RESEARCH REPORT

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Modulation of striatal dopamine dynamics by cocaine self-administration and amphetamine treatment in female rats

Cody A. Siciliano[1,2] | Madelyn I. Mauterer[1] | Steve C. Fordahl[1,3] | Sara R. Jones[1]

[1]Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, North Carolina

[2]Department of Brain and Cognitive Sciences, The Picower Institute for Learning and Memory, Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts

[3]Department of Nutrition, University of North Carolina at Greensboro, Greensboro, North Carolina

Correspondence
Sara R. Jones, Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157.
Email: srjones@wakehealth.edu

Abstract

Despite decades of research into the neurobiological basis of cocaine abuse, pharmacotherapeutic treatments for cocaine addiction have been largely ineffective. Converging evidence from preclinical research and from outpatient clinical trials suggest that treatment with amphetamine is efficacious in reducing cocaine intake. Although it has been suggested that amphetamine treatment reduces cocaine intake as an agonist replacement therapy, we have shown recently that multiple aspects of dopamine signaling are altered by cocaine self-administration and returned to pre-cocaine function by amphetamine treatment in the nucleus accumbens of male rats. Here, we sought to determine if these effects were also evident in female subjects, and across regions of the striatum. Female rats performed 5 days of cocaine self-administration (1.5 mg kg^{-1} inj^{-1}, 40 inj/day) and were treated with a single amphetamine (0.56 mg/kg) or saline infusion 1 hr prior to killing. We then used ex vivo fast-scan cyclic voltammetry in the nucleus accumbens core or dorsolateral caudate-putamen to examine dopamine signaling and cocaine potency. We found that in the nucleus accumbens core, cocaine self-administration decreased dopamine uptake rate and cocaine potency, and both alterations were restored by amphetamine treatment. In the dorsolateral caudate-putamen, neither cocaine self-administration nor amphetamine treatment altered dopamine uptake; however, cocaine potency was decreased by self-administration and returned to control levels by amphetamine. Together, these findings support a role for amphetamine treatment for cocaine addiction outside of agonist replacement therapy, and suggest that the development of cocaine tolerance is similar across sexes.

KEYWORDS
cocaine potency, dopamine transporter, dopamine uptake, releasers, voltammetry

1 | INTRODUCTION

Currently available pharmacotherapeutic treatments for cocaine addiction are largely ineffective in reducing cocaine use

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Abbreviations: AMPH, amphetamine; DAT, dopamine transporter; DLC, dorsolateral caudate-putamen; NAc, nucleus accumbens.

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and relapse; thus, much effort is aimed at identifying compounds that may be efficacious treatments. Recently, there has been mounting evidence supporting the hypothesis that amphetamine (AMPH)-based dopamine releasing agents may be useful in the treatment of cocaine addiction. Although the canonical therapeutic applications of AMPH are in the treatment of ADHD, narcolepsy and obesity disorders, more recent studies have demonstrated that AMPH decreases cocaine intake across a range of modalities. For example, in rodent studies, continuous treatment with low-dose AMPH reduces breakpoints for intravenous cocaine on a progressive ratio schedule

of reinforcement (Chiodo, Lack, & Roberts, 2008). Numerous studies in nonhuman primate models have also demonstrated that continuously infused AMPH can selectively reduce responding for cocaine. Indeed, AMPH produces a rightward and downward shift in the dose-effect function for cocaine, when cocaine self-administration is maintained on a progressive ratio (Negus & Mello, 2003a) or second-order schedule of reinforcement (Negus & Mello, 2003b) in rhesus monkeys. Further, under more clinically relevant conditions where monkeys were treated continuously with AMPH and allowed to self-administer cocaine every eighth day, AMPH produced reliable decreases in the reinforcing efficacy of cocaine (Czoty, Gould, Martelle, & Nader, 2011). These effects appear to be specific to responding maintained by cocaine, as AMPH had little effect on food consumption or food-maintained responding (Chiodo et al., 2008; Czoty, Martelle, & Nader, 2010; Negus & Mello, 2003a, 2003b), and any alterations to food responding were transient, while effects on responding for cocaine were long-lasting (Czoty et al., 2011). Finally, in choice self-administration procedures, where animals are given the option to allocate responding toward either cocaine or food, AMPH treatment shifts responding away from cocaine and toward food (Banks, Hutsell, Schwienteck, & Negus, 2015; Negus, 2003).

The success of AMPH in decreasing cocaine taking in animal studies has largely translated into studies in humans, both in terms of controlled laboratory experiments and outpatient double-blind placebo-controlled clinical trials (Herin, Rush, & Grabowski, 2010). Similar to animal studies, AMPH maintenance in cocaine-dependent individuals shifted choice away from cocaine in a cocaine versus money choice procedure (Rush, Stoops, Sevak, & Hays, 2010). Further, AMPH maintenance treatment reduced several of the self-reported effects of cocaine in cocaine-dependent individuals, including self-reported assessment of the "willing to pay for" and "good effects" aspects of cocaine and (Rush, Stoops, and Hays, 2009). Shearer, Wodak, van Beek, Mattick, and Lewis (2003) found that maintenance on AMPH treatment in a small placebo-controlled trial improved several facets of treatment outcomes, including large reductions (~40%) in benzoylecgonine (cocaine metabolite)-positive urine tests during the treatment period as compared to the placebo control group. Self-reported use of cocaine, criminal activity, and cocaine craving were also reduced as compared to pre-treatment levels (Shearer et al., 2003). These findings were supported and extended by two larger clinical trials conducted by Grabowski and colleagues in which AMPH was administered on an outpatient basis for 12 (Grabowski et al., 2001) or 24 (Grabowski, Rhoades, et al., 2004; Grabowski, Shearer, Merrill, & Negus, 2004) weeks to cocaine-dependent individuals seeking treatment. Both studies saw reductions in benzoylecgonine-positive urine tests (but see Mooney et al., 2015). Together, these studies, in combination with the preclinical literature, support the notion that AMPH treatment is a viable option

for treating cocaine dependence and addiction. However, the large majority of preclinical studies have not included female subjects, and while clinical trials have included female subjects, sex was often not reported as a variable.

The effects of cocaine are often sexually dimorphic, and in both animal and human studies, female subjects often show greater acute effects of cocaine (e.g., self-reported effects in humans, or conditioned place preference in animals) as well as higher rates of cocaine self-administration (Becker, 2016; Calipari et al., 2017; and Dow-Edwards, 2010; Roth & Carroll, 2004). A recent study from our lab indicated that AMPH's effects may go beyond agonist replacement therapy, and may be due instead to AMPH-induced reversal of cocaine-evoked plasticity. Ferris et al. (2015) demonstrated that 5 days of binge cocaine self-administration resulted in reduced cocaine potency in the nucleus accumbens (NAc) core; however, when animals were given a single AMPH infusion following cocaine self-administration, cocaine potency was restored back to control levels. The ability of AMPH to modulate cocaine-induced plasticity has only been examined in the NAc core, ignoring other dopamine terminal regions, and in male subjects. The aim of the current study was to further explore the phenomenon of AMPH-mediated reversal of cocaine-induced plasticity in female subjects, and across dopaminergic terminal region. Here, we find a similar phenomenon in females suggesting that AMPH may serve as an effective pharmacological intervention in both males and females.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult female Sprague-Dawley rats (200–250 g; Harlan Laboratories), were maintained on a 12:12 hr reverse light/dark cycle (3:00 a.m. lights off; 3:00 p.m. lights on) with food and water ad libitum. All animals 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.

2.2 | Self-administration

Rats were anesthetized (100 mg/kg ketamine, 10 mg/kg xylazine, i.p.) and implanted with chronic indwelling jugular catheters as previously described (Siciliano, Ferris, & Jones, 2015). Immediately following surgery, animals were given warm ringer's solution with 5 mg/kg ketoprofen subcutaneously (1 ml total volume) and placed on a heating pad until recovered. Animals were singly housed, and each 6-hr session took place in the home cage during the active/dark cycle (09:00–15:00 hr). The first self-administration session began 3 days post surgery. Without any prior operant

training, animals were given access on a fixed-ratio one schedule to a cocaine-paired lever; which, upon responding, initiated an intravenous injection of cocaine (1.5 mg/kg, infused over ~4 s, depending on animal weight). After each response/infusion, the lever was retracted and a stimulus light was illuminated for a 20-s timeout period. Sessions lasted 6 hr or until 40 injections were taken. Acquisition (Day 1) was counted when the animal reached 35 or more responses with a stable and consistent inter-injection interval. Following acquisition of cocaine maintained responding, the animals were given access to 40 injections per day (1.5 mg kg^{-1} infusion^{-1}) for a period of 5 consecutive days. The morning following completion of the final self-administration session (~18 hr withdrawn), animals were given a single experimenter-delivered infusion of AMPH (0.1 ml, 0.56 mg/kg, i.v.) or saline (0.1 ml, i.v.). Animals were killed exactly 1 hr post AMPH or saline infusion for voltammetry experiments. Control animals were naive rats housed under the same reversed light–dark light cycle.

2.3 | Ex vivo voltammetry

Fast-scan cyclic voltammetry (FSCV) was used to examine dopamine signaling kinetics and the ability of cocaine to inhibit dopamine uptake in the NAc core and DLC. Animals were briefly anesthetized with isoflurane before decapitation was performed in a ventilated area free of any blood or tissue from previous animals. A vibrating tissue slicer was used to prepare 400-μm thick coronal brain sections containing the NAc core, as previously described (Siciliano, Calipari, Ferris, & Jones, 2014). The tissue was immersed in oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), NaH$_2$PO$_4$ (1.2), CaCl$_2$ (2.4), MgCl$_2$ (1.2), NaHCO$_3$ (25), glucose (11), L-ascorbic acid (0.4) and pH was adjusted to 7.4. Once sliced, the tissue was transferred to the testing chambers containing bath aCSF (32°C), which flowed at 2 ml/min. A carbon fiber microelectrode (100–150 μM length, 7 μM diameter) and bipolar stimulating electrode were placed into the core of the NAc or DLC. Dopamine release was evoked by a single electrical pulse (350 μA, 4 msec, monophasic) applied to the tissue every 3 min. Extracellular dopamine was recorded by applying a triangular waveform (−0.4 to +1.2 to −0.4 V vs. Ag/AgCl, 400 V/s). Once the extracellular dopamine response was stable, cocaine (0.3–30 μM) was applied cumulatively to the brain slice.

2.4 | K$_i$ values

As described previously (Siciliano, Calipari, & Jones, 2014), inhibition constants (K$_i$) were calculated by the equation: [(K$_m$)/(S)] where K$_m$ is equal to the K$_m$ of dopamine for the dopamine transporter, or 1.6 μM, and S is equal to the slope of the linear concentration-response regression for cocaine.

The K$_i$ is reported in μM and is a measure of the cocaine concentration that is necessary to decrease the rate of dopamine–DAT interactions to 50% of their uninhibited rate.

2.5 | Data analysis

For all analysis of FSCV data, Demon Voltammetry and Analysis software was used (Yorgason, España, & Jones, 2011). Recording electrodes were calibrated by recording responses (in electrical current; nA) to a known concentration of dopamine (3 μM) using a flow-injection system. This was used to convert electrical current to dopamine concentration. Michaelis–Menten modeling parameters were used to determine the maximal rate of dopamine uptake and cocaine-induced uptake inhibition (Wightman et al., 1988). Michaelis–Menten modeling provides parameters that describe the amount of dopamine released following electrical stimulation, the maximal rate of dopamine uptake (V_{max}), and alterations in the ability of dopamine to bind to the DAT, or apparent K$_m$. For pre-drug modeling, we followed standard voltammetric modeling procedures by setting the baseline K$_m$ parameter to 160 nM, based on the affinity of dopamine for the DAT, whereas V_{max} values were allowed to vary as the pre-drug measure of the rate of dopamine uptake. Following drug application, apparent K$_m$ was allowed to vary to account for changes in drug-induced dopamine uptake inhibition while the respective V_{max} value determined for that subject at baseline was held constant. The apparent K$_m$ parameter models the amount of dopamine uptake inhibition following a particular concentration of drug.

2.6 | Statistics

Graph Pad Prism (version 6) was used to statistically analyze data sets and create graphs. Baseline kinetics (release and uptake) and K$_i$ values were compared using a one-way analysis of variance (ANOVA). Concentration-response curves for cocaine were subjected to a mixed-model two-way repeated measures ANOVA with concentration as the within subject factor, and experimental group as the between subject factor. Differences between groups were tested using a Bonferroni post hoc test. All p values of <0.05 were considered to be statistically significant.

3 | RESULTS

3.1 | Cocaine self-administration-induced deficits in dopamine system function are rescued by a single infusion of AMPH

Animals were allowed access to cocaine until they completed 5 days of 40 infusions (Figure 1a). This self-administration procedure holds intake constant, but results in an escalation
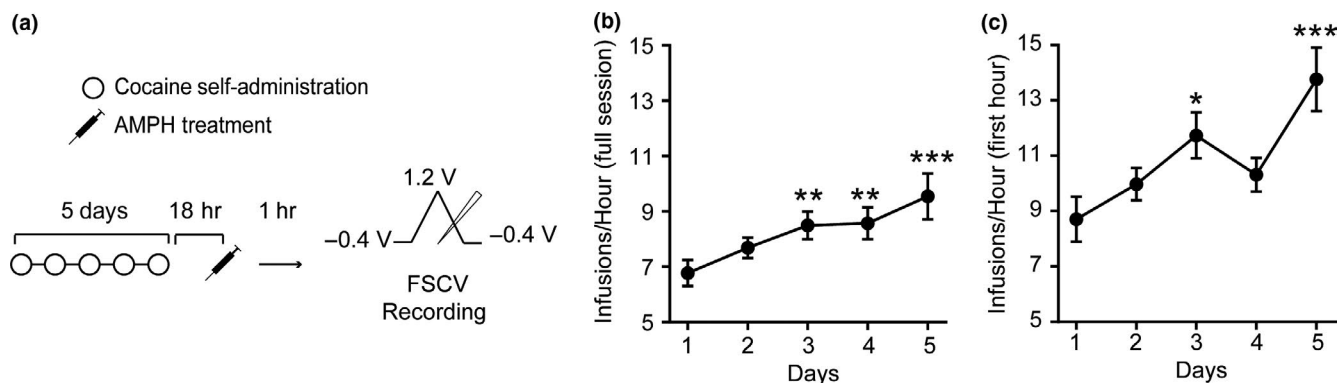
(a)

○ Cocaine self-administration

↗ AMPH treatment

5 days   18 hr   1 hr

○—○—○—○—○   ↗   →

1.2 V

−0.4 V ⟋⟍ −0.4 V

FSCV Recording

(b)

(c)

FIGURE 1 Timeline of cocaine self-administration and voltammetric recordings. (a) Experimental design depicting 5 days of cocaine self-administration, followed by a single AMPH or saline infusion ~18 hr after the final self-administration session. Animals were killed for ex vivo voltammetry experiments 1 hr following infusion. (b) Rate of cocaine intake over the 5 days of cocaine self-administration. (c) Rate of cocaine intake during the first hour of each session. **$p < 0.01$; ***$p < 0.001$ versus Day 1

in the rate of cocaine intake over days in males (Siciliano, Ferris, & Jones, 2015). Similarly, a one-way ANOVA revealed an escalated rate of cocaine intake in female rats (Figure 1b), measured by infusions/hour over the 5 days of cocaine self-administration ($F_{4,24} = 8.44$, $p = 0.0002$; Bonferroni post hoc test: Day 3 vs. Day 1, $p < 0.01$; Day 4 vs. Day 1, $p < 0.01$; Day 5 vs. Day 1, $p < 0.001$). Additionally, animals exhibited escalation of front-loading behavior as shown by a sharp increase in the number of cocaine infusions taken during the first hour of each session (Figure 1c; one-way ANOVA, $F_{4,24} = 6.627$, $p = 0.001$; Bonferroni post hoc test: Day 3 vs. Day 1, $p < 0.05$; Day 5 vs. Day 1, $p < 0.001$).

Following completion of cocaine self-administration and treatment with either saline (Cocaine SA + Saline group) or AMPH (Cocaine SA + AMPH group), we found that there was no effect of either treatment group on dopamine release compared to control animals (Figure 2a,b). However, a one-way ANOVA ($F_{2,25} = 2.834$, $p = 0.08$) and Bonferroni post hoc test revealed that dopamine uptake rate was decreased in the Cocaine SA + Saline group, compared to controls ($p < 0.05$), an effect that was not present in AMPH-treated animals (Figure 2a,c). We then bath-applied cumulative concentrations of cocaine to striatal slices to determine alterations in cocaine potency at the DAT (Figure 3a). A two-way ANOVA revealed a main effect of concentration ($F_{4,100} = 170.8$, $p < 0.0001$) and group ($F_{2,25} = 6.57$, $p = 0.005$), on cocaine potency, as well as a concentration × group interaction ($F_{8,100} = 4.60$, $p < 0.0001$; Figure 2b). Bonferroni post hoc analysis indicated decreased cocaine-induced uptake inhibition in Cocaine SA + Saline animals as compared to controls at the 10 ($p < 0.05$) and 30 μM ($p < 0.001$) concentrations (Figure 3b). Cocaine SA + AMPH animals showed increased cocaine-induced uptake inhibition compared to Cocaine SA + Saline animals at 10 ($p < 0.05$) and 30 μM
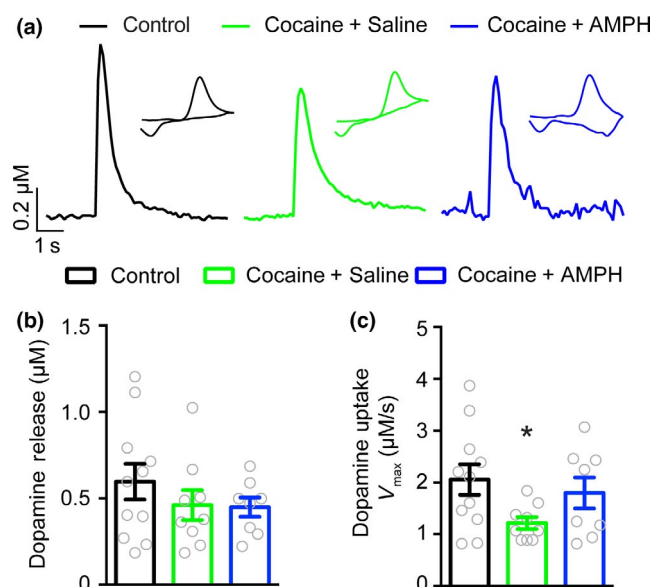
(a) — Control — Cocaine + Saline — Cocaine + AMPH

□ Control □ Cocaine + Saline □ Cocaine + AMPH

(b)

(c)

FIGURE 2 Cocaine self-administration-induced reductions in dopamine uptake in the NAc core are rescued by a single AMPH infusion. (a) Representative traces and cyclic voltammograms. (b) Dopamine release in the NAc core is unchanged by cocaine self-administration or AMPH treatment. (c) Maximal rate of dopamine uptake (V_{max}) is decreased by cocaine self-administration and rescued by AMPH treatment. *$p < 0.05$. Control, $n = 11$; Cocaine + Saline, $n = 9$; Cocaine + AMPH, $n = 8$

($p < 0.001$) concentrations, but did not differ at any concentration as compared to control animals (Figure 3b).

Further, from the concentration-response curves we calculated inhibitory constants (K_i values) for cocaine-induced inhibition of the DAT. K_i values are the concentration of cocaine needed to produce a 50% reduction in dopamine uptake, and are inverse to cocaine potency. A one-way ANOVA found a main effect of group on K_i values ($F_{2,25} = 7.24$, $p = 0.003$), and post hoc analysis revealed that Cocaine SA + Saline animals exhibited increased K_i values, compared to controls ($p < 0.05$;
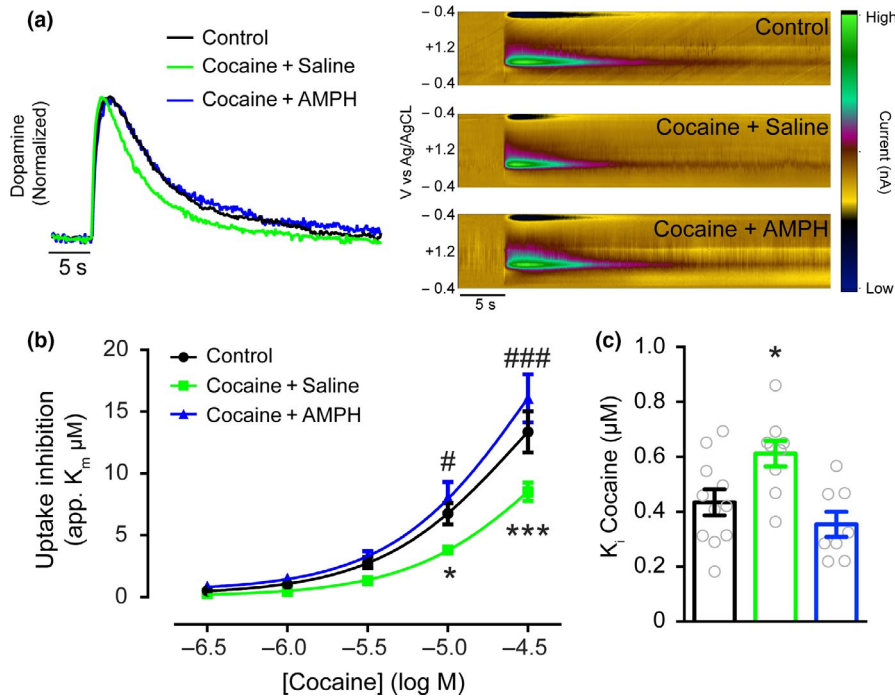
FIGURE 3 Cocaine self-administration-induced reductions in cocaine potency in the NAc core are rescued by a single AMPH infusion. (a) Representative traces (left) and pseudo-color plots (right) after 30 μM cocaine. (b) Concentration-response curves for cocaine reveal a downward shift in cocaine effect on dopamine uptake in cocaine self-administration animals, which are returned to control levels by an AMPH infusion. (c) K_i values (inhibitory constant) for cocaine at the dopamine transporter are increased by self-administration, indicative of decreased cocaine potency, and returned to control levels by an AMPH infusion. $*p < 0.05$; $***p < 0.001$ versus control; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ versus cocaine SA + Saline. Control, $n = 11$; Cocaine + Saline, $n = 9$; Cocaine + AMPH, $n = 8$

Figure 3c). However, there was no difference between control and Cocaine SA + AMPH animals. These results demonstrate that cocaine self-administration reduces both dopamine uptake rate and cocaine potency in the NAc core, and that both of these effects are rescued by AMPH treatment.

3.2 | Cocaine self-administration decreased dopamine uptake rate and produced tolerance to the DAT-inhibiting effects of cocaine in the DLC

We next sought to determine the effects of cocaine self-administration and AMPH treatment on dopamine signaling kinetics and cocaine potency in the DLC (Figure 4a). We found that, similar to the NAc core, dopamine release was unaltered in either treatment group (Figure 4a,b). In contrast to the NAc core, we found that dopamine uptake rate was also not changed in Cocaine SA + Saline or Cocaine SA + AMPH animals (Figure 4a,c).

Following bath application of cocaine (Figure 5a), a two-way ANOVA revealed main effects of concentration ($F_{4,84} = 206.2, p < 0.0001$) and group ($F_{2,21} = 5.17, p = 0.015$) on cocaine potency, as well as a concentration × group interaction ($F_{8,84} = 4.74, p < 0.0001$; Figure 5b). Bonferroni post hoc analysis indicated decreased cocaine-induced uptake

inhibition at 30 μM cocaine in Cocaine SA + Saline animals, compared to controls ($p < 0.01$; Figure 5b). Further, in Cocaine SA + AMPH animals, cocaine-induced inhibition was increased at 10 ($p < 0.05$) and 30 μM ($p < 0.001$) as compared to Cocaine SA + Saline animals, and at 30 μM ($p < 0.01$) as compared to control animals. This demonstrates that tolerance to cocaine in the DLC, induced by cocaine self-administration, is reversed by AMPH. A main effect of treatment group was found on K_i values in the DLC ($F_{2,21} = 4.26, p = 0.028$), but no significant differences were detected between groups following post hoc analysis (Figure 5c).

3.3 | Cocaine actions at the DAT are similar across striatal subregion

To determine if cocaine effects are different between the DLC and NAc core, we compared cocaine effects on uptake inhibition across the regions within each group (Figure 6). In control animals, a two-way ANOVA revealed a main effect of concentration on cocaine potency ($F_{4,72} = 112.9, p < 0.0001$), but no effect of region or a concentration × region interaction was detected (Figure 6a). Similarly, we found the main effects of concentration on cocaine potency in Cocaine SA + Saline ($F_{4,60} = 138.4, p < 0.0001$; Figure 6b) and Cocaine SA + AMPH ($F_{4,52} = 131.6, p < 0.0001$;
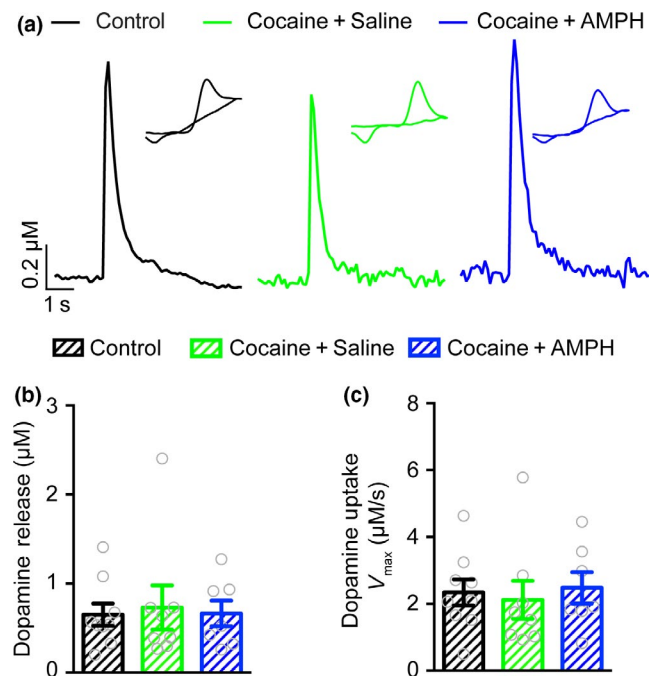
FIGURE 4 Cocaine self-administration and AMPH treatment do not change dopamine release or uptake in the DLC. (a) Representative traces and cyclic voltammograms. Both dopamine release (b) and uptake (c) in the DLC are unchanged in either treatment group as compared to control animals. Control, $n = 9$; Cocaine + Saline, $n = 8$; Cocaine + AMPH, $n = 7$

Figure 6c) animals, but no effect of region or interaction. These data demonstrate that cocaine potency is the same between the NAc core and DLC in female rats, and that these regions respond similarly to cocaine self-administration and AMPH treatment.

4 | DISCUSSION

Here we show that high intake cocaine self-administration results in several distinct alterations in striatal DAT function of female rats. We found that the effects of cocaine self-administration were different across striatal subregion, resulting in decreased dopamine reuptake in the NAc core, whereas dopamine reuptake in the DLC was preserved. Conversely, we found that cocaine self-administration-induced alterations to cocaine potency were similar across regions, as cocaine self-administration resulted in tolerance to the DAT-inhibiting effects of cocaine in both regions tested. Importantly, a single infusion of AMPH restored all of these measures to at least control levels, and increased cocaine potency in the DLC beyond control levels in animals treated with AMPH. These findings have implications for the development of dopamine system deficits in female cocaine users, as well as for the use of AMPH as a treatment for cocaine addiction and dependence.

The work of our lab and many others has highlighted alterations in dopamine system function, specifically at terminal projection regions in the striatum, as a consequence of cocaine use that is critical to the development of addiction in male subjects (see Siciliano, Calipari, Ferris, & Jones, 2015 for review). However, the effects of cocaine can be sexually dimorphic, and in both animal and human studies, female subjects often show greater acute effects of cocaine (e.g., self-reported effects in humans, or conditioned place preference in animals) as well as higher rates of cocaine self-administration (Becker, 2016; Becker & Koob, 2016; Calipari et al., 2017; and Dow-Edwards, 2010; Kasperski et al., 2011; Roth & Carroll, 2004). Furthermore, there are complex interactions between the estrous cycle and cocaine self-administration, whereby fluctuating ovarian hormones can influence cocaine effects (Calipari et al., 2017) and long-term cocaine administration can interfere with the natural hormonal cycle, factors which could converge to influence cocaine-induced neural plasticity. However, in the case of cocaine self-administration effects on the dopamine system, we did not see differences between males and females in the development of tolerance. Females that had self-administered cocaine still showed decreased cocaine potency at the dopamine transporter, similar to what we have shown previously in males (Ferris, Mateo, Roberts, & Jones, 2011; Siciliano, Fordahl, & Jones, 2016). These data suggest that in regard to dopamine terminal adaptations induced by chronic cocaine self-administration, effects are not sex-specific and that treatments that target the reversal of these effects could be efficacious across sexes. While the impact of cocaine self-administration on cocaine potency appears to be similar between sexes, estrous cycle can also heavily modulate cocaine effects on the dopamine system acutely (Calipari et al., 2017; Cummings, Jagannathan, Jackson, & Becker, 2014), as well as the acute effects of other psychostimulants such as AMPH (Becker, 1990; Becker & Cha, 1989; Castner, Xiao, & Becker, 1993). Thus, it will be important to examine interactions between cycle and chronic cocaine exposure in future studies.

We have previously shown that cocaine self-administration produces tolerance to the DAT-inhibiting actions of cocaine in the NAc core and shell of male subjects (Ferris et al., 2011; Mateo, Lack, Morgan, Roberts, & Jones, 2005; Siciliano, Ferris, & Jones, 2015). Here, we show that DAT in the DLC also undergoes alterations which result in a similar degree of tolerance. Further, the fact that cocaine potency was decreased in both regions, but uptake was only altered in the NAc core demonstrates that tolerance is independent of changes in dopamine uptake. The ubiquitous presence of tolerance also suggests that decreased cocaine potency is a product of direct DAT-cocaine interactions irrespective of originating input (i.e., projections arising from ventral tegmental area vs. substantia nigra) or local microenvironment surrounding dopamine terminals. Instead, we hypothesize
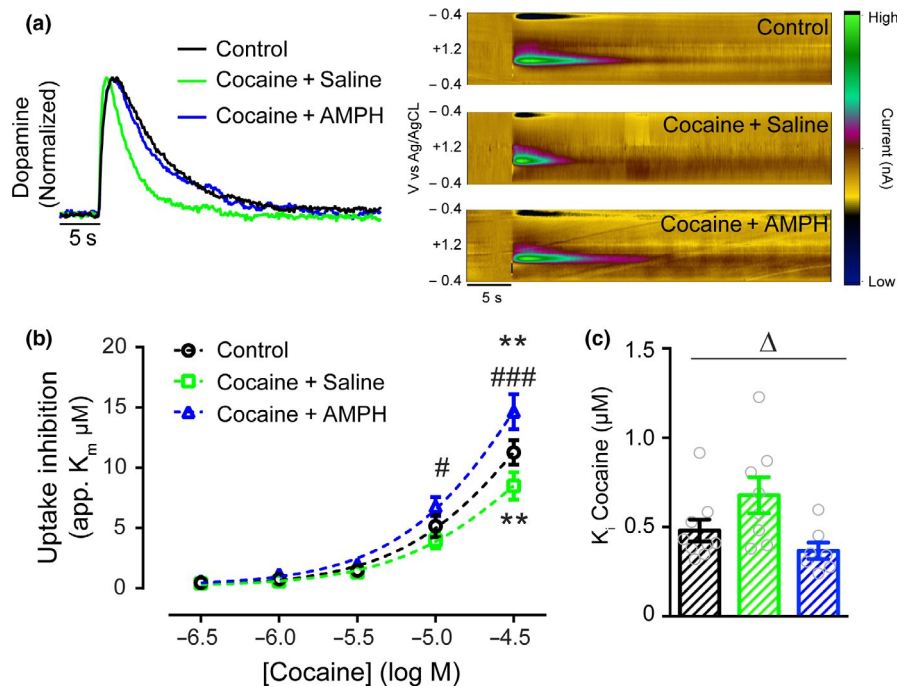
FIGURE 5 Cocaine self-administration produces AMPH-reversible cocaine tolerance in the DLC. (a) Representative traces (left) and pseudo-color plots (right) after 30 μM cocaine. (b) Similar to the NAc core, we found that cocaine self-administration decreased cocaine effects on dopamine uptake rate in the DLC. Further, cocaine potency was increased greater than control levels in cocaine self-administration animals that received a single infusion of AMPH. (c) There was a main effect of group on K_i values, although post hoc analysis did not reach significance. Δ, main effect of ANOVA; **$p < 0.01$ versus control; [#]$p < 0.05$, [###]$p < 0.001$ versus cocaine SA + Saline. Control, $n = 9$; Cocaine + Saline, $n = 8$; Cocaine + AMPH, $n = 7$
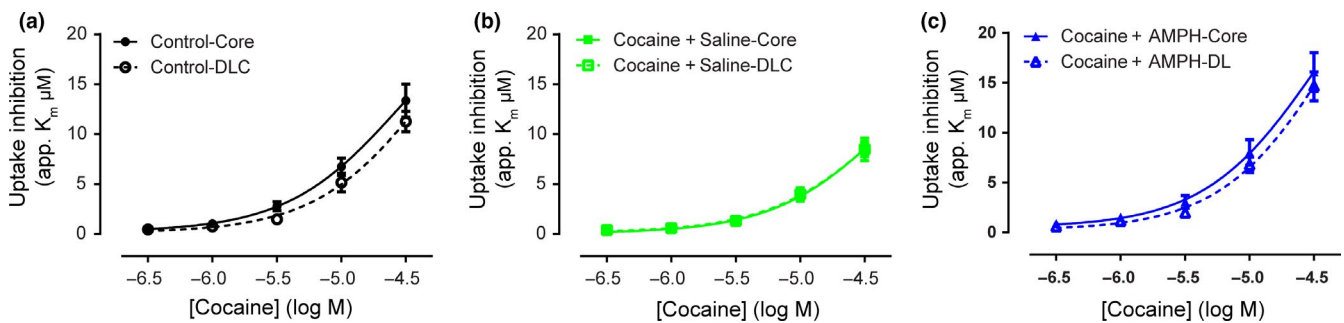

FIGURE 6 Cocaine tolerance and rescue by AMPH are similar across striatal subregion. Cocaine-induced inhibition of dopamine uptake is similar between the DLC and NAc core in (a) control animals, (b) cocaine self-administration animals treated with saline, and (c) cocaine self-administration animals treated with AMPH

that decreased cocaine potency results from alterations, such as conformational changes or deviations in DAT binding partners, which can alter cocaine potency independent of up-take rate/DAT expression (Hong & Amara, 2010).

One notable difference between these findings and previous studies in male subjects is the lack of effect of cocaine self-administration on dopamine release in the NAc core. Indeed, we have reported decrease dopamine release following the same cocaine self-administration procedure in male subjects (Ferris et al., 2012; Siciliano, Ferris, & Jones, 2015). Here, we did observe a small decrease in dopamine release in cocaine self-administration animals with and without AMPH

treatment, though neither reached statistical significance compared to control animals. It is possible that the lack of effect observed here is due to insufficient statistical power. Notably, cycle stage was not observed in the current; given that estrous has been shown to modulate cocaine effects at the DAT in mice (Calipari et al., 2017) it is possible that cycle stage may have effected some of the results reported here. However, the NAc core experiments in the current study ($n = 8-11$ per group) had greater statistical power than any of our previous studies in males ($n = 4-8$ per group). Thus, while it is possible that a statistically significant effect of cocaine self-administration on dopamine release in female

subjects may emerge with increased study size, at the very least the effect size is smaller than seen in previous studies with male subjects which reached statistical significance with fewer subjects.

Alterations to dopamine uptake and cocaine potency have been linked to escalated cocaine intake and increased motivation to administer cocaine (Siciliano, Calipari, et al., 2015). Thus, AMPH-induced restoration of dopamine system function may be contributing to the well-documented reducing effect of AMPH treatment on cocaine intake. Although the effects of AMPH on cocaine intake have been described in several species and models, until recently it was thought that AMPH was acting as an agonist replacement therapy (a compound that acts similarly on the same neurotransmitter system as the abused drug). This study provides additional evidence to support the recent hypothesis that the efficacy of AMPH stems, at least in part, from reversal of cocaine induced plasticity of dopamine terminals (Ferris et al., 2015). These are, to our knowledge, the first studies examining the neurochemical underpinnings of AMPH's efficacy as a pharmacotherapeutic agent in the treatment of cocaine addiction, and call for a re-classification of AMPH's mechanism of action.

Although AMPH-based monoamine releasers have been effective at reducing cocaine intake and the reinforcing efficacy of cocaine in preclinical and clinical settings, results with monoamine uptake inhibitors have been less promising, suggesting that these effects are not simply due to the ability of the compound to increase synaptic dopamine levels. Methylphenidate, a non-substrate dopamine transporter inhibitor and the active compound in the ADHD medication Ritalin, has been examined as a possible medication in the treatment of cocaine addiction. Similar to AMPH, methylphenidate has been shown to decrease some of the positive self-reported effects of cocaine (Collins, Levin, Foltin, Kleber, & Evans, 2006; Winhusen et al., 2006). Results examining the effects of methylphenidate on the reinforcing effects of cocaine have been variable with some human studies showing methylphenidate-induced decreases in cocaine choice during a cocaine versus money choice procedure (Collins et al., 2006), while animals studies have shown no change or increases in cocaine self-administration during methylphenidate treatment (Czoty, Martelle, Gould, & Nader, 2013; Hiranita, Soto, Newman, & Katz, 2009). Furthermore, clinical trials of methylphenidate treatment of cocaine abuse have also produced mixed results with some studies showing small decreases in cocaine use (Khantzian, Gawin, Kleber, & Riordan, 1984) while others have shown no benefit of treatment (Grabowski et al., 1997). These results suggest that the ability of monoamine system-targeting treatments to decrease cocaine intake is not entirely a product of their ability to elevate monoamine levels, and that AMPH's efficacy may be dependent on some other

characteristic of the drug, possibly a function of its properties as a releaser agent, which may allow for reversal of cocaine-induced plasticity as shown in the current study.

Together, these data outline a neural interaction between cocaine-induced plasticity of dopamine nerve terminals and AMPH treatment, whereby AMPH has the unique ability to restore dopamine terminal function. These findings expand a growing literature highlighting the unique actions of dopamine releasing agents, and provide a putative neurochemical mechanism for the increased efficacy of these compounds in reducing cocaine intake as compared to monoamine blocker compounds. Future research will aim to determine the molecular mechanisms underlying AMPH-induced reversal of cocaine-induced plasticity of dopamine system function. Elucidating these mechanisms may open avenues for the development of novel pharmacotherapeutic compounds with similar effects on cocaine intake, without the inherent abuse liability of AMPH.

CONFLICT OF INTEREST

The authors have no conflicts to report.

DATA ACCESSIBILITY

Data collected for this manuscript can be provided upon request from the corresponding authors.

AUTHOR CONTRIBUTIONS

C.A.S. and S.R.J designed the research, C.A.S., S.C.F., and M.I.M. performed the research, C.A.S. and S.C.F. analyzed the data, C.A.S. and S.R.J. wrote the paper.

ORCID

Cody A. Siciliano https://orcid.org/0000-0001-9871-2089

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