Published in final edited form as:

Behav Brain Res. 2014 March 15; 261: 71–78. doi:10.1016/j.bbr.2013.12.012.

Chronic wheel running affects cocaine-induced c-Fos expression in brain reward areas in rats

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Abstract

Emerging evidence from human and animal studies suggests that exercise is a highly effective treatment for drug addiction. However, most work has been done in behavioral models, and the effects of exercise on the neurobiological substrates of addiction have not been identified. Specifically, it is unknown whether prior exercise exposure alters neuronal activation of brain reward circuitry in response to drugs of abuse. To investigate this hypothesis, rats were given 21 days of daily access to voluntary wheel running in a locked or unlocked running wheel. Subsequently, they were challenged with a saline or cocaine (15 mg/kg, ip) injection and sacrificed for c-Fos immunohistochemistry. The c-Fos transcription factor is a measure of cellular activity and was used to quantify cocaine-induced activation of reward-processing areas of the brain: nucleus accumbens (NAc), caudate putamen (CPu), medial prefrontal cortex (mPFC), and orbitofrontal cortex (OFC). The mean fold change in cocaine-induced c-Fos cell counts relative to saline-induced c-Fos cell counts was significantly higher in exercising compared to control rats in the NAc core, dorsomedial and dorsolateral CPu, the prelimbic area, and the OFC, indicating differential cocaine-specific cellular activation of brain reward circuitry between exercising and control animals. These results suggest neurobiological mechanisms by which voluntary wheel running attenuates cocaine-motivated behaviors and provide support for exercise as a novel treatment for drug addiction.

Keywords

cocaine; exercise; wheel running; c-Fos; neurobiology; reward; environmental enrichment

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1. Introduction

A growing body of clinical and preclinical research has shown that physical exercise is effective in preventing and treating drug abuse. Controlled laboratory studies in humans demonstrated that exercise decreased cravings for alcohol [1], cigarettes [2], and cannabis [3] and alleviated symptoms of tobacco withdrawal [2, 4, 5, 6, 7]. Further, rodent models of the human drug abuse process have shown that exercise in the form of voluntary wheel running delayed acquisition of cocaine self-administration [8], prevented the escalation of cocaine intake [9, 10], and attenuated reinstatement of cocaine-seeking behavior (relapse) [11, 12, 13]. Long-term daily wheel running also prevented behavioral sensitization to cocaine [14, 15] and decreased motivation to lever-press for cocaine [16] and heroin [17] under a progressive ratio schedule.

However, despite promising evidence for exercise in the treatment of drug abuse, not much is known regarding the effects of exercise on the neurobiological substrates of addiction. Drug reward involves activation of the mesolimbic dopamine system, which consists of dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and areas of the limbic forebrain such as the prefrontal cortex (PFC) [18, 19, 20]. Repeated exposure to drugs of abuse such as cocaine or natural rewards such as physical exercise can produce plasticity in this pathway. For example, both chronic cocaine and voluntary wheel running upregulated the transcription factor FosB in the NAc [21, 22, 23], tyrosine hydroxylase in the VTA [23, 24], and dynorphin in the medial caudate putamen (CPu) [25]. However, while wheel running can activate and elicit changes in brain areas implicated in the pathophysiology and treatment of addiction, it has not been yet associated with altered neuronal responses to drugs of abuse.

The present study investigated the hypothesis that prior long-term voluntary wheel running would influence cocaine-induced activation of brain reward areas. Rats were given 21 days of voluntary access to a locked or unlocked running wheel before being challenged with a cocaine or saline injection and then sacrificed for immunohistochemical labeling of c-Fos. C-Fos is a transcription factor and a product of *c-fos*, a member of the immediate early gene family [26]. C-Fos expression is a measure of neuronal activation in the central nervous system, and stimuli such as drugs of abuse [26], exercise [27], and sexual behavior [28] elicit its expression in reward processing areas of the brain. We quantified c-Fos immunoreactivity in the NAc, CPu, mPFC, and OFC to investigate whether a history of chronic exercise would alter the activation of this reward circuitry in response to cocaine.

2. Material and methods

2.1 Subjects

Thirty-one female Wistar rats approximately 90 days of age were the subjects in this experiment. Female rats were studied, as they readily acquire voluntary wheel running [29], they run more than males [30, 31, 32, 33], and they are more sensitive than males to the attenuating effects of wheel running on cocaine-maintained behaviors [9, 31, 34,]. Rats were purchased from Harlan Sprague-Dawley, Inc. (Madison, WI, USA), and upon arrival in the laboratory, they were pair-housed in standard polycarbonate cages for a minimum of 3 days

before the experimental protocol began. Once behavioral testing commenced, animals were singly-housed in operant conditioning chambers for 21 days. Following behavioral testing, rats were singly-housed in polycarbonate cages for 7 days before immunohistochemical processing of brain tissue. Water was available ad libitum at all times; rat chow (Harlan Teklad 2018, Harlan Sprague-Dawley, Inc., Madison, WI, USA) was available ad libitum except during behavioral testing, when it was only freely available outside of daily experimental sessions. Body weights were taken once/week. All laboratory conditions were temperature- and humidity-controlled with a 12-h light/dark cycle with lights on at 6 am.

2.2 Drugs

Cocaine HCl was provided by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC, USA). Cocaine was dissolved in sterile saline at a concentration of 8 mg/ml and refrigerated until use.

2.3 Wheel running

During behavioral testing, rats were housed in custom-built, octagonally-shaped operant conditioning chambers with access to an adjoining running wheel (Med Associates, Inc., St. Albans, VT, USA) through a guillotine-style door. Within these chambers, rats had daily access to either a locked or unlocked running wheel for 21 days. The locked running wheel provided daily access to environmental stimulation of the wheel without the ability to run. At session onset (9 am), a house light (4.76 W) illuminated, and the experimenter opened the door to the attached running wheel. Rats had free access to the locked or unlocked running wheel from 9 am until 3 pm (6-h session), when the house light extinguished and the wheel door was closed by the experimenter. Running wheel access was given during the light phase of the light-dark cycle to correspond to earlier work by our laboratory investigating the effects of wheel running on cocaine-motivated behaviors during this same daily time frame [10, 11, 31, 36].

2.4 Acute cocaine exposure

After 21 days of wheel access, rats underwent a “rest” period of 7 days to allow the acute effects of wheel running to diminish [35] before being challenged with a cocaine (15 mg/kg, ip) or saline (equivalent volume, ip) injection. The cocaine dose was chosen because it is in the range of doses that elicited differential reinstatement of cocaine seeking in rats screened for high vs. low wheel running [36], and it was sensitive enough to evoke differential c-Fos activation in brain reward areas in rat models of individual differences in drug abuse vulnerability [37]. Each cohort consisted of 4-8 rats and included at least one rat from each group. The combination of wheel running and cocaine treatment yielded 4 randomly-assigned treatment groups: unlocked/cocaine (N = 9), unlocked/saline (N = 7), locked/cocaine (N = 10), and locked/saline (N = 5).

2.5 c-Fos immunohistochemistry

Between 8-10 am, rats were injected with cocaine or saline (i.p.), and 90 min later, they were anesthetized with Sleepaway (26% sodium pentobarbital, 7.8% isopropyl alcohol, 20.7% propylene glycol, and distilled water; 0.5-0.75 ml/animal, i.p., Fort Dodge

Laboratories, Fort Dodge, IA) and intracardially perfused with 25 mM phosphate-buffered saline (PBS, pH 7.6) for 2 min followed by 4% paraformaldehyde in 25 mM PBS for 20 min. Brains were post-fixed in 4% paraformaldehyde for 2 h and then transferred to 10% sucrose in PBS overnight. Using a freezing microtome, 40 μ m coronal sections were taken at the level of the orbitofrontal cortex (OFC), medial prefrontal cortex (mPFC), caudate-putamen (CPu), and nucleus accumbens (NAc). Alternate tissue slices were used for immunohistochemical processing. Slices were rinsed in 25 mM PBS with 0.1% bovine serum albumin (BSA) then incubated in rabbit antibody to c-Fos (sc-52, Santa Cruz Biotechnologies, Inc., Santa Cruz, CA, USA) at 1:3000 in 25 mM PBS with 0.1% BSA and containing 0.03% Triton X-100 for a total of 48 h (24 h at room temperature followed by 24 h at 4° C). After primary antibody incubation and a series of three 10 min rinses, sections were incubated for 45 min in biotinylated anti-rabbit IgG (1:200, Vector Laboratories, Burlingame, CA, USA) in 25 mM PBS with 0.1% BSA, rinsed in PBS with BSA, then incubated in avidin-biotin horseradish peroxidase complex (Vector Laboratories) at 1:50 in 25 mM PBS with 0.1% BSA for 45 min with each incubation separated by a series of three 10 min rinses. Sections were then rinsed for 10 min in 0.05 M Tris buffer (pH 7.6) and incubated in 0.08% diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich Chemicals, St. Louis, MO, USA) in Tris buffer with 0.003% hydrogen peroxide for 5 min. Nickel chloride (0.03%) was added to the DAB solution to enhance staining. Sections were put through a final series of rinses with Tris and then deionized water. Finally, they were mounted on Adhesion Superfrost Plus slides (Brain Research Labs, Waban, MA, USA) and left to dry overnight. After sections were dry, they were dehydrated, cleared, and cover-slipped using DPX (Sigma-Aldrich Chemicals, St. Louis, MO, USA).

2.6 Data analysis

Daily wheel revolutions were recorded for the groups with access to unlocked running wheels, and mean revolutions over the 21 days were analyzed with a 2-tailed Student's t-test. Mean age and body weight were also compared among the 4 groups using separate 2-factor ANOVA with GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD, USA). Differences were considered significant if $p < 0.05$.

Brain regions from all groups were included in the c-Fos analysis. As previously described [37], a single tissue section was selected for each brain area to ensure that our cell counts were taken from the same rostral-caudal level for all animals. To validate the rostral-caudal location of the tissue section, we took measurements of the distance between landmarks in which this measurement changes monotonically through the nucleus. For example, at the level of the nucleus accumbens the medial-lateral distance between the medial edge of the anterior commissure and the tip of the lateral ventricle shrinks with the progression through tissue sections in the caudal direction. For the nucleus accumbens and caudate analyses, tissue sections for analysis had to have the same measurement between these structures. The brain regions selected for analysis, with the level relative to Bregma as determined from Paxinos and Watson [38], included subregions of the mPFC, such as the cingulate cortex (Cg1) (+2.20 mm), prelimbic area (PrL) (+2.20 mm), infralimbic area (IL) (+2.20 mm); the OFC (+3.70 mm); the dorsomedial and dorsolateral CPu (+1.28 mm); and the NAc shell and core (+1.28 mm). Rectangular counting boxes (300 $\times$ 637 μ m) were positioned on digital

images of each brain area without regard to c-Fos labeling. Representative examples of the coding box placement for the the NAc and the CPu and also the mPFC subregions and the OFC are shown in Figures 1 and 2, respectively. These digital images then were coded and counted by an individual blind to the coding scheme. Basal c-Fos cell counts among the saline-treated groups were analyzed by a 2-factor (wheel access X brain region) ANOVA to assess potential unstimulated differences in c-Fos labeling. Because significant differences were detected among the saline treated animals, a mean fold change was calculated by dividing all of the individual counts for the rats in the cocaine-treated groups by the mean count for their respective saline-treated group. Subsequently for each brain area, fold changes for unlocked and locked wheel access groups were compared using 2-tailed Student's t-tests.

In order to investigate the possible relationship between exercise exposure and c-Fos immunoreactivity in response to cocaine in these brain areas, wheel revolutions for the unlocked wheel access groups (e.g., unlocked/cocaine, unlocked/saline) were correlated with the total c-Fos cell counts for each brain region using separate Pearson's product-moment correlations (R Package 2.13.1, R-project.org).

3. Results

3.1 Wheel running, age, and body weight

Groups with access to unlocked running wheels did not differ in mean ($\pm$ SEM) wheel revolutions (unlocked/saline: 770 ± 315 ; unlocked/cocaine: 940 ± 244) over the 21 days of access (Fig. 3). Further, none of the groups differed significantly in mean age at the start (unlocked/saline: 92 ± 1 ; unlocked/cocaine: 92 ± 1 ; locked/saline: 91 ± 1 ; locked/cocaine: 91 ± 2) or mean body weight (unlocked/saline: 282 ± 8.22 g; unlocked/cocaine: 285 ± 5.70 g; locked/saline: 289 ± 6.66 g; locked/cocaine: 306 ± 8.00 g) over the course of the experiment.

3.2 Acute cocaine-induced c-Fos expression

Mean total c-Fos cell counts differed among the saline-treated locked and unlocked wheel access groups (baseline conditions; Table 1). There was a main effect for the locked wheel access groups to have greater total c-Fos counts than the unlocked wheel access groups throughout areas of the striatum and the cortex, including NAc shell ($F_{1,30} = 53.7$, $p < 0.0001$), NAc core ($F_{1,37} = 13.3$, $p = 0.002$), dorsomedial CPu ($F_{1,29} = 10.5$, $p = 0.003$), dorsolateral CPu ($F_{1,29} = 5.75$, $p = 0.02$), PrL ($F_{1,30} = 5.17$, $p = 0.03$), IL ($F_{1,30} = 5.73$, $p = 0.02$), and also the OFC ($F_{1,29} = 4.87$, $p = 0.04$). Further, there was a main effect of greater c-Fos cell counts in cocaine-treated vs. saline-treated groups in the NAc shell ($F_{1,30} = 4.85$, $p = 0.04$), NAc core ($F_{1,37} = 9.04$, $p = 0.008$), and dorsolateral CPu ($F_{1,29} = 20.8$, $p = 0.0001$) of the striatum. However, there was no wheel access X treatment interaction.

Given baseline differences in c-Fos-reactive cell counts (Table 1), mean fold change in c-Fos cell counts (i.e., increase in cocaine-induced c-Fos cell counts over saline-induced c-Fos cell counts) were analyzed. Mean fold change in response to cocaine differed among animals with access to wheel running compared to controls (Fig. 4). The unlocked wheel

access group had significantly greater mean fold change in c-Fos expression in the NAc core ($t_{17} = 2.14$, $p = 0.0473$), dorsomedial CPu ($t_{17} = 2.48$, $p = 0.0239$), and dorsolateral CPu ($t_{17} = 3.35$, $p = 0.0038$) subregions of the striatum. Also, while there was only a trend for greater c-Fos expression in the Cg1 ($t_{17} = 2.09$, $p = 0.0516$), there was significantly larger mean fold change in the PrL ($t_{17} = 1.06$, $p = 0.0339$) and OFC ($t_{17} = 4.02$, $p = 0.0009$) in rats with unlocked vs. locked wheel access.

C-Fos cell counts were not significantly correlated with mean wheel revolutions in either the cocaine-treated or the saline-treated groups with access to an unlocked running wheel (data not shown), perhaps due to small sample sizes.

4. Discussion

The results of the present study indicated that prior chronic exercise (e.g., voluntary wheel running) exposure specifically increased the neuronal response to acute cocaine compared to control conditions (e.g., locked running wheel). Increases in cocaine-induced cellular activation following 21 days of voluntary wheel running occurred in the NAc core, the dorsomedial and dorsolateral CPu, the PrL subregion of the mPFC, and the OFC. As these brain areas are critical for the development of drug abuse [18, 19, 20] the results indicate that chronic exercise induces neuroplasticity that may impact the progression and treatment of addiction.

Exercise-mediated changes in the neuronal response to cocaine likely resulted from the considerable overlap among the neurobiological substrates engaged by exercise and cocaine. Initial cocaine reward is hypothesized to depend on dopamine release in the NAc [20], and the NAc serves a critical role in the acute reinforcing effects of drugs of abuse by integrating input from the PFC, amygdala, and hippocampus and sending output to motor areas [39, 40]. Wheel running was shown to increase c-Fos [27] and delta FosB in the NAc core [22], or both the core and shell [35, 23]. While the NAc shell appears to be more responsive to the primary reinforcing effects of drugs [41, 42], the core is thought to mediate responding to cues associated with drugs or natural rewards [43, 44, 45]. Further, the NAc core has also been shown to contribute to cocaine-induced reinstatement. The dorsal mPFC - NAc core - ventral pallidum circuit is critical for the reinstatement of cocaine-seeking behavior elicited by cocaine priming injection [46], and the present results indicate changes in cocaine-induced activation of both the NAc core and the PrL, a dorsal subregion of the mPFC. Given prior work demonstrating an attenuation of cocaine-induced reinstatement with access to a voluntary wheel running [11, 13], the effect of exercise on reinstatement and other relapse-related behaviors may be partly mediated by its actions in these brain areas.

Related to its role in cocaine-induced reinstatement in animal models, the mPFC is activated during drug- and cue-induced craving in humans [47]. Adaptations such as increased phosphorylation of ERK in the mPFC have been implicated in the incubation of cue-induced cocaine seeking in rats [48], and both pERK and cue-induced drug seeking were reduced by daily wheel running [12]. Activation of the circuitry from the OFC to the dorsal CPu also appears to be important for drug craving in humans [47, 49, 50, 51], and its disruption has been associated with binge-like drug consumption [52,53] and the

development in compulsivity disorders [49]. While the results of the current study revealed increases in cocaine-induced activation of the PrL subregion of the mPFC, the OFC, and also the dorsal CPu following exercise vs. control conditions, the nature of this activation is unknown. Many studies investigating chronic drug exposure cite impairments in executive functioning [20], and binge intake of psychostimulants resulted in poorer performance on PFC-dependent working memory and sustained attention tasks [54, 55] and OFC-dependent reversal learning tasks [56, 57]. A recent study demonstrated decreased excitability of PrL pyramidal neurons following compulsive cocaine intake in rats [58], and reversal of this hypoactivity prevented compulsive cocaine self-administration. Interestingly, chronic wheel running reduced damage to serotonergic nerve terminals in the dorsal mPFC subregions Cg1 and PrL and the OFC and to dopaminergic terminals in the dorsolateral and dorsomedial CPu (but not NAc) induced by binge-like administration of methamphetamine [59]. Further, self-administration studies have demonstrated that access to wheel running prevented compulsive or binge intake of cocaine [9, 10]. Thus, exercise may exert its attenuating effects on drug-maintained behaviors by protecting against the neurotoxic effects of drug exposure, and this may help to preserve the integrity of executive control systems including those involving the mPFC and OFC.

The CPu is the brain area perhaps that receives the most overlapping activation from exercise and cocaine. The dorsal striatum, or the CPu, receives convergent input from the motor cortex and thalamus and is the origin of the basal ganglia circuits that are important for motor control [60]. While the CPu has no apparent role in the acute rewarding effects of drugs of abuse, it is involved in habit learning [61, 62] and is recruited during the development of compulsive drug seeking [63] and drug craving [64, 65]. The results of the present experiment demonstrating an increase in cocaine-elicited c-Fos immunoreactivity in the dorsomedial CPu in exercising vs. control rats are supported by earlier studies that showed greater Fos-positive [35] and dynorphin-positive [25] cell counts in the dorsomedial CPu following chronic wheel running. Other reports indicated that exercise training elicited adaptations in motor circuits and increased the efficiency of activation of the CPu [66], including increasing its oxidative capacity [67]. Together, evidence suggests that chronic exercise may induce plasticity in the CPu that alters its reactivity to stimuli such as drugs of abuse.

Overall, the present investigation found evidence that chronic exercise altered baseline neuronal activity and affected cocaine's activation of brain areas critically important for the development of addiction and the precipitation of relapse. Specifically, raw measures of c-Fos immunoreactivity were lower in exercising compared to control rats following both saline and cocaine priming injections. In the rats with exercise exposure, this implies there was either overall lower basal activity or perhaps lower reactivity to the injection. When counts were normalized to account for different group baselines, acute cocaine elicited a greater increase in c-Fos immunoreactivity in areas of the mesolimbic dopamine pathway in exercising compared to control rats. Taking into account existing work, it appears that chronic cocaine and chronic wheel running affect the c-Fos response to an acute cocaine exposure differently. Interestingly, although an acute cocaine injection will elicit c-Fos activation in a naïve animal, it has been shown that an acute cocaine injection following a period of chronic cocaine exposure does not significantly induce c-Fos in areas of the

striatum, indicating that tolerance develops in this response [79, 80, 81]. However, in the present experiment, we found significant levels of c-Fos activation following an acute cocaine exposure in the wheel running groups. Therefore, the effects of chronic wheel running on cocaine-induced c-Fos activation may be mediated by different mechanisms than those that mediate the effects of chronic cocaine.

Based on prior behavioral work, it is unlikely that the increase in cellular activation in exercising rats confers general vulnerability to cocaine addiction. While one study found that chronic wheel running increased cocaine conditioned place preference [16], this result is complicated by reports of decreased cocaine conditioned place preference with chronic treadmill running [34] and may be influenced by the timing of exercise, since the immediate after-effects of wheel running itself also can induce place preference [68]. Additionally, an investigation of behaviorally-selected high vs. low wheel runners found greater cocaine self-administration and reinstatement of cocaine-seeking behavior in the high runners compared to the low runners [36]. These results are consistent with other animal models of individual differences where the high extreme phenotype showed greater susceptibility for addiction [69, 70, 71, 72]. However, the results of the present study reflect on the effects exercise experience (locked vs. unlocked running wheel access) rather than avidity for exercise, making direct comparisons difficult.

In contrast, a number of investigations argue for a protective role for exercise in regard to susceptibility to drug-motivated behaviors. Results from self-administration dose-response analyses using a progressive ratio schedule of reinforcement demonstrated that voluntary wheel running decreased the reinforcing effects of cocaine [16] and heroin [17] by shifting the dose-response curves downward. Additionally, voluntary wheel running reduced intake of cocaine [31], amphetamine [73], methamphetamine [74], and alcohol [75, 76, 77] in addition to preventing the acquisition [8] and escalation of cocaine self-administration [9, 10] and the reinstatement of cocaine seeking [11, 12, 13]. Therefore, at the behavioral level of analysis, there is much evidence for attenuating effects of exercise on addiction.

Collectively, prior work and current results demonstrate a role for exercise in mediating changes in brain reward circuitry that influence its response to cocaine. Although the present study delineated some of the brain structures affected, further investigations will be needed to determine the mechanism of the effects of exercise. Nevertheless, these results provide support for the neurobiological basis of exercise as a treatment for addiction.

Acknowledgments

National Institute on Drug Abuse (NIDA) grants R01 DA 013680 (RLM), R01 DA003240 (MEC), and K05 DA015267 (MEC) supported this research. The authors would like to thank Paul Regier and Alex Claxton for technical assistance.

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Highlights

- Examination of effects of exercise on cocaine-induced activation of reward pathways.
- Rats are given access to voluntary wheel running for 21 days.
- After an acute cocaine injection, rats are sacrificed for c-Fos immunohistochemistry.
- Wheel running increases cocaine-induced c-Fos activation of reward areas.
- Results may suggest neurobiological mechanisms for exercise's effects on addiction.

Figure 1. Counting box placement for c-Fos-reactive cells of the dorsal and ventral striatum: (a) dorsal medial (DM) CPu, (b) dorsal lateral (DL) CPu, and (c) NAc shell (left box) and core (right box). (d) Image of cresyl violet-stained coronal brain slice at +1.28 mm from bregma (Jones, brainmaps.org database [78]).

Figure 2.

Counting box placement for c-Fos-reactive cells of the OFC and mPFC: (a) Image of cresyl violet-stained coronal brain slice at +3.70 mm from bregma (Jones, brainmaps.org database, [78]) where c-Fos-reactive cell counts were taken for (b) OFC. (c) Image of cresyl violet-stained coronal brain slice at +2.20 mm from bregma where c-Fos-reactive cell counts were taken for (d) Cg1, (e) IL, and (f) PrL.

Figure 3.

Mean wheel revolutions during daily 6-h sessions over the 21-day access period did not differ among the unlocked wheel access groups.

Figure 4.

Because there were basal differences in c-Fos labeling between treatment conditions, c-Fos responses to cocaine were normalized to the appropriate control level. In this case no difference between saline and cocaine is indicated by a fold change of 1. Unlocked wheel access rats had greater mean fold change in c-Fos-reactive cell counts in response to cocaine than locked wheel rats in regions of the striatum, such as the NAc core, dorsomedial (DM) CPu, and dorsolateral (DL) CPu (* $p < 0.05$, ** $p < 0.01$). Further, cocaine-induced c-Fos expression was significantly higher in unlocked vs. locked wheel access groups in the PrL subregion of the mPFC and the OFC (* $p < 0.05$, ** $p < 0.01$).

Table 1

Mean ($\pm$ SEM) total c-Fos-reactive cell counts

Wheel Access	Treatment	NAc		CPu		mPFC		OFC	
		Shell	Core	DM	DL	CgI	PrL	IL	
Unlocked	Saline	40.0 (10.5)	28.3 (3.9)	10.9 (4.9)	3.9 (2.4)	68.9 (12.6)	77.0 (16.3)	69.1 (15.7)	108.7 (36.0)
Unlocked	Cocaine	59.4 (8.9) [†]	43.8 (5.5) [†]	30.7 (6.7)	45.8 (10.4) [†]	91.9 (14.7)	93.9 (11.8)	63.1 (5.2)	149.9 (14.5)
Locked	Saline	66.0 (11.2) [*]	72.6 (4.7) [*]	39.8 (7.4) [*]	23.5 (10.5) [*]	118.0 (21.3) [*]	135.2 (15.8) [*]	112.2 (14.6) [*]	215.4 (16.7) [*]
Locked	Cocaine	87.0 (5.9) ^{†*}	81.9 (4.7) ^{†*}	51.3 (7.6) [*]	75.5 (10.0) ^{†*}	98.2 (14.5) [*]	106.3 (15.2) [*]	79.3 (12.2) [*]	154.9 (24.3) [*]

[†] Main effect: cocaine > saline, $p < 0.05$ ^{*} Main effect: locked wheel > unlocked wheel, $p < 0.05$