Eight-week-old male Fischer rats (Charles River, Kingston, NY, USA) were individually housed in standard cages and maintained on a 12 h light/dark cycle with free access to food and water. Behavioral testing took place during the light cycle. Rats were allowed to acclimate for 7 days before any experimental procedures began, and were handled once per day beginning 4 days prior to testing. Animal care and use were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD, USA) and approved by the Hunter College, CUNY, Institutional Animal Care and Use Committee.

2.2. Materials

2.2.1. Drugs and antibodies

Cocaine hydrochloride and MK-801 were purchased from Sigma Chemical Co. (St. Louis, MO). Primary antibodies for pERK (9101), ERK (9102), SIRT1 (2493), SIRT2 (12672), FosB (5G4) and CREB (9197) were purchased from Cell Signaling Technologies (Beverly, MA). The primary antibody against pCREB (06-519) was purchased from Millipore (Billerica, MA, USA) and α-tubulin (sc-8035) was purchased from Santa Cruz Technologies (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-rabbit (NA-934) and anti-mouse (NA-931) IgG were purchased from Amersham Phamacia (Piscataway, NJ).

2.2.2. CPP apparatus

The place preference apparatus (purchased from Med Associates, Georgia, VT) previously described [7,9,36] consisted of a rectangular cage with three chambers: 2 square conditioning chambers (28 cm in length) separated by a neutral rectangular chamber (12 cm long and 4 cm wide). The two conditioning chambers were differentiated by tactile and visual cues; in one, the floor was a stainless steel mesh and the walls were white, and in the other, the floor was made up of a grid of stainless steel rods and the walls were black. The middle chamber had grey walls and a smooth PVC floor. The chambers were separated by computer-automated guillotine doors, allowing free access among all three chambers during pre-test and preference testing. Locomotor responses were measured with a computerized photo-beam system and MED-PC software, which recorded time spent in each chamber, total locomotor behavior (sum of all horizontal counts), entrances into each chamber (multiple beams broken between two chambers) and exploratory behavior (a single broken beam between two chambers without entrance).

2.3. Procedures

2.3.1. CPP procedure

After 4 days of handling, rats were placed into the neutral middle chamber of the CPP apparatus with the guillotine doors open and allowed to freely explore all three chambers for 15 min (pre-test). Rats were randomly assigned to one of three treatment groups as follows: saline/saline, saline/cocaine, or mk-801/cocaine treatment groups (n = 9–10 animals/group). Conditioning occurred over the next four days consisting of alternating drug/saline treatments on alternate days (2 cocaine/mk-801 treatments and 2 saline treatments on alternating days). On the first day of conditioning rats were pretreated with an i.p. injection of saline and received another saline injection 30 min later and were immediately confined to one of the conditioning chambers for 30 min. On the second day, rats were pretreated with i.p. injections of saline (0.9%) or MK-801 (0.25 mg/kg) followed 30 min later by an i.p. injection of saline or cocaine (20 mg/kg) and immediately confined for 30 min to the chamber opposite from conditioning day one (counterbalanced so that half of the rats received cocaine in black and saline and white and vice versa). Control rats received both saline pretreatment and saline again 30 min later and were confined to alternating chambers on alternating days. CPP testing was conducted in a drug-free state the day after the last conditioning session and followed the same procedure as the preconditioning test.

2.3.2. Protein preparation

Immediately after the drug-free CPP test, rats were briefly exposed to CO_2 (less than 30 s) and euthanized by rapid decapitation. Brains were removed and flash frozen in 2-methylbutane (−40 °C). Tissue punches of the NAc and CPu [Fig. 3E, +2.0 to +1.8] were dissected out of each brain on a cold glass plate and homogenized with a Polytron handheld homogenizer (Kinematica, Luzern, Switzerland) in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 10% Glycerol, 1% Triton X-100, 1% sodium deoxycholic acid) containing a phosphatase inhibitor cocktail. Homogenates were incubated for 30 min and then centrifuged for 15 min (13,000 rpm,