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ORIGINAL INVESTIGATION

Sex differences in behavioral and PKA cascade responses to repeated cocaine administration

Luyi Zhou[1,2,3] · **Wei-Lun Sun**[3,4,5] · **Karen Weierstall**[4,5] · **Ana Christina Minerly**[4,5] · **Jan Weiner**[4,5] · **Shirzad Jenab**[4,5] · **Vanya Quinones-Jenab**[2,4,5]

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Abstract Previous studies have shown sex different patterns in behavioral responses to cocaine. Here, we used between-subject experiment design to study whether sex differences exist in the development of behavioral sensitization and tolerance to repeated cocaine, as well as the role of protein kinase A (PKA) signaling cascade in this process. Ambulatory and rearing responses were recorded in male and female rats after 1 to 14 days of administration of saline or cocaine (15 mg/kg; ip). Correspondent PKA-associated signaling in the nucleus accumbens (NAc) and caudate-putamen (CPu) was measured at each time point. Our results showed that females exhibited higher cocaine-induced behavioral responses and developed behavioral sensitization and tolerance faster than males. Whereas females developed behavioral sensitization to cocaine after 2 days and tolerance after 14 days, male rats developed sensitization after 5 days. In addition, cocaine induced a sexual dimorphic pattern in the progression of neuronal adaptations on the PKA cascade signaling in region (NAc vs. CPu) and time (days of cocaine administration)-dependent manners. In general, more PKA signaling cascade changes were found in the NAc of males on day 5 and in the CPu of females with repeated cocaine injection. In addition, in females, behavioral activities positively correlated with FosB levels in the NAc and CPu and negatively correlated with Cdk5 and p35 in the CPu, while no correlation was observed in males. Our studies suggest that repeated cocaine administration induced different patterns of behavioral and molecular responses in the PKA cascade in male and female rats.

Keywords Cocaine · Female · PKA · FosB · Addiction · Sex differences

Luyi Zhou and Wei-Lun Sun are equal contributors.

✉ Vanya Quinones-Jenab
vanya.quinones@hunter.cuny.edu

[1] Department of Biological Sciences, Hunter College of the City University of New York, New York, NY 10065, USA

[2] Department of Biology PhD Program, Graduate School and University Center of the City University of New York, New York, NY 10016, USA

[3] Present address: Department of Drug Discovery and Biomedical Sciences, University of South Carolina, Columbia, SC 29208, USA

[4] Department of Psychology, Hunter College of the City University of New York, New York, NY 10065, USA

[5] Department of Biopsychology and Behavioral Neuroscience PhD Subprogram, Graduate School and University Center of the City University of New York, New York, NY 10016, USA

Introduction

Women account for one third of the 1.5 million Americans who used cocaine in 2013 (National Survey on Drug Use and Health 2013). The current gender differences in cocaine use reflect differences in opportunity, rather than vulnerability to abuse of psychostimulants (Van Etten and Anthony 1999, 2001). As more attention is paid to sex-specific effects on drug abuse, it is becoming apparent that males and females react differently to cocaine. For instance, compared with men, women experience more nervousness after intranasal administration of cocaine, take longer to feel the subjective effects of cocaine, report less euphoria and dysphoria, take have more severe drug use at greater amounts of drugs, and have stronger craving in response to cocaine-associated cues (Kosten et al. 1996). One's sex also affects treatment outcome and/or relapse rates; women report shorter abstinence periods between cocaine uses than do men (Kosten et al. 1993). Women are also more likely than men to relapse after stressful or depressive life events (Back et al. 2005; Elman et al. 2001). However, mixed results have been found on

relapse after exposure to cocaine-paired environmental cues (Avants et al. 1995; Robbins et al. 1999; Sterling et al. 2004).

In rodents, sex differences are also present in cocaine-induced behavioral responses. For instance, females exhibit exaggerated locomotor responses to cocaine, develop behavioral sensitization more rapidly and with lower doses of cocaine, (reviewed in Becker and Hu 2008; Festa et al. 2004; Quinones-Jenab and Jenab 2010). Most of these studies use within-subject measure of sensitization, which compares the first injection with successive injections. Few studies have examined sex differences in cocaine sensitization using between-subject measure, in which animals were subjected to saline (acute cocaine group) or cocaine (chronic cocaine group) administration history with an injection of cocaine during the test. Previously, we used 14-day between-subject settings and observed sensitization in males but tolerance in females (Nazarian et al. 2009). However, the progression of sex difference of sensitization or tolerance development has not been fully characterized.

Compared to the relatively well-defined sex difference in behavioral response to cocaine, the underlying mechanism for the behavioral dimorphism is still unclear. Protein kinase A (PKA) cascade is critical for cocaine addiction. In the striatum, repeated cocaine administration increases the activation of PKA, phosphorylation of CREB (p-CREB), and expression of immediate-early genes, i.e., Fos, ΔFosB, and Jun (see McClung and Nestler 2008). Repeated cocaine administration also increases Cdk5, a downstream target of ΔFosB, and p35, a Cdk5 regulatory subunit (Bibb et al. 2001; Scheggi et al. 2004). While induction of p-CREB and Cdk5 is postulated to associate with tolerance to cocaine, PKA and FosB are known to associate with cocaine's behavioral sensitization (Benavides et al. 2007; Bibb et al. 2001; Carlezon et al. 1998; Hiroi et al. 1997; Miserendino and Nestler 1995; Pliakas et al. 2001). In the above studies, only male animals were used. The role of the PKA cascade in females or sex differences in cocaine addiction is mainly unexplored.

Some evidence has pinpointed the PKA pathway as a contributor to females' hypersensitivity to cocaine. In the nucleus accumbens (NAc), but not the caudate putamen (CPu), females have higher PKA protein levels both basally and after cocaine administration, while cocaine produced a long lasting CREB phosphorylation in males (Nazarian et al. 2009). However, the time course of the PKA cascade that changes along with the development of behavioral sensitization or tolerance is yet to be determined.

In this study, we use a multi-days between-subject experimental design to investigate the hypothesis that female rats develop a more robust and rapid locomotor sensitization and tolerance to cocaine. We also hypothesize that cocaine induces a sexual (male vs. female), regional (NAc vs. CPu), and temporal (days of cocaine administration) dimorphic pattern in the progression of neuronal adaptions of the PKA cascade.

Methods

Animals A total of 175 male and female Fischer rats of 60-day-old (Charles River, Raleigh, NC) were individually housed in Plexiglas chambers (20 × 20 × 41 cm) layered with beta chips. Rats were given free access to food and water and maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). All rats were weighed and handled for 7 days prior to testing. Animals were randomly assigned to experimental groups (n = 8–11/group). Animal care and use were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care Use Committee of Hunter College of CUNY.

Drug administration Cocaine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO). Cocaine solutions were prepared daily by dissolving the product in physiological saline (0.9 %). Throughout the study, all administrations were conducted in each rat's home cage. In the saline and repeated cocaine groups, all rats received once daily saline or cocaine (15 mg/kg; ip) injections for different administration lengths (2, 5, or 14 days). In acute cocaine groups, animals received daily saline injections, except on the day of test when cocaine was injected.

Locomotor activities measurements Ambulatory and rearing behaviors were measured in the home cage for each rat for 60 min after the last drug administration. Behaviors were monitored using a Cage Rack Photobeam Activity System from San Diego Instruments (San Diego, CA). The system consists of two frames placed around the rat's home cage. Ambulatory activity was determined by total counts of two consecutive photobeam interruptions in the lower frame. Rearing activity was represented as total counts of vertical motions detected by the upper frame.

Brain tissue dissection and protein preparation Right after the locomotor measurements, rats were decapitated (following a brief 20-s exposure to CO_2). After the brains were removed, they were flash frozen in 2-methylbutane (−40 °C) and stored at −80 °C until used. Brains were placed in a brain matrix (Braintree Scientific, Braintree, MA), and a 2 mm-thick coronal slice (2.7 to 0.7 mm anterior to Bregma) was cut. The CPu (dorsolateral region) and NAc (including NAc core and shell regions) were bilaterally dissected using 3- and 2-mm micropunchers, respectively. Brain tissues were then homogenized using a Polytron handheld homogenizer (Kinematica, Luzern, Switzerland) in a lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 2 mM EDTA, 10 % glycerol, 1 % Triton X-100, 1 % Igepal CA-630, 1 % sodium deoxycholic acid containing protease inhibitors mixture). The protein concentration was determined using a Bradford kit from Bio-Rad Laboratories (Hercules, CA).

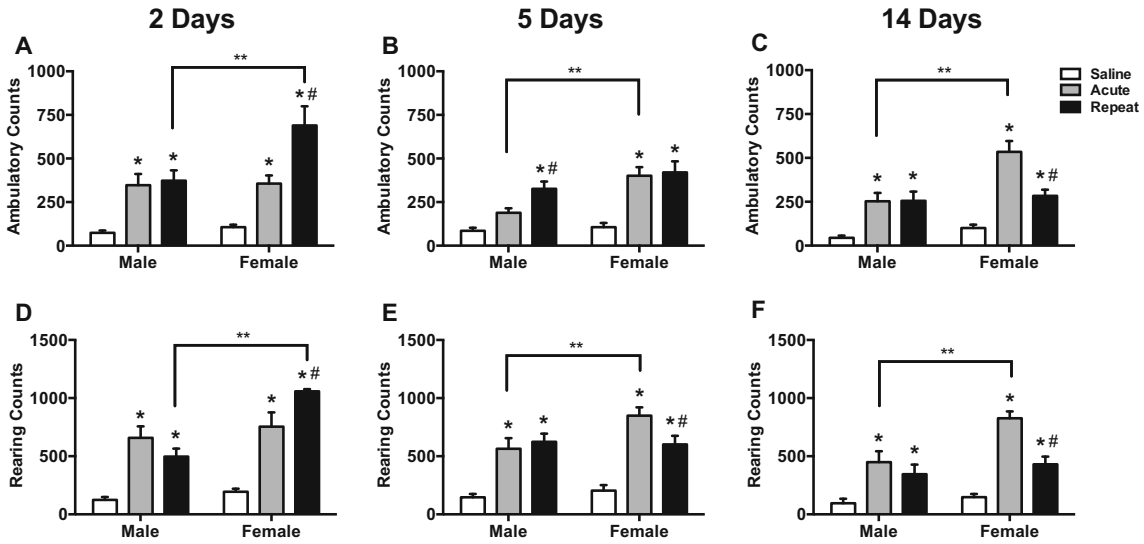


Fig. 1 Ambulatory (**a**–**c**) and rearing (**d**–**f**) responses to acute and repeated cocaine (15 mg/kg) administration after 2 (**a**, **d**), 5 (**b**, **e**), and 14 days (**c**, **f**). Data presented as mean ± SEM of the sum of ambulatory or rearing counts 1 h after injection. *Single asterisk* represents significant differences as compared with saline groups. *Number sign* represents significant differences between acute and repeated groups. *Double asterisks* represent significant differences between male and female rats for the same treatment groups ($p < 0.05$) ($n = 9$–11 per group)

Antibodies The antibody against PKA catalytic subunit (PKAc) was purchased from BD Biosciences (San Jose, CA), and p-CREB antibody was purchased from Millipore (Billerica, MA). Antibody recognizing both FosB and ΔFosB was purchased from Cell Signaling Technologies (Beverly, MA). Cdk5, p35, and α-tubulin antibodies were purchased from Santa Cruz Technologies (Santa Cruz, CA). All appropriate secondary antibodies were purchased from Amersham Pharmacia (Piscataway, NJ).

Western blot analysis Protein samples were boiled in Lammeli buffer containing 1 % β-mercaptoethanol and loaded onto 10 % SDS-PAGE gels. Gels were electrophoresed, transferred to nitrocellulose membranes, and blocked for 30 min with 5 % non-fat dry milk in tris-buffer-saline-tween (TBST) at room temperature. Membranes were then probed with primary antibodies overnight at 4 °C. After three washes with TBST, membranes were incubated with their appropriate secondary antibody for 60 min at room temperature, followed by three more washes with TBST. For normalization of protein levels, all membranes were re-probed with α-tubulin antibody followed by the secondary antibody. Antibody binding was detected using an enhanced chemiluminescence kit (Amersham Pharmacia, Piscataway, NJ). Films were then quantified using a computer densitometer and Image Quant Program (Molecular Dynamics).

Statistical analysis of data Behavioral data are presented as either mean total behavioral counts per 60 min ± SEM or mean of the count differences between acute and repeated groups ± SEM. Briefly, for calculating the count differences, the average behavioral counts of the corresponding group of acute cocaine-injected rats were subtracted from ambulatory or rearing counts of individual rats in the repeated cocaine-injected group. Protein levels are presented as mean % saline ± SEM after being normalized to their corresponding α-tubulin levels. Analysis of variance (ANOVA), followed by Tukey's post hoc tests, was used for both behavioral and protein-level analysis. A 95 % confidence interval was used to compare the behavior count differences with zero. Pearson correlation coefficient (r) was used for correlation between behavioral activities and protein levels. Determination of statistically significant differences was considered at the 0.05 probability level ($p < 0.05$).

Results

Sex differences in cocaine-induced locomotor activities As shown in Fig. 1a–c, significant main effects of drug in ambulatory responses in all three experimental manipulations were seen (2 days $F_{(2,50)} = 24.89$, $p < 0.001$; 5 days $F_{(2,54)} = 25.90$, $p < 0.001$; and 14 days $F_{(2,53)} = 30.49$, $p < 0.001$). Overall, regardless of sex or duration of drug administration, cocaine administration significantly increased ambulatory activity as compared with activity in saline controls ($p < 0.05$ for all comparisons). Significant main effects of sex in ambulatory responses were also seen (2 days $F_{(1,50)} = 5.33$, $p < 0.05$; 5 days $F_{(1,54)} = 11.20$, $p < 0.01$; and 14 days $F_{(1,53)} = 12.82$, $p < 0.001$), in which females had a higher number of ambulatory responses to cocaine than did males. Significant interactions between drug and sex were obtained after 2 and 14 days of injection, but not 5 days (2 days $F_{(2,50)} = 3.82$, $p < 0.05$; and 14 days $F_{(2,53)} = 5.53$, $p < 0.01$; but 5 days $F_{(2,54)} = 2.83$, $p = 0.068$). Post hoc tests indicated that females had higher ambulatory responses

after acute cocaine administration (in the 5- and 14-day paradigms; $p < 0.05$). Females also had higher ambulatory responses after 2 days of cocaine administration than did males ($p < 0.05$). In addition, in females, 2 days of cocaine injection produced a significantly higher number of ambulatory activities than in the corresponding acute cocaine-injected groups ($p < 0.05$), but after 14 days of drug injection, ambulatory activity was significantly lower than in those receiving acute cocaine administration ($p < 0.05$). However, no differences between acute and repeated cocaine-injected females were observed after 5 days of administration. In male rats, ambulatory activity was significantly higher only after 5 days of repeated cocaine administration as compared with the acute cocaine-injected group ($p < 0.05$).

Similar to ambulatory responses, significant main effects of drug on rearing responses were observed (2 days $F_{(2,50)} = 42.68$, $p < 0.001$; 5 days $F_{(2,54)} = 35.63$, $p < 0.001$; and 14 days $F_{(2,53)} = 32.32$, $p < 0.001$; see Fig. 1d–f). Regardless of sex or length of drug administration, rearing activity was significantly increased in animals receiving cocaine administration as compared with saline controls ($p < 0.05$ for all comparisons). Significant main effects of sex were seen after 2 and 14 days of drug injection, but not after 5 days of drug administration (2 days $F_{(1,50)} = 16.59$, $p < 0.001$; 14 days $F_{(1,53)} = 10.59$, $p < 0.01$; but 5 days $F_{(1,54)} = 3.799$, $p = 0.056$), which in general, female rats reared more than male rats. Moreover, significant interactions between drug and sex were seen for rearing responses after 2 and 14 days of administration, but not after 5 days

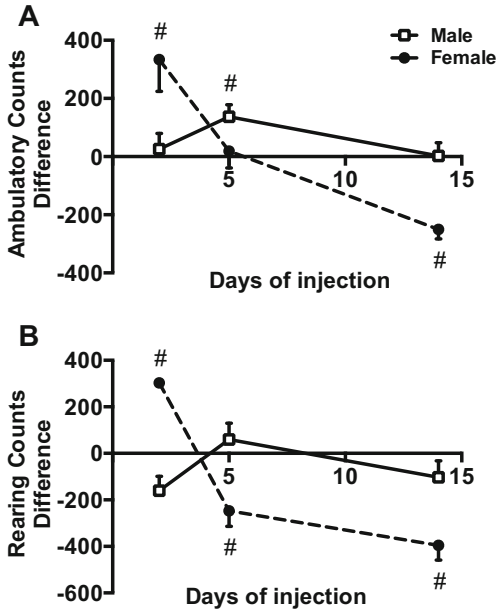


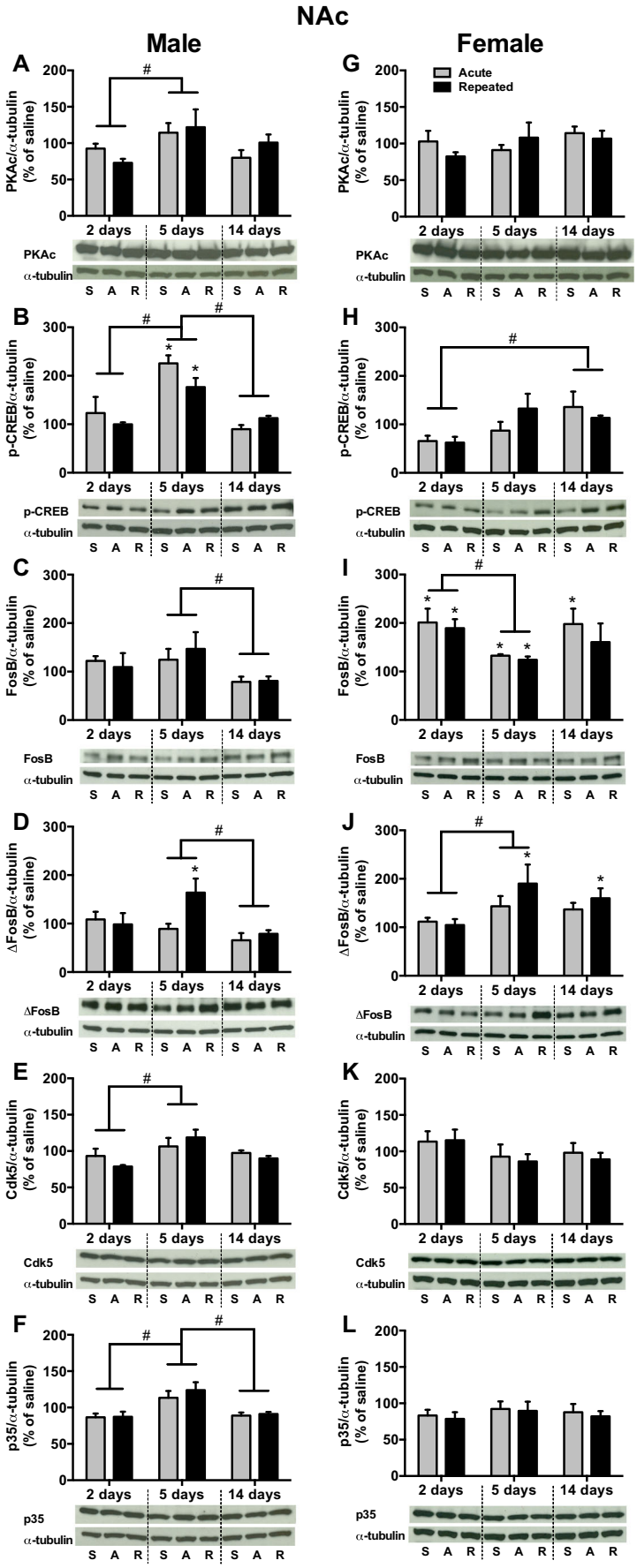
Fig. 2 Ambulatory (**a**) or rearing (**b**) counts differences between the acute and the repeated cocaine (15 mg/kg)-injected groups after 2, 5, and 14 days. Data presented as mean ± SEM of individual repeated counts—average acute counts. *Number sign* represents significant difference between acute and repeated groups at $p < 0.05$ level. *Solid line* and *squares* represent males' average responses. *Broken lines* and *solid triangles* represent females' average responses. nN = 9–11 per group; $p < 0.05$

Fig. 3 Protein induction levels of PKAc (**a**, **g**), p-CREB (**b**, **h**), FosB (**c**, **i**), ΔFosB (**d**, **j**), Cdk5 (**e**, **k**), and p35 (**f**, **l**) in the NAc of male (**a**–**f**) and female (**g**–**l**) rats after acute and repeated cocaine administration (15 mg/kg). Protein induction levels are expressed as mean % to saline ± SEM values. $N = 4$ per group. Representative immunoblots of respective proteins and α-tubulin are included in *below graphs*. *Number sign* represents significant difference between different lengths of cocaine administration. *Double asterisks* represent significant difference between acute and repeated cocaine administration. *Single asterisk* represents significant difference as compared with saline controls. *Gray bars* represent acute cocaine administration. *Black bars* represent repeated cocaine-injected groups. [*S* saline, *A* acute cocaine administration, *R* repeated cocaine administration; $p < 0.05$]

of administration (2 days $F_{(2,50)} = 7.46$, $p < 0.01$; 14 days $F_{(2,53)} = 3.88$, $p < 0.05$; but 5 days $F_{(2,54)} = 2.78$, $p = 0.070$). Post hoc tests showed that acute cocaine-induced higher rearing activities in female rats than male rats, but only in the 5- and 14-day paradigms ($p < 0.05$ for all comparisons). Females also reared more than males after 2 days of repeated cocaine injection ($p < 0.05$). Additional comparisons indicated that in female, 2 days of drug administration induced significantly higher rearing responses than acute cocaine administration ($p < 0.05$). However, after 5 and 14 days of cocaine administration, rearing responses were lower than with acute cocaine administration ($p < 0.05$). Male rats showed no differences in rearing responses between acute and repeated cocaine exposures.

As illustrated in Fig. 2, female rats developed behavioral sensitization (counts differences higher than 0) and tolerance (counts difference lower than 0) earlier than males. Specifically, after 2 days of cocaine, female rats developed sensitized ambulatory activities, but male rats did not develop ambulatory sensitization until after 5 days of cocaine. In addition, female rats developed ambulatory tolerance after 14 days, while no tolerance was observed in males within the longest length of cocaine injection. Compared to ambulatory activities, female rats develop tolerance of rearing response faster than both 5- and 14-day repeated cocaine administration induced tolerance, although 2 days of cocaine administration still induced sensitization. However, male rats did not develop sensitization or tolerance in rearing responses throughout the time course of drug exposure.

Sex differences in cocaine-induced responses in the PKA-signaling pathway in NAc As shown in Fig. 3, significant main effects for length of drug exposure were observed for PKAc, p-CREB, ΔFosB, Cdk5, and p35 protein induction levels in the NAc of male rats (PKAc $F_{(2,18)} = 3.84$, $p < 0.05$; p-CREB $F_{(2,18)} = 19.72$, $p < 0.001$; ΔFosB $F_{(2,18)} = 4.41$, $p < 0.05$; Cdk5 $F_{(2,18)} = 6.04$, $p < 0.01$; p35 $F_{(2,18)} = 12.18$, $p < 0.001$). However, the main effect for length of cocaine exposure in FosB protein induction levels was marginally significant at the $p = 0.054$ level ($F_{(2,18)} = 3.45$, $p = 0.054$). Post hoc analysis indicated that PKAc and Cdk5 induction levels were higher after 5 days of cocaine administration than after 2 days of drug injection; FosB and ΔFosB


NAc
Male
Female
Acute
Repeated
A
B
C
D
E
F
G
H
I
J
K
L
PKAc/α-tubulin (% of saline)
p-CREB/α-tubulin (% of saline)
FosB/α-tubulin (% of saline)
ΔFosB/α-tubulin (% of saline)
Cdk5/α-tubulin (% of saline)
p35/α-tubulin (% of saline)
2 days
5 days
14 days
PKAc
p-CREB
FosB
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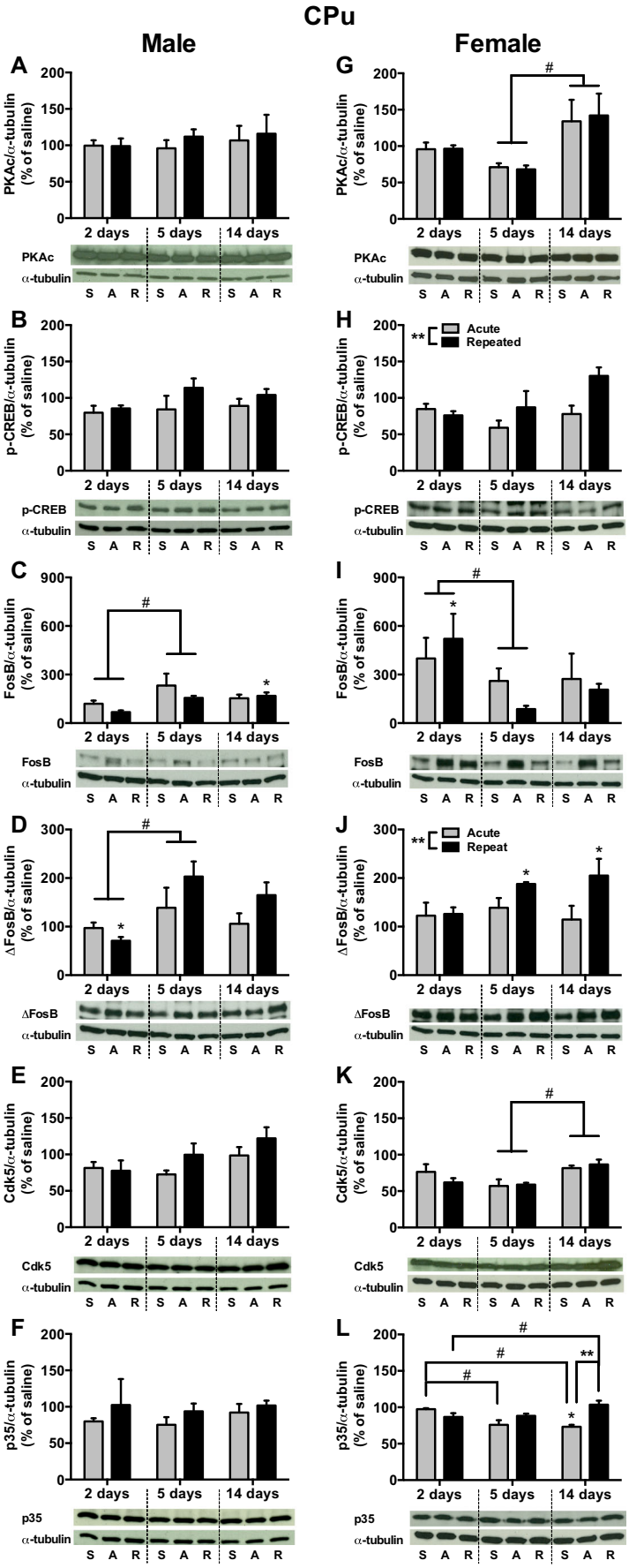
p35
α-tubulin
S A R S A R S A R


CPu
Male
Female
A
G
PKAc/α-tubulin (% of saline)
2 days
5 days
14 days
PKAc
α-tubulin
S A R S A R S A R
B
H
p-CREB/α-tubulin (% of saline)
Acute
Repeated
p-CREB
C
I
FosB/α-tubulin (% of saline)
FosB
D
J
ΔFosB/α-tubulin (% of saline)
Acute
Repeat
ΔFosB
E
K
Cdk5/α-tubulin (% of saline)
Cdk5
F
L
p35/α-tubulin (% of saline)
p35

◀ **Fig. 4** Protein induction levels of PKAc (**a**, **g**), p-CREB (**b**, **h**), FosB (**c**, **i**), ΔFosB (**d**, **j**), Cdk5 (**e**, **k**), and p35 (**f**, **l**) in the CPu of male (**a**–**f**) and female (**g**–**l**) rats after acute and repeated cocaine administration (15 mg/kg). Protein induction levels are expressed as mean % to saline ± SEM values. $N = 4$ per group. Representative immunoblots of respective proteins and α-tubulin are included in *below graphs*. *Number sign* represents significant difference between different lengths of cocaine administration. *Double asterisks* represent significant difference between acute and repeated cocaine administration. *Single asterisk* represents significant difference as compared with saline controls. *Gray bars* represent acute cocaine administration. *Black bars* represent repeated cocaine-injected groups. [*S* saline, *A* acute cocaine administration, *R* repeated cocaine administration; $p < 0.05$]

levels were higher after 5 days than after 14 days of repeated cocaine administration ($p < 0.05$ for all comparisons). For the other regulators—p-CREB and p35, protein induction levels were higher after 5 days than after 2 or 14 days ($p < 0.05$ for all comparisons). In addition, both acute and repeated cocaine administration increased p-CREB protein induction levels, but only repeated cocaine elevated ΔFosB levels, as compared with saline controls in the 5-day paradigm ($p < 0.05$ for all comparisons).

In the NAc of female rats, significant main effects for length of drug exposure were observed in p-CREB, FosB, and ΔFosB protein induction levels (p-CREB $F_{(2,18)} = 4.67$, $p < 0.05$; FosB $F_{(2,18)} = 3.96$, $p < 0.05$; ΔFosB $F_{(2,18)} = 3.88$, $p < 0.05$; see Fig. 3). p-CREB induction levels were lower after 2 days of cocaine administration than after 14 days of injection ($p < 0.05$ for both comparisons). On the other hand, after 2 days of drug administration, FosB induction levels were higher, while ΔFosB levels were lower than after 5 days of injection ($p < 0.05$ for both comparisons). In addition, regardless of cocaine administration length, both acute and repeated cocaine exposure elevated FosB protein induction levels as compared with their saline controls, except for 14-day repeated cocaine injection. ΔFosB levels were elevated after 5- and 14-day repeated cocaine administration as compared with their saline controls ($p < 0.05$ for all comparisons).

Sex differences in cocaine-induced responses in the PKA-signaling pathway in CPu As shown in Fig. 4, in the CPu of male rats, significant main effects for length of drug exposure were observed for FosB and ΔFosB protein induction levels (FosB $F_{(2,18)} = 4.37$, $p < 0.05$; ΔFosB $F_{(2,18)} = 5.75$, $p < 0.02$). FosB and ΔFosB levels were higher after 5 days of cocaine injection than after 2 days ($p < 0.05$ for both comparisons). In addition, 14 days of repeated cocaine administration increased the protein induction levels of FosB as compared with saline controls ($p < 0.05$). However, 2-day repeated cocaine administration decreased ΔFosB levels as compared with saline controls ($p < 0.05$).

In the CPu of female rats, significant main effects for length of drug exposure were observed for PKAc, FosB, and Cdk5 proteins (PKAc $F_{(2,18)} = 7.34$, $p < 0.01$; FosB $F_{(2,18)} = 3.73$, $p < 0.05$; Cdk5 $F_{(2,18)} = 7.26$, $p < 0.01$). Specifically, PKAc and Cdk5 protein induction levels were higher after 14 days of injection than after 5 days; while FosB levels were higher after 2 days of drug administration than after 5 days ($p < 0.05$ for all comparisons). Significant main effects for drugs were seen for p-CREB and ΔFosB (p-CREB $F_{(1,18)} = 5.42$, $p < 0.05$; ΔFosB $F_{(1,18)} = 6.19$, $p < 0.05$), in which repeated cocaine exposure increased p-CREB and ΔFosB levels as compared with acute cocaine. Furthermore, a significant drug main effect and interaction of drug × length were seen in p35 ($F_{(1,18)} = 8.93$, $p < 0.01$; $F_{(2,18)} = 11.05$, $p < 0.001$). Post hoc analysis indicated that after 14-day repeated cocaine injection, p35 protein induction levels were higher than with administration of acute cocaine on day 14 and higher than after 2 days of repeated administration ($p < 0.05$ for all comparisons). In addition, acute cocaine injection on day 5 and day 14 had significantly lowered p35 levels than acute cocaine administration on day 2, and the p35 level in animals receiving acute cocaine on day 5 was even lower than in saline controls ($p < 0.05$ for all comparisons). Lastly, after 5- and 14-day repeated cocaine exposure, ΔFosB protein induction levels were significantly higher than in the saline controls ($p < 0.05$ for both comparisons), while FosB levels were significantly increased after 2 days of repeated cocaine administration as compared with saline controls ($p < 0.05$ for both comparisons).

Sex differences in correlation between locomotor activities and protein levels As shown in Table 1, in the NAc, among all proteins examined, only the FosB protein levels were significantly correlated with ambulatory and rearing counts in female rats ($r = 0.49$, $p < 0.01$; $r = 0.50$, $p < 0.01$, respectively). FosB levels in the CPu of female rats also positively correlated with behavioral activities (ambulatory $r = 0.48$, $p < 0.01$; rearing $r = 0.55$, $p < 0.001$). Conversely, the Cdk5 protein levels in the CPu of female rats negatively correlated with locomotor activities (ambulatory $r = -0.36$, $p < 0.05$; rearing $r = -0.44$, $p < 0.01$). In addition, the protein levels of p35 also negatively correlated with locomotor activities (ambulatory $r = -0.41$, $p < 0.02$; rearing $r = -0.39$, $p < 0.02$). However, in male rats, no correlation between behavioral activities and levels of the studied proteins was observed in NAc or CPu (data not shown).

Discussion

This study reveals several novel findings. First, female rats develop behavioral sensitization more rapidly than males in a between-subject measurement setting. Second, only female rats are able to develop tolerance using an intermittent (once a day) cocaine administration paradigm. Third, cocaine-induced changes in the PKA cascade are sex, time, and region dependent. Finally, behavioral and PKA signaling response correlation was only observed in females, but not males.

Table 1 Correlation analysis between behavioral and PKA signaling responses after repeat cocaine administration in female rats

Area	Protein	Ambulatory	Rearing
NAc	PKAc	$r = 0.033$, $p = 0.846$	$r = -0.117$, $p = 0.498$
	p-CREB	$r = -0.017$, $p = 0.922$	$r = -0.098$, $p = 0.571$
	FosB	**$r = 0.489$, $p = 0.002$***	**$r = 0.445$, $p = 0.007$***
	ΔFosB	$r = 0.232$, $p = 0.173$	$r = 0.153$, $p = 0.373$
	Cdk5	$r = -0.018$, $p = 0.919$	$r = 0.077$, $p = 0.656$
	p35	$r = -0.178$, $p = 0.299$	$r = -0.089$, $p = 0.606$
CPu	PKAc	$r = -0.024$, $p = 0.889$	$r = -0.033$, $p = 0.847$
	p-CREB	$r = -0.175$, $p = 0.308$	$r = -0.213$, $p = 0.213$
	FosB	**$r = 0.480$, $p = 0.003$***	**$r = 0.552$, $p = 0.000$***
	ΔFosB	$r = 0.193$, $p = 0.260$	$r = 0.161$, $p = 0.348$
	Cdk5	**$r = -0.361$, $p = 0.031$***	**$r = -0.435$, $p = 0.008$***
	p35	**$r = -0.409$, $p = 0.013$***	**$r = -0.391$, $p = 0.018$***

*and bolded entries represent statistical significance

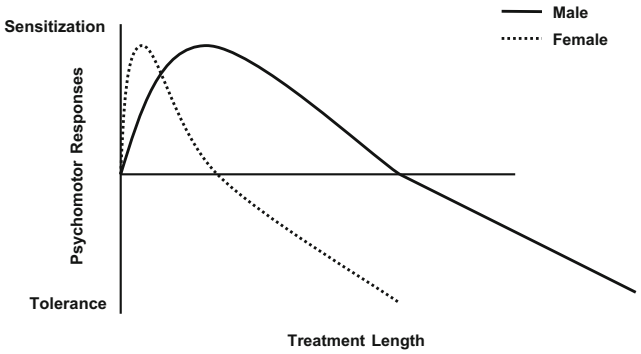


Fig. 5 Conceptual model of the locomotor response changes to repeated cocaine in males and females. *Solid line* represents hypothetical behavioral responses of males; *broken line* represents hypothetical behavioral responses in female rats. *Area above* the X-axis represents sensitization to behavioral responses. *Area below* the X-axis represents tolerance. As shown in this model, females develop behavioral sensitization and tolerance earlier than males, indicating their hypersensitivity to cocaine

Consistent with previous reports (reviewed in Festa et al. 2004), female rats in this study setting had higher behavioral responses to acute cocaine administration and more quickly developed behavioral sensitization to repeated cocaine injection than did males. We also observed that females developed behavioral tolerance to cocaine, while males did not develop tolerance in the length of cocaine treatment used in this study. It is noticeable that in male rats, behavioral tolerance to cocaine is achieved only via continuous (e.g., minipump) cocaine administration (Izenwasser and French 2002; King et al. 1992). The finding that female rats are able to develop tolerance using an intermittent (once a day) cocaine administration paradigm provides further evidence that females may be more sensitive to the behavioral and rewarding effects of cocaine. Based on these results, we propose a model (Fig. 5), in which females develop faster behavioral sensitization and tolerance to intermittent repeated cocaine administration, indicating different behavioral sensitivities in response to cocaine in male and female rats. However, behavioral tolerance in males is hypothetical, whether it can be achieved with this intermittent administration paradigm or how long it takes is still to be determined.

On the molecular level, we observed that repeated cocaine administration induced striatal PKA-mediated responses, including the induction of p-CREB, the expression of immediate-early genes (Fos and ΔFosB), and other regulatory proteins such as Cdk5 and p35 in both male and female rats. We also showed that the temporal pattern of cocaine-induced alterations and the location of where these changes occur (NAc or CPu) in this pathway are sexually dimorphic. Overall, in males, PKA signaling cascade was mostly changed in the NAc (5 out of 5 proteins) relative to CPu (2 out of 5 proteins), with consistent increases on day 5 when behavioral sensitization was observed. In females, more changes in the PKA signaling cascade was found in CPu (5 out of 5 proteins) than NAc (3 out of 5 proteins) but with no consistent changes on a particular day.

Prominent theories of addiction postulate that molecular and cellular adaptations in the striatal PKA signaling pathway may underlie changes from cocaine-seeking behaviors to addiction (Chen et al. 2009; Self 2004). Specifically, induction of PKA and FosB is known to associate with cocaine's behavioral sensitization. Previous studies have shown that intra-NAc infusion of PKA activator potentiates locomotor activity to repeated cocaine, and FosB mutant mice failed to develop behavioral sensitization after repeated cocaine administration (Hiroi et al. 1997; Miserendino and Nestler 1995). Our finding that increased induction of PKAc and FosB was observed on the day of behavioral sensitization in male rats is consistent with previous findings. However, on the same day, increased p-CREB, Cdk5, and p35 levels were also observed. Induction of p-CREB and Cdk5 is postulated to associate with tolerance to cocaine. For instance, greater activation of p-CREB in the NAc is associated with blunted psychomotor and rewarding effects in response to chronic cocaine (Carlezon et al. 1998; Pliakas et al. 2001). On the other hand, both pharmacological and genetic inhibitions of Cdk5 activity in the NAc of rodents resulted in super-sensitivity to locomotor and rewarding effects of repeated cocaine exposure (Benavides et al. 2007; Bibb et al. 2001). Thus, the increased induction of p-CREB, Cdk5, and p35 levels in the NAc of males may represent a positive feedback mechanism to counteract the induction of PKA and FosB. However, lack of correlation between PKA signaling response and behavioral response in males suggests that these molecular changes might only be compensatory phenomenon rather than critical causal effect on behavioral response. In female rats, we observed positive correlation between FosB levels and locomotor activities and negative correlation between Cdk5, p35 levels, and locomotor activities. Although in females, the directions of correlation are consistent with previous theories, the causal relationship still needs

further investigation. These findings suggest that in females, the combined opposite alterations in FosB and Cdk5/p35 may associate with the development of behavioral sensitization and tolerance.

There are several limitations in this study. First, in this study, cocaine was administered in the home cage. Evidences show that cocaine sensitization is easier to achieve when cocaine is injected in novel environment (Badiani et al. 1995). Thus, whether females still develop faster sensitization and tolerance or whether tolerance to cocaine in males could be achieved in novel environment needs further exploration. Second, direct comparison of PKA cascade protein levels between males and females is lacking. Previous studies have shown that females have higher PKA protein levels in the NAc both basally and after cocaine administration, which may contribute to their hypersensitivity to cocaine (Nazarian et al. 2009). It will be helpful to examine the role of sex difference in PKA cascade protein levels, in addition to the time course of protein induction in the behavioral response to chronic cocaine. Last, the gross dissection of brain tissues was used in the current study, which does not differentiate subregions or neuron subtypes associated with differential PKA cascade responses. For instance, a previous study indicated NAc subregion-specific up-regulation of ΔFosB after chronic cocaine treatment (Brenhouse and Stellar 2006). In addition, it has been shown that induction of ΔFosB by chronic cocaine was only observed in dopamine receptor 1-enriched medium spiny neurons (Lobo et al. 2013). Whether females shown a different pattern from males in these measurements is still to be determined. Answers to these questions are necessary for a more comprehensive understanding of the biological basis of sex differences in cocaine abuse and to develop sex-specific treatments for cocaine addiction.

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Compliance with ethical standards Animal care and use were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care Use Committee of Hunter College of CUNY.

Conflict of interest The authors declare that they have no conflict of interest.

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