Published in final edited form as:

Behav Brain Res. 2012 August 1; 233(2): . doi:10.1016/j.bbr.2012.05.021.

Cocaine-induced c-Fos expression in rats selectively bred for high or low saccharin intake and in rats selected for high or low impulsivity

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Abstract

Sweet preference and impulsivity are predictors of cocaine self-administration; however no research has been conducted to investigate neuronal activation in key brain reward areas after first time exposure to cocaine in rats that differ in their propensity for cocaine-seeking and – taking behavior. In this study we used rats that that been selectively bred for high vs. low saccharin intake and rats selected for high vs. low impulsivity for food. The goal of this study was to investigate whether there are differences of c-Fos reactivity between high and low phenotypes and determine whether these differences are similar between the two animal models. A group of rats was bred for either high or low saccharin intake. Another group of rats was selected as high or low impulsive based on performance in a delay-discounting task. Subsequently, rats were given an acute injection of cocaine or saline and then c-Fos expression was observed and analyzed in several brain regions. The low reward-seeking phenotypes showed higher cocaine-induced c-Fos expression in several of these regions. Low saccharin preferring rats showed higher cocaine-induced c-Fos expression in the nucleus accumbens shell, and low impulsive rats showed higher cocaine-induced c-Fos expression in the orbitofrontal cortex and cingulate gyrus 1 area. In addition, both low impulsive and low saccharin rats had higher cocaine-induced c-Fos in the dorsal medial and dorsal lateral caudate putamen. The results indicate that individual differences in neuronal reactivity exist prior to chronic exposure to drugs of abuse. Furthermore, similar differences between the two animal models may be indicative of a common mechanism underlying vulnerability to drugs of abuse.

Keywords

c-Fos; Cocaine; Addiction; Sweet Preference; and Impulsivity

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1. Introduction

Selecting for individual differences in laboratory rat models of reward-seeking behavior have emerged in recent years as powerful predictors of drug abuse [1,2,3]. These animal models are invaluable, as it is difficult and time consuming to conduct such prospective studies in humans, and because the findings in animals appear to be paralleled in humans (e.g., [4,5]). Biological factors such as age (adolescent vs. adults), genetic strain (Lewis vs. Fisher), sex (females vs. males), and hormonal factors (high estrogen vs. high progesterone) are all important predictors in animal models of drug abuse (e.g., [1,2,3]). Further, rats that are selectively bred for high (vs. low) sweet intake [1,6], high shock avoidance [7], and reactivity to a novel environment [8] have a greater vulnerability to drug-seeking behaviors than their low-performing counterparts. Similarly, groups of rats that are selected from random outbred groups for individual differences in high (vs. low) sweet intake [9], novelty reactivity [10,11,12], more impulsive choices on a delay discounting task [3,13], or a proclivity for physical activity [14], also express drug-seeking behavior that is greater than their counterparts who perform lower on the respective measures. Collectively, these vulnerability characteristics in rats have parallels in humans and serve as animal models of genetically-determined externalizing disorders that are associated with drug abuse and other disruptive behaviors [16].

Individual differences that have been particularly well characterized with respect to models of drug abuse are found in rats selectively bred for high vs. low (HiS, LoS) sweet intake [1] and rats selected from an outbred group for high vs. low (HiI, LoI) impulsivity [3,13]. Despite procedural differences in selecting the HiS vs. LoS and HiI vs. LoI rats, there are striking similarities in their patterns of drug-seeking behavior. For example, both HiS and HiI rats initiate cocaine self-administration faster than their low counterparts and in greater numbers per group [17,19,20]. Both HiS and HiI animals escalate their intake more than their low counterparts during long access to cocaine [21,22], and compared to their LoS and LoI counterparts they both show greater reinstatement of responding on a lever previously associated with cocaine self-administration after cocaine access has been discontinued [20,21].

Despite the procedural differences in selective breeding for saccharin preferring animals compared with outbred rats differing in levels of impulsivity, the similarities in drug-seeking behavior suggest common neural mechanisms underlying behaviors motivated by drug and nondrug (e.g., food) rewards. However there is little information regarding converging neural mechanisms underlying innate responsiveness to cocaine. Furthermore, there is little research that seeks to explore underlying mechanisms of a trait before the animal receives chronic drug exposure. Using an acute drug challenge in drug naïve animals allows us to determine whether a system is primed for differential reaction to drugs, allowing us to better assess the vulnerability that animals possess prior to chronic exposure.

We chose c-Fos activation as our indicator of neuronal activity, as this has proven to be a useful tool in characterizing neuronal activation in brain regions related to addiction circuitry [23,24], and researchers have observed individual differences in acutely-induced c-Fos expression [25,26] in animal that show divergent drug-seeking behavior observed during chronic exposure to drugs of abuse [8,27]. Previous studies have shown that c-Fos expression is increased by stimulants such as cocaine and amphetamine, and this expression has shown to be mediated by dopamine receptors [24]. Therefore, we investigated areas that receive dopaminergic projections, such as the ventral and dorsal striatum and the prefrontal cortex [23,28]. We were also interested in regions of the PFC, such as the orbitofrontal cortex (OFC) and medial PFC (mPFC), because of the role of these areas in impulsivity [29,30,31,32]. Thus, it was our goal to compare neural patterns of c-Fos expression in these

areas in both HiS vs. LoS and HiI vs. LoI animals receiving their initial exposure to an acute injection of cocaine.

2. Methods

2.1. Experimental Subjects

Male rats selectively bred for high or low saccharin intake (HiS or LoS) or male rats selected as HiI and LoI, based on behavioral criteria on a delay discounting task [17], were used in this study. Rats were aged matched for each phenotype within each cohort. Table 1 shows a summary of mean ages and weights for each group. The breeding and experimental rooms were temperature- (24° C) and humidity-controlled with a 12-h light/dark schedule (lights on at 6:00 am.). The University of Minnesota Institutional Animal Care and Use Committee approved the use of these rats for the procedures in this study under protocol number 1008A87754. Laboratory facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and principles of laboratory animal care [18] were followed.

2.2. Procedure for selection of rats in each model

2.2.1. Selection of high and low saccharin rats—One set of rats was experimentally-naïve Sprague-Dawley derived from rats that had been propagated through pairings of rats with extreme HiS or LoS phenotype scores [6]. Progenitor LoS and HiS rats were obtained from a stock from Occidental College (Los Angeles, CA, USA) that has been maintained at the University of Minnesota [33]. Prior to experimental testing, rats were pair-housed in plastic cages. Rats had access to *ad libitum* food and water throughout experimental testing. At least 5 days prior to sacrifice, HiS and LoS rats were housed singly then tested for a saccharin consumption phenotype score. This test was conducted to verify the breeding of the HiS/LoS lines and is described in greater detail in previous publications [6,33]. Briefly, evaluation of an individual animal's preference for 0.1% saccharin solution was compared by measuring the intake of saccharin over a 24 hr period when both saccharin and water are available. This amount was compared to a previously established baseline of water intake when only water was available [(24 h saccharin intake ml – 24 h baseline water intake ml)/body weight g × 100]. A positive score indicated a preference for saccharin, a negative score indicated an aversion to saccharin, and a score of zero indicated no preference for saccharin. Table 1 summarizes the groups' saccharin scores. The scores reported for both HiS and LoS rats were consistent with scores that have been previously reported for many other studies using these selectively-bred lines [1,34,35,36].

2.2.2. Selection of high and low impulsive rats—A second group of experimentally-naïve outbred Wistar rats (Harlan-Sprague Dawley Indianapolis, IN) was selected as HiI or LoI based on a delay discounting task. Prior to experimental sessions, rats had access to *ad libitum* food (Purina Laboratory Chow, Purina Mills, Minneapolis, MN) and water and were pair-housed in plastic cages. During the experimental testing, and after being designated as HiI or LoI, they were maintained on a mild food-restricted diet in which they were fed 20 g of food daily until sacrifice. Selection of HiI and LoI rats took place in operant conditioning chambers consisting of an octagonal structure with alternating Plexiglas and stainless steel walls. On one of the stainless steel walls, a 45 mg pellet feeder was attached to a pellet delivery trough (Coulbourn Instruments, Allentown, NJ, USA), and the stainless steel walls on either side of the pellet receptacle were mounted with 2 response levers 4 cm above the cage floor. Tri-colored (red, green, and yellow) stimulus lights (4.6 W) were mounted above each lever. In addition, a white house light (4.6 W) was mounted at the top of each chamber above the feeder and delivery trough. Operant conditioning chambers were enclosed by wooden sound-attenuating boxes, and MED-PC software (Med Associates, St. Albans, VT,

USA) was used to program experiments and collect data. Rats were tested in 2-h daily delay discounting sessions. Each rat was tested once per day at the same time each day. Sessions consisted of 15 blocks of 4 trials each, with each block consisting of 2 forced-choice trials and 2 free-choice trials. Sessions began with 2 forced-choice trials first on the left lever then on the right lever both signaled by stimulus lights above the lever. After the forced-choice trials, stimulus lights above both levers were lit, indicating free-choice trials. In all trials a response on one lever (delay side) produced three 45 mg pellets after an adjustable delay. The other lever (immediate side) produced one 45 mg pellet immediately. After a lever response and delivery of the pellet(s), the house light and stimulus lights were turned off, starting the intertrial interval, which was programmed to maintain a time of 60 sec.

An initial starting delay of 6 sec was used at the beginning of the experiment and changed depending on the response of the rat. A response on the immediate side decreased the delay by 1 sec; whereas, a response on the delay side increased the delay by 1 sec. After the session ended, the final delay was recorded, and the ending delay served as the starting delay for the following day. Each day the average delay (mean adjusted delay or MAD) for the free choice trials was calculated by dividing the sum of total delay time on the free choice trials by the number of free choice trials completed. When the MAD for a particular rat was stable for 5 days (i.e., within 5 sec with no increasing or decreasing trends across the 5 days), the delay-discounting portion of the experiment ended for that rat and it was assigned to the HiI or LoI groups based on its MAD score. If the average MAD over the 5 days of stable behavior was greater than or equal to 13 sec, the rat was designated as LoI. If the average MAD over the 5 days of stable behavior was less than or equal to 9 sec, the rat was designated as HiI. Only a few rats fell into the 9 to 13 sec range, and they were not used in the study. The rationale for this classification of HiI and LoI by these criteria is discussed by Perry et al. [17] who developed the procedure and initially reported a bimodal distribution of MADs with a large sample size. Table 1 shows the MAD scores for HiI and LoI groups. The range of MAD scores was similar to those previously reported [3,17,20,34].

2.3. Cocaine Treatment

Cocaine treatments were identical for the experiments with the HiS/LoS and HiI/LoI comparisons. Cohorts of 4 rats (e.g., HiS/cocaine, HiS/saline, LoS/cocaine, LoS/saline) were tested at one time. Within each of the HiS/LoS or HiI/LoI conditions, two rats (1 HiS/HiI and 1 LoS/LoI) received 15 mg of cocaine per kg body weight i.p. with the other two rats (1 HiS/HiI and 1 LoS/LoI) receiving an i.p. injection of saline equivalent in volume to that used for the 15 mg/kg dose of cocaine. After the injection of cocaine or saline, rats were placed back in their home cages until sacrifice. A single acute dose of cocaine was chosen based on its ability to produce individual behavioral differences in these HiS/LoS and HiI/LoI animal models [20,21].

2.4. c-Fos immunohistochemistry

Approximately 1.5 hr after the i.p. injection, rats 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 they were then 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 hr and then moved to 10% sucrose in PBS overnight. Using a freezing microtome, 40 μ m coronal sections were taken at the level of the medial prefrontal cortex (mPFC), the caudate-putamen (CPu), and the nucleus accumbens (NAc). For the HiI and LoI rats, more rostral sections at the level of the orbitofrontal cortex (OFC) were also taken. Alternate sections were used for immunohistochemical processing. Sections were rinsed in 25 mM PBS with 0.1% BSA then incubated in rabbit antibody to c-Fos (sc-52,

Santa Cruz Biotechnologies, Inc, Santa Cruz, CA), 1:3000 in 25 mM PBS with 0.1% BSA and containing 0.03% Triton X-100 for a total of 48 hrs (24 hr at room temperature followed by 24 hr 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) 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) in Tris buffer with 0.003% hydrogen peroxide for 5 min. Nickel chloride (0.03% concentration for the HiS and LoS rats, 0.015% concentration for the HiI and LoI rats) was added to the DAB to intensify the staining (different nickel concentrations were used to optimize staining for each strain of rats). However, one cohort of saccharin animals (1 rat each for the HiS and LoS males receiving either cocaine or saline) inadvertently received the lower concentration of nickel. Since the cell counts for these rats were within the range for the others in their respective groups, the data for these animals were included in the data analysis. Sections were put through a final series of rinses with Tris then deionized water and mounted on Superfrost plus slides (Brain Research Labs, Waban, MA) and left to dry overnight. After sections were dry, they were dehydrated, cleared, and cover-slipped using DPX (Sigma-Aldrich Chemicals, St. Louis, MO).

2.5. Drugs

Cocaine HCL was supplied by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC, USA).

2.6. Data Analysis

Brain regions from 17 HiS, 18 LoS, 14 HiI, and 13 LoI rats were included in the c-Fos analysis. Areas analyzed included the OFC, regions of the mPFC [cingulate cortex (Cg1), pre-limbic (PrL), and infra-limbic areas (IL)], as well as the dorsal medial (DM) CPu and dorsal lateral (DL) CPu and the NAc core and shell. For each set of regions, a single section was selected that matched the appropriate template determined from the Paxinos and Watson [37] rat atlas. Rectangular counting boxes (300 × 637 μm) were positioned on digital images obtained from the identified sections for all the areas of interest. The boxes were positioned in the same location on c-Fos labeled sections without regard to patterns of c-Fos labeling. The sampling procedure insured that the regions counted were consistent across all the animals. Measurements of specific landmarks in the brain section for each designated region (e.g., at the level the nucleus accumbens the medial-lateral distance from the tip of the ventricle to the medial edge of the anterior commissure) were used to verify that the brain slices included in the study were taken at the same rostral to caudal level across treatment groups. Figure 1 shows the rostral to caudal levels where both OFC and mPFC sections were obtained as well as showing examples of c-Fos expression in each area. Figure 2 shows the rostral to caudal level where the NAc and dorsal CPu sections were obtained as well as giving examples of c-Fos expression for each area.

After sampling regions were digitally acquired, the image files were coded, and labeled cells were counted by two individuals unaware of the coding scheme. Prior to counting cells inter-rater reliability between individuals performing the cell counts was independently confirmed on a separate set of 15 images (Pearson's $r > 0.9$).

After counting, pictures of the brain regions were decoded and cell counts analyzed. As our goal was to investigate relative changes in c-Fos expression in response to cocaine, rather than basal differences among treatment conditions, a magnitude of change (i.e., fold

differences) for cocaine-induced c-Fos expression over saline-induced c-Fos expression was calculated for each specific brain region in both the HiS and LoS groups and the HiI and LoI groups. The magnitude of change was compared in the statistical analysis across the different phenotypes (e.g., HiS vs LoS) in each specific brain region by analysis with 2-tailed Student's t test. Saccharin scores, MAD values, age, and weight between the Hi and Lo phenotypes by cocaine or saline treatment were analyzed with 2×2 ANOVAs. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Saccharin scores of HiS and LoS rats

Table 1 shows the saccharin phenotype test scores obtained prior to cocaine exposure and sacrifice. Positive scores indicate higher intake of saccharin (available with concurrent water) than water measured during a previous day when only water was available. Scores close to zero indicate no preference, and negative scores indicate saccharin aversion. In these samples both the HiS and LoS groups had saccharin scores above zero which was consistent with many other HiS and LoS rats that had been tested in this lab and others [1], the mean saccharin score of the HiS rats was significantly higher than that of the LoS rats $F(1,34) = 16.20, p < 0.001$.

3.2. Mean adjusted delay (MAD) values of HiI and LoI rats

Table 1 also shows the MAD values for each group. Results were compared using the MAD score that was calculated by averaging the selected delays over the 30 choice trials. Lower scores indicate higher impulsivity levels. The mean of the MAD values for the HiI rats was significantly less than for the LoI rats $F(1,26) = 78.11, p < 0.001$. These scores were consistent with those recently reported in other studies[3,13].

3.3 Acute cocaine-induced c-Fos expression in each rat model

3.3.1. HiS vs. LoS rats—Cocaine-induced c-Fos expression data for HiS and LoS rats were analyzed by transformation as a magnitude of change over baseline c-Fos levels. This was accomplished by taking individual cocaine-induced c-Fos counts and dividing by mean baseline (saline-induced) c-Fos counts, which resulted in a fold change value of cocaine induction over baseline levels. There were no group differences in the Cg1, PrL, or IL PFC areas (Figure 3a). Results showed significantly more cocaine-induced c-Fos expression in LoS rats compared to HiS rats in the NAc shell ($t_{14} = -2.78, p < 0.05$) but not the NAc core (Figure 3b). Finally, there were significant differences in cocaine-induced c-Fos expression in the DM CPu ($t_{14} = -2.58, p < 0.05$), and the DL CPu ($t_{14} = -2.71, p < 0.05$) between HiS and LoS rats (Figure 3c).

3.3.2. HiI vs. LoI rats—Cocaine-induced c-Fos expression data in HiI and LoI rats were analyzed by transformation as a magnitude of change over baseline c-Fos levels as described above. Results were compared by 2-tailed Student's t-tests. A single injection of cocaine induced significantly more c-Fos expression in LoI rats compared to HiI rats in the OFC ($t_{12} = -2.84, p < 0.05$) and the Cg1 ($t_{12} = -2.96, p < 0.05$) but not in the PrL or IL (Figure 4a). There were no significant group differences between the HiI and LoI rats in the NAc shell or core (Figure 4b). Lastly, results indicated significant differences in cocaine-induced c-Fos expression in the DM CPu ($t_{12} = -4.72, p < 0.01$) and the DL CPu ($t_{12} = -2.64, p < 0.05$) between HiI and LoI rats (Figure 4c).

4. Discussion

Overall, the results of this study indicated that low drug abuse vulnerable phenotypes exhibited a greater induction of c-Fos expression compared to their high-vulnerable counterparts when challenged with an acute dose of cocaine. This study used an experimenter-administered single dose of cocaine, while previous studies on these lines of selected (HiI, LoI) and selectively-bred (HiS, LoS) rats have examined chronic self-administration or cocaine-rewarded behavior. The neurobiological consequences of an acute administration of cocaine, and the differences obtained suggest that groups that had less impulsivity for food [3], less sweet preference [1], and lower avidity for cocaine self-administration [1,3] demonstrated more neuronal activity after the acute dose of cocaine. As in previous comparisons of these different phenotypes using self-administration models, the present study revealed similarities and differences between the high vs. low impulsive and sweet-preferring or nonpreferring phenotypes in the brain areas showing c-Fos expression, suggesting that the impulsivity and sweet preferring phenotypes are not simply replicates of other more basic differences (e.g., novelty seeking). For example, while both LoS and LoI rats had higher cocaine-induced c-Fos expression compared to HiS and HiI rats, respectively, in the DM CPu, and DL CPu. LoS rats showed higher cocaine-induced c-Fos expression than HiS rats in the NAc shell, and LoI rats showed more cocaine-induced c-Fos expression than HiI rats in the OFC and Cg1.

That the c-Fos results from these two behavioral phenotypes were only partially overlapping indicates that they may have similarities as well as differences with respect to drug-seeking and -taking behavior. For example both the HiI and HiS rats have exhibited greater reactivity to the rewarding effects of drugs than the LoS and LoI rats, respectively [1,3]. However it is more difficult to interpret the results of previous studies that examined differences between these high and low phenotypes in response to negative or aversive events. For example, LoI and LoS rats show a greater response to punishment [38], conditioned taste aversion [39], and food-restricted (stress)-induced wheel running [40]. In contrast, a recent study reported that LoS rats may be less vulnerable to negative emotional effects of withdrawal to morphine compared to HiS rats [41]. Previous studies have shown that expression of c-Fos can be induced by both rewarding [24] and aversive [25] stimuli. Furthermore, a recent study found that the NAc shell can encode both rewarding and aversive events [42], and as our results indicated, LoS and HiS animals differed in reaction to acute cocaine in the NAc shell. Thus, it is not known whether the differential c-Fos activity in the low vs. high phenotypes could be due to greater sensitivity to the rewarding effects of drugs, such that the low phenotypes reach a greater physiological response at a dose that is too low for the high phenotypes, which results in lower drug intake. In contrast, the low phenotypes may also experience a greater aversive effect from drugs of abuse, resulting in lower drug intake.

The differences in brain regions that were activated may reflect behavioral differences that are expressed in the different phenotypes, explaining the partial overlap in results. For example HiS and LoS rats show differences in impulsivity for food, but not cocaine in delay discounting tasks. In contrast, HiI and LoI rats show no differences in saccharin preference scores [1]. Other studies indicate that HiI and LoI rats do not differ in aversive effects such as food-deprivation-induced wheel running, but LoS rats show significantly higher running during food deprivation than HiS rats. Thus, the HiI/LoI and HiS/LoS phenotypes share some traits, but their unique phenotypes (impulsive, sweet preferring) are not completely reciprocal [1,3,39,43]. When comparing the HiS vs. LoS and HiI vs. LoI it should be noted that the former were food satiated prior to the c-Fos determinations, while the latter had operant behavior experience and mild food restriction for several weeks. Chronic food restriction (75-80% body weight) has been shown to increase c-Fos reactivity to

amphetamine in dorsal and ventral striatum but not the cingulate cortex [54]. In the present study, we argue that the direction of change would remain the same, but the magnitude of change for both HiI and LoI animals may have been intensified by mild food restriction (~90% body weight) in the striatum but not Cg1. It is unknown whether food restriction would affect c-Fos measures in other mPFC areas; however, it is worth noting that in a different study, Cabeza de Vaca and Carr [55] reported daily intake (10 g/day) was approximately 50% of ad libitum intake (using Sprague-Dawley rats). In this study we used Wistar rats in the HiI/LoI groups and previous studies report 20 g/day to be about 80-85% of normal free-feeding weight for Wistar rats [56,57]. Therefore, it is unknown whether 20 g/day in the HiI and LoI animals would affect c-Fos reactivity at all. In addition, the rats selected for saccharin intake and impulsivity were also derived from two different rat strains, Sprague Dawley and Wistar, respectively. However, despite these strain and experiential differences, similarities in c-Fos reactivity emerge, strengthening the argument for commonalities among rats selected for nondrug reward seeking and corresponding differences in drug seeking in terms of underlying neuronal processes.

Previous studies using animal models have focused on individual differences in drug self-administration and have been extremely valuable in providing information about behavioral mechanisms underlying this differential drug self-administration. The goal of our experiment was to initiate an examination of the neurobiological underpinnings of individual differences in responsiveness to drugs of abuse. We used two different animal models, one in which rats were selectively bred for their preference for saccharin and another in which rats from an outbred population were culled based on their performance on a delay-discounting task (a measure of impulsiveness). Despite the genetic and phenotypic differences of these models, they have partially overlapping attributes in some areas (e.g. reactivity to aversive events) and unique differences in others (e.g. saccharin preference), as noted above. Perhaps the most striking overlapping characteristic is in regard to the self-administration of cocaine, where the Hi rats within each model self-administer cocaine more readily than do the Lo rats. We used this common phenotype of high vs. low cocaine self-administration within these models to test the hypothesis that the high and low drug-seeking phenotypes would also show differential neural activation following acute exposure to cocaine. In this regard, we measured neuronal c-Fos following cocaine administration in two brain regions (including subnuclei) related to the behavioral demands of saccharin preference and/or impulsiveness, i.e., the PFC and the CPu, as well as in the NAc. The rationale is that the individual behavioral differences in responsiveness to cocaine exist because of intrinsic properties that are extensions of the mechanistic qualities underlying the selected behavioral phenotypes in these animals.

One of these behavioral properties is impulsiveness. As discussed above, higher impulsivity scores in rats predict greater vulnerability to drug abuse. In humans impulsivity expressed as high delay discounting rates [44], and impulsive disorders such as ADHD, predict greater vulnerability to substance abuse [45]. Higher impulsivity scores reflect decisions related to more immediate results (less rewarding overall) rather than results that are delayed and last longer (more rewarding overall). In regards to addiction, one example in humans is when addicts relapse to experience immediate pleasure thereby avoiding the aversive effects of withdrawal from drugs or alcohol. However, they give little thought to the life-long benefits that would accrue in the future by remaining abstinent [46].

The neurobiology of impulsivity has been examined in rats primarily by experimentally damaging specific brain regions thought to be involved in impulsive choice such as delay discounting tasks [31,32,47,48] or impulsive action such as the 5-choice serial reaction time task (5-CSRT) (e.g., [29,30]). From these studies, the OFC was identified as a key brain area regulating impulsive choice [31,32,48,49]. From this literature it follows that this region

might be differentially responsive to cocaine in the HiI and LoI animals. In fact, the LoI rats had greater cocaine-induced c-Fos activity in the OFC compared to HiI rats, confirming the importance of this region and lending support to the body of evidence for the interrelationship between the OFC's role in impulsivity and drug self-administration.

Similarly, impulsive behavior has been shown to be mediated by the mPFC. Lesions of the anterior cingulate cortex increase premature responses in the 5-CSRT task [29,30]. Premature responses are thought to be a type of impulsive action. Our results showed that LoI rats had greater cocaine-induced c-Fos expression in the Cg1 (part of the anterior cingulate cortex) compared to HiI rats. Though our HiI and LoI animals were not tested on an impulsive action-type task, such as the 5-CSRT task, the HiI rats also showed shorter latencies from the start of each delay discounting trial to each lever press, suggesting impulsive action. Because LoI animals had greater cocaine-induced c-Fos expression in the mPFC and OFC due to lower baseline levels of c-Fos, and keeping in mind lesioning studies that generally show that decreased activity in the mPFC and OFC increase impulsive behavior, differences of cocaine-induced c-Fos expression in these areas may be indicative of a greater potential for top-down cognitive processing in LoI rats giving them an intrinsically greater capacity to inhibit impulsive behaviors such as those shown in the delay discounting task and also in drug self-administration.

In addition to intrinsic qualities such as differences of subjective experience (i.e. rewarding or aversive effects of cocaine) or trait-like qualities (impulsivity) the differential reactivity of c-Fos may be related to individual differences in performance of an instrumental task. Striatal areas have been found to be particularly important for both learning and habit [50,51,52]. Rats initially learn to self-administer cocaine in the acquisition phase. High and low saccharin-preferring phenotypes and impulsive phenotypes consistently show high vs. low rates of acquisition of drug self-administration both in terms of speed of acquisition and in the percentage of animals per group that meet the acquisition criteria [1]. In this regard, the DM CPu is a vital area that underlies instrumental learning [52], and our results in the present study show differences in the DM CPu between high and low drug-seeking phenotypes. These common patterns of c-Fos induction between high and low drug-seeking phenotypes represent a common underlying neurobiological factor that may be driving similar differences of animal behavior during an operant task. Interestingly, our results point to a potential relation between the DM CPu in a way that differences in c-Fos activity should correlate with performance on a self-administration acquisition learning task, a hypothesis that remains to be tested.

After an animal has learned the task, habitual action may play a role in the differences observed in previous studies in high vs. low drug-seeking phenotypes in a self-administration task. As it has been discovered that one of the key neural substrates of habitual action is the DL CPu [51], one could posit that differences in neuronal activity in this area to acute stimuli could relate to differences in habit formation after extended experience on a task, thereby affecting behaviors associated with the habitual actions, such as drug use. Since acutely-induced c-Fos predicts the presence of chronically-induced neuropeptides that regulate dopaminergic projections and subsequent dopamine release [53], chronic changes in dopaminergic mechanism functioning affecting behavior may be predicted by acute c-Fos expression, as well. As noted, our results showed that low drug-seeking phenotypes (LoS and LoI) had greater cocaine-induced c-Fos expression compared to high drug-seeking phenotypes (HiS and HiI) in the DL CPu. Again our results point to a potential common attribute that would affect performance during an operant task for HiS/LoS and HiI/LoI animals, as well as a common attribute that may underlie similar drug-seeking behavior.

5. Conclusions

Because only a minority of people who use drugs become addicted (e.g. about 10%) [15], an important question in drug abuse research is what factors may confer vulnerability to addiction in humans. One answer lies in individual traits that can affect the neurobiological responsiveness to drugs the first time a person chooses to try a drug that can ultimately lead to addiction. The results of this study show that in two different rat models of individual differences in addiction vulnerability, brain regions associated with the behavioral components of the traits of these animals (e.g., saccharin preference or impulsiveness) are differentially activated the first time these animals are administered cocaine. These findings provide initial insight into the neural mechanisms underlying individual differences in drug addiction.

Acknowledgments

This work was supported by NIDA grants P20 DA024196, R01 DA003240, and K05 DA015267 (MEC) and by NIDA grant R01 DA013680 (RLM). The authors want to thank Nathan Holtz and Alex Claxton for their assistance in the immunohistochemistry and cell counting procedures.

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Abbreviations

HiS	High saccharin preferring rats
LoS	Low saccharin preferring rats
HiI	High impulsive rats
LoI	Low impulsive rats
MAD	Mean adjusted delay (in the delay-discounting task)
CPu	Caudate putamen
DL CPu	Dorsal lateral caudate putamen
DM CPu	Dorsal medial caudate putamen
NAc	Nucleus accumbens
OFC	Orbitofrontal cortex
PFC	Pre-frontal cortex
CG1	Cingulate gyrus 1 area
PrL	Prelimbic area
IL	Infralimbic area

Highlights

- We utilized two animal models that display high and low cocaine self-administration.
- We compared neuronal activity in selected brain regions related to drug reward
- Individual differences exist with first-time exposure to cocaine in both animal models.
- Low drug-seeking phenotypes exhibited greater cocaine-induced c-Fos expression.
- A potential common underlying mechanism between models was observed in dorsal striatum.

Figure 1.

a) Cresyl violet image at the rostral to caudal level (boxes on the cresyl violet image correspond to the outlined counting regions in **b**) where slices were taken to obtain **b**) the orbitofrontal cortex. **c)** Cresyl violet image at the rostral to caudal level (boxes on the cresyl violet image correspond to the outlined counting regions in **d-f**) where slices were taken to obtain the **d**) cingulate gyrus 1 area, **e**) pre-limbic area, and the **f**) infralimbic area.

Figure 2.

a) Cresyl violet image at the rostral to caudal level (boxes on the cresyl violet image correspond to the outlined counting regions in b-d) where slices were taken to obtain the **b)** nucleus accumbens core (left box) and shell (right box), **c)** dorsal lateral striatum, and the **d)** dorsal medial striatum.

Figure 3.

Mean ($\pm$ SEM) fold change value of cocaine-induced c-Fos expression levels over baseline (saline-induced) c-Fos expression levels for HiS and LoS animals in **a)** mPFC, **b)** NAc core and shell, and **c)** Dorsal medial and dorsal lateral CPu. HiS (high saccharin preference rat), LoS (low saccharin preference rat), mPFC (medial prefrontal cortex), Cg1 (cingulate gyrus 1 area), PrL (pre-limbic area), IL (infra-limbic area), NAc (nucleus accumbens), CPu (caudate putamen), DM (dorsal medial), DL (dorsal lateral). * indicates a difference between HiS and LoS animals of $p < 0.05$

Figure 4.

Mean ($\pm$ SEM) fold change value of cocaine-induced c-Fos expression levels over baseline (saline-induced) c-Fos expression levels for HiI and LoI animals in **a)** OFC and mPFC, **b)** NAc core and shell, and **c)** DM and DL CPu. HiI (high impulsive rat), LoI (low impulsive rat), OFC (orbitofrontal cortex), mPFC (medial prefrontal cortex) Cg1 (cingulate gyrus 1 area), PrL (pre-limbic area), IL (infra-limbic area), NAc (nucleus accumbens), CPu (caudate putamen), DM (dorsal medial), DL (dorsal lateral). * indicates a difference between HiI and LoI animals of $p < 0.05$.

Table 1

Summary of selection characteristics

Phenotype	Treatment	Age (Days)	Weight (g) [#]	MAD Value (sec) [*]	Saccharin Score ^{**}
HIS	Cocaine	113 (±6.2)	462 (±18.6)	-	22.21 (±2.9)
HIS	Saline	109 (±6.23)	502 (±26.9)	-	18.00 (±2.9)
LoS	Cocaine	105 (±3.8)	533 (±19.7)	-	9.90 (±1.7)
LoS	Saline	109 (±5.1)	545 (±20.5)	-	10.23 (±1.6)
Hil	Cocaine	152 (±8.58)	418 (±17.4)	4.75 (±0.9)	-
Hil	Saline	140 (±5.7)	384 (±9.1)	4.83 (±0.6)	-
LoI	Cocaine	159 (±11.9)	438 (±25.8)	30.49 (±1.6)	-
LoI	Saline	158 (±12.1)	444 (±27.2)	25.93 (±3.7)	-

A 2 × 2 ANOVA was used to compare age, weight, MAD, and saccharin score.

[#]Significant main effect of weight for HIS and LoS (but not Hil and LoI), $F(1,34) = 7.10$, $p < 0.05$.

^{*}Significant main effect of MAD score for Hil and LoI.

^{**}Significant main effect of saccharin score for HIS and LoS.