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# Effects of withdrawal from chronic escalating-dose binge cocaine on conditioned place preference to cocaine and striatal preproenkephalin mRNA in C57BL/6J mice

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ABSTRACT

Relapse is a serious problem for the effective treatment of cocaine addiction.
*Rationale:* Examining cocaine re-exposure-induced behavioral and neurobiological alterations following chronic escalating-dose binge cocaine administration and withdrawal may provide insight into the neurobiological basis of cocaine relapse.
*Objectives:* Our goal was to determine how exposure to chronic escalating-dose cocaine affects development of subsequent cocaine-induced conditioned place preference (CPP) and changes in endogenous opioid systems.
*Methods:* Mice were injected with either escalating-dose binge cocaine (15–30 mg/kg/injection × 3/day) or saline for 14-days and conditioned with 15 mg/kg of cocaine or saline (once per day for 10-days), starting either 1 or 14-days after the last day of binge injections.
*Results:* Mice exposed to chronic escalating cocaine did not develop CPP to cocaine when conditioning commenced on the first day of withdrawal (CPP test on day 10 of withdrawal). By contrast, mice did develop CPP to cocaine when conditioning started on the 14th day of withdrawal (CPP test on day 24 of withdrawal). Furthermore, preproenkephalin (*Penk*) mRNA levels in caudate putamen were significantly higher in mice that received 14-day withdrawal from escalating-dose binge cocaine before the CPP procedure (tested 24 days post-binge) than those that received 1-day withdrawal (tested 10 days post-binge).
*Conclusions:* The rewarding effect of cocaine was blunted in early withdrawal from chronic escalating exposure, but recovered in more prolonged withdrawal. Time-dependent elevations in *Penk* mRNA levels may be part of the underlying mechanisms of this effect.



## 1. Introduction

Cocaine addiction is a chronic, relapsing disease (e.g. Leshner, 1997; Ridenour et al., 2005). As drug use progresses from early experimentation and occasional use toward the compulsive drug-seeking characteristic of addiction (e.g. Vanderschuren and Everitt, 2004), tolerance to the rewarding effects of the drug tends to develop and an escalation in the daily dose of drugs consumed appears in animal studies (e.g. Ahmed and Koob, 1998; Mantsch et al., 2004; Picetti et al., 2010). Over time, patients with addictive diseases typically undergo periods of withdrawal, followed by relapse (e.g. Stewart, 2000; Belin and Everitt, 2008). Relapse to cocaine abuse may occur after extended periods of withdrawal, indicating long-term persistent alterations in neurochemistry as a result of chronic exposure to cocaine. These persistent effects may alter the rewarding effects, or the incentive valence, of cocaine on subsequent re-exposure (e.g. Gawin, 1991; Shaham et al., 2003).

It is well established that striatal opioid neuropeptides and their receptors are altered following cocaine exposure in rodents (e.g. Hammer, 1989; Hurd and Herkenham, 1993; Przewlocka and Lason, 1995; Daunais et al., 1997; Adams et al., 2000). However, little direct information is available about their impact over the course of cocaine withdrawal. Binge pattern administration of cocaine to rats leads to a significant decrease in the mean level of kappa opioid receptor (KOP-r) mRNA in the substantia nigra, with no significant change in the mean level of KOP-r mRNA in the caudate putamen (CPu) (Spangler et al., 1996). Ten-day withdrawal after 10-day cocaine administration resulted in a significant reduction of prodynorphin mRNA levels in the ventrorostral striatum in rats (Svensson and Hurd, 1998). After repeated cocaine administration (20 mg/kg × 3, for 5 days) to rats either no acute changes (at 3 h), or a decrease in the binding of delta opioid receptor (DOP-r) in the

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nucleus accumbens (NAc) and striatum after 24 and 48 h withdrawal were observed (Turchan et al., 1999).

We hypothesize that the duration of withdrawal from repeated cocaine exposure is a critical factor influencing the behavioral and neurobiological outcomes of subsequent cocaine re-exposure. To test our hypothesis, we determined, in parallel, behavioral and neurobiological consequences of re-exposure to cocaine in a CPP paradigm, beginning at short-term (1-day) and extended (14-day) withdrawal from chronic escalating-dose binge cocaine administration. This was achieved with a paradigm of non-contingent chronic escalating-dose binge cocaine (14-days), followed by a short-term (1-day) or long-term (14-day) withdrawal (i.e. cocaine-free and injection-free interval), then followed by cocaine re-exposure during conditioned place preference (CPP) training in mice.

Escalating-dose binge cocaine was administered in the current study, since both rodents (Ahmed and Koob, 1998; Mantsch et al., 2004; Picetti et al., 2010) and human addicts self-administer escalating doses of cocaine over time. We therefore determined whether previous cocaine exposure and the length of withdrawal would affect subsequent cocaine-induced CPP in mice. The neurobiological assays in the current study focused primarily on the endogenous opioid receptors and neuropeptides in the CPu and NAc, terminal regions of the nigrostriatal and mesolimbic dopaminergic pathways, in order to determine the involvement of endogenous opioid components in the rewarding effect of cocaine during subsequent re-exposure. To our knowledge, this is the first study applying escalating-dose binge cocaine administration followed by a CPP procedure to study behavioral and molecular neurobiological consequences of chronic re-exposure to cocaine after acute and more protracted cocaine-free periods.

## 2. Materials and methods

### 2.1. Subjects

Male adult (10 weeks old on arrival) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were single-housed with free access to food and water in a light-(12:12 h light/dark cycle, lights on at 7:00 am) and temperature-(25 °C) controlled room. Animal care and experimental procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996). The experimental protocols used were approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

### 2.2. Escalating-dose binge cocaine

Cocaine-HCl (NIH-NIDA) was dissolved in physiological saline and administered intraperitoneally three times per day in the home cage. Each injection was separated by 1 h and the first was given 30 min after the start of the daily light cycle. We studied the behavior of mice in the light phase, because a common pattern of human cocaine use involves multiple doses in the early evening, which corresponds to the start of the daily light cycle in nocturnal animals. To model the human pattern of increasing doses of cocaine over time, the dose of cocaine was increased every 3 days for the first 9 days, from 15 mg/kg/injections (45 mg/kg/day) to 25 mg/kg/injections (75 mg/kg/day). Mice then received three 30 mg/kg/injections (90 mg/kg/day) for the last 5 days, for a total of 14 days of binge cocaine administration. This dosing schedule models the dose range self-administered by rats given long access (6–10 h) to cocaine (for review, see Koob and Kreek, 2007). Control mice were injected with saline three times per day for 14 days with the same schedule of administration.

### 2.3. Conditioned place preference

#### 2.3.1. Mouse place preference chambers

Mouse place preference chambers (model ENV-3013) were purchased from Med Associates (St. Albans, VT). Each chamber has three distinct compartments that can be separated by removable doors. Automated data collection is accomplished by individual infrared photobeams on a photobeam strip, with six beams in the white and black compartments and two beams in the smaller central gray compartment with a smooth solid floor. The center compartment has a neutral gray floor. The black compartment is 16.8 × 12.7 × 12.7 cm with a stainless steel grid rod floor. The white compartment (also 16.8 × 12.7 × 12.7 cm) has a stainless steel mesh floor.

#### 2.3.2. Conditioned place preference and locomotor activity determinations

One or 14 days after the last day of escalating-dose binge cocaine or saline injection, a 10-day CPP procedure was initiated in these mice. Experiments were performed in a dimly-lit, sound-attenuated chamber. The study used an unbiased, counterbalanced design in which six mice were randomly assigned to either the cocaine or saline compartment. Half the animals had white and half had black as the cocaine-paired side. During the pre-conditioning session, each animal was placed in the center compartment with free access to the black and white compartments and the time spent in each compartment was recorded for 30 min. During the conditioning sessions, mice were placed into and restricted to the appropriate compartment for 30 min after cocaine (15 mg/kg, i.p.) or saline injection. Locomotor activity was assessed as the number of "crossovers" defined as breaking the beams at either end of the conditioning compartment. The animals were injected with cocaine and saline on alternate days, for a total of eight conditioning sessions with four cocaine and four saline trials for each animal. Control mice were conditioned with saline in both the black and white compartments. Conditioning sessions were conducted daily. The post-conditioning test session was performed on the day after the last conditioning session, and was identical to the pre-conditioning session: each mouse had free access to both white and black compartments without cocaine or saline injection. The difference between the pre- and post-conditioning sessions in the amount of time spent on the drug-paired compartment was used to determine whether the mice had developed a conditioned place preference to the cocaine-associated compartment.

### 2.4. In situ hybridization

#### 2.4.1. Hybridization probes

Brain sections were subjected to an *in situ* hybridization protocol as described earlier (Mathieu-Kia and Besson, 1998). $^{32}$P-labeled antisense riboprobes were used to detect preproenkephalin (*Penk*) and preprodynorphin (*Pdyn*) mRNA levels. The DNA templates for the probes were generated from cDNA fragments of *Penk* (containing sequences from nucleotides 342–1025, GenBank accession number 13227) and *Pdyn* (containing sequences from nucleotides118- 614, GenBank accession number U64968) (Fig. 1).

#### 2.4.2. Detection and localization of Penk and Pdyn mRNA by in situ hybridization histochemistry

Mice were decapitated following the post-conditioning test, either 10 or 24 days after the last binge injections, and their brains were frozen at −80° C. Sectioning was performed in mice from each treatment group (six from each group). Coronal sections (20 μm) were cut at −16 °C and mounted onto Superfrost/Plus glass slides (Fisher Scientific, Pittsburgh, PA). The brain sections were fixed for 10 min at 4° C in 4% paraformaldehyde/0.1 M sodium phosphate buffer (pH 7.2) and washed for 10 min in 0.1 M sodium phosphate buffer. Sections were then acetylated at room temperature for 10 min with 0.25% acetic anhydrite dissolved in acetylation buffer. Slides were washed twice in $dH_2O$ for 1 min and air dried. Sections were hybridized overnight at 65° C in a humidified environment with a solution consisting of Denhart's solution, 50% formamide, 10% dextran sulfate, 10 mM DTT, 100 μg/ml of sheared and denatured salmon sperm DNA, 400 μg/100ul of tRNA, 10% SDS and 1 mM EDTA and $6 \times 10^6$ cpm/ml oligonucleotide probe. The hybridization solution was applied in a volume of 100 μl and the slide was coverslipped. The next day, sections were soaked in 4 × SSC/1 mM DTT solution to remove the cover-slips. Slides were subsequently washed in 4 × SSC/1 mM DTT solution at RT for 60 min, 50% formamide/2× SSC, pH 7 at 65 °C for 30 min followed by RNase digestion (20 μg/ml) at room temperature for 60 min. A final 15-min wash in 1 × SSC at 60 °C was carried out followed by a 15-min wash in 0.1 × SSC at 60 °C. The slides were quickly dehydrated in ascending ethanol concentrations and air dried at room temperature. The slides were then exposed to X-ray films (Bmax, Kodak, Rochester, NY) for 4 days for *Penk* and 7 days for *Pdyn*. The specificity of mRNA hybridization in the brain sections was tested by two controls: the absence of labeling after use of unlabeled probes and after use of sense strand probes. The coronal sections and levels relative to Bregma analyzed in the current study are shown in Fig. 2B.

#### 2.4.3. Quantification of Penk and Pdyn mRNA levels

Quantitative analysis of messenger RNA was performed on autoradiographic films by quantifying gray levels on the exposed film in regions corresponding to the CPu, NAc core and shell (Fig. 2A). The hybridization signals were quantified on both hemispheres by optical density measurement with a computerized image processing system (MCID Imaging Research, St. Catharines, Ontario, Canada). Four to five sections per animal were quantified and the values were averaged to generate an optical density value that corresponds to mRNA abundance in each region in each mouse.

### 2.5. Autoradiography

Mu opioid receptor (MOP-r) densities in sections were measured by autoradiography as described earlier (Unterwald et al., 1989). Briefly, brain sections were pre-incubated in 50 mM Tris–HCl (pH 7.4) at room temperature for 30 min and then in 50 mM Tris–HCl (pH 7.4) containing tritiated opioid ligands for 60 min at 4 °C. MOP-