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Fos expression induced by cocaine-conditioned cues in male and female rats

Luyi Zhou

Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425

Carla Pruitt, Christina B. Shin, Arturo D. Garcia, and Arturo R. Zavala

Department of Psychology, California State University, Long Beach, 1250 Bellflower Boulevard, Long Beach, CA 90840

Ronald E. See

Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425

Abstract

Previous studies have shown that female rats exhibit different patterns of drug seeking during multiple phases of cocaine addiction when compared with males. However, the underlying mechanisms for these sex differences remain largely unknown. Here, we used a cocaine self-administration/reinstatement model to examine neuronal activation, as determined by Fos expression, following cue-induced reinstatement of cocaine seeking in male and female rats. Fos expression revealed both similarities between sexes in some brain regions, as well as selective sexually dimorphic patterns. As compared to no cue control subjects, conditioned cues induced higher Fos expression in the Cg1 region of the anterior cingulate cortex, but lower expression in the nucleus accumbens in both males and females. Females exhibited higher Fos expression than males in multiple brain regions, including the agranular insular cortex, dorsal medial caudate-putamen, nucleus accumbens shell, ventral tegmental area, dorsal subiculum, and ventral CA1 and CA3 regions of the hippocampus. Notably, only Fos expression in the prelimbic cortex, nucleus accumbens shell, basolateral amygdala, and ventral subiculum correlated positively with lever responding in response to conditioned cues across males and females. These findings indicate that while sexually dimorphic Fos activation does occur, the relationship between cue-induced cocaine seeking and neuronal activation may be similar for males and females in key brain regions of the relapse circuit.

Keywords

Cocaine; Cues; Fos; Sex differences

Address correspondence to: Dr. Luyi Zhou Department of Neurosciences Medical University of South Carolina 173 Ashley Avenue, Charleston, SC 29425 TEL: 843-792-6333 FAX: 843-792-4423 zhou@musc.edu.

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Introduction

Important gender (human) or sex (animal) differences have been characterized in the development of addiction and relapse to drugs of abuse. Clinical studies have found significant gender differences in cocaine addiction, whereby women may be more vulnerable to some aspects of cocaine dependence. For example, women meet the criteria of drug dependence more quickly and enter treatment earlier than men and cocaine-dependent women report higher rates of cocaine use and shorter periods of abstinence (McCance-Katz et al. 1999; Westermeyer and Boedicker 2000; Griffin et al. 1989). Studies also suggest that higher frequent drug use can be triggered by negative situations in women, but positive situations in men (Waldrop et al. 2007). It is thus not surprising that women are more susceptible to craving and relapse resulting from stressful events or depression (McKay et al. 1996; Elman et al. 2001). However, studies on gender differences in response to cues have been mixed, with results showing that cocaine dependent women are more (Elman et al. 2001; Robbins et al. 1999), equally (Avants et al. 1995; Waldrop et al. 2010), or less (Sterling et al. 2004) reactive to cocaine associated cues than man.

Preclinical animal models of addiction have examined sex differences both during and after repeated cocaine self-administration. During the acquisition phase, female rats acquire cocaine self-administration faster than males (Jackson et al. 2006; Lynch and Carroll 1999). Greater motivation for cocaine has also been noted in that females exhibit higher responding (but not cocaine intake) than males on short-access (2 h/day) schedules of reinforcement (Kippin et al. 2005; Fuchs et al. 2005), enhanced cocaine intake on long access (6 h/day) schedules of reinforcement (Lynch and Taylor 2004; Roth and Carroll 2004), and higher breakpoints on progressive ratio schedules of reinforcement (Mello et al. 2007; Roberts et al. 1989). Females also exhibit greater resistance to extinction (Fuchs et al. 2005) and enhanced cocaine-primed reinstatement of cocaine seeking (Kippin et al. 2005), but fairly similar cue-induced reinstatement when compared to males (Fuchs et al. 2005; Feltenstein et al. 2011).

Two laboratory studies on gender differences in brain activity in human cocaine abusers under conditions of stimulated craving have been published. In one study that used PET, cerebral blood flow in response to cocaine-related imagery showed that cocaine dependent women had less activation of the amygdala, insula, ventral cingulate, and orbitofrontal cortex, but higher activation in other frontal cortical areas as compared to cocaine dependent men (Kilts et al. 2004). A more recent study using functional MRI showed that corticostriatal-limbic hyperactivity was linked to stress cues in cocaine dependent women and cocaine-associated cues in cocaine dependent men (Potenza et al. 2012). These clinical findings suggest neural circuit activation differences between cocaine dependent men and women under conditions that precipitate relapse to cocaine use.

Although sex differences in cocaine seeking in animal models of relapse have been characterized across various models of reinstatement, preclinical studies on sex differences in neural function in response to cocaine conditioned-cues (or any other relapse factor) have not yet been conducted. One recent study conducted only in male rats showed that cocaine-conditioned cues after a history of cocaine self-administration altered the expression of the immediate early gene product, Fos, in several brain areas (Kufahl et al. 2009). Several of

these regions (e.g., nucleus accumbens and hippocampus) have previously been shown to exhibit sex differences in several physiological parameters (Walker et al. 2000; Walker et al. 2006; Juraska 1991; Isgor and Sengelaub 2003, 1998; Parducz and Garcia-Segura 1993). In order to directly compare sexual dimorphisms in neural circuits that underlie relapse, we examined Fos activation in a large number of brain regions immediately following cue-induced reinstatement to cocaine seeking in female and male rats with a prior history of cocaine self-administration.

Materials and methods

Subjects

Age-matched male (initial weight 250–300 g) and female (initial weight 175–225 g) Sprague Dawley rats (Charles River Laboratories, Raleigh, NC, USA) were single housed in a temperature- and humidity-controlled animal facility on a reversed 12-h light–dark cycle (lights off at 06:00). All experimental procedures occurred during the dark cycle between 07:00 and 15:00. Animals were given *ad libitum* access to water and maintained on a diet of standard rat chow (Harlan, Indianapolis, IN, USA). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina and complied with federal guidelines in the “Guide for the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources on Life Sciences, National Research Council.

Surgery

Rats were anesthetized (IP) using a mixture of ketamine hydrochloride (Vedco Inc., St. Joseph, MO) and xylazine (Lloyd Laboratories, Shenandoah, IA) at 66 and 1.33 mg/kg, followed by Equithesin (4 mg/kg sodium pentobarbital, 17 mg/kg chloral hydrate, and 21.3 mg/kg magnesium sulfate heptahydrate dissolved in 44% propylene glycol and 10% ethanol solution) at 0.5 ml/kg. Ketorolac (Sigma-Aldrich, St. Louis, MO) was administered (2 mg/kg, IP) as a preoperative analgesic. One end of a silastic catheter was implanted into the right jugular vein and the other end ran subcutaneously and exited from a small incision just below the scapula and was attached to an infusion harness (Instech Solomon, Plymouth Meeting, PA) that provided access to an external port for IV drug delivery. To help maintain patency, daily IV infusion of 0.1 ml antibiotic cefazolin (100 mg/ml) and 0.1 ml heparinized saline (70 U/ml) was given after surgery and maintained throughout the length of self-administration (given after each session). An additional 0.1 ml heparinized saline (10 U/ml) was infused immediately prior to each self-administration session. Catheter patency was periodically verified with 0.1 ml (10 mg/ml, IV) methohexital sodium (Eli Lilly & Co., Indianapolis, IN), a short-acting barbiturate that produces a rapid loss of muscle tone.

Cocaine self-administration

Self-administration sessions were conducted in standard operant conditioning chambers housed in sound-attenuating cubicles and controlled by a computerized data collection program (MED-PC, Med-Associates, St Albans, VT). The chambers were equipped with two retractable levers, two stimulus lights (one above each lever), a tone generator, and a house light. Additionally, each chamber had a balanced metal arm and spring leash attached

to a swivel (Instech Solomon, Plymouth Meeting, PA). Tygon tubing extended through the leash and was connected to a 10 ml syringe mounted on an infusion pump located outside the cubicle. Cocaine hydrochloride (National Institute on Drug Abuse, Research Triangle Park, NC) was dissolved in 0.9% sterile saline. Prior to the start of each session, the catheter was connected to the tubing and the house light signaled the initiation of the session and remained on throughout the entire session. Presses on the active (i.e., cocaine-paired) lever resulted in a 2-s activation of the infusion pump for a 50 μ l infusion of cocaine at 0.20 mg (males) or 0.15 mg (females), resulting in approximately 0.6 mg/kg/infusion. However, daily cocaine intake was individually calculated for each animal based on the actual body weight. Cocaine infusions were paired with a 5-s presentation of a stimulus light and tone (4.5 kHz, 78 dB) complex. After each infusion, a 20-s time-out period occurred, during which responses on the active lever were recorded, but resulted in no programmed consequences. Presses on the inactive lever had no consequences. Rats self-administered cocaine during daily 2-h sessions along an FR1 schedule of reinforcement. Self-administration sessions continued for at least 10 sessions, with the criterion of 10 infusions per session.

Extinction and reinstatement of cocaine seeking

Following self-administration, animals underwent daily 2-h extinction sessions, during which responses on either lever had no programmed consequences. Extinction sessions continued for 7 sessions prior to the 2-h test session, for which males and females were assigned to either a Cues group or a No-Cues group with $n = 7$ for each group. In the Cues groups, rats underwent a cue-induced reinstatement test, whereby responses on the previously cocaine-paired lever resulted in a 5-s presentation of the light+tone cues in the same manner as during self-administration. If rats failed to press the active lever within the first minute of reinstatement, a single light+tone cue was presented to indicate the availability of cues. In the No-Cues controls, rats underwent an extra extinction session, whereby active lever presses had no consequences. In females, estrous cycle stages were monitored via vaginal lumen samples as previously described (Zhou et al. 2012), but were not differentiated based on a lack of evidence for estrous cycle differences.

Fos protein immunocytochemistry

Immediately following the test, rats were deeply anesthetized with Equithesin and then transcardially perfused with 150 ml ice-cold 0.9% saline, followed by 500 ml of 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 24 h, and then cryoprotected by submersion in 20% sucrose for at least 24 h. Brains were then sectioned into 40 μ m thick tissue sections, centered at anatomical locations from bregma at +3.24, +1.68, -2.52, and -5.88 mm (Paxinos and Watson, 1998). Fos was visualized by incubating the sections in rabbit anti-Fos (1:5,000, sc-52, Santa Cruz Biotechnology, Dallas, TX) overnight at room temperature, followed by incubation in biotinylated donkey anti-rabbit secondary antibody (1:500, Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 h, and incubation in avidin-biotin complex (1:500, Vector Laboratories, Burlingame, CA) for 1.5 h. Finally, sections were incubated in 3,3'-diaminobenzidine and nickel ammonium sulfate for 7 min, producing a purple-black reaction product in the nucleus. The

sections were mounted onto glass slides, air-dried, dehydrated in alcohols, and cover slipped for light-microscopic inspection.

Fos immunoreactivity was examined using an Olympus BX40 (Olympus Imaging America Inc., Center Valley, PA) microscope set at 200 $\times$ magnification and counted by an observer blind to treatment conditions. The anatomical locations and boundaries of each region were determined using a rat brain atlas (Paxinos and Watson, 1998) and are illustrated in Fig. 1. Sections taken at +3.24 mm from bregma contained the Cg1 region of the anterior cingulate cortex (Cg1), prelimbic (PrL), infralimbic (IL), orbital (Orb), and agranular insular (AgI) cortices. Sections taken at +1.68 mm from bregma contained the Cg2 region of the anterior cingulate cortex (Cg2), dorsal medial and dorsal lateral regions of caudate-putamen (dmCPu and dlCPu), and core and shell regions of the nucleus accumbens (NAcC and NAcS). Sections taken at -2.52 mm from bregma contained the lateral hypothalamus (LH), basolateral amygdala (BLA), and central amygdala (CeA). Sections taken at -5.88 mm contained the CA1 and CA3 regions of the ventral hippocampus (vCA1 and vCA3), dentate gyrus (DG), dorsal and ventral subiculum (dS and vS), and ventral tegmental area (VTA). The sections were selected such that the rostral-caudal extent of each region of interest was sampled (360 μ m). Fos immunoreactivity was counted and identified by a blue-black oval-shaped nucleus. Representative photomicrographs of Fos protein expression in the PrL and NAcS are shown in Fig. 2. Each region of interest was analyzed in both hemispheres from three-tissue sections/rat. There were a total of 6 sample areas counted (i.e., 1 sample area/2 hemispheres/3 sections). The counts from all of the sample areas within a given region were averaged to provide a mean number of immunoreactive cells per sample area. The area of each photographed sample was 0.0825 mm². The counts from all the sample areas from a given region were scaled to provide a mean number of Fos-positive cells per mm².

Data analysis

To evaluate sex differences in cocaine self-administration, a two-tailed t-test was used to analyze the average active lever presses and cocaine intake for the mean of the last 3 self-administration sessions between male and female rats. Mixed-factors, repeated-measures analysis of variance (ANOVA) was used to analyze active lever responses during extinction, with sex and session as the between- and within-subjects factors, respectively. Two-way ANOVAs were used to analyze active lever presses and regional Fos cell counts for cue-induced reinstatement, with sex and test as between-subject factors. Post hoc analyses were conducted using Bonferroni tests. All data were presented as the mean $\pm$ S.E.M., and α was set at $p < 0.05$, except for linear regression between active lever responding and Fos cell counts in response to conditioned-cues combining both males and females, where the α was set at $p < 0.01$. Data points were excluded from analysis if the Box plot method revealed them as extreme outliers with greater than 3 interquartile range (IQR) from the upper quartile, or smaller than 3 IQR from the lower quartile. With this criterion, a total of only 6 data points were excluded from the entire Fos data set. Based on the consistently low responses in all groups and no significant differences between groups, data for inactive lever presses are not presented.

Results

Both females and males exhibited typical patterns of stable cocaine self-administration, a reduction in responding across extinction, and robust cue-induced reinstatement. However, female rats showed modest, but consistently higher cocaine seeking across these different stages of the addiction cycle when compared to males. The total period of daily cocaine self-administration for all rats was 10.75 ± 0.13 sessions, with no differences between sexes. During the last 3 days of cocaine self-administration, female rats had higher active lever pressing ($t_{26}=3.93$, $p<0.001$) and cocaine intake ($t_{26}=3.27$, $p<0.01$) as compared to males (Fig. 3A and 3B). Across the 7 days of extinction (same duration in males and females), mixed-model ANOVA for lever pressing across the extinction sessions revealed a significant main effect for session ($F_{(6,156)}=41.94$, $p<0.001$), with a typical extinction curve seen in both males and females (Fig. 3C). A significant main effect for sex ($F_{(1,26)}=6.56$, $p<0.05$) was also observed. While a sex $\times$ session interaction failed to reach statistical significance, the data showed a clear pattern of higher responding in the females for the first three days of extinction. Females also reinstated their cocaine seeking more than the males when re-exposed to cocaine-paired cues. As shown in Fig. 3D, a two-way ANOVA for active lever presses indicated significant main effects for sex ($F_{(1,24)}=6.88$, $p<0.05$) and test ($F_{(1,24)}=68.52$, $p<0.001$), although the sex $\times$ test interaction just failed to reach statistical criteria ($F_{(1,24)}=4.22$, $p=0.051$).

As shown in Fig. 4, Fos expression revealed both similarities between the sexes in some brain regions, as well as sexually dimorphic patterns in other regions. A significant main effect of test was observed for Cg1 ($F_{(1,24)}=4.31$, $p<0.05$), NAcC ($F_{(1,22)}=16.66$, $p<0.001$), and NAcS ($F_{(1,23)}=17.97$, $p<0.001$). However, the direction of changes varied in that conditioned cues significantly increased Fos expression in Cg1, but decreased Fos expression in the NAcC and NAcS of both males and females as compared to the no-cues groups. In the NAcS, a significant main effect of sex was also observed ($F_{(1,23)}=14.10$, $p<0.01$), with higher Fos cell counts in females than males. Similar significant main effects of sex were observed in AgI ($F_{(1,24)}=9.60$, $p<0.01$), VTA ($F_{(1,23)}=6.08$, $p<0.05$), dS ($F_{(1,23)}=15.44$, $p<0.001$), vCA1 ($F_{(1,24)}=13.01$, $p<0.01$), and vCA3 ($F_{(1,24)}=9.15$, $p<0.01$). In all of these regions, females showed higher Fos expression than males. In the dmCPu, a significant main effect of sex ($F_{(1,23)}=7.45$, $p<0.05$) and a sex $\times$ test interaction ($F_{(1,23)}=4.50$, $p<0.05$) was seen. Post-hoc analyses revealed that females had higher Fos expression, but only following cue exposure. In addition to the changes shown in Fig. 4, Fos expression in a variety of other brain areas revealed no sex differences or effects of cue-induced reinstatement (Table 1).

Since changes in Fos activity by themselves may not necessarily be linked to cue driven cocaine seeking, we further studied the relationship of brain Fos activation and cue-induced reinstatement. We combined the data from both males and females in the subjects that underwent cue-induced reinstatement and conducted correlation analyses of Fos expression and cue paired lever responding. As shown in Fig. 5, significant correlations between active lever presses and Fos cell counts were only observed in four distinct brain regions, including the PrL ($r^2=0.50$, $p<0.01$), NAcS ($r^2=0.56$, $p<0.01$), BIA ($r^2=0.50$, $p<0.01$), and VS ($r^2=0.46$, $p<0.01$). Notably, all of these regions have been strongly implicated in the

reinstatement circuitry. All of the other examined brain regions failed to show significant positive or negative correlations with lever responding. Additionally, analyses of Fos expression and active lever responding in males and females of the No-Cues group indicated no significant correlation in any of the examined brain areas, suggesting that Fos expression in these four brain areas was specifically correlated to cue-induced reinstatement, but not lever responding in general.

Discussion

In the current study, several findings emerged regarding differences and similarities in Fos expression between females and males in response to cocaine-conditioned cues. Notably, male and female rats showed broadly similar Fos activation patterns in response to cocaine conditioned-cues, which induced higher Fos expression in the Cg1 region, but lower Fos expression in the NAcC and NAcS in both sexes. However, females also exhibited generally higher Fos activation than their male counterparts in a number of brain regions, including AgI, dmCPu, NAcS, VTA, dS, vCA1, and vCA3. Finally, although cue-induced Fos expression in the PrL, BIA, and vS did not differ from extinction levels, Fos in these three regions (as well as the NAcS) was strongly correlated with cue-induced cocaine seeking for both males and females. All four of these regions have been extensively implicated in reinstatement of cocaine seeking (McLaughlin and See 2003; Sun and Rebec 2003; See et al. 2007; Feltenstein and See 2008). Together, the current data suggest that while sex differences in Fos activation occur in some brain regions, the relationship between Fos activation and cocaine seeking in several key structures of the relapse circuit is similar for males and females.

In the present study, females exhibited slightly higher responding for cocaine reinforcement and cocaine intake as compared to males. The current results are consistent with previous studies in which females had consistently higher cocaine seeking, but equal (Kippin et al. 2005; Fuchs et al. 2005) or enhanced (Lynch and Taylor 2004; Roth and Carroll 2004) cocaine intake as compared to males. We also found that females exhibited more responding during early extinction and slightly higher responding during cue-induced reinstatement, reflecting an overall pattern of somewhat heightened motivation for cocaine seeking in females (Anker and Carroll 2011).

Males and females generally showed similar Fos patterns in response to cocaine conditioned-cues. Higher Fos activation was only observed in the Cg1, while lower Fos activation was observed in the NAcC and NAcS in both males and females in the Cues group when compared to the No-cues control of the same sex. Fos activation after cue-induced reinstatement was less robust than predicted, perhaps due to the more limited impact of the cue stimulus presentation. Compared to the FR1 schedule of reinforcement used in the current study, rats with a history of cocaine self-administration along an FR11 schedule of reinforcement reinstated to a higher degree during cue-induced cocaine seeking (Acosta et al. 2008). In a previous study (Kufahl et al. 2009), male rats trained on this more demanding FR11 schedule of reinforcement followed by a longer 17-day withdrawal period not only showed a higher number of active lever presses (mean of 178), but also exhibited greater Fos activation in several brain regions in response to conditioned cues as compared

to the current results. These differences are likely due to the different reinforcement value of the cues as a result of the prior history of reinforcement. However, it should be noted that we found both significant cue-induced reinstatement and activation of multiple brain areas following a history of reinforcement along an FR1 schedule, which is typically used in most reinstatement models of relapse. We have previously shown that the Cg1 region is a critical area for cue-induced reinstatement (McLaughlin and See 2003), although it is unclear why other regions did not show an increased Fos response. Notably, cocaine-experienced monkeys showed sustained neuronal firing in the anterior cingulate throughout the duration of discrete cue presentation (Baeg et al. 2009). The synchronization between Cg1 activation and cue presentation further suggests that it is a key site of cue mediation of cocaine seeking.

In the NAc, we found that Fos expression decreased in response to conditioned cues in both males and females. Fos changes in the NAc after cocaine-associated cues depends upon the method of prior conditioning and the type of cue presentation (Zavala et al. 2007; Kufahl et al. 2009; Miller and Marshall 2005, 2004; Ciccocioppo et al. 2001). While it is not clear as to why Fos decreased in the NAc, it may be related to alterations in glutamatergic receptor function upon cue exposure. Cue exposure attenuated withdrawal-induced surface expression of GluA1/2-containing AMPA receptors in the NAc (Boudreau et al. 2007), suggesting decreased glutamate signaling mediated by GluA1/2-containing AMPA receptors. More direct evidence comes from a recent study by LaLumiere et al. (2012), which hypothesized a possible decrease in AMPA signaling after cue-induced cocaine seeking. They showed that suppression of cue-induced cocaine seeking by IL activation was reversed by intra-NAcS microinjection of an AMPA receptor antagonist (LaLumiere et al. 2012). This finding suggests that cue-induced cocaine seeking potentially decreases AMPA-mediated signaling in the NAc, thereby reducing Fos expression. On the other hand, most neurons in the NAc are GABAergic interneurons. The reduced Fos response may have reflected a reduction in inhibitory GABAergic neuronal activity. Future assessment with double labeling procedures will be necessary to clarify the role of specific neuronal types.

Across males and females, Fos expression in the PrL, NAcS, BIA, and vS positively correlated with active lever responding in response to conditioned cues. Previous studies have shown that the PrL, BIA, and vS are all crucial for cue-induced reinstatement, as inactivation of these regions decreased cocaine seeking induced by conditioned cues (McLaughlin and See 2003; Sun and Rebec 2003). Notably, these three brain regions communicate extensively with each other. The vS projects directly to the BIA and PFC and receives input from the BIA and PFC (Groenewegen et al. 1990; Witter and Groenewegen 1990; Jay and Witter 1991; Pennartz et al. 1994; French and Totterdell 2003). In addition, projections exist between the BIA and PrL (Hoover and Vertes 2007; Gabbott et al. 2005). Thus, the positive correlations between Fos cell counts and cocaine-paired lever presses may reflect engagement of this circuit during the relapse event. The finding of a positive correlation in the NAcS was surprising, as inactivation of the NAcC, but not NAcS, abolished cue-induced reinstatement of cocaine seeking (Fuchs et al. 2004). However, inhibition of the NAcS can disrupt learning mechanisms engaged during a relapse event, suggesting that the NAcS plays an important role in goal-directed cocaine seeking (See et al. 2007). In further support of its role in drug seeking, the NAcS has been shown to be

necessary for contextual and drug-primed reinstatement to drug seeking (Anderson et al. 2003; Bossert et al. 2007; Bossert et al. 2012).

Females exhibited higher Fos expression than males in a number of regions, including the AgI, dmCPu, NAcS, VTA, DS, vCA1, and vCA3 regions. While the functional significance of these sex differences is unclear, such sexually dimorphic patterns have been seen for other measures of neuronal function, particularly dopamine. Compared with males, females have higher accumbal dopaminergic tone, with increased basal dopamine release and uptake (Walker et al. 2000; Walker et al. 2006). Females also show enhanced sensitivity to dopamine D1 receptor agonists (Schindler and Carmona 2002; Festa et al. 2006), as well as higher basal protein levels in D1 receptor-associated PKA/DARPP-32 cascades (Nazarian et al. 2009; Zhou et al. 2009), which may provide the physiological basis for higher activation by dopamine release. Taken together, the heightened dopamine system in females may be related to their increased Fos activation upon cue exposure in specific mesocorticolimbic areas.

Gonadal hormones may also play an important role in the noted sex differences. The basal firing activity of VTA dopamine neurons has been shown to be estrous cycle dependent, with highest activation in estrus and lowest in proestrus (Zhang et al. 2008). As compared to ovariectomized female rats, sham rats had higher dopamine neuron number and density, and greater inhibition of dopamine uptake in the VTA (Walker et al. 2012). However, estrogen treatment in ovariectomized rats promoted dopamine neuronal sensitivity to cocaine (Zhang et al. 2008). Notably, the effect of estrogen on stimulant-induced dopamine function appears to be sex specific, as estrogen treatment increased nicotine-evoked dopamine release in the striatum in females, but not males (Dluzen and Anderson 1997). Thus, gonadal hormones, especially estrogen, may play a modulatory role in the higher Fos expression in females.

In conclusion, the current data show unique patterns of neuronal activation in response to cocaine-conditioned cues in males and females. The direction of Fos induction by cocaine-associated cues was generally similar in males and females (including activation in the Cg1 and inhibition in the NAc), and brain regions that showed positive correlations between Fos activation and cocaine seeking were the same in both sexes. Females did show higher neuronal activation than males in several brain regions, including areas previously implicated in cue-induced reinstatement. These findings thus suggest that the magnitude and/or patterns of neuronal activation, rather than different neuroanatomical locations, might play a key role in the previously noted sex differences in cocaine seeking. Further examination of sexually dimorphic neuronal activity in males and females may provide new insights for potential gender-based treatment strategies in drug addiction.

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Fig. 1.

Schematic representation of coronal sections of the rat brain taken at +3.24, +1.68, -2.52, and -5.88 mm from Bregma (Paxinos and Watson, 1998). Encircled numbers in the sections represent the regions analyzed for Fos expression as follows: (1) Cg1 region of the anterior cingulate cortex (Cg1); (2) prelimbic cortex (PrL); (3) infralimbic cortex (IL); (4) orbital cortex (Orb); (5) agranular insular cortex (AgI); (6) Cg2 region of the anterior cingulate cortex (Cg2); (7) dorsal medial caudate-putamen (dmCPu); (8) dorsal lateral caudate-putamen (dlCPu); (9) nucleus accumbens core (NAcC); (10) nucleus accumbens shell (NAcS); (11) lateral hypothalamus (LH); (12) basolateral amygdala (BLA); (13) central amygdala (CeA); (14) dorsal subiculum (dS); (15) dentate gyrus (DG); (16) ventral CA1 region of the hippocampus (vCA1); (17) ventral CA3 region of the hippocampus (vCA3); (18) ventral tegmental area (VTA); (19) ventral subiculum (vS).

Fig. 2.

Representative photomicrographs of Fos protein expression in the PrL (**A**) and NAcS (**B**). Magnified samples of Fos-labeled PrL and NAcS taken at 200× magnification are separately shown from males and females in the No-Cues and Cues group. Fos protein expressions were identified as dark ovals (highlighted by arrows).

Fig. 3.

Sex differences in cocaine seeking during self-administration, extinction, and reinstatement. Data are shown for active lever presses (**A**) and cocaine intake (**B**) during cocaine self-administration, and lever presses during extinction (**C**) and cue-induced reinstatement (**D**). Significant differences are indicated for females compared to males ($*p < 0.05$) or conditioned cues compared to the corresponding no-cues controls of the same sex ($^{\dagger}p < 0.05$).

Fig. 4.

Fos expression in brain regions with sex or test differences. Fos cell counts in the No-Cues and Cues group of both male and female rats in the Cg1, AgI, dmCPu, NAcC, NAcS, VTA, dS, vCA1 and vCA3 regions. Significant differences are indicated for females compared to males ($*p < 0.05$) or conditioned cues compared to the corresponding no-cues controls of the same sex ($†p < 0.05$).

Fig. 5. Correlations between Fos expression and cue-induced cocaine seeking. Fos cell counts in PrL, NAcS, BIA and vS were plotted against active lever presses of both male and female rats in the Cues groups.

Table 1

Fos cell counts in brain areas that did not show sex or test differences.

Brain Region	Male No-Cues	Male Cues	Female No-Cues	Female Cues
PrL	177.9 ± 25.1	166.1 ± 25.8	179.9 ± 23.6	217.1 ± 21.1
IL	131.1 ± 27.0	162.3 ± 30.9	172.3 ± 27.7	231.9 ± 39.9
Orb	297.3 ± 39.2	315.5 ± 38.5	375.3 ± 72.8	410.4 ± 70.0
Cg2	214.6 ± 21.2	218.8 ± 30.0	229.2 ± 15.9	248.2 ± 20.7
d1CPu	13.6 ± 3.7	11.4 ± 4.8	19.9 ± 7.5	12.6 ± 3.9
B1A	78.8 ± 17.7	107.7 ± 19.8	133.4 ± 19.1	130.5 ± 25.4
CeA	53.1 ± 14.5	51.0 ± 16.3	58.9 ± 11.2	39.5 ± 12.8
LH	60.2 ± 14.2	66.8 ± 7.5	98.7 ± 12.1	79.4 ± 16.7
DG	37.2 ± 4.8	36.1 ± 6.4	41.4 ± 7.3	50.7 ± 3.8
vS	339.2 ± 66.4	245.6 ± 39.2	225.2 ± 26.2	294.3 ± 32.6

Data are presented as mean ± SEM.