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ARTICLE

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PET imaging of dopamine transporters and D2/D3 receptors in female monkeys: effects of chronic cocaine self-administration

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Brain imaging studies using positron emission tomography (PET) have shown that long-term cocaine use is associated with lower levels of dopamine (DA) D2/D3 receptors (D2/D3R); less consistent are the effects on DA transporter (DAT) availability. However, most studies have been conducted in male subjects (humans, monkeys, rodents). In this study, we used PET imaging in nine drug-naïve female cynomolgus monkeys to determine if baseline measures of DAT, with [^{18}F]FECNT, and D2/D3R availability, with [^{11}C] raclopride, in the caudate nucleus, putamen and ventral striatum were associated with rates of cocaine self-administration and if these measures changed during long-term (~13 months) cocaine self-administration and following time-off (3–9 months) from cocaine. Cocaine (0.2 mg/kg/injection) and 1.0 g food pellets were available under a multiple fixed-interval (FI) 3-min schedule of reinforcement. In contrast to what has been observed in male monkeys, baseline D2/D3R availability was positively correlated with rates of cocaine self-administration only during the first week of exposure; DAT availability did not correlate with cocaine self-administration. D2/D3R availability decreased ~20% following cumulative intakes of 100 and 1000 mg/kg cocaine; DAT availability did not significantly change. These reductions in D2/D3R availability did not recover over 9 months of time-off from cocaine. To determine if these reductions were reversible, three monkeys were implanted with osmotic pumps that delivered raclopride for 30 days. We found that chronic treatment with the D2/D3R antagonist raclopride increased D2/D3R availability in the ventral striatum but not in the other regions when compared to baseline levels. Over 13 months of self-administration, tolerance did not develop to the rate-decreasing effects of self-administered cocaine on food-reinforced responding, but number of injections and cocaine intake significantly increased over the 13 months. These data extend previous findings to female monkeys and suggest sex differences in the relationship between D2/D3R availability related to vulnerability and long-term cocaine use.

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INTRODUCTION

Worldwide estimates indicate that substance use disorders (SUDs) continue to be a major public health problem [1]. In the United States, the estimated cost of SUD is $1.3 trillion annually [2]. As it relates to cocaine use disorders (CUD), there is an estimated 5.7 million Americans that are current cocaine users [3]. Although there has been extensive research and clinical trials conducted to address this problem, at present, there are no FDA-approved treatments for CUD [4–7]. Much of the research has involved male subjects, despite the epidemiological evidence of sex differences in vulnerability, maintenance and treatment strategies related to cocaine use disorders [8–10]. The present study used female cynomolgus monkeys, brain imaging using positron emission tomography (PET), and cocaine self-administration to replicate and extend earlier work conducted in male monkeys [11].

Cocaine is a monoamine transport blocker, elevating extracellular concentrations of brain dopamine (DA), serotonin and norepinephrine, although the reinforcing effects have largely been attributed to DA [12]. The primary mechanism of action mediating cocaine reinforcement is blocking DA transporters (DAT), resulting in elevated synaptic DA which binds to two families of DA receptors, D1-, with subtypes designated D_1 and D_5, and D2-like, with subtypes D_2, D_3 and D_4. A considerable amount of PET imaging studies have focused on D2-like receptors (referred to in this paper as D2/D3 receptors; D2/D3R) and vulnerability across several substances of misuse. For example, several studies found that non-alcoholic people with a family history of alcoholism, had higher levels of D2/D3R availability [13–15]; one hypothesis put forth was that high levels of D2/D3R were protective of future excessive substance use.

With regard to stimulants, it has also been hypothesized that there is an inverse relationship between D2/D3R availability and vulnerability to stimulant reinforcement, as well as poorer treatment outcomes [11, 16–19]. However, those studies were conducted almost exclusively in male subjects. For example, in one study, D2/D3R availability was initially assessed in 12 experimentally naïve male monkeys prior to being given the opportunity to self-administer cocaine [11] and it was found that

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over the first 10 weeks of self-administration, there was an inverse relationship between baseline D2/D3R availability and rates of cocaine self-administration. This finding is consistent with studies in non-cocaine abusing men in which positive subjective effects of methylphenidate were more likely in individuals with low measures of D2/D3R availability [16]. One aim of the present study was to extend this characterization of baseline D2/D3R availability and vulnerability to cocaine reinforcement to experimentally naïve female monkeys. A second aim was to examine how long-term cocaine self-administration by females affected D2/D3R availability and if these receptor levels would recover after cocaine access was terminated. In male monkeys, there was greater than 20% reduction in D2/D3R availability after self-administering cocaine for ~1 year, and recovery of D2/D3R occurred in only half of the male monkeys [11]. No such characterization of D2/D3R plasticity has been conducted in female subjects. We also examined the effects of chronic raclopride on the plasticity of the D2/D3R, which has previously been shown to increase D2/D3R availability in cocaine-naïve monkeys [20].

The present study further characterized the relationship between vulnerability to cocaine reinforcement and another measure of DA neurotransmission, baseline DAT. Previous PET studies in individually and socially housed cocaine-naïve female monkeys showed that measures of DAT availability were associated with changes in social rank and inversely related to cocaine vulnerability [21]. As it relates to the long-term effects of cocaine self-administration, an early receptor autoradiography study using [^{3}H]WIN 35,428, found two- to three-fold higher DAT densities in the striatum of cocaine overdose victims, compared to age-matched and drug-free control subjects [22]. This finding has been replicated in male monkeys with an extensive history of cocaine self-administration [23]. Human (primarily men) PET imaging studies assessing DAT availability have been mixed, with reports of no effects of cocaine use [24], as well as increases in DAT availability due to cocaine exposure [25]. In monkeys, acquisition of cocaine self-administration did not affect DAT availability, even though the same low-dose cocaine intake decreased D2/D3R availability [26]. The present study extended this characterization to within-subject longitudinal studies of long-term cocaine self-administration and time-off from cocaine on DAT availability. How DAT and D2/D3R measures changed in the same subjects over extended periods of cocaine self-administration was also examined. For these studies, [^{11}C] raclopride was used to measure D2/D3R availability and 2-carbomethoxy-3-(4-chlorophenyl)-8-(2-fluoroethyl)-nortropane ([^{18}F]FECNT), was used to quantify DAT availability [27, 28].

MATERIALS AND METHODS

Subjects

The subjects were nine experimentally naïve adult female cynomolgus monkeys (*Macaca fascicularis*). Monkey weights ranged from 2.78 to 3.04 kg and they lived in stainless steel cages (0.71 × 1.73 × 1.83 m; Allentown Caging Equipment, Co., Allentown, NJ) with removable wire mesh partitions that separated monkeys into quadrants (0.71 × 0.84 × 0.84 m). For these studies, because social status can influence DA receptor measures [21] and the primary goal was to examine DA receptor function during and following long-term cocaine self-administration, all monkeys were individually housed with visual and auditory access to the other subjects. Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit calmly in a standard primate chair (Primate Products). Monkeys were weighed weekly and fed enough food daily (Purina Monkey Chow and fresh fruit and vegetables) to maintain a healthy body weight and appearance as determined by daily inspection and periodic veterinary examinations. Feeding occurred at least 1 h after return to the homecage. Water was available *ad libitum* in the homecage. Menstrual cycle phase was assessed by daily vaginal swabs [29, 30] and was approximately 28 days. The first day of bleeding was indicative of menses and was counted as day 1 of the cycle. Follicular phase was considered days 2–10 and luteal phase days 19–28 of the menstrual cycle. Behavioral studies were conducted 5-days per week and in both phases of the menstrual cycle, while PET imaging studies were conducted only in the follicular phase. Animal housing, handling and all experimental procedures were performed in accordance with the 2011 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Wake Forest University Non-Human Primate Environmental Enrichment Plan.

Surgery

Each monkey was prepared with a chronic indwelling venous catheter in a major vein (femoral, internal or external jugulars) and subcutaneous vascular port (VAP; Access Technologies, Skokie, IL) under sterile surgical conditions, as described previously (e.g. [11]).

Apparatus

For the behavioral sessions, monkeys were placed into ventilated, sound-attenuating chambers (1.5 ×0.74 ×0.76 m; Med Associates, East Fairfield, VT) designed to accommodate a primate chair. On one side of the chamber were two photo-optic switches (Model 117-1007; Stewart Ergonomics, Inc., Furlong, PA) with a horizontal row of three stimulus lights 14 cm above each switch. Between the two switches was a food receptacle, which was connected with tygon tubing to a pellet dispenser (Gerbrands Corp., Arlington, MA) located on the top of the chamber. Food-reinforced responding was maintained by the delivery of 1.0-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ). A peristaltic infusion pump (Cole-Parmer Instrument Co., Niles, IL) was also located on top of the chamber for intravenous drug delivery at a rate of ~1.5 mL per 10 s. Reinforcers were accompanied by illumination of a red light inside the chamber associated with food presentations (1 s) and drug injections (10 s).

Before every session, the area on the monkey's back containing the VAP was prepared with an antiseptic povidone-iodine (PVP) scrub (Medline Industries, Mundelein, IL) followed by isopropyl alcohol (Fisher Scientific, Fair Lawn, NJ). The area was given a final preparation with PVP scrub before a 22-gauge Huber needle (Access Technologies) was inserted into the monkey's port, connecting the catheter to the infusion pump on the chamber. Before the start of each session, the pump was manually activated for approximately 3 s to fill each monkey's port with saline or concentration of cocaine available.

Procedure: Cocaine self-administration

Monkeys were trained to respond under a multiple fixed-interval (FI) 3-min schedule of reinforcement. Under the FI 3-min contingency, the first response after 3 min resulted in the presentation of a 1.0 g banana-flavored food pellet, followed by a 10-s timeout (TO). Each session consisted of four 20-min components, which alternated between left and right switches, and were signaled by illumination of the lights above that switch. Components were separated by a 2-min TO. Baseline PET scans were conducted after establishing food-maintained responding. After obtaining all baseline PET scans, monkeys were surgically implanted with an indwelling intravenous catheter, as described above.

After completion of the baseline scan, the schedule was changed to a multiple FI 3-min schedule of food and drug reinforcement. Food presentation was contingent on responding on one side, in the presence of a stimulus light illuminated above that switch, while drug injections were available on the other switch and in the presence of that side's stimulus light. Food-maintained responding occurred in components 1 and 3 (referred to as Fd1 and Fd2, respectively), and drug was available during components 2 and 4 (referred to as Coc1 and Coc2, respectively). Food components lasted 20 min or until 5 reinforcers were obtained, while drug components were 60 min or until 15 reinforcers were received. First, saline was made available in components 2 and 4 for at least 5 consecutive sessions and until responding was deemed extinguished (i.e., mean response rate decreased by at least 80% of food-reinforced responding for 3 consecutive sessions with no trends in responding). After re-establishing food-maintained responding, 0.03–0.1 mg/kg/injection cocaine HCl (National Institute on Drug Abuse, Bethesda, MD, dissolved in sterile 0.9% saline) was made available for 3–6 sessions. The dose was then changed to 0.2 mg/kg/injection cocaine for the remainder of the study.

PET imaging

Baseline PET scans with both radiotracers were conducted in all nine monkeys. Six of these monkeys were also scanned several times over the course of cocaine self-administration sessions (see below). Prior to any PET imaging, a structural magnetic resonance imaging (MRI) was obtained in each monkey under ketamine (15–20 mg/kg, i.m.) anesthesia with a 1.5-Tesla GE Signa NR scanner (GE Medical Systems). T1-weighted whole brain images were used to anatomically define spherical regions of interest (ROIs), including the right and left caudate nucleus (Cd), putamen (Pt) and ventral striatum (Vs), all at 0.5 cm diameter and cerebellum (Cb; 0.8 cm diameter), for later co-registration with PET images. PET studies used the DAT radioligand [^{18}F] 2-carbomethoxy-3-(4-chlorophenyl)-8-(2-fluoroethyl)-nortropane (FECNT) [28, 31, 32] and the D2/D3R radioligand [^{11}C] racloporide [33]. PET scans only occurred when the monkey was in the follicular phase of her menstrual cycle [34].

On the day of a PET scan, the monkey was anesthetized with 10 mg/kg ketamine, transported to the PET Center and maintained under 1.5% isoflurane anesthesia. We had previously shown that ketamine-induced anesthesia did not affect D2/D3R availability [35]. At the PET Center, a percutaneous venous catheter was inserted for tracer injection and fluid replacement throughout the PET scan. No behavioral sessions were conducted on the day of the PET study, in order to further decrease the likelihood of cocaine-induced elevations in DA. Body temperature was maintained at 40 °C and vital signs were monitored throughout the scanning procedure (see [34]). PET scans were acquired using a Siemens/CTI Concorde Primate microPET P4 scanner specifically designed for small-animal imaging, with approximately 2 mm resolution.

At the start of the PET scan, approximately 0.148 GBq of [^{11}C]raclopride or [^{18}F]FECNT was injected intravenously, followed by 3 mL of saline. The activity ranged from 0.15 to 0.22 GBq of [^{11}C]raclopride or [^{18}F]FECNT Monkeys underwent dynamic brain PET scans for 90 min ([^{11}C]raclopride) or 120 min ([^{18}F]FECNT) under baseline conditions (i.e., before cocaine exposure; $N = 9$), following self-administration of ~100 mg/kg cocaine ($N = 5$), ~1000 mg/kg cocaine ($N = 5$) and 4–9 months of time-off from cocaine ($N = 4$). Post scanning, the raw dicom data were binned as 5 min × 18 frames (90 min) for ([^{11}C]raclopride or 0.5 min × 30 frames, 5 min × 9 frames, and 20 min × 3 frames (120 min) for [^{18}F]FECNT. Tissue-time-activity curves were generated for radiotracer concentrations in each ROI and distribution volume ratios (DVR) for the Cd, Pt and Vs were calculated using the Cb as the reference region using PMOD. Representative tissue time activity curves for both radiotracers are shown in the Supplemental File (Supplementary Fig. S1).

Effects of chronic raclopride on D2/D3 receptor availability

Three monkeys that did not show recovery of D2/D3 receptor measures after 1000 mg/kg cocaine self-administration and 9 months time-off from cocaine were implanted, under sterile surgical conditions, with an Alzet osmotic pump (Durect Corp., Cupertino, CA). Each pump had a reservoir volume of 2 ml and a flow rate of 2.5 µl/h, and thus could dispense contents for a maximum of 33 days. Raclopride, in a concentration calculated to deliver a dose of 0.01 mg/kg/h has been shown to increase D2/D3 receptor availability in monkeys [20]. To implant the osmotic pump, the monkey was anesthetized with 10 mg/kg ketamine (i.m.) and a small incision was made in the interscapular region of the back. The pump was inserted into a subcutaneous pocket formed by blunt dissection. During chronic raclopride treatment, monkeys continued to participate in behavioral experiments 5 days per week, but only responding in the presence of the food reinforcer. The osmotic pump was removed after 29–32 days. PET studies were again conducted within 48–72 h of pump removal, to better assure elimination of residual raclopride.

Statistical analysis

A repeated measures linear random effects model was used to determine if there were differences in the three brain regions of interest (caudate nucleus, putamen, and ventral striatum) and D2/D3R and DAT availability. Baseline bivariate correlations between D2/D3R and DAT availability in the caudate nucleus, putamen, and ventral striatum were determined. Moreover, bivariate correlations between mean food and cocaine-maintained response rates and D2/D3R and DAT availability in the same three brain regions was determined using Bonferroni's correction for multiple comparisons. The effects of cocaine self-administration on DAT availability were analyzed using a repeated measures linear random effects model with region (caudate nucleus, putamen, and ventral striatum) and condition (BL, 100 mg/kg, 1000 mg/kg) as factors. Since there was no effect of cocaine self-administration on DAT availability no statistics were run with DAT and abstinence data. The effects of cocaine self-administration on D2/D3R availability were analyzed using a repeated measures linear random effects model with region (caudate nucleus, putamen, and ventral striatum) and condition (BL, 100 mg/kg, 1000 mg/kg, 4-months time-off, 6-months time-off, and 9-months time-off) as factors. Finally, a repeated measures linear random effects model was used to determine if there was recovery of D2/D3R availability to baseline levels following 30 days of treatment with raclopride, with region (caudate nucleus, putamen, and ventral striatum) and condition (BL, 1000 mg/kg, 9 months time-off, and 30-days treatment) as factors. A significant test was followed by pairwise multiple comparisons (Holm-Sidak) *post-hoc* tests.

For analyses of behavior, the primary dependent variables were response rates (total responses divided by component time) and total number of reinforcers delivered in each component. To determine if food- and cocaine-maintained responding changed across time, we calculated mean data (±1 SD) over consecutive months for each monkey and conducted an analysis on the linear and quadratic trends in the data. Moreover, a repeated measures linear random effects model was used to compare reinforcement frequency across the 13 months to baseline levels. Data are shown as group means ± SEM. All statistics were conducted using SPSS or PRISM and significance was set at $p < 0.05$.

Drugs

(-)-Cocaine HCl was supplied by the National Institute on Drug Abuse (Bethesda, MD) and raclopride was purchased from Sigma-Aldrich (St. Louis, MO); both were dissolved in 0.9% saline. All drug doses are expressed as the salt form. Cocaine doses were changed by adjusting the concentration of drug, calculated for a pump duration of 10 s (~1.5 ml/10 s).

RESULTS

Baseline measures of D2/D3R and DAT availability in female monkeys

Nine experimentally naïve, normally cycling, female cynomolgus monkeys were scanned twice in the follicular phase: once with [^{11}C] raclopride and once with [^{18}F]FECNT (Table 1, Supplementary Table S1, Supplementary Fig. S2). D2/D3R availability was significantly higher in the putamen when compared to the caudate nucleus ($t(8) = 5.44$, $p = 0.0006$) and the ventral striatum ($t(8) = 7.15$, $p < 0.0001$). Moreover, the caudate nucleus had significantly higher D2/D3R availability than the ventral striatum ($t(8) = 4.90$, $p = 0.0012$). DAT availability was also significantly higher in the putamen when compared to the caudate nucleus ($t(8) = 5.72$, $p = 0.0004$) and the ventral striatum ($t(8) = 7.25$, $p < 0.0001$). The caudate nucleus also had significantly higher D2/D3R availability than the ventral striatum ($t(8) = 4.40$, $p = 0.0023$) (Supplementary Table S1). In each region examined, the correlation between DVRs for D2/D3R and DAT was non-significant ($p > 0.05$).

Correlation between cocaine self-administration and D2/D3R and DAT availability

When cocaine was made available to each monkey in components 2 and 4, the first 3–6 sessions involved increasing the cocaine dose from 0.03 to 0.1 until the final dose of 0.2 mg/kg/injection was available. Week 1 was considered the first week of 0.2 mg/kg/injection cocaine self-administration. There were five dependent variables considered in the correlations each week: mean response rates for Fd-1, Fd-2, Coc-1, Coc-2 and Mean-Coc. The only significant correlations between DVRs and behavior were observed in the ventral striatum in Week 1 (Supplementary Table S2). In that region, Coc-1 response rates ($r = +0.77$; $p < 0.02$) and overall mean rates of cocaine self-administration ($r = +0.76$; $p < 0.02$) were directly related to baseline D2/D3R availability; rates of food-maintained responding were not correlated with D2/D3R availability at any time point (Supplementary Table S2). There were no significant relationships between DAT availability and food- or cocaine-maintained responding over the first 10 weeks of cocaine self-administration (Supplementary Table S3).

Table 1. Recovery of D2/D3R ([^{11}C]Raclopride) availability following long-term cocaine self-administration in female monkeys.

Caudate nucleus							
		Chronic cocaine SA		**Time-off from cocaine SA**			**+ Chronic Rac**
Subject	**BL**	**100 mg/kg (% BL)**	**1000 mg/kg (% BL)**	**4 mo (% BL)**	**6 mo (% BL)**	**9 mo (% BL)**	**30 d Tx (% BL)**
C-7434	4.58	89	80	80	–	78	72
C-7440	3.54	–	50	–	–	83	–
C-7441	3.46	83	86	72	76	73	90
C-7453	4.30	73	63	56	97	–	–
C-7457	4.88	88	68	47	72	57	68
Mean	**4.15**	**83.25**	**69.40**	**63.75**	**81.67**	**72.75**	**76.67**
SEM	0.32	4.23	7.10	8.65	7.75	6.50	8.29
Putamen							
C-7434	5.41	94	82	75	–	78	80
C-7440	4.19	-	50	–	–	96	–
C-7441	4.48	86	73	68	75	68	89
C-7453	5.11	80	60	60	96	–	–
C-7457	5.01	95	68	51	65	53	70
Mean	**4.84**	**88.75**	**66.60**	**63.50**	**78.67**	**73.75**	**79.67**
SEM	0.25	4.09	6.12	5.97	9.13	10.42	6.72
Ventral Striatum							
C-7434	3.23	103	77	70	–	81	99
C-7440	3.26	-	61	–	–	98	–
C-7441	3.22	94	72	72	88	76	91
C-7453	3.27	92	64	68	94	–	–
C-7457	3.78	90	63	52	55	46	59
Mean	**3.35**	**94.75**	**67.40**	**65.50**	**79.00**	**75.25**	**83.00**
SEM	0.12	3.31	3.40	5.28	12.12	12.50	14.97

SA Self-administration.
The bold values show group mean data (differentiating group mean data from the individual subject data).

Effects of cocaine self-administration on D2/D3R and DAT availability

Monkeys self-administered cocaine for approximately 13 months, followed by up to 9 months of time-off from cocaine, using a longitudinal, within-subject design with PET studies conducted at various time points. On average, it took the monkeys 4 months to self-administer 100 mg/kg of cocaine and 12.4 months to self-administer 1000 mg/kg of cocaine. The repeated measures linear random effects model demonstrated that in the caudate nucleus, there was a significant reduction in D2/D3R availability from baseline at both the 100 mg/kg cocaine timepoint ($p = 0.03$) and the 1000 mg/kg cocaine timepoint ($p = 0.0002$) (Fig. 1, left panel, top graph). In the putamen, there was a significant decrease in D2/D3R availability from baseline at the 1000 mg/kg cocaine timepoint ($p < 0.001$), but not at the 100 mg/kg timepoint ($p = 0.16$) (Fig. 1, left panel, middle graph). Similarly, in the ventral striatum, D2/D3R availability was only significantly reduced from baseline at 1000 mg/kg of cocaine ($p \leq 0.001$; Fig. 1, left panel, lower graph). In contrast to the effects observed on D2/D3R availability with [^{11}C]raclopride, there were no significant effects of 100 and 1000 mg/kg cocaine self-administration on DAT availability, as assessed with [^{18}F] FECNT, in any region examined (Fig. 1, right panels). Changes in D2/D3R and DAT availability for each monkey are shown in Supplementary Tables S4 and S5.

In addition to longitudinal PET studies, we also examined how behavior changed over approximately 13 months of access to 0.2 mg/kg/injection cocaine in female monkeys. Each session began with food reinforcement available in the first component (Fd1), and monkeys typically received the maximum of 5 food reinforcers each session over the 13 months of the study, with no significant increasing or decreasing trends ($F(1,78) = 0.03$, $p = 0.8597$), (Fig. 2A). Moreover, food reinforcement frequency in Fd1 was only significantly lower than baseline levels in Months 7 ($p = 0.008$) and 8 (0.002). For Fd2, a cubic trend model showed a significant decreasing trend until Month 4, followed by a significant increasing trend until month 10, followed by a significant decreasing trend for the remainder of the study ($b = -0.01346$ (0.00326), $t(76) = -4.13$, $p < 0.0001$). Furthermore, average reinforcement frequency for every month was significantly lower than baseline for Fd2 demonstrating that tolerance did not develop to the rate-decreasing effects of cocaine (Fig. 2B).

Cocaine self-administration was studied in two components. Because 0.2 mg/kg/injection cocaine is a relatively high dose, we did not expect monkeys to earn the maximum number of 10 injections per component. Over the 13 months of cocaine self-administration, the number of cocaine injections received in the first component (Fig. 2C) increased from 7.08 (±0.68) in Month 1 to 8.14 (±1. 21) in Month 13 and followed a significant linear increasing trend over time ($b = 0.1276$ (0.02572), $t(76) = 4.96$, $p < 0.0001$); every month resulted in significantly higher reinforcement frequency when compared to month 1 for Coc1. The number of cocaine injections received in the second component (Fig. 2D) increased from 7.07 (±0.69) in Month 1 to 8.00 (±1.16) in

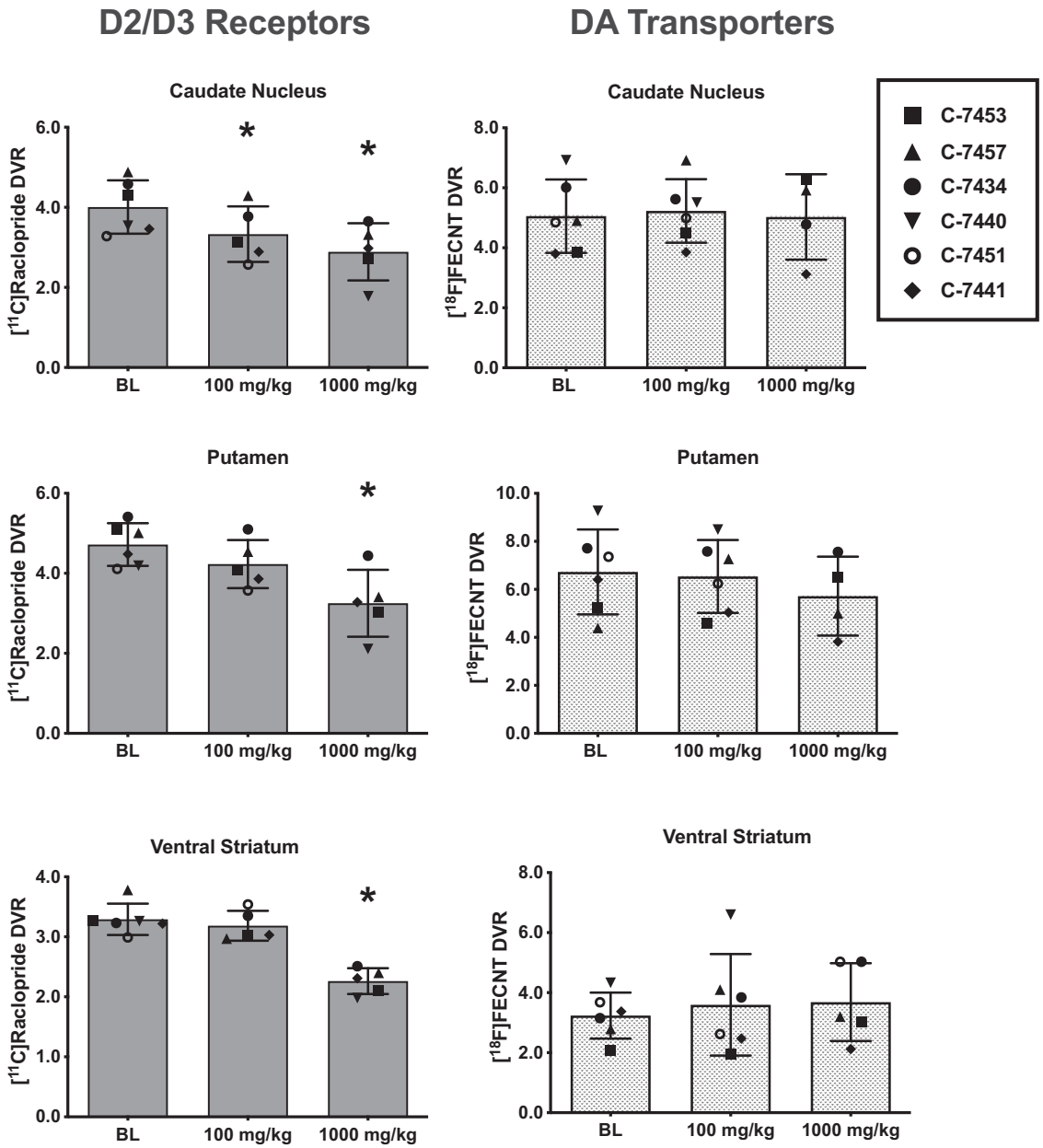

Fig. 1 Changes in [^{11}C]raclopride (left panels) and [^{18}F]FECNT (right panels) DVRs as a function of cocaine intake. Data represent the mean (±SEM) from $N = 5$ at both 100 and 1000 mg/kg cocaine intakes for D2/D3 receptors and $N = 6$ and 4 for DA transporters following 100 and 1000 mg/kg self-administration, respectively. Baseline (BL) scans occurred prior to any cocaine exposure. Data points within each bar represents individual subjects. *$p < 0.05$.

Month 13 and also followed a significant linear increasing trend over time (b = 0.1124 (0.02692), t(78) = 4.17, $p < 0.0001$); reinforcement frequency was significantly higher for all months when compared to baseline. Finally, total session injections increased from 14.15 (±1.33) in Month 1 to 16.15 (±2.27) in Month 13; a quadratic trend for total cocaine injections was significant and showed a significant increasing trend until 9 months and then a decreasing trend (b = −0.03424 (0.01377), t(77) = −2.49, $p = 0.0151$) (Fig. 2E).

Recovery of D2/D3R availability during abstinence and following pharmacological challenges

In four monkeys that had self-administered 0.2 mg/kg/injection cocaine for one year, PET scans with [^{11}C]raclopride were conducted 4-, 6- and 9-months after the last cocaine self-administration session. In all three regions of interest, there was no recovery of D2/D3R availability to baseline following 4-, 6- or 9-months of abstinence ($p < 0.05$) (Fig. 3, Table 1, Supplementary Fig. S2).

In order to determine if these reductions were permanent, we implanted three monkeys with osmotic pumps that delivered raclopride for 30 days and then redetermined D2D3R availability with [^{11}C]raclopride. When compared to D2/D3R availability at the 9-month time-off from cocaine, 30 days of treatment with raclopride did not result in significant increases in receptor availability in the caudate nucleus ($p = 0.255$) and in the ventral striatum ($p = 0.093$), but did produce significant increases in the putamen ($p = 0.023$) (Fig. 4). Only in the ventral striatum did chronic raclopride result in changes in D2/D3R availability such that DVRs were not different from baseline.

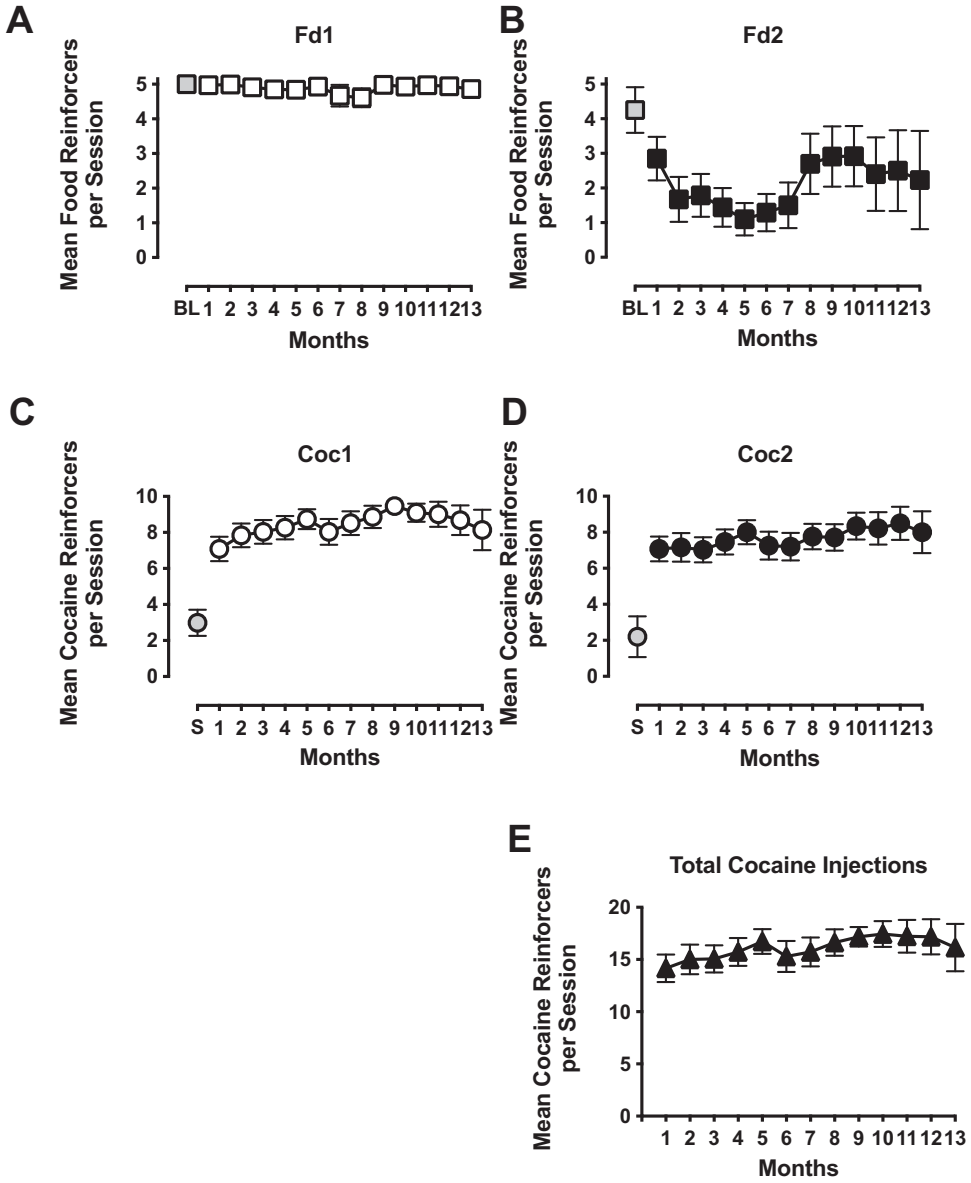

Fig. 2 Effects of cocaine self-administration on food- and cocaine-maintained responding. Changes in behavior over 13 months of food- and cocaine-reinforced responding (**A**–**E**). Mean (±SEM) number of food reinforcers (**A**, **B**) and cocaine injections (**C**, **D**) per component and total session injections (**E**). Each point is the mean of the last 5 sessions, each month, over 13 consecutive months. The first data points, shaded gray, are the mean ± SEM effects when saline was available in components 2 and 4. Each point represents the mean (±SEM) from 6 to 7 female cynomolgus monkeys. *$p < 0.05$ compared to the data from the first time point.

DISCUSSION

The purpose of the present study was to examine the relationship between DA receptor and transporter availability on cocaine reinforcement in female monkeys. While previous studies in male monkeys showed an inverse relationship between D2/D3R availability and rates of cocaine self-administration over the first 10 weeks of cocaine availability [11], no such relationship was observed in female monkeys responding under a similar schedule of reinforcement, except in Week 1 and only in the ventral striatum. As was observed in male monkeys, long-term cocaine self-administration resulted in reductions in D2/D3R availability in female monkeys and individual differences in rates of recovery following time-off from cocaine. For three of the females that did not show recovery of D2/D3R measures out to 9 months after their last cocaine exposure, 1-month of chronic raclopride resulted in a return to baseline only in the ventral striatum which suggests that the reductions were not permanent or neurotoxic in that brain region. However, D2/D3R availability remained low in the putamen and caudate nucleus even after chronic raclopride treatment. There was no relationship between DAT availability and rates of cocaine- or food-maintained responding, nor did DAT availability change with chronic cocaine self-administration. Finally, similar to what was observed in male monkeys, no evidence of tolerance to the rate-decreasing effects on self-administered cocaine on food-reinforced responding was observed and the number of cocaine injections received in the session changed modestly, although statistically significant over a 13-month period of self-administration.

D2/D3R and vulnerability

Previous studies examining the relationship between D2/D3R and vulnerability to stimulant use, have documented inverse relationships in male subjects: human [16], monkey [11, 17] and rodent [18]. In contrast, in the only PET study involving female monkeys and cocaine self-administration [21], the relationship between

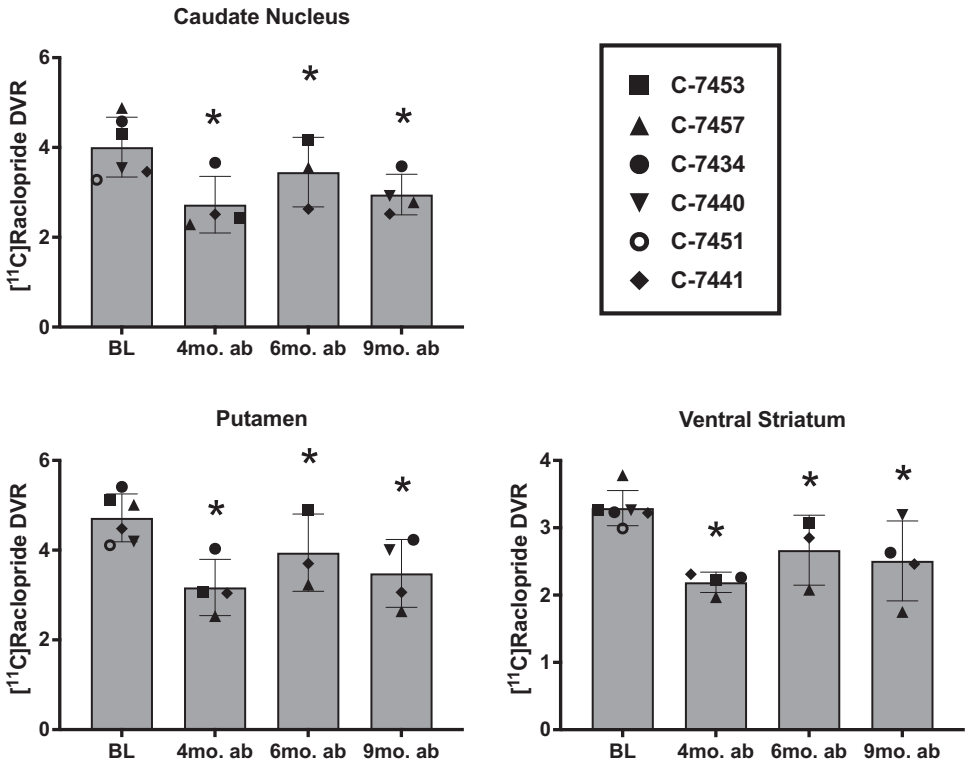

Fig. 3 Changes in [^{11}C]raclopride as a function of time-off from cocaine self-administration. Data represent the mean (±SEM) from $N = 5$ for D2/D3 receptors. Baseline (BL) scans occurred prior to any cocaine exposure. Data points within each bar represents individual subjects. *$p < 0.05$.

D2/D3R availability and vulnerability was direct. That is, the dominant female monkeys in the social group had higher levels of D2/D3R than subordinate females and were more sensitive to the reinforcing effects of cocaine. In male monkeys, the dominant animals had higher D2/D3R availability and were initially protected from the reinforcing effects of cocaine. In both of those studies, reinforcement was studied under a fixed-ratio schedule. Another schedule had been used in male monkeys that allowed for more variability in responding – a fixed-interval schedule of reinforcement [11]. In order to further characterize the relationship between D2/D3R availability and cocaine self-administration and possible sex differences, the present study was a replication of that earlier study in male monkeys [11], but in female monkeys. Unlike previous studies, with the exception of Week 1 of access being positively correlated with D2/D3R availability in the ventral striatum, no relationships between D2/D3R availability and rates of cocaine self-administration were observed in the present study. In the earlier study in which female monkeys responded under a fixed-ratio schedule of reinforcement [21], the primary dependent variable that correlated with D2/D3R availability was the lowest dose of cocaine that functioned as a reinforcer. In the present study, female monkeys self-administered a relatively high dose of cocaine under a fixed-interval schedule of reinforcement, with response rates being the primary dependent variable that was compared with baseline D2/D3R availability. The use of FI schedules has the advantage that only one response is necessary (at the end of the interval) to receive a cocaine injection, so direct effects of cocaine on response rates (whether increases or decreases) do not necessarily affect cocaine intake. It appears that high-dose cocaine-maintained response rates are not influenced by baseline D2/D3R availability. Future studies will need to further characterize cocaine self-administration under other conditions, including lower cocaine doses under the FI schedule and the use of progressive-ratio and concurrent schedules, in order to assess the relationships between D2/D3R and reinforcing strength of cocaine.

Other receptor systems

A second testable hypothesis as to why there was no relationship between D2/D3R availability and rates of cocaine self-administration in female monkeys is that other CNS targets are more relevant to vulnerability in females compared with males. One target that has received considerable recent attention is the kappa opioid receptor (KOR) system [36, 37]. PET studies from our lab recently reported sex and social rank differences in KOR availability which are more consistent with vulnerability in females than the relationship with D2/D3R availability [38]. How KOR availability changes with long-term cocaine self-administration and time-off from cocaine, will be important information, potentially revealing interactions between KOR and the DA receptor system and the consequences of long-term cocaine self-administration [39].

D2/D3R and long-term cocaine use

There is an extensive literature documenting lower measures of D2/D3R availability using PET in human subjects (e.g. [19, 40–42]) and in animal models (e.g. [11]). Receptor autoradiography findings suggest that the lower measures from PET are due to reductions in D2/D3R densities [43–45]. Importantly, all of these studies have utilized primarily male subjects. The present study extended these findings to females and noted no sex differences in the effects of long-term cocaine self-administration and time-off from cocaine on D2/D3R availability. No human-subjects PET imaging studies have had enough women with CUD participating to allow for assessments of the long-term consequences of cocaine use on DA receptor function in women compared with men. The findings reported here suggest that the effects on D2/D3R availability are more dependent on cocaine history than the sex of the individual.

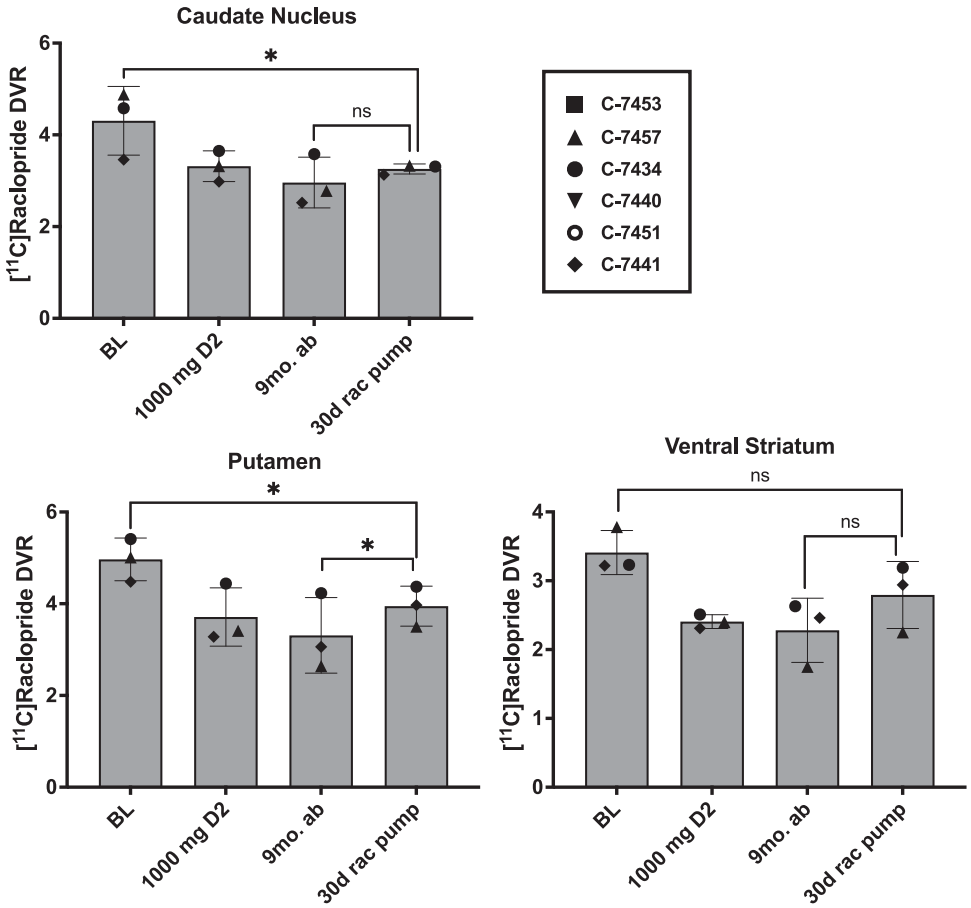

Fig. 4 Changes in [^{11}C]raclopride as a function of cocaine intake, time-off from cocaine abstinence, and 30-days chronic treatment with raclopride. Data represent the mean (±SEM) from $N = 5$. Data points within each bar represents individual subjects. *$p < 0.05$.

DAT and cocaine self-administration

The present study also extended within-subject assessments of changes in DA receptor system function to include the DA transporter. In an earlier study in which D2/D3R availability did not change in female monkeys who became subordinate (the less sensitive phenotype to cocaine reinforcement), DAT availability significantly decreased in the caudate nucleus and putamen in those subordinate female monkeys [21]. To better determine the role of the DAT in cocaine self-administration in female monkeys, the DAT-selective radiotracer [^{18}F]FECNT [28, 32, 46] was studied in cocaine-naïve monkeys and following long-term cocaine self-administration. No relationship between DAT availability and rates of cocaine self-administration or food-maintained responding were observed in any region of interest. Similarly, long-term cocaine self-administration did not significantly affect DAT availability. These findings are in contrast to other studies documenting the importance of the DAT to cocaine reinforcement (e.g. [47–49]). In a study using an interval schedule of drug self-administration [47], rhesus monkeys were trained to respond under a second-order FI 10-min (fixed-ratio 20: S) schedule of reinforcement. Complete self-administration dose-response curves were determined for cocaine and the DAT blocker RTI-113. At another time, PET scans with [^{18}F]FECNT were conducted in the same monkeys and cocaine and RTI-113 doses were administered to determine DAT occupancy. The investigators noted that at doses of cocaine that maintained maximum rates of responding, DAT occupancy was between 65–75%, while DAT occupancy for the RTI-113 doses that maintained maximum response rates were much greater, ranging between 94–99%. These findings suggest that how cocaine interacts at the DAT is fairly unique and not sufficient, on its own, to account for its high reinforcing value (see also [12]). Clearly, additional work is needed to better understand how long-term cocaine exposure on DAT function can be used as a target for novel medication development (see [7]).

Significance of the research

The significance of the present study in female monkeys, documenting within-subjects decreases in DA receptor function, involves the development of personalized medicine strategies for treating CUD. Perhaps the best example of this is from Martinez et al. [19]. In that study, people with CUD (22 men and 3 women) were studied twice with [^{11}C]raclopride and PET: once as a baseline and once following administration of the DAT blocker methylphenidate. Methylphenidate should elevate DA and decrease the binding potential of [^{11}C]raclopride at D2/D3R. Following the two PET scans, all the subjects were enrolled in a behavioral treatment intervention involving contingency management and a community reinforcement approach. The investigators noted subjects who did not respond to the behavioral treatment were the ones with lowest changes in D2/D3R availability following the methylphenidate challenge. That is, low methylphenidate-induced DA effects, as determined with PET, were associated with treatment failure. Consistent with the Martinez et al. [19] findings, Wang et al. [50] noted that methamphetamine users with lower D2/D3R availability and less response to a methylphenidate challenge (which should further decrease the PET signal) were more likely to relapse. The present study in female monkeys, conducted in a very similar manner to a

study in male monkeys [11], allows for hypotheses related to the influence of D2/D3R availability in cocaine reinforcement. While still an empirical question, based on our nonhuman primate imaging studies, we would hypothesize that DA changes are a more relevant target for males, while other neurotransmitter systems, including KOR, may be more relevant for identifying novel treatments for women with CUD.

Limitations

There are some limitations to the present study. While our primary goal was to replicate the earlier study in male monkeys [11], there were substantial differences between the two studies that should be acknowledged. The male monkeys previously studied were rhesus macaques, while the female monkeys in the present study were cynomolgus macaques. While we are not aware of differences in the behavioral and neuropharmacological effects of cocaine in these two macaque species, it remains a possibility. A second, and more significant differences in the studies, is based on the PET camera. In the male monkey study, the resolution of the PET camera was 9 mm and only the "basal ganglia" was assessed. In contrast, the current PET camera used for the studies in females had a spatial resolution of ~2 mm and the ROIs were much more detailed. It is possible that if portions of the basal ganglia could have been identified with PET in the study with male monkeys, that the inverse relationship previously reported would not be as robust or predictive of self-administration. Finally, the radiotracer used in the male monkey study was [^{18}F]fluoroclebopride, while the present study used [^{11}C]raclopride; both have similar affinities for the D2/D3R. Just as with the different species, we do not consider this a major limitation, but is one that should be noted.

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AUTHOR CONTRIBUTIONS

MAN designed the experiments. AND, AA-N and SHN performed the behavioral studies, including intravenous catheterization. AND and SHN analyzed the PET data, HDG provided technical guidance with initial PET analyses, KKSS, RJV, AM and MMG were involved in the synthesis of both radiotracers and BAR and MIA were responsible for the statistical analyses. The manuscript was written by MIA and MAN. All authors read a draft of this manuscript and provided critical edits to the content.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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