Neuropharmacology 67 (2013) 259–266

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Chronic escalating cocaine exposure, abstinence/withdrawal, and chronic re-exposure: Effects on striatal dopamine and opioid systems in C57BL/6J mice

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ARTICLE INFO

Article history:
Received 11 August 2012
Received in revised form
23 October 2012
Accepted 25 October 2012

Keywords:
Escalating-dose binge cocaine
Preprodynorphin
Striatal dopamine
Mice
Cocaine addiction

ABSTRACT

Cocaine addiction is a chronic relapsing disease with periods of chronic escalating self-exposure, separated by periods of abstinence/withdrawal of varying duration. Few studies compare such cycles in preclinical models. This study models an "addiction-like cycle" in mice to determine neurochemical/molecular alterations that underlie the chronic, relapsing nature of this disease. Groups of male C57BL/6J mice received acute cocaine exposure (14-day saline/14-day withdrawal/13-day saline + 1-day cocaine), chronic cocaine exposure (14 day cocaine) or chronic re-exposure (14-day cocaine/14-day withdrawal/14-day cocaine). Escalating-dose binge cocaine (15–30 mg/kg/injection × 3/day, i.p. at hourly intervals) or saline (14-day saline) was administered, modeling initial exposure. In "re-exposure" groups, after a 14-day injection-free period (modeling abstinence/withdrawal), mice that had received cocaine were re-injected with 14-day escalating-dose binge cocaine, whereas controls received saline. Microdialysis was conducted on the 14th day of exposure or re-exposure to determine striatal dopamine content. Messenger RNA levels of preprodynorphin (*Pdyn*), dopamine D1 (*Drd1*) and D2 (*Drd2*) in the caudate putamen were determined by real-time PCR. Basal striatal dopamine levels were lower in mice after 14-day escalating exposure or re-exposure than in those in the acute cocaine group and controls. *Pdyn* mRNA levels were higher in the cocaine groups than in controls. Long-term adaptation was observed across the stages of this addiction-like cycle, in that the effects of cocaine on dopamine levels were increased after re-exposure compared to exposure. Changes in striatal dopaminergic responses across chronic escalating cocaine exposure and re-exposure are a central feature of the neurobiology of relapsing addictive states.

1. Introduction

1.1. Models of cocaine addiction

Cocaine addiction is a chronic relapsing disease characterized by escalation of cocaine doses, intermittent withdrawal and, in many cases, relapse to cocaine use (e.g. Leshner, 1997). Recurrences of cocaine use (i.e., relapse) lead to neurobiological changes persisting for extended periods of time and result in behavioral alterations upon subsequent re-exposure to cocaine. Preclinical models that allow rodents to self-administer cocaine at sufficiently large doses for prolonged daily sessions (mimicking human exposure) show robust escalation of daily intake (Ahmed and Koob, 1998; Mantsch et al., 2004; Picetti et al., 2011). Certain rodent studies (e.g., "re-instatement" paradigms) have been used to model the first stages of a relapse episode, but less is known about the processes underlying the severe and protracted relapse periods that occur in addicted persons (e.g. Ahmed and Koob, 1998). Overall, the effects of substantial cocaine re-exposure after drug-free intervals have not been well studied in preclinical models. Determining neurobiological alterations induced by chronic escalating-dose binge cocaine administration and re-exposure after a drug-free interval could provide novel insight into the underlying mechanisms of cocaine addiction.

1.2. Pharmacological effects of cocaine

The main pharmacological effect of cocaine is to bind to monoamine (primarily dopamine) transporters and block reuptake. Cocaine produces its acute rewarding effects principally by increasing synaptic dopamine concentrations in the nigrostriatal and mesolimbic dopaminergic pathways, with impact on the nigrostriatal pathways in the chronic phases of the addiction

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http://dx.doi.org/10.1016/j.neuropharm.2012.10.015

process (e.g. Porrino et al., 2004). The opioid receptors (mu, kappa and delta) and their endogenous ligands (beta-endorphin, dynorphins and enkephalins) are highly expressed in the nigrostriatal and mesolimbic dopaminergic pathways (Mansour et al., 1995) and play an important role in mediating the rewarding and behavioral effects of cocaine.

Numerous studies have found that striatal opioid neuropeptides and their receptors are altered following chronic cocaine exposure in rodents (e.g. Hammer, 1989; Daunais et al., 1993; Hurd and Herkenham, 1993; Spangler et al., 1993; Przewlocka and Lason, 1995). Furthermore, extracellular concentrations of dopamine in the striatum change following acute and chronic cocaine administration (Akimoto et al., 1989; Di Chiara and Imperato, 1988; Maisonneuve and Kreek, 1994; Maisonneuve et al., 1995; Zhang et al., 2001; Zhang et al., 2003). We and others have reported that binge-pattern administration (e.g., injections at hourly intervals) results in particular neurobiological alterations (e.g. Unterwald et al., 1994).

In the current study, a paradigm of chronic escalating-dose binge cocaine was administered (non-contingently) in mice to mimic escalating self-exposure in addicted persons, attaining a controlled level of drug exposure (Ahmed and Koob, 1998; Mantsch et al., 2004; Picetti et al., 2011). We hypothesized that chronic escalating-dose binge cocaine exposure and re-exposure (modeling "full relapse" versus the more commonly studied "reinstatement" stage) lead to differential behavioral and neurochemical outcomes. Thus, locomotor activity was monitored during the course of initial 14-day cocaine exposure, a 14-day drug-free interval and secondary 14-day cocaine re-exposure to compare effects of chronic cocaine exposures and re-exposures on behavior. Striatal dopamine, dopamine D1, D2 receptor, DAT mRNA and opioid gene expression, known to be altered in response to acute and chronic cocaine exposure, were examined and compared between the chronic exposure and re-exposure groups.

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-controlled (25 °C) 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. Each injection was separated by 1 h and the first was given 30 min after the start of the daily light cycle. We study 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/injection (45 mg/kg/day) to 25 mg/kg/injections (75 mg/kg/day). Mice finally received three 30 mg/kg/injection (90 mg/kg/day) for the last 5 days, for a total of 14 days of binge cocaine administration. Control mice were injected with saline three times per day for 14 days with the same schedule of administration.

2.3. Experimental groups

After a one-week acclimation to the environment, mice were randomly assigned to five groups (Table 1). In the 14-day escalating-dose binge cocaine exposure groups, mice were either injected with escalating-dose binge cocaine (chronic cocaine) or saline (chronic saline) for 14 consecutive days.

In the 14-day escalating-dose binge cocaine re-exposure groups, one group of mice was injected with escalating-dose binge cocaine while two groups of mice were injected with saline for 14 consecutive days. After a 14-day drug-free interval, the mice that had received 14-day escalating-dose binge cocaine before, again received 14-day escalating-dose cocaine injections (chronic cocaine re-exposure) whereas mice that had received saline before received binge saline injections (chronic saline re-exposure) in the same pattern. Further, one saline re-exposure group was injected with one binge of cocaine (15 mg/kg × 3, i.p.) on the last day of 14-day re-exposure period (acute cocaine).

Table 1
Description of treatment received by the individual groups used in the study.

Groups	Treatment
Chronic saline	14 day saline
Chronic cocaine	14 day cocaine
Acute cocaine	14 day saline/14 day withdrawal/ 13 day saline + 1 day cocaine
Re-exposure saline	14 day saline/14 day withdrawal/ 14 day saline
Re-exposure cocaine	14 day cocaine/14 day withdrawal/ 14 day cocaine

2.4. Locomotor activity

2.4.1. Apparatus

Locomotor behavior was measured using the high density SmartFrame Motor-Monitor System (Kinder Scientific, Paway, CA). The high density SmartFrame chassis has 15 infrared (IR) photobeams along the *Y* axis and 7 IR photobeams along the *X* axis. As the experimental animal moves in its cage it breaks photobeams on this XY grid. Beam breaks are recorded by a dedicated microprocessor in each chassis and transmitted to a PC via serial communication. Data is compiled using Kinder Scientific MotorMonitor software. Total distance traveled was measured.

2.4.2. Locomotor activity measurement

A cage with clean bedding material was placed into each chassis of the SmartFrame motor monitor system. Behavioral measurement was monitored only in the mice that received the entire addiction-like cycle of 14-day escalating-dose binge cocaine/saline, 14-day withdrawal and 14-day escalating-dose binge cocaine/saline re-exposure. During the cocaine/saline administration period, including initial exposure and re-exposure, each mouse was removed from its home cage, then injected with either escalating-dose cocaine or saline and immediately placed into the cage inside the chassis after each injection, one mouse in each cage. Locomotor activity was monitored after each cocaine or saline injection and for an additional 2 h after the last injection. Specifically, the total distance traveled was recorded in 10 min bins for a total of 5 h (300-min) beginning immediately following the first daily injection.

During the 14-day withdrawal period, mice were also placed into the cages inside the chassis without cocaine or saline injections. The total distance traveled was recorded in 10 min bins for a total of 5 h (300-min) at an analogous time during withdrawal.

On day 9 of the escalating-dose binge cocaine (or saline) exposure (chronic cocaine/saline groups) or re-exposure period (chronic cocaine/saline re-exposure groups), mice were anaesthetized with a combination of xylazine (8.0 mg/kg i.p.) and ketamine (80 mg/kg i.p.) and were placed in a Kopf stereotaxic frame modified for the mouse (David Kopf, Topanga, CA) for implantation of a guide cannula. A guide cannula (CMA/7: CMA, North Chelmsford, MA) was implanted into the caudate putamen (coordinates from Bregma: $A = 0.65$ mm, $L = \pm 2.00$ mm and $V = 3.00$ mm (Franklin and Paxinos, 1997). In half of the mice, the guide cannula was implanted into the right side, while in the other half it was inserted in the same brain region on the left side. The guide cannula was fixed to the skull by dental acrylic (Den-Mat, Santa Maria, CA), according to the manufacturer's instructions.

2.5. In vivo microdialysis

Dialysis probes (2 mm in length, o.d. = 0.24 mm) (CMA/7) were calibrated for dopamine recovery *in vitro* before each experiment, as described previously (e.g. Maisonneuve and Kreek, 1994; Zhang et al., 2001, 2003). On day 13 of escalating-dose binge exposure or re-exposure to cocaine or saline, mice were individually placed into microdialysis chambers (CMA/120) with free access to food and water to allow them to acclimate to the microdialysis chamber. Dialysis probes were then lowered into the caudate putamen and were perfused with artificial cerebrospinal fluid (aCSF) (146 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$) at 1.0 μl/min overnight. Following the overnight stabilization period (15–16 h), basal levels of dialysate were collected from the freely-moving mice every 20 min for 1 h at a flow rate of 1.0 μl/min. After collection of baseline samples, each mouse received binge-pattern cocaine or saline administration. Dialysate samples were collected every 20 min throughout the binge cocaine administration period and for 1 h after

the last injection. All collecting tubes contained perchloric acid and EDTA as preservatives. Samples (20 μl each) were immediately frozen and stored at −80 °C until used for dopamine analysis. All experiments were performed between 8:00 a.m. and 2:00 p.m. in the light portion of the light/dark cycle. Mice were sacrificed 90 min after the last cocaine or saline injection.

2.6. Determination of dialysate dopamine levels

HPLC with electrochemical detection (ESA, North Chelmsford, MA) was used to measure dopamine concentration in the dialysates. The HPLC system consisted of an ESA 540 autosampler, an ESA 582 solvent delivery system, a reverse phase C18 column and an ESA microdialysis cell (Model 5014B). The mobile phase was delivered at a rate of 0.5 ml/min. Chromatograms were integrated and compared with standards using the ESA 501 chromatography system. With this method, the detection limit for dopamine is 500 fg.

2.7. Histological verification of probe placement

At the end of each microdialysis study, the mouse was decapitated following brief CO_2 exposure, and its brain was removed and hemisected. The half of the brain with microdialysis cannula implanted was used for histological evaluation of probe placement. Frozen sections were cut to verify the correct placement of dialysis probes following acetyl cholinesterase labeling (see Franklin and Paxinos, 1997). Fig. 2A is a photomicrograph of a tissue section from a mouse used in this study and shows the location of the microdialysis probe in the brain.

2.8. Real-time PCR

The caudate putamen from each mouse was dissected from the half of the brain without dialysis probe implantation and homogenized in Qiazol (Qiagen, Valencia, CA). Total RNA was isolated from homogenates of the caudate putamen using the miRNeasy kit (Qiagen). DNase (Turbo DNA-free, Ambion, Austin, TX) was used to treat the isolated RNA. The quality of RNA from each sample was determined spectrophotometrically. Complementary DNA was synthesized from 1 μg of total RNA using the Super Script III first strand synthesis kit (Invitrogen, Carlsbad, CA).

Real-time PCR was used to measure the relative mRNA levels of prodynorphin (*Pdyn*), dopamine transporter (*Slc6a3*, DAT), dopamine D1 (*Dadr1*) and D2 (*Dadr2*) receptors using commercially available primers and master mix (RT2qPCR™ primer assays and RT2 Real Time™ SYBR® Green PCR Master Mix; Qiagen) in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). In the real-time PCR assay, water controls containing primers of target genes were included. Any sample with a cycle threshold (Ct) greater than that of the water control or a Ct ≥35 was not included in the analysis according to manufacturer's instructions. The relative expression of each gene was calculated by subtracting the CT value of the target gene from the CT value of the house keeping gene GAPDH to determine the ΔCt. The final measure of relative expression was calculated as $2^{-\Delta Ct}$.

2.9. Data analysis

For clarity of presentation, since there was no significant difference between the chronic saline group and the chronic saline re-exposure group, the two saline groups were combined into a single saline control group (saline/saline re-exposure). To examine the significance of differences among the four groups, mice that received only one binge cocaine exposure following 14-day saline, 14-day withdrawal and 13-day saline (the acute cocaine group), mice that received 14-day escalating-dose binge cocaine exposure for the first time, mice that were re-exposed to 14-day escalating-dose binge cocaine administration after a 14-day withdrawal, and the combined saline control group, one- or two-way analyses of variance (ANOVA) were used, with repeated measures as needed. Following

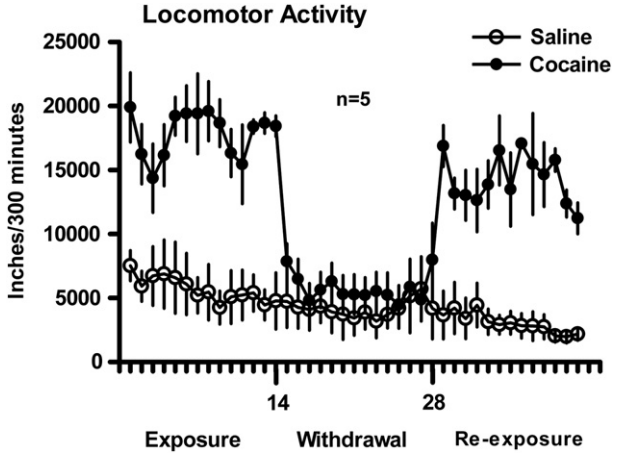

Fig. 1. The mean (±SEM) locomotor activity of mice that received escalating-dose binge cocaine or saline administration in each period (exposure, withdrawal and re-exposure) is shown. Cocaine resulted in significantly increased horizontal locomotor activity on all days of injection compared to saline controls, $p < 0.000001$.

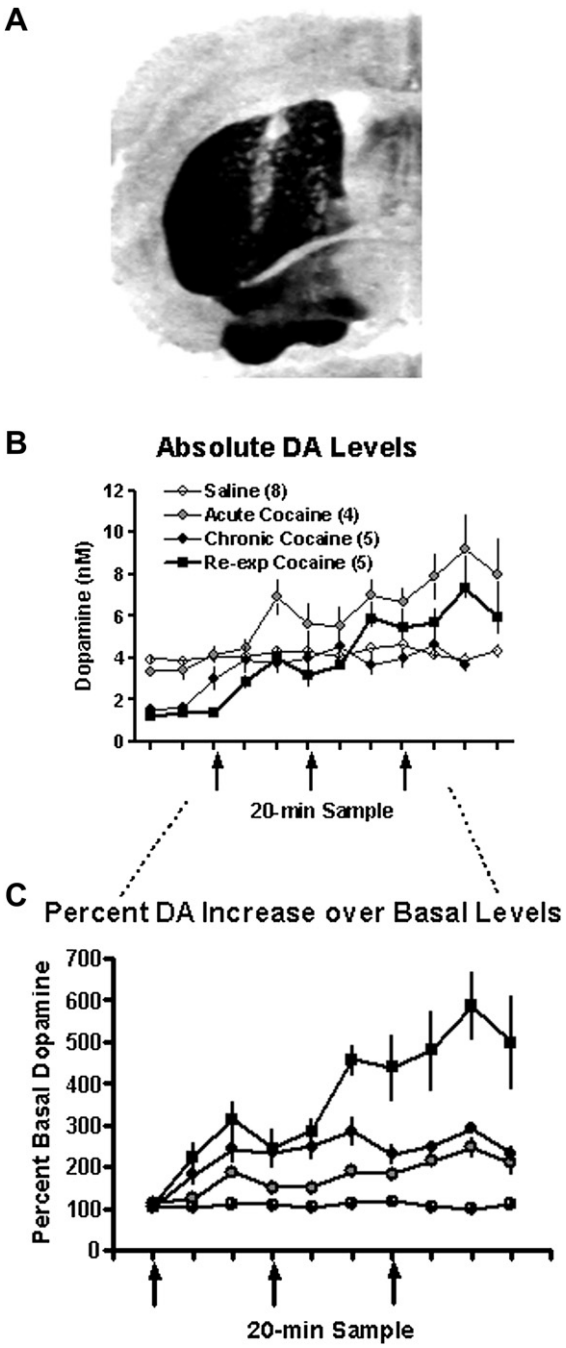

Fig. 2. A. A photomicrograph of a tissue section from a mouse used in this study shows the location of the microdialysis probe in the brain. The mean (±SEM) levels of dopamine in the dialysate from the striatum during the baseline, in the hour after each injection of mice which received either binge cocaine or saline injections, are shown in B. The arrows indicate cocaine or saline injections. Basal (pre-injection) dopamine levels in both the chronic cocaine and chronic cocaine re-exposure groups were significantly lower than in the saline/saline re-exposure controls. Levels of dopamine expressed as percent increases over baselines in the 3 h after cocaine or saline injections are shown in C.

ANOVAs, Newman–Keuls *post hoc* tests or planned comparisons (primarily between exposure and re-exposure groups) were made as appropriate.

3. Results

3.1. Locomotor activity

To assess the behavioral stimulatory effects of acute cocaine exposure, chronic cocaine exposure and re-exposure, two groups of mice were studied. One group received 14-day escalating-dose binge cocaine administration, 14-day withdrawal and then 14-day re-exposure to escalating-dose binge cocaine, while the other group received saline injections in the same pattern. Escalating-dose binge cocaine administration resulted in significantly increased horizontal locomotor activity on all days of injection compared to saline controls as can be seen in Fig. 1. A two-way ANOVA of the mean locomotor activity of each mouse in each period (exposure, withdrawal and re-exposure), Condition × Period, with repeated measures showed a significant main effect of Condition, $F(1, 8) = 18.42$, $p < 0.005$, of Period, $F(2, 16) = 52.13$, $p < 0.000001$ and a significant Condition × Period interaction effect, $F(2, 16) = 38.77$, $p < 0.000002$. A planned comparison of the locomotor activity level in the two 14-day escalating-dose cocaine periods showed a slight but significantly lower locomotor response to cocaine in the re-exposure period, $p < 0.01$. However, since the saline animals also showed slightly lower locomotor activity in the re-exposure period, $p < 0.05$, this change in locomotor activity did not appear to be specific to the cocaine-treated mice.

3.2. Effect of escalating-dose binge cocaine administration on dialysate dopamine in the striatum

The mean (±SEM) levels of dopamine in the dialysate from the striatum of C57BL/6J mice during the baseline, and in the hour after each injection of mice which received either binge cocaine administration or saline injections, are shown in Fig. 2B.

3.2.1. Changes in absolute dopamine levels

Examining dopamine levels from dialysates of the three baseline samples before the first injection, it is clear that each of the chronic cocaine groups (i.e., chronic cocaine or chronic cocaine re-exposure) had lower dopamine levels than those from the acute cocaine and saline controls (Fig. 2B). Two-way ANOVA, Condition × 20-min Sample, detected a significant effect of chronic escalating-dose cocaine administration on basal dopamine levels, $F(2, 15) = 83.09$, $p < 0.000001$. Basal dopamine levels in both the chronic cocaine group and the chronic cocaine re-exposure group were significantly lower than in the acute cocaine and saline/saline re-exposure groups, $p < 0.0002$, Newman–Keuls *post hoc* test. As can be seen in the figure, dopamine levels increased after cocaine injections (indicated by the arrows) but not after saline injections. In a separate group, we have found that absolute dialysate levels had returned to normal by the beginning of the re-exposure period (that is, at the end of 14-day withdrawal, data not shown).

3.2.2. Percent change in dopamine over basal levels

Since 14-day escalating-dose binge cocaine administration led to significantly reduced basal levels of extracellular dopamine in the striatum, it was important to examine the magnitude of the increase in dopamine in response to binge cocaine administration expressed as percent increase over basal levels, shown in Fig. 2C.

The mean (±SEM) percent increase in dopamine over the 3 h after cocaine (or saline in controls) injections for each group are shown in Fig. 3. One-way ANOVA showed that there was a significant main effect of Condition, $F(3, 18) = 23.46$, $p < 0.000005$. Newman–Keuls *post hoc* tests showed that the increase in striatal dopamine levels over basal dopamine levels was significantly higher in the mice that had chronic cocaine exposure ($p < 0.01$) and chronic cocaine re-exposure ($p < 0.0002$) than in saline/saline re-exposure controls. Furthermore, mice re-exposed to cocaine showed a greater percent increase in dopamine compared to that observed in the chronic exposure group ($p < 0.002$). Thus, there is evidence of sensitization of the relative increase in dopamine levels during chronic cocaine re-exposure. Interestingly, the percent increase in dopamine induced by acute binge cocaine just missed significance when compared to saline controls, $p = 0.058$.

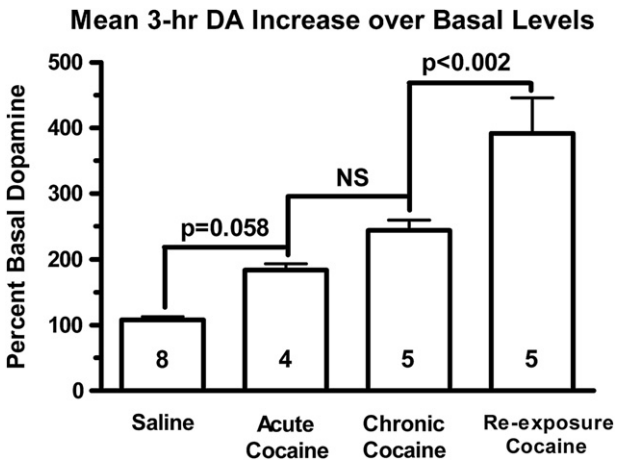

Fig. 3. The mean (±SEM) percent increases in dopamine over the 3 h after cocaine or saline injections are shown. The increase in striatal dopamine levels over basal levels was significantly higher in the mice from the chronic cocaine group and chronic cocaine re-exposure group than in saline/saline re-exposure controls. Mice in the chronic cocaine re-exposure group showed a greater percent increase in dopamine compared to that observed in the chronic cocaine group.

The mean concentrations of absolute dopamine levels of the three baseline samples, and absolute dopamine levels in response to each cocaine injection across the binge are shown in Fig. 4A. Since basal levels were significantly lower in the chronic cocaine and chronic cocaine re-exposure groups, it was important to examine the percent increase over basal dopamine in response to each injection of cocaine (Fig. 4B). Two-way ANOVA, Condition × Injection Order (three injections of cocaine or saline), with repeated measures showed that there was a significant main effect of Condition, $F(3, 18) = 11.18$, $p < 0.0005$, a significant effect of Injection Order, $F(2, 36) = 11.29$, $p < 0.0002$ and a significant Condition × Injection Order interaction, $F(6, 36) = 3.01$, $p < 0.02$. There was no change in dopamine across the three hourly "binge-pattern" saline injections. In contrast, the acute cocaine group showed a gradual increase in dopamine in response to cocaine injections across the binge. Further, both chronic cocaine and chronic cocaine re-exposure groups showed at least a 200% increase of basal levels (Fig. 4B), after the first injection. Then, while the chronic cocaine group showed no further elevation in dopamine response after the second and third injection, the mice in the chronic cocaine re-exposure group showed an increasing response over the course of the binge, so that by the second and third injections, the percent increase in striatal dopamine was greater in the chronic cocaine re-exposure group than in the chronic cocaine group, $p < 0.001$ and $p < 0.05$ respectively (planned comparisons). Furthermore, in the chronic cocaine re-exposure group, the increase in percent basal dopamine was significantly greater on the third injection than in the first, $p < 0.0005$. This again is evidence of sensitization, here observed within the binge administration of cocaine.

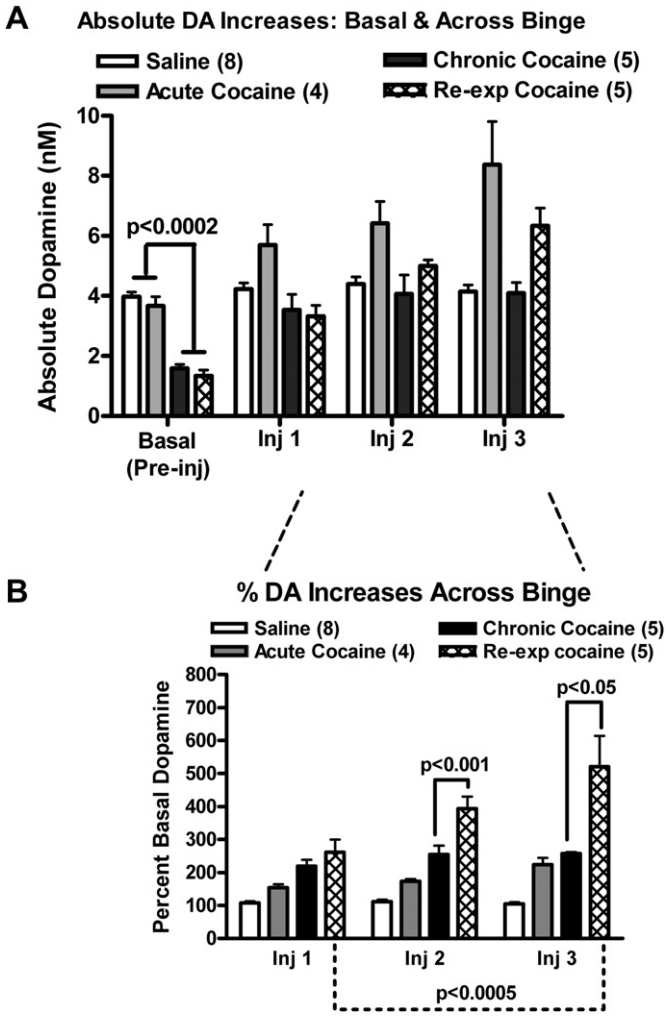

Fig. 4. The increase of dopamine in response to each cocaine or saline injection across the binge is shown. A shows absolute dopamine increases whereas B shows percent increases over pre-injection basal levels. Both the chronic cocaine and chronic cocaine re-exposure groups showed increases in dopamine over basal levels after the first injection. The chronic cocaine group showed no further elevation after the second and third injections. The chronic cocaine re-exposure group showed an increasing response over the course of the binge. The dopamine levels in the chronic cocaine re-exposure group were significantly higher than those of the chronic cocaine group.

3.3. Effects of escalating-dose binge cocaine on mRNA levels

3.3.1. Preprodynorphin mRNA

The mean (+SEM) levels of *Pdyn* mRNA in the caudate putamen in response to saline, acute cocaine, chronic cocaine exposure and re-exposure are shown in Fig. 5A. One-way ANOVA showed a significant main effect of condition on *Pdyn* mRNA, $F(3, 22) = 15.48$, $p < 0.00002$. Newman–Keuls *post hoc* tests showed that mice that had acute and chronic cocaine exposure or re-exposure had significantly higher levels of *Pdyn* mRNA compared to the saline control mice, $p < 0.01$, $p < 0.001$ and $p < 0.0005$, respectively. There was no significant difference in *Pdyn* mRNA between the chronic cocaine exposure and re-exposure groups. In a separate group, we found that *Pdyn* levels returned to saline control levels by the end of the 14-day drug-free period (i.e., before the start of 14-day re-exposure; data not shown).

3.3.2. Dopamine transporter (Slc6a3; DAT) mRNA

The mean (+SEM) levels of DAT mRNA in the caudate putamen in response to saline, acute cocaine and chronic cocaine exposure and re-exposure are shown in Fig. 5B. One-way ANOVA showed that there was no significant main effect of Drug Condition on DAT mRNA, $F(3, 22) = 2.26$, $p = 0.109$. However, a planned comparison showed that dopamine transporter in the caudate putamen was significantly lower in the 14-day cocaine re-exposure group than in the 14-day cocaine exposure group, $p < 0.05$.

3.3.3. Dopamine receptor mRNA

The mean (+SEM) levels of the dopamine receptor *Drd1* and *Drd2* mRNAs are shown in Fig. 5C and D, respectively. There were no significant differences in the mRNA levels of either the *Drd1* or *Drd2* gene among mice in the 14-day cocaine exposure group, the re-exposure group or the saline/saline re-exposure controls.

4. Discussion

The current experiments modeled chronic escalating cocaine exposure, abstinence/withdrawal, and chronic escalating re-exposure, in order to determine behavioral, underlying neurobiological and molecular changes that may have a role in the chronic relapsing nature of cocaine addiction. These changes may not be evident in more commonly used (re-instatement) paradigms, in which typically only a single day of re-exposure (or extinction responding) occurs.

4.1. Locomotor activity

Mice injected with daily escalating-dose binge pattern cocaine showed increases in locomotor activity, compared with saline controls, on all days of exposure and re-exposure. Such a finding is similar to the locomotor effect induced by steady-dose cocaine administration reported earlier (e.g. George and Ritz, 1990; Koff et al., 1994; Kuzmin et al., 2000; Morse et al., 1993; Schlussman et al., 2003a; Tolliver and Carney, 1994). Surprisingly, no increases in daily total locomotor activity across the escalating regimen were found (i.e., between each of the daily binge injections). Furthermore, no sensitization in locomotor activity was observed in mice that had received escalating-dose binge cocaine. It is known that the initial cocaine dose (15 mg/kg) produces robust behavioral and neurobiological effects in this mouse strain (e.g., Schlussman et al., 2003a; Zhang et al., 2001). We have also previously reported that C57BL/6J mice do not develop sensitization to the locomotor stimulating effects of chronic (14-day) "binge" pattern cocaine, and instead show a tolerance of this effect (Schlussman et al., 2003a). Therefore, it is possible that the locomotor-stimulating effect of cocaine had reached a plateau with the initial cocaine dose in this strain of mouse. This is in contrast to another study in C57BL/6J mice that found an initial sensitization to the locomotor effects of cocaine followed by a dose-dependent increase in the duration of the locomotor effect of cocaine (Bailey et al., 2008). The differences between these behavioral findings may be related to methodological differences concerning habituation to the activity monitors, or the different parameters of dosing.

4.2. Dopamine levels

Activation of the dopaminergic system in the dorsal striatum (caudate putamen), in addition to the ventral striatum (nucleus accumbens), has been shown to be critical for some behavioral effects of cocaine, especially after repeated or chronic exposure, modeling stages of addiction, rather than initial drug experimentation (e.g., Ito et al., 2002; Zhang et al., 2004a, 2004b). Basal striatal dopamine levels were lower in mice that had either the initial 14-day cocaine exposure or re-exposure compared with those of the saline control mice, supporting the hypothesis that chronic cocaine

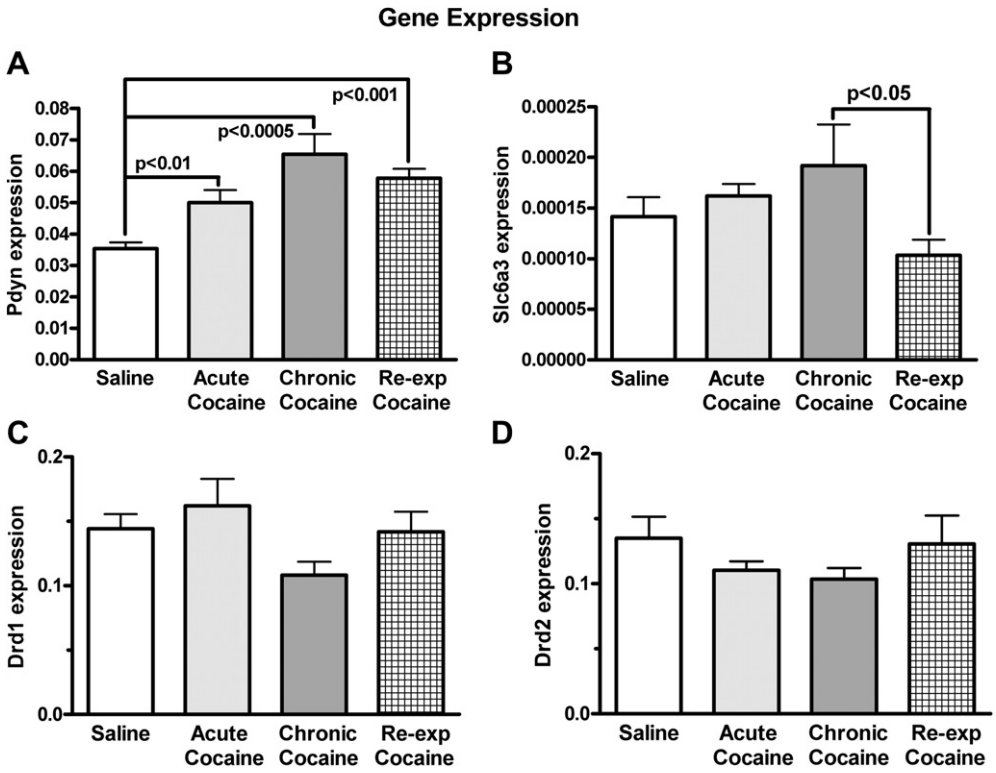

Fig. 5. The mean (+SEM) levels of *Pdyn* (A), DAT (B), *Drd1* (C) and *Drd2* (D) receptor mRNA levels in the caudate putamen in response to saline, chronic cocaine exposure and re-exposure are shown. Mice that had acute cocaine exposure, chronic cocaine exposure or chronic cocaine re-exposure had significantly higher levels of *Pdyn* mRNA compared to the saline/saline re-exposure control mice with no significant difference in *Pdyn* mRNA between chronic cocaine and chronic cocaine re-exposure groups (A). Although one-way ANOVA showed no significant main effect of cocaine on DAT mRNA, a planned comparison found that dopamine transporter in the caudate putamen was significantly lower in the chronic cocaine re-exposure group than in the chronic cocaine group (B).

administration results in persistently lower basal striatal dopaminergic tone (Chefer and Shippenberg, 2002; Imperato et al., 1992; Maisonneuve et al., 1995; Parsons et al., 1991; Rossetti et al., 1992; Zhang et al., 2003). This may, at least in part, underlie continuing anhedonia and dysphoria that persists even after prolonged abstinence.

As expected, acute binge cocaine administration led to increases in striatal dopamine levels, with the starting dose of escalating cocaine (15 mg/kg). In response to binge cocaine on day 14 of chronic cocaine exposure or re-exposure, striatal dopamine levels also increased significantly compared with those of the saline controls. Interestingly, the increases in dopamine levels induced by acute binge cocaine administration were significantly lower than those of the chronic cocaine exposure and re-exposure groups. The lower dose (15 mg/kg, i.p.) administered in the first binge cocaine exposure in the acute group may contribute to this finding. Even more strikingly, cocaine-induced increases in dopamine levels differed substantially between chronic cocaine exposure and re-exposure groups. Dopamine levels in response to the last binge cocaine during re-exposure were significantly higher than those induced by the last binge cocaine during initial the 14-day exposure. Furthermore, such alterations in striatal dopamine levels were also found within the binge in response to each cocaine injection in the re-exposure group. These data add to findings that the frequency and pattern of cocaine administration affects cocaine-induced neurochemical alterations in the brain, as we suggested previously when comparing single cocaine injections to binge cocaine injections (Unterwald et al., 2001). These findings are of particular interest in the context of the addiction-like cycle model, in that history prior to chronic re-exposure in this paradigm resulted in robust changes in the dopaminergic system, even after a relatively prolonged injection-free period that models abstinence/withdrawal. Follow-up studies with the "no-net flux" technique (Chefer et al., 2006), could further elucidate how an addiction-like cycle of chronic cocaine exposure and re-exposure influences dopamine re-uptake dynamics.

4.3. Cocaine effects on mRNA levels

4.3.1. Pdyn mRNA

The dynorphin/kappa opioid system in the striatum is thought to be involved in modulating the rewarding properties of cocaine. Cocaine administration has been found to increase *Pdyn* mRNA levels in the striatum (Fagergren et al., 2003; Schlussman et al., 2003b; Spangler et al., 1993; Spangler et al., 1997). The dynorphin/kappa opioid receptor system modulates dopamine levels in the same brain region (e.g. Zhang et al., 2004a, 2004b). In response to acute binge cocaine, 14-day cocaine exposure or re-exposure, *Pdyn* mRNA levels in the caudate putamen were significantly increased compared with those of the saline/saline re-exposure controls; however, no significant difference in *Pdyn* mRNA levels was found between the chronic cocaine exposure and re-exposure groups. Taken together, these findings support the conclusion that increased dynorphin gene expression is a recurrent counter-regulatory process to cocaine exposure. Current research suggests that dynorphin/kappa up-regulation underlies varied neuropsychiatric-like sequelae, including anhedonia, depression-like, and anxiety-like behaviors (e.g. Bruchas et al., 2009). Of interest, dynorphin up-regulation in dorsal striatal areas has been detected after chronic, but not short-term, cocaine self-administration in non-human primate models (e.g. Fagergren et al., 2003; Porrino et al., 2004).

4.3.2. Dopamine transporter (Slc6a3; DAT) mRNA

The dopamine transporter is the main direct pharmacodynamic target of cocaine and other psychostimulants (including methamphetamine), and governs cocaine-induced neurobiological effects by regulating extracellular dopamine levels (see above). We identified low, but measurable levels of DAT mRNA in the caudate putamen. This is in agreement with an earlier report from our laboratory demonstrating the presence of DAT mRNA in dopaminergic terminal fields (Maggos et al., 1997). The potential relevance of mRNA at axon terminals versus cell bodies has been investigated in earlier studies (e.g., Melia et al., 1994). Altered dopamine transporter function and phosphorylation in the caudate putamen have been reported in rats following chronic cocaine self-administration and extinction (Ramamoorthy et al., 2010). Since larger amounts of DAT mRNA are present in the midbrain where dopamine cell bodies are located, it would be of interest to measure Slc6a3 mRNA from samples in the midbrain.

It should be noted that some mice in the chronic cocaine/saline re-exposure groups were used for behavioral analysis while the mice in the chronic cocaine/saline exposure group were not. The differences observed in cocaine-induced dopamine levels and DAT mRNA between these groups are unlikely to be the result of subtle differences in the environmental context associated with daily removal from the home cage for behavioral testing. At the time of sacrifice, the chronic re-exposure group habituated to the testing environments during the 41 days of behavioral measurements, as is evident from the reduction of locomotor behavior of saline animals across the 41 days (Fig. 1). Furthermore, in the chronic cocaine re-exposure group, there were no significant differences in dopamine levels and gene expression between mice with or without locomotor measurement (data not shown).

4.4. Conclusions

In summary, an “addiction-like” cycle of chronic escalating-dose binge cocaine exposure, abstinence/withdrawal, and chronic re-exposure can help to delineate behavioral and underlying neurobiological and molecular adaptations that persist in the course of these stages, and crucial adaptations that have occurred specifically as a result of a stage modeling “full relapse”. This is one of the few experimental paradigms that have formally compared consecutive escalating cycles of cocaine exposure, separated by substantial injection-free periods (“abstinence”) (see also Ahmed and Koob, 1998; Dalley et al., 2005; with intriguing behavioral findings). Our findings point to significant differences in components of the dopaminergic system following chronic cocaine exposure versus chronic cocaine re-exposure. These differences may be related to the neurobiological roots of relapse to addictive diseases in human patients.

Disclosure/conflict of interest

The authors declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Acknowledgment

This work was supported by NIH-NIDA P60 DA05130 to Dr. Mary Jeanne Kreek.

Cocaine-HCl was generously provided by the NIH-NIDA Division of Drug Supply and Analytical Services.

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