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Withdrawal from extended-access cocaine self-administration results in dysregulated functional activity and altered locomotor activity in rats

Erin S. Calipari^{1,†}, Thomas J.R. Beveridge^{1,2,†}, Sara R. Jones^{1,2}, and Linda J. Porrino^{1,2,*}

¹Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston Salem, North Carolina 27157

²Center for Neurobiology of Addiction Treatment (CNAT), Wake Forest School of Medicine, Winston Salem, North Carolina 27157

Abstract

Much work has focused on determining the consequences of cocaine self-administration on specific neurotransmitter systems, thus neglecting the global changes that occur. Previous imaging studies have focused on the effects of cocaine self-administration in the presence of high blood levels of cocaine, but have not determined the functional effects of cocaine self-administration after cocaine has cleared. Extended-access cocaine self-administration, where animals administer cocaine for 6 hours each day, results in escalation in the rate of cocaine intake and is believed to model the transition from recreational use to addiction in humans. We aimed to determine the functional changes following acute (48 hours) withdrawal from an extended-access, defined intake self-administration paradigm (5 days, 40 inj/day, 6hrs/day), a time point when behavioral changes are present. Using the 2-[¹⁴C]deoxyglucose method to measure rates of local cerebral glucose metabolism, an indicator of functional activity, we found reductions in circuits related to learning and memory, attention, sleep, and reward processing, which have important clinical implications for cocaine addiction. Additionally, lower levels of functional activity were found in the dorsal raphe and locus coeruleus, suggesting that cocaine self-administration may have broader effects on brain function than previously noted. These widespread neurochemical reductions were concomitant with substantial behavioral differences in these animals, highlighted by increased vertical activity and decreased stereotypy. These data demonstrate that behavioral and neurochemical impairments following cocaine self-administration are present in the absence of drug and persist after cocaine has been cleared

Keywords

Rat; metabolism; striatum; cocaine self-administration; withdrawal

Introduction

The neuroadaptations that occur following cocaine administration have been studied extensively in order to determine both the consequences of cocaine abuse and to find potential targets for addiction treatment. Previous work using non-contingent cocaine

*Corresponding author: Linda J. Porrino, Department of Physiology and Pharmacology, Center for the Neurobiological Investigation of Drug Abuse, Wake Forest University School of Medicine, Winston Salem, NC 27157-1083, USA, lporrino@wakehealth.edu, Tel.: 336-7168575, Fax: 336-7168501.

[†]ESC and TJRB contributed equally to this work.

exposure has shown significant neuroadaptations in gene expression, protein function, neurotransmitter release and uptake, and concomitant behavioral changes (Vanderschuren & Pierce, 2010; Mu et al., 2010). However it is important to choose a model that accurately mimics human drug taking (Hemby et al., 1994, 1997; Porrino 1993; Lecca et al., 2007). Rodent self-administration is a translational model of human cocaine abuse, and much of the current literature on cocaine self-administration has focused on extended-access self-administration, during which animals have access to cocaine self-administration for 6 hours (h) per day (Ahmed & Koob, 1998). This paradigm allows animals to administer high levels of cocaine consistently over the session and has been reported to model some aspects of human cocaine consumption patterns (Dackis and O'Brien, 2001). Extended-access cocaine self-administration has been shown to reproduce many of the neurochemical hallmarks of cocaine addicts and is characterized by reduced basal dopamine levels (Mateo et al., 2005; Ferris et al., 2011), behavioral and neurochemical tolerance to cocaine (Ferris et al., 2011, 2012; Calipari et al., 2012), and increased motivation to administer cocaine (Zimmer et al., 2012; Wee et al., 2008). However, it has been shown that escalation of cocaine intake is not a result of changes in cocaine's ability to elevate striatal dopamine levels, suggesting that cocaine self-administration has effects that extend beyond the dopamine system (Ahmed et al., 2003).

Most of the current literature on the effects of chronic cocaine self-administration on the brain has focused on the striatal dopamine system, thus neglecting the contribution from other neurotransmitters and circuits. Neuroimaging techniques allow for a global view of the effects of cocaine self-administration on the brain. Previous work has demonstrated that immediately following 21 days of self-administration, while blood levels of cocaine are still high, there are reductions in functional activity in a number of brain regions (Macey et al., 2004); however, the question remained of whether these changes persisted beyond the self-administration session. Most functional activity studies determine the effects of a drug challenge; however, the present study focused on rates of local cerebral glucose utilization in the absence of drug, in order to determine its residual effects. These data are important because determining the persistent effects of cocaine self-administration on functional activity can point to changes in specific brain regions and circuits which may be predictive of behavioral deficits in cocaine-addicted individuals.

We show that cocaine self-administration results in functional reductions in brain regions involved in reward, learning, and memory that are present 48 h after the final cocaine session. We also see reduced function of the dorsal raphe and locus coeruleus, which has implications for global brain function as these nuclei have an extensive network of projections. Using behavioral activity analysis after cocaine self-administration we report alterations which could be predicted based on decreased serotonergic and dopaminergic functioning, demonstrating that these neural changes have behavioral implications. The reduced functional activity in selected regions suggests that even limited cocaine self-administration is capable of producing reductions in regional brain activity that may have adverse consequences for normal functioning.

Materials and Methods

Animals

Male, Sprague-Dawley rats (375–400 g; Harlan Laboratories, Frederick, MD) were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine.

Self-Administration

Rats ($n = 14$) were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), implanted with chronic indwelling jugular catheters, and trained for i.v. self-administration as previously described (Liu et al., 2005). Following surgery, animals were singly housed, and all self-administration sessions took place in the home cage. Each animal was maintained on a reverse light cycle (3:00 am lights off; 3:00 pm lights on), and all self-administration procedures occurred during the active/dark cycle. Sessions were six hours in length and were terminated at the end of the six hours or after 40 injections of drug. Animals self-administered cocaine (1.5 mg/kg/inj over 4 sec) on a fixed-ratio 1 schedule of administration. Concurrent with the start of each injection, the lever retracted and a stimulus light was activated for 20 seconds to signal a time-out period. Under these conditions, animals acquired a stable pattern of cocaine self-administration within 1 to 5 days. The criterion for acquisition was self-administration of 35 or more infusions in one session (this was then considered Day 1). Following acquisition, the animals were given access to a maximum of 40 injections per day for a period of 5 consecutive days (i.e. 4 more days after acquisition of self-administration).

Control animals were either drug-naïve rats housed under the same reverse light-dark light cycle for at least one week prior to all experimental manipulations or instrumented animals that had undergone the same surgery, handling and housing conditions as cocaine self-administering animals. We have previously addressed the effects of operant responding and surgery controls on neurochemical outcomes, and several previous studies from our lab have confirmed that there are no significant differences in dopamine neurochemistry between naïve controls, surgery controls, and many paradigms of operant responding (Ferris et al., 2011, Calipari et al., 2013).

Locomotor Analysis

Locomotor analysis was performed as previously described (Läck et al. 2008) the day following completion of cocaine self-administration. Locomotor analysis was performed on a different group of animals from the functional activity experiments. On the test day, prior to locomotor recording, animals were allowed to habituate in the testing room, in their home cages for 60 minutes. Following habituation to the room, rats (control, $n=7$; cocaine self-administration, $n=7$) were placed in the locomotor chamber (MedAssociates) and baseline activity recorded for 30 min. Rats then received an intraperitoneal (IP) injection of saline, and activity was recorded for 90 min. Locomotor recordings were performed in two separate groups (control and cocaine self-administration) and data was compared across groups. Outcome measures were distance travelled (cm), stereotypy (total beam breaks while animal is stationary) and vertical activity (number of periods of continuous vertical beam breaks).

Measurement of local cerebral glucose utilization

Twenty-four h after their final self-administration session, animals underwent femoral artery catheterization surgeries, as previously described (Macey et al., 2004). Animals were allowed 24 h to recover from surgery. Rates of local cerebral glucose utilization (LCGU) in rat brain were quantified 48 h after their last cocaine self-administration session according to the method of Sokoloff et al. (1977), as adapted for use in freely moving animals (Crane and Porrino 1989). As part of a separate study, both cocaine self-administration animals ($n=7$) and controls ($n=6$) were administered saline (1 ml/kg, IP) 30 min prior to initiation of the [^{14}C]-2-deoxyglucose (2DG) procedure. One control animal was dropped from analysis due to an occluded femoral catheter. Control animals consisted of both drug-naïve ($n=3$) and instrumented animals ($n=3$) that had undergone exactly the same surgical procedures as the cocaine self-administering animals, as well as housing conditions, light/ dark cycle and handling. Rates of local cerebral glucose utilization in the brains of these three animals in

response to saline injections were no different from the drug-naïve controls, which were housed in similar conditions and handled in the same fashion; thus their data were combined. The 2DG procedure was conducted in the animal's home cage and was initiated by means of an intravenous infusion of a pulse of 2-[¹⁴C]DG (75 µCi/kg; specific activity 55 mCi/mmol; New England Nuclear, Boston, MA) through the jugular venous catheter (via which self-administration had previously occurred). Timed femoral arterial blood samples were collected over the next 45 minutes and immediately centrifuged. Rates of LCGU in cocaine self-administering rats were compared with those obtained from control rats. Plasma concentrations of 2-[¹⁴C]DG were determined by liquid scintillation counting (Beckman Instruments, CA), while plasma glucose levels were determined by means of a Beckman Glucose Analyzer II (Beckman Instruments, CA). Immediately after the 45-minute sample, animals were killed by administration of intravenous pentobarbital (100 mg/kg). Brains were rapidly removed, frozen in isopentane at -45°C, and stored at -80°C until sectioned. Brains were sectioned coronally (20µm) in a cryostat maintained at -20°C, collected on glass coverslips and immediately transferred to a hot plate (60°C) to dry. Coverslips were apposed to Kodak EMC film for 13–17 days along with a set of calibrated [¹⁴C]methylmethacrylate standards (Amersham, IL) previously calibrated for their equivalent wet weight ¹⁴C concentrations. Films were developed in GBX developer (Kodak, NY).

Autoradiograms were analyzed by quantitative densitometry with a computerized image analysis system (MCID, Imaging Research, Ontario). Tissue ¹⁴C concentrations were determined from densitometric analysis of autoradiograms of the calibrated standards. Rates of glucose utilization were then calculated using the optical densities and a calibration curve obtained from local ¹⁴C tissue concentrations, time-courses of the plasma glucose and ¹⁴C concentrations, and the constants according to the operational equation of the 2DG method (Sokoloff et al. 1977). Glucose utilization measurements were determined for 20 discrete brain regions according to the rat brain atlas of Paxinos and Watson (Paxinos and Watson 1998). Each brain region was analyzed bilaterally in a minimum of five brain sections per animal.

Statistics

Graph Pad Prism (version 4, La Jolla, CA, USA) was used to statistically analyze data sets and create graphs. Locomotor data was subjected to a two-way ANOVA with experimental group and time as the factors, followed by planned Bonferroni's tests for multiple comparisons. Rates of local cerebral glucose utilization were analyzed in specific functional groups of brain regions (cortex, basal ganglia, limbic-related and brainstem) using 2-way analyses of variance (ANOVA), brain region group X treatment (control vs. cocaine self-administration) with brain region group as a repeated measure. These were followed by planned Bonferroni's tests for multiple comparisons. Statistical significance was considered as $p < 0.05$.

Results

Extended-access to cocaine self-administration results in escalation in the rate of daily intake

Animals self-administered cocaine (1.5 mg/kg/inj) for five consecutive days with a daily maximum of 40 total injections (all animals self-administered the maximum each day). Injections were limited to ensure that all animals had the same intake over each self-administration session in order to control for total intake as a factor in the functional effects of cocaine. Because of this limit on the number of daily injections, therefore, animals quickly reached the maximum total daily intake (60 mg/kg) and could not exceed that. However, animals could control the rate of intake over the sessions (Figure 1). Similar to

previous studies this rate increased over the 5 day period (Mateo et al., 2005; Calipari et al., 2012; Ferris et al., 2012). One way ANOVA revealed a main effect of session ($F_{4, 56} = 14.93, p < 0.001$). Tukey's post hoc analysis revealed an increase in rate versus the first session on sessions two ($q = 4.216, p < 0.05$), three ($q = 5.843, p < 0.01$), four ($q = 8.734, p < 0.001$), and five ($q = 9.673, p < 0.0001$).

Behavioral analysis shows a differential baseline locomotor profile following 48 h withdrawal from cocaine self-administration

Following cocaine self-administration, behavioral activity was assessed using automated monitors. Our a priori hypothesis was that locomotor activity in response to a novel environment would be reduced following cocaine self-administration (Koeltzow and White, 2003). Two-way ANOVA revealed a main effect of cocaine self-administration on the response to a novel environment ($F_{(1, 75)} = 4.04, p < 0.05$). In addition, there was a main effect of time ($F_{(5, 75)} = 73.53, p < 0.0001$) as animals habituated to the activity chamber. There were no significant differences between the groups in either stereotypy or vertical activity. Bonferroni's tests for multiple comparisons were performed to compare across treatment groups, and a reduction in locomotor activity was observed at the 15 minute time point in animals that had undergone cocaine self-administration ($t = 3.219, p < 0.05$; Figure 2). Alternatively, we found a significant effect of time ($F_{(17, 204)} = 17.64, p < 0.001$), but not of treatment ($F_{(1, 204)} = 0.05, \text{NS}$) or an interaction ($F_{(17, 204)} = 8.93, \text{NS}$) on saline-induced locomotion indicating that normal forward locomotion was not impaired by a prior cocaine self-administration history (Figure 3).

In order to determine whether other measures of locomotor behavior had changed as a consequence of cocaine self-administration and withdrawal, we assessed vertical activity and stereotypy-like behavior. Student's t-test revealed that cocaine self-administration animals had higher vertical counts as compared to controls ($t_{12} = 7.604, p < 0.0001$; Figure 4). In addition, cocaine self-administration animals had lower stereotypy counts as compared to controls ($t_{12} = 3.988, p < 0.0001$; Figure 4). These results, taken with the lack of difference in distance traveled, highlight a qualitative change in spontaneous locomotor activity, distinct from forward activity.

Rates of local cerebral glucose utilization are lower following 48 h withdrawal from cocaine self-administration

Baseline plasma glucose concentrations prior to the initiation of the 2DG procedure were not different between drug-naïve controls and cocaine-experienced animals (controls, $144.2 \pm 6.7 \text{ mg/mL}$; 48 h withdrawal from cocaine, $153.4 \pm 17.0 \text{ mg/mL}$). Rates of local cerebral glucose metabolism were measured in 20 brain regions and data are shown in Table 1. These rates were globally lower in animals with a history of cocaine self-administration measured 48 h after the final self-administration session as compared to drug-naïve controls ($84.5 \pm 4.7 \text{ } \mu\text{mol/100 g/min}$ controls versus $74.6 \pm 4.4 \text{ } \mu\text{mol/100 g/min}$ cocaine-withdrawal, $t_{11} = 2.245, p < 0.05$). This pattern was observed in all 20 of the regions in which glucose utilization rates were measured. In the cortex, two-way analysis of variance revealed a main effect of treatment ($F_{(1, 11)} = 5.95, p < 0.05$) and brain region ($F_{(2, 22)} = 151.9, p < 0.001$), but no interaction. In the basal ganglia, there was a main effect of treatment ($F_{(1, 11)} = 8.10, p < 0.05$) and brain region ($F_{(5, 55)} = 125.67, p < 0.001$), but no interaction. In limbic brain areas, there was a main effect of treatment ($F_{(1, 11)} = 6.10, p < 0.05$) and brain region ($F_{(7, 77)} = 110.3, p < 0.001$), and an interaction ($F_{(7, 70)} = 3.041, p < 0.05$). Finally, in the brainstem, there was a main effect of treatment ($F_{(1, 11)} = 12.48, p < 0.01$) and brain region ($F_{(2, 22)} = 75.21, p < 0.001$), but no interaction. Planned multiple comparisons showed that 48 h after cocaine self-administration functional activity was lower in the anterior cingulate cortex (-12%), dorsal caudate putamen (-16%), nucleus accumbens (-16%), basolateral amygdala

(– 16%), medial nucleus of the thalamus (– 12%), hippocampal CA1 region (– 24%), dorsal raphe (– 18%), locus coeruleus (– 13%) and cerebellum (– 15%), when compared to controls.

Discussion

Here we demonstrate that there are functional and behavioral reductions present 48 h after 5-day cocaine self-administration. The functional alterations were characterized by reduced brain activity, as indicated by lower rates of cerebral glucose utilization, in circuits involved in learning and memory, attention, sleep, and reward processing. These data are consistent with human studies that have demonstrated marked reductions in functional brain activity, in particular prefrontal cortical and striatal regions, which occurs early in the withdrawal period and lasts for up to four months following cocaine abuse (Volkow et al., 1992, 1993). Previously, we have shown that cocaine self-administration resulted in reductions in functional activity, but these effects were measured immediately following the final infusion at a time when cocaine levels were still high (Macey et al., 2004). The present study extended these findings and demonstrated that there were functional alterations 48 hours following the final self-administration session, indicating that neuroadaptations associated with cocaine administration may persist and result in reduced activity. In addition, we found that there were also behavioral changes, as indicated by increased vertical and reduced stereotypical behavior, suggesting that these functional alterations may have direct behavioral consequences even in the absence of drug. These results highlight the fact that even a relatively short (five day) exposure to cocaine, which resulted in escalation of the rate of daily intake, can lead to neurochemical (Mateo et al., 2005; Calipari et al., 2012; Ferris et al., 2012), functional and behavioral changes that can be detected during withdrawal. While cumulative daily intake could not escalate beyond the maximum 40 injections per day, unlike the classic self-administration procedure introduced by Ahmed and Koob (1998), the present modified self-administration procedure shares many of the same characteristics. For example, one key observation of the traditional long access paradigm is escalated intake during the first hour of daily self-administration sessions. Similar increases in first hour intake are also characteristic of the current paradigm (as depicted in Figure 1). Although the maximum session length was 6 h, rats completed their 40 injections in shorter and shorter time periods over the course of 5 days of access. Another similarity is total intake. Typical total intake in many escalation studies is 60–90 infusions of 0.75 mg/kg/injection (approximately 45–70 mg/kg/session), whereas in the present study total intake of 40 daily infusions of 1.5 mg/kg/injection results in an intake of 60 mg/kg/session.

While previous reports have focused on the neurochemical changes that occur when drug is still present or in response to a challenge dose of cocaine (Macey et al., 2004; Kornetsky, Huston-Lyons, & Porrino, 1991; Zocchi, Conti, & Orzi, 2001), the present study focused on measuring functional activity 48 hours after the last self-administration session. At this time point, we found that functional activity was lower than controls in the nucleus accumbens, anterior cingulate cortex, basolateral amygdala, hippocampus, dorsal caudate, medial thalamus, dorsal raphe, and locus coeruleus – brain areas involved in vital processes such as learning, memory, reward and reinforcement. In contrast to these data, our previous work has examined the effects of five days of cocaine self-administration on functional activity, while cocaine was present, and found no changes in any of these regions. In fact, most of the regional differences from controls were higher, not lower, levels of functional activity (Macey et al., 2004). Macey et al. (2004) also compared rates of glucose utilization from this five-day group to those from a 30-day self-administration group that had a similar cumulative intake to the current study (≈ 100 mg cocaine over the five days in the current study versus ≈ 150 mg cocaine in the 30-day group). The animals with the 30-day self-administration history had similar changes to those observed in the present study, suggesting

that cumulative intake, and not necessarily duration of drug exposure, may be the major determinant of the consequent neuroadaptations. This observation is consistent with previous work from our lab indicating that total intake, not the length of the self-administration history, is responsible for the neurochemical changes that occur following cocaine self-administration (Calipari et al., 2013). Because glucose utilization was assessed immediately after the final reinforcer in the 30-day self-administration group from Macey et al. (2004) and rates were significantly lower than controls, it suggests that not only are the circuits depressed in the absence of cocaine, but cocaine failed to produce sufficient effects to ‘normalize’ circuits. Continued drug-taking in addicted individuals has been suggested to occur as compensatory behavior to “normalize” a baseline dysregulated state (Koob, 2009; Koob and Le Moal, 1997). It is important to differentiate the effects of cocaine-induced alterations of neural networks while cocaine is present with those of cocaine self-administration on functioning in the absence of drug, as they have very different implications for the functioning of the brain at baseline.

The mesocorticolimbic dopamine system mediates many of the reinforcing and rewarding effects of cocaine (Pierce and Kumaresan, 2006), and because neuroadaptations resulting from chronic drug exposure are often opposite from the acute effects, it is not surprising that there were reductions in the activity of these regions following cocaine self-administration. Previous work has demonstrated reduced function of the striatal dopamine system at a similar time point following cessation of cocaine self-administration, as well as the development of tolerance to the neurochemical effects of cocaine (Ferris et al., 2011). Further, these functional impairments are present 18 hours following the final cocaine self-administration session (Mateo et al., 2005; Ferris et al., 2011, 2012; Calipari et al., 2012), and persist for up to two weeks following cocaine exposure (Ferris et al., 2012). These reductions in dopamine function have been observed using both fast scan cyclic voltammetry and microdialysis where it was found that 18–24 hours of withdrawal from cocaine self-administration resulted in reduced dopamine release and uptake, as well as reduced baseline dopamine overflow, respectively (Ferris et al., 2011, 2012; Calipari et al., 2012, 2013; Maisonneuve et al., 1995; Weiss et al., 1992; but see, Hooks et al., 1994; Meil et al., 1995). The current data agree with these observations in that functional activity was significantly reduced in the terminal fields of the VTA, namely the nucleus accumbens and caudate putamen (Koeltzow and White, 2003). Moreover, the neurochemical changes previously reported concur with the behavioral data, which demonstrated a decreased locomotor response to a novel environment following withdrawal from cocaine self-administration.

Previous studies have demonstrated the influential role of striatal dopamine levels on the locomotor response to a novel environment; for example, animals can be separated into two groups (high and low response) according to their locomotor activity in reaction to a novel environment. Ferris et al., (2013) recently demonstrated that high and low response to novelty can predict both the tolerance that develops to cocaine directly at the dopamine transporter as well as the rate of acquisition of cocaine self-administration. Low novelty responders have been shown to have lower extracellular dopamine levels, in line with the present data where we observed reduced functional activity in dopaminergic regions following 48 h withdrawal from cocaine self-administration (Verheij and Cools, 2008; Verheij et al., 2008). Previous work on withdrawal from cocaine self-administration has found depression of locomotor activity during similar time-points, an effect that is indicative of reductions in dopamine levels and VTA cell firing (Koeltzow and White, 2003; Gauvin et al., 1997). Thus, the lower levels of locomotor activity would be predicted based on the reduced functional activity in dopaminergic nuclei. These alterations suggest that there could be changes in reward processing at baseline, which could play an important role in the reinstatement of cocaine-seeking (Schmidt and Pierce, 2010). Because these regions are

involved in the processing of salient stimuli, these data also suggest that the processing of other alternate rewards, such as food, may also be impaired at baseline (Carelli, 2002; Schultz, 2010). In addition, there may be differential effects of cocaine on dopaminergic systems involved in motor and reward processing, an effect that was highlighted in the behavioral data indicating that there was no difference in baseline forward locomotion following cocaine self-administration. It is important to note that there were reductions in stereotypic behaviors, indicating that although forward locomotion does not differ between groups, there may be inherent differences in motor control following cocaine self-administration, although it is not clear as to the meaning of these results. Together, these data suggest that the alterations in functional activity are not general changes that occur in all dopaminergic terminal fields, but rather are specific to those associated with reward and reinforcement and selective aspects of motor control.

In line with reductions in functional activity in dopaminergic regions 48 hours following cocaine self-administration, electrophysiological recordings have demonstrated reduced action potential firing in nucleus accumbens neurons both *in vitro* and *in vivo* after withdrawal from cocaine self-administration (Dong et al., 2006; Ishikawa et al., 2009; Kourrich and Thomas, 2009; Mu et al., 2010; White et al., 1995). The lower rates of glucose metabolism in the brain regions reported here, together with the reductions in cell firing could suggest a reduced/ depressive state following acute withdrawal from self-administration. Indeed, Markou and Koob (1991) have demonstrated elevations in intracranial self-stimulation thresholds in rats, indicating a depressed or anhedonic state in animals following self-administration. The elevated thresholds were reversed by a dopamine D2 receptor agonist, suggesting that the effects were due, at least in part, to reduced dopamine system activity following a cocaine self-administration history (Markou & Koob, 1992). A similar phenomenon has also been demonstrated in mice, where withdrawal from cocaine delivered via osmotic mini-pump also resulted in elevated reward thresholds 72 hours following treatment (Stoker & Markou, 2011). Because functional activity measurements were made 48 hours following the final cocaine self-administration session, these modifications may be indicative of the changes that occur during early withdrawal periods and may coincide with the alterations in reward thresholds. Further, it is well documented that, similar to the anhedonic-like behavior seen in rodents, human addicts going through early withdrawal from cocaine exposure also report depression and anhedonia (Markou & Kenney, 2002). It is possible, therefore, that the widespread decreased functional activity may be associated with depression and anhedonia during the early withdrawal periods. Although it is possible that these changes may also underlie the neuroadaptations that occur during later stages of withdrawal, the time points measured in this study can only confirm that this state is present 48 hours following cocaine self-administration. Future studies that look at the time course of both the functional and behavioral effects of cocaine withdrawal are necessary to confirm whether the effects observed here are persistent.

Although reward and reinforcement are an essential part of the early stages of the addiction process, drug addiction is dependent on neural plasticity associated with drug-induced reward learning mechanisms (Jones and Bonci, 2005). Within the current paradigm, it is important to distinguish between escalation and task- learning, as they likely have different neural mechanisms driving the behavioral processes. Prior to acquisition, animals have inconsistent responding which is characterized by unevenly spaced inter-injection intervals and sporadic intake over sessions (Ferris et al., 2013). However, following acquisition, animals space their injections evenly in a dose-dependent fashion (Calipari et al., 2012, 2013; Ferris et al., 2011, 2012, 2013), suggesting that they have associated active lever responding with cocaine administration (Norman et al., 2004). Previous work, which includes similar doses with similar inter-injection intervals (~7 min), has demonstrated a linear relationship between dose and inter-injection interval. Because of the linear

relationship between dose and inter-injection interval, it is clear that the animals are aware that the lever press is associated with the reinforcer (cocaine) and are not learning this relationship (Norman and Tsibulsky, 2006). As a result the changes observed here are not associated with the early stages of goal-reward associations, but rather the changes that occur following repeated drug use. Following cocaine self-administration, we observe functional reductions in activity in brain regions involved with drug-induced reward learning mechanisms. Specifically, the reductions in the prefrontal cortex (PFC) and nucleus accumbens activity suggest that there may be suppression of cortico-striatal loops. Goal-directed learning is reliant on the dorsomedial striatum through loops that project from the cortex to the striatum (Alexander et al. 1986; Lawrence et al. 1998; Haber and Calzavara 2009; McFarland and Haber 2002). Here we show reductions in functional activity in these areas, implying that this type of learning may also be impaired. These data suggest that 1) individuals may be less able to learn new goal-directed behaviors, and 2) that they also may be less able to replace already formed associations. Replacing associations that occurred during the development of drug addiction is a process that is essential for continued abstinence and the prevention of relapse in abstinent individuals. In addition, the motivational loop, comprised of the ventral striatum, orbitofrontal and anterior cingulate cortex, hippocampus, and amygdala (Lawrence et al. 1998), seemed to be particularly affected. These reductions in regional functional activity may also potentially lead to drug-taking in order to restore these brain areas to the functional state that was present before the drug-taking was initiated (Koob and Le Moal, 1997).

In additions to reductions in areas involved in reward learning and motivational behaviors, there were also reductions in regions involved in learning and memory. Reductions in functional activity were observed in the hippocampus, medial thalamus, and basolateral amygdala. Reduced activity in these regions has important implications for normal functioning and the learning capacity at baseline after the cessation of drug consumption. Even more important for cocaine abusers is the role that learning plays in cue-reinforcement pairings during drug abuse. It is well established that cue conditioning plays a role in the effects of drugs and on relapse, where cues alone are sufficient to reinstate drug taking/seeking after periods of prolonged abstinence (Lu et al., 2004; Shaham et al., 2003; Schmidt and Pierce, 2010). The basolateral amygdala has also been shown to be a major modulator of the extinction of conditioned place preference, further suggesting that the reductions in functional activity, and perhaps learning, may lead to a decreased ability to replace associations between drugs and cues (Schroeder & Packard, 2003, 2004). In addition to extinction of conditioned preference, the basolateral amygdala has been shown to be integral to the neural circuits involved in fear conditioning (Berlau & McGaugh, 2006; LaLumiere et al., 2003; Sacchetti et al, 1999; Vazdarjanova & McGaugh, 1999), thus reductions in the region have wider implications for associative learning in general, and not just reward based learning.

Here we demonstrate that there are large reductions in rates of glucose utilization in the dorsal raphe and locus coeruleus following withdrawal from cocaine self-administration. Our previous study, which investigated the effect of cocaine self-administration while cocaine was still on board, found no differences in functional activity in the locus coeruleus and actually higher levels of metabolism in the dorsal raphe, compared to controls (Macey et al., 2004). Because these areas are cell body nuclei for catecholamine projections that are widespread throughout the brain, these data demonstrate that cocaine self-administration affects a broad expanse of the brain, certainly well beyond the dopamine system that is typically investigated. Our data of alterations of functional activity in the dorsal raphe are particularly intriguing, since the 5-HT system has been shown to play a role in locomotor activity. Specifically, 5-HT levels have been shown to be inversely related to vertical activity (Brookshire and Jones, 2009); thus, it is tempting to speculate that reduced

serotonergic activity (as indicated by the lower levels of functional activity in the dorsal raphe) may have had direct behavioral consequences (increased vertical activity at baseline). In addition, if the alterations in raphe activity that we see in rodents are also present in human users, they may account for the sleep disturbances that are often reported by addicts following cocaine abuse during the first three weeks of abstinence (Morgan & Malison, 2007; Schierenbeck et al., 2008). Also, dysfunction of both the dorsal raphe and locus coeruleus has been directly related to anxiety and depression during acute (one week) and long-term (six-week) withdrawal (Carrasco and Van de Kar, 2003; Graeff et al., 1996; Itoi and Sugimoto, 2010; Weiss et al., 2001). Further, the locus coeruleus system has been shown to mediate shifts in attention, thus in addition to stress and anxiety, these reductions could have effects on basic attention, which could in turn lead to additional learning and memory deficits (Aston-Jones et al., 1999; Rajkowski et al., 1994; Usher et al., 1999). These functional alterations could be due to the ability of cocaine to inhibit both the norepinephrine and serotonin transporters (Rothman and Baumann, 2003), thus, these changes during withdrawal may be compensatory effects due to the sustained elevated levels of these transmitters during cocaine self-administration. Taken together, these data demonstrate that the alterations that occur following cocaine self-administration are clearly not limited to just the dopamine system, but include other neurotransmitter systems as well, and suggest that cocaine has a widespread influence on the brain even when the drug is no longer present.

In summary, the present study demonstrated that cocaine self-administration resulted in robust alterations in both functional and behavioral activity long after cocaine has been cleared. These data suggest that there are reductions in the functionality of a number of critical circuits involved in reward processing, memory, attention, sleep, and stress processing. The reductions in these areas have important implications for individuals that abuse cocaine as these data indicate that even a short (five-day) self-administration history can result in functional reductions in activity that are present up to 48 h later. These deficits were accompanied by behavioral changes as well, indicating that these metabolic changes are functionally relevant in the behaving animal. It is important to determine the functioning of neural networks after cocaine self-administration, as it is possible that the reductions in some of these regions persist for longer periods of time and could facilitate the continued use of drugs in the face of negative consequences, and facilitate continued drug administration in the face of robust tolerance, as well as potentiate drug seeking after periods of prolonged abstinence.

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Abbreviations

LGGU	local cerebral glucose utilization
2-DG	[¹⁴ C]-2-deoxyglucose
ACC	anterior cingulate cortex
NAc	nucleus accumbens
DA	dopamine
IP	intraperitoneal

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Figure 1. Cocaine self-administration results in escalation of rate of cocaine intake

(A) Event record of a representative cocaine self-administration animal. Each line represents a daily self-administration (SA) session. Each tick mark represents an injection of cocaine (1.5 mg/kg/inj). Animals are allowed 40 injections or 6 hours, whichever comes first. Animals demonstrate regular inter-injection intervals, and while injections stay constant, they take less time over sessions to complete the 40 injections. (B) The animals showed escalation of drug intake by self-administering 40 injections each day in successively shorter time periods. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; SA, self-administration)

A.

B.

Figure 2. Cocaine self-administration results in reduced locomotor response to a novel environment

Animals were placed in automated locomotor activity monitors and their behavior on the first exposure to the environment and the subsequent habituation was monitored for 30 minutes. (A) Locomotor activity, represented as distance traveled over time. Cocaine self-administration animals (open circles) habituated to the novel environment faster than their control counterparts (closed circles). (B) Total locomotor distance traveled in the novelty session. Animals with a history of cocaine self-administration have significantly reduced responses to a novel environment. (* $p < 0.05$; SA, self-administration)

Figure 3. Cocaine self-administration does not alter baseline locomotion

Animals were placed in automated locomotor activity monitors and their behavior following a saline injection to determine the effects of cocaine self-administration on forward locomotor activity. (A) Locomotor activity, represented as distance traveled over time. Cocaine self-administration animals (open circles) were not significantly different in their response to a saline injection than control animals (closed circles). (B) Total locomotor distance traveled during the saline session. No differences were observed in normal baseline forward locomotion following cocaine self-administration. (SA, self-administration)

Figure 4. Altered locomotor activity following cocaine self-administration

Animals were placed in automated locomotor activity monitors and their behavior following a saline injection to determine the effects of cocaine self-administration on stereotypy and vertical activity (A) Stereotypy as represented as average stereotypy counts per five minute bin over the 90 minute session. Cocaine self-administration animals (blue) had reduced stereotypy counts as compared to controls (black). (B) Vertical activity as represented as average stereotypy counts per five minute bin over the 90 minute session. Cocaine self-administration animals (blue) had increased vertical counts as compared to controls (black). (***) $p < 0.001$; SA, self- administration)

Figure 5. Cocaine self-administration results in reduced functional activity in a number of brain regions

Pseudocolor enhanced autoradiograms showing rates of local cerebral glucose metabolism in the striatum (top row) and hippocampus (bottom row) of a representative control rat (panel A) and a rat 48 h following cocaine self-administration (panel B).

Table 1

Effect of 48 h withdrawal from a five-day binge cocaine self-administration on rates of local cerebral glucose utilization in rat brain.

Brain Region	Drug-naïve Controls (n = 6)	Cocaine-exposed (n = 7)
<i>Cortex</i>		
Medial prefrontal	76.2 ± 1.9	67.7 ± 3.2
Anterior cingulate	105.9 ± 2.9	93.1 ± 4.2 *
Motor	108.9 ± 2.9	97.3 ± 5.7
<i>Basal Ganglia</i>		
Dorsal caudate	111.0 ± 5.9	93.7 ± 4.0 *
Ventral caudate	96.8 ± 3.5	85.7 ± 4.7
Nucleus accumbens	94.4 ± 4.1	79.7 ± 3.8 **
Globus pallidus	53.8 ± 3.7	45.2 ± 2.8
Substantia nigra pars compacta	65.2 ± 2.8	58.7 ± 4.0
Substantia nigra pars reticulata	56.2 ± 3.3	50.1 ± 2.7
<i>Limbic</i>		
Basolateral amygdala	81.3 ± 4.5	68.1 ± 3.4 *
Central amygdala	42.9 ± 3.5	39.8 ± 2.1
Lateral thalamus	95.1 ± 6.2	83.0 ± 5.9
Medial thalamus	97.7 ± 4.2	84.8 ± 3.7 *
Ventromedial hypothalamus	56.6 ± 5.9	46.1 ± 3.0
Hippocampal CA1 region	78.6 ± 7.2	60.0 ± 3.9 *
Hippocampal CA3 region	81.2 ± 6.9	66.6 ± 3.7
Dentate gyrus	64.5 ± 6.6	52.4 ± 2.4
<i>Brainstem</i>		
Dorsal raphe	117.5 ± 2.3	96.4 ± 7.5 *
Locus coeruleus	73.8 ± 2.0	64.0 ± 2.8 *
Cerebellum	70.6 ± 3.7	60.3 ± 2.4 *

Values are local rates of glucose utilization expressed as mean (μmol/100g/min)±S.E.M.

* P<0.05

** P<0.001 versus drug-naïve controls.