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PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys

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Dopamine neurotransmission is associated with high susceptibility to cocaine abuse. Positron emission tomography was used in 12 rhesus macaques to determine if dopamine D2 receptor availability was associated with the rate of cocaine reinforcement, and to study changes in brain dopaminergic function during maintenance of and abstinence from cocaine. Baseline D2 receptor availability was negatively correlated with rates of cocaine self-administration. D2 receptor availability decreased by 15–20% within 1 week of initiating self-administration and remained reduced by ~20% during 1 year of exposure. Long-term reductions in D2 receptor availability were observed, with decreases persisting for up to 1 year of abstinence in some monkeys. These data provide evidence for a predisposition to self-administer cocaine based on D2 receptor availability, and demonstrate that the brain dopamine system responds rapidly following cocaine exposure. Individual differences in the rate of recovery of D2 receptor function during abstinence were noted.

Cocaine abuse remains a major public health problem worldwide[1]. In the United States, recent estimates indicate substantial increases in the annual number of new cocaine users[2,3]. Furthermore, estimates reported in 2002 indicate that hospital emergency room visits related to cocaine use have increased by over 22% between 1994 and 2001 (ref. 4). At present, there are no clinically effective therapies for cocaine addiction[5], and an understanding of the biological and environmental mediators of vulnerability to cocaine abuse remain elusive[6]. A great deal of research has been directed toward understanding how cocaine affects brain function, with the ultimate goal of developing new treatment strategies[6,7]. The present study used drug self-administration, which is an animal model of drug abuse with high face and predictive validity for the human condition[8–10], allowing for the systematic evaluation of variables that affect vulnerability, maintenance and relapse to drug abuse. In addition, the noninvasive brain imaging technique positron emission tomography (PET) was used to examine the role of brain dopamine (DA) systems in conferring vulnerability to cocaine abuse and to determine if changes occurred following chronic cocaine exposure in subjects that were previously cocaine naïve. Such information would aid in the development of treatment strategies for cocaine-abusing individuals.

Cocaine is an indirect-acting monoamine agonist, binding with approximately equal affinity at the DA, serotonin and norepinephrine transporters[11,12]. The downstream effects of DA mediate the subjective and reinforcing effects of cocaine leading to its high abuse potential. Within the DA system, experimental data have been presented to implicate a role for D2-like DA receptors[13,14]. For example, in non-drug-abusing individuals, the subjective effects of the psychostimulant methylphenidate (Ritalin) are related to D2 receptor availability, such that individuals with low D2 measures report the drug to be pleasant, whereas individuals with high D2 receptor availability find the psychostimulant to be aversive[15]. In nonhuman primates, the formation of social hierarchies produces differences in D2 receptor availability, with subordinate monkeys having lower D2 measures compared to dominant monkeys[16]. When given access to cocaine, subordinate monkeys self-administer cocaine at higher rates, resulting in larger intakes compared to dominant monkeys. Taken together, there seems to be an inverse relationship between D2 receptor availability and 'vulnerability' to the use of psychostimulants. Research has also indicated that cocaine abusers have lower D2 receptor availability compared to age-matched non-drug-abusing individuals[17,18]. However, it is not clear if these lower measures of D2 receptor availability are a result of cocaine exposure or a predisposing trait present before the initiation of drug use[19]. The present study used a nonhuman primate model of cocaine abuse and PET imaging to examine questions related to three stages of addiction: whether D2 receptor availability in cocaine-naïve monkeys was a trait variable related to vulnerability; whether cocaine exposure decreased D2 receptor availability; and whether these cocaine-induced changes in D2 receptor availability were transient or long lasting.

RESULTS

Baseline measures of D2 receptor availability

Twelve experimentally naïve adult male rhesus monkeys (*Macaca mulatta*) were initially trained to use a lever in order to receive food

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Table 1 Baseline [^{18}F]FCP DVRs for individual monkeys with and without 1.0 mg lorazepam per kg body weight

Subject	BL	BL-retest	Loraz	Loraz-test	Percent diff¶
R-1241	2.50	2.50	2.73	2.93	5.0%
R-1246	3.00	3.77	2.81	3.10	6.9%
R-1247	2.18	3.16	2.40	2.64	6.7%
R-1249	2.97	2.82	2.92	3.01	2.1%
R-1276	2.47	2.07	2.29	2.01	9.2%
R-1284	2.66	2.78	2.71	2.54	4.6%
R-1289	2.58	2.57	2.08	2.62	16.2%
R-1277	3.20	3.09	2.54	2.53	0.3%
R-1274	2.28	2.69	2.45	2.48	0.9%
R-1278	2.81	2.47	2.01	2.25	8.0%
Mean	2.66	2.79	2.49	2.61	6.0%
s.e.m.	(0.11)	(0.16)	(0.10)	(0.11)	(1.6)

¶Represents effects of 1.0 mg lorazepam per kg body weight on test-retest variability and was calculated as follows: (s.d. divided by mean) * 100. BL, baseline.

Table 2 Correlation between baseline D2 DVR and food- and cocaine-maintained response rates

Week	Fd1	Coc1	Fd2	Coc2	Cocaine¶
1	ND	ND	ND	ND	ND
2	–0.27	–0.41	–0.46	–0.45	–0.41
3	–0.23	–0.47	–0.18	–0.50	–0.55
4	–0.35	–0.77**	–0.50	–0.51	–0.79**
5	–0.53	–0.67*	–0.52	–0.79**	–0.76**
6	–0.53	–0.78**	–0.58	–0.67*	–0.81**
7	–0.64*	–0.74*	–0.26	–0.47	–0.69*
8	–0.60	–0.70*	–0.22	–0.47	–0.66*
9	–0.65*	–0.79**	–0.29	–0.58	–0.72*
10	–0.73*	–0.81**	–0.09	–0.76*	–0.78**

Numbers were calculated from mean values of the five sessions each week, for each of 12 monkeys, and were log-transformed before analyses. *$P < 0.05$; **$P < 0.01$. ¶Mean response rates for Coc1 and Coc2.

reinforcement, and initial PET scans were conducted to measure D2 receptor availability using the DA D2 radioligand [^{18}F]fluoroclebopride (FCP; refs. 20,21). To minimize the influence of differences in synaptic DA concentrations, which may affect binding of the radioligand to D2 receptors[22], we also examined the effect of 1.0 mg lorazepam per kg body weight. As reported previously[16,23], there was a high amount of uptake of [^{18}F]FCP and a linear rate of washout from the basal ganglia. In the reference region, the cerebellum, there was a low amount of [^{18}F]FCP uptake and a high rate of washout. We determined distribution volume ratios (DVRs) by calculating the ratio of uptake in the basal ganglia to that in the cerebellum[24]. In ten monkeys, we examined the test-retest variability of this radioligand, as well as the effects of 1.0 mg lorazepam per kg body weight on D2 receptor availability. Consistent with a study in humans using [^{11}C]raclopride[25], DVRs of [^{18}F]FCP were not statistically affected by lorazepam (**Table 1**). In the lorazepam condition, mean DVRs were between 2.49 (± 0.10, s.e.m.) and 2.61 (± 0.11), and varied by approximately 6% (**Table 1**). These data extend previous measures of low between-scan variability with this radiotracer from cynomolgus monkeys[23] to rhesus monkeys.

Correlations between D2 receptor availability and behavior

To determine if baseline levels of D2 receptor availability (that is, in terms of DVRs) were related to vulnerability to cocaine reinforcement, all monkeys were allowed to self-administer cocaine (while food reinforcement was also available) by responding under a multiple fixed-interval (FI) 3-min schedule. Food and cocaine reinforcement were available twice during daily sessions. The food reinforcement components of the experiment (Fd1 and Fd2) were 20 min in duration and the cocaine components (Coc1 and Coc2) were 60 min in duration. The food and cocaine components were presented in the following order: Fd1, Coc1, Fd2, Coc2. Because the dose of cocaine available was relatively high (0.2 mg per kg body weight per injection), several training sessions at lower doses were necessary to ensure the safety of the monkeys; consequently, week 1 of self-administration was not included in the correlative analysis. Mean rates of cocaine self-administration were inversely related to baseline D2 receptor availability from weeks 4 through 10 ($r = –0.66$ to $–0.80$, $P < 0.05$; **Table 2**). This correlation was most evident in the first cocaine component and in the mean response rates over the entire week. The first cocaine component may be more indicative of relative reinforcing effects as rates in the second component may have been influenced by the direct effects of cocaine on responding[5,10,26]. There were significant ($P < 0.05$) correlations between baseline D2 receptor availability and food-reinforced response rates in Fd1 in weeks 7, 9 and 10 only, whereas there

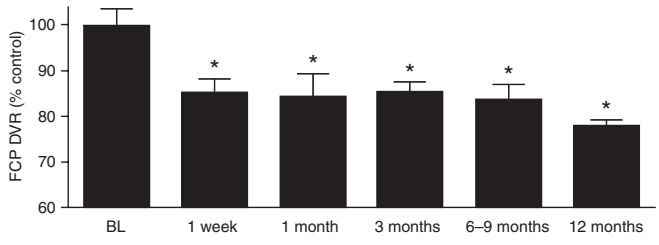

Figure 1 Changes in [^{18}F]FCP DVR as a function of cocaine exposure. Data represent the mean (± s.e.m.) from 7–9 monkeys, except at the 12-month time point ($n = 5$). The baseline scans (BL) occurred before any cocaine exposure and represent the mean of two determinations, with lorazepam, for each monkey (**Table 1**). *$P < 0.05$ compared to (BL). D2 DVRs were significantly decreased at all time points.

Table 3 Effects of cocaine self-administration on [^{18}F]FCP DVRs†

Subject	BL§	1 week (%)	1 month (%)	3 months (%)	6–9 months (%)	12 months (%)
R-1241	2.83 (0.14)	–	–	84	74	75
R-1246	2.96 (0.21)	–	–	91	82	76
R-1247	2.52 (0.17)	–	79	82	86	80
R-1249	2.97 (0.06)	–	–	79	77	79
R-1276	2.15 (0.20)	–	80	91	98	79
R-1284	2.63 (0.12)	88	87	93	77	–
R-1289	2.35 (0.38)	74	76	83	91	–
R-1277	2.54 (0.01)	88	100	82	86	–
R-1274	2.47 (0.02)	84	–	–	–	–
R-1278	2.13 (0.17)	81	–	–	–	–
R-1325	2.13 (0.33)	87	–	–	–	–
R-1350	2.43 (–)¶	85	–	–	–	–
Mean		84	84	86	84	78
s.e.m.		(2.0)	(4.8)	(2.0)	(3.0)	(1.1)

†Represents percent of mean DVR. §Mean DVRs (± s.d.) are from the test and retest studies with lorazepam (see **Table 1**). ¶Two studies were conducted but data acquisition and analysis failed on one of them.

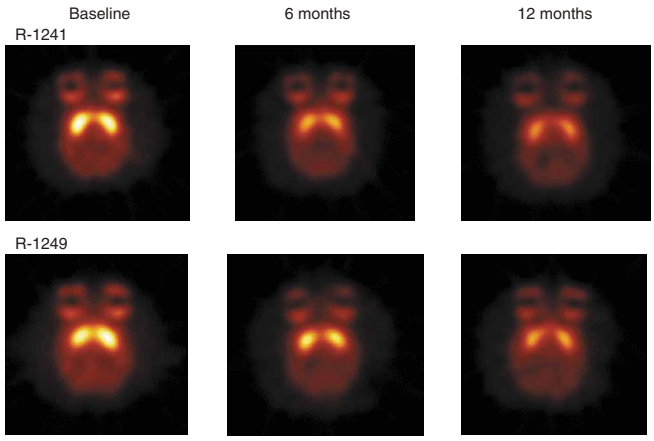

Figure 2 Normalized, coregistered PET images (percent injected dose per cm^3) of [^{18}F]FCP binding in the basal ganglia of two representative rhesus monkeys, when cocaine naïve, after approximately 6 months, and after 12 months of cocaine self-administration. Before the PET studies, at 6 months and 12 months, monkey R-1241 had self-injected approximately 508 mg and 750 mg cocaine per kg body weight, respectively, and monkey R-1249 had self-injected approximately 382 mg and 687 mg cocaine per kg body weight, respectively.

were no correlations noted with Fd2 performance (all $P > 0.05$). There were no significant correlations between the number of food presentations or cocaine injections and baseline D2 DVRs in any component across the first 10 weeks of self-administration (all $P > 0.05$; data not shown).

Effects of cocaine reinforcement on D2 DVRs

Irrespective of baseline levels, exposure to cocaine significantly reduced D2 receptor availability ($F_{5,71} = 13.3$, $P < 0.0001$; **Fig. 1**). An average decrease of 16% was evident after 1 week of cocaine self-administration, with a maximum decrease of approximately 22% after 1 year of self-administration (**Table 3**). This is the first documentation, using a within-subjects design and beginning with cocaine-naïve monkeys, of robust decreases in D2 receptor availability as a result of cocaine self-administration (**Fig. 2**).

We also investigated how behavior changed with continued exposure to cocaine. In each session, the first food component (Fd1) occurred before the cocaine component, and monkeys typically received the maximum number of food reinforcers (**Fig. 3a**). In contrast, Fd2 occurred immediately after cocaine availability and was significantly disrupted throughout the 1 year of exposure ($F_{12,92} = 22.43$, $P < 0.0001$; **Fig. 3b**). Regarding cocaine self-administration, because a relatively high cocaine dose was self-administered, monkeys did not typically earn the maximum number of injections per session; this allowed us to assess tolerance development to the reinforcing effects of cocaine, which would be manifested by an increase in the number of earned injections. The number of cocaine injections in the first component (Coc1) significantly increased over time compared to the mean number of injections received during the first month ($F_{11,99} = 5.58$, $P < 0.0001$; **Fig. 3c**), whereas there were no changes in the number of Coc2 injections from that in the first month (**Fig. 3d**). The mean number of cocaine injections per session (that is, Coc1 and Coc2) did not change from month 1 to month 12 of exposure (**Fig. 3e**). Overall, these findings indicate very little tolerance to the reinforcing effects of cocaine, as measured by the total number of injections per session, and no apparent tolerance to the behaviorally disruptive effects of cocaine on non-drug-reinforced behaviors.

Recovery of D2 DVRs during abstinence

In a final set of experiments, we determined the plasticity of D2 receptor DVRs by examining the rate of recovery during abstinence from cocaine. For three monkeys given only 1 week of cocaine exposure, with an average of 20 mg cocaine per kg body weight and 15% reductions in D2 receptor availability, recovery was complete within 3 weeks (**Table 4**). Of the 12 monkeys that self-administered cocaine for 12 months, 5 monkeys were studied in abstinence after 1 year of cocaine self-administration; these monkeys averaged 22% reductions in D2 receptor availability due to cocaine exposure. Three of the five monkeys showed complete recovery of D2 DVR within 3 months of abstinence, whereas two monkeys did not recover D2 receptor availability even after 12 months of abstinence (**Table 4**). Rate

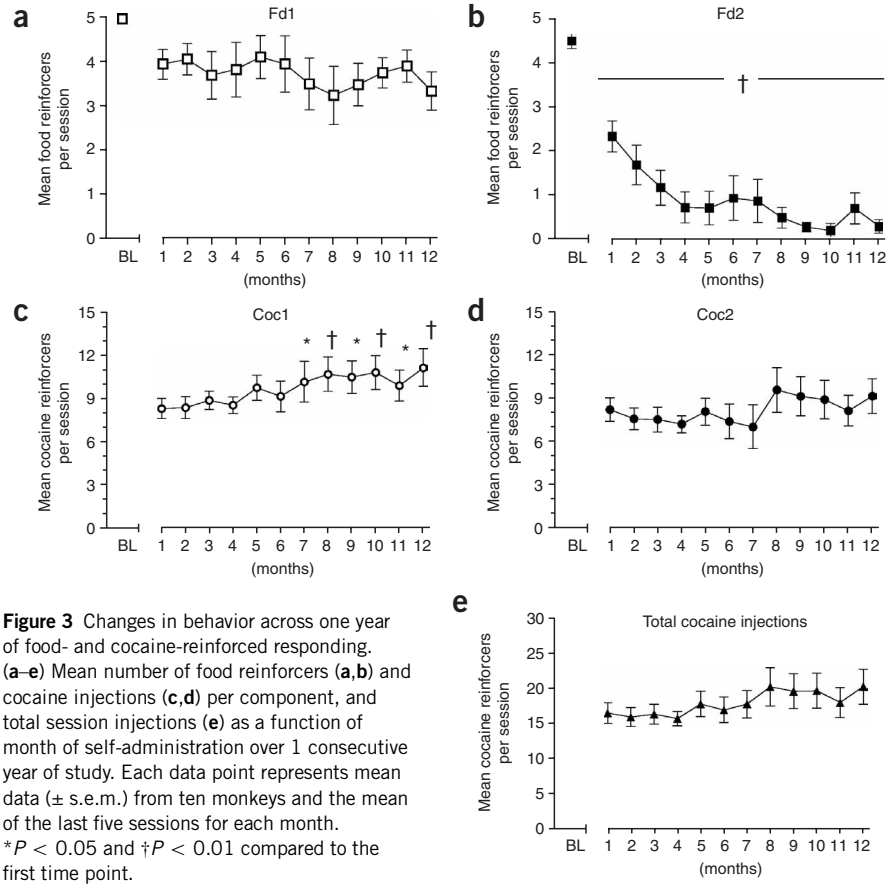

Figure 3 Changes in behavior across one year of food- and cocaine-reinforced responding. (**a**–**e**) Mean number of food reinforcers (**a**,**b**) and cocaine injections (**c**,**d**) per component, and total session injections (**e**) as a function of month of self-administration over 1 consecutive year of study. Each data point represents mean data (± s.e.m.) from ten monkeys and the mean of the last five sessions for each month. *$P < 0.05$ and †$P < 0.01$ compared to the first time point.

Table 4 Recovery of [^{18}F]FCP DVR after 1 week (top) or 1 year (bottom) of cocaine self-administration†

One week history of cocaine self-administration (SA)

Subject	Intake (mg per kg)	1 week SA	1 week Abstinence	3 weeks Abstinence
R-1278	13	81%	101%	
R-1325	23	87%	97%	
R-1350	20	85%	88%	100%

One year history of cocaine self-administration (SA)

Subject	Intake (mg per kg)	1 year SA	1 week Abstinence	1 month Abstinence	3 months Abstinence	12 months Abstinence
R-1241	776	75%	75%	79%	79%	83%
R-1249	703	79%	72%	81%	81%	76%
R-1246	1,011	76%	78%	94%	–	
R-1247	710	80%	84%	88%	101%	
R-1276	739	79%	97%	96%	–	

†Monkeys self-administered cocaine 5 d per week; PET studies were conducted 24 h after a self-administration session. During abstinence, monkeys responded under a food-reinforced FI 3-min schedule. Values are percent of baseline and represent DVRs relative to each monkey's baseline (see **Table 1**).

of recovery was not related to the total cocaine intake over the approximately 12-month period of cocaine self-administration (**Table 3** and **Fig. 4**). However, in the two monkeys that did not recover D2 DVRs by the 12th month of abstinence, examination of self-administration data indicated that previous food-reinforced responding at the beginning of each session (Fd1) was substantially decreased and that Fd1 reinforcement frequency was affected to a greater degree than in the three monkeys that did show recovery (**Fig. 4**). The disruption in Fd1 responding occurred before the first cocaine self-administration injection of each session.

DISCUSSION

The primary goals of the present study were to determine the relationship between DA D2 receptor availability and vulnerability to cocaine self-administration; to determine if cocaine reinforcement would reduce D2 receptor availability; and to examine the rate of recovery of D2 receptor availability during abstinence from cocaine. In monkeys that were previously cocaine naïve, there was a marked inverse relationship between baseline D2 receptor availability and rates of cocaine self-administration over the first 10 weeks of exposure. Furthermore, cocaine self-administration decreased D2 receptor availability in all monkeys. Finally, in 60% of the monkeys studied (3 of 5), there was evidence that D2 receptor availability could recover in abstinence even after 1 year of cocaine exposure. Overall, these findings provide unequivocal evidence for a role of DA D2 receptors in cocaine abuse and suggest that treatments aimed at increasing the number of D2 receptors may have promise for alleviating drug addiction.

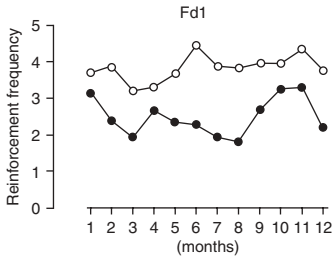

Figure 4 Mean number of reinforcers in the first food component (Fd1) as a function of time over 1 consecutive year of self-administration. Each point represents mean data from two monkeys who did not show recovery of D2 receptor function during abstinence (closed circles) and from three monkeys who did show recovery of D2 DVR during abstinence (open circles).

The present findings relating D2 receptor availability to drug abuse vulnerability are in agreement with findings reported in humans using another psychostimulant, methylphenidate[15]. A strength of the present study lies in the within-subjects design beginning with cocaine-naïve individuals and assessing the relationship between baseline D2 receptor availability and vulnerability to the reinforcing effects of cocaine. Furthermore, these data are consistent with earlier work from this laboratory showing that environmentally induced changes in D2 receptor availability can influence rates of cocaine self-administration[16], and confirm an inverse relationship between D2 receptor availability and rates of cocaine self-administration.

Previous investigations in humans have reported lower D2 receptor availability in cocaine abusers compared to controls[17,18]. However, it could not be determined from those studies whether low D2 receptor availability was a predisposing trait to cocaine abuse or a consequence of cocaine exposure[19]. The present findings in monkeys suggest that both factors are likely to be true. That is, low D2 receptor availability is a predisposing trait, and cocaine exposure produces robust decreases in D2 receptor availability. Our earlier work in socially housed monkeys suggests that environmental variables can alter levels of D2 receptor availability and influence vulnerability to cocaine reinforcement, in a manner consistent with the present inverse relationship between D2 receptor availability and cocaine reinforcement. The present findings also suggest that more vulnerable individuals are even more likely to continue using cocaine because of the cocaine-induced reductions in the number of D2 receptors.

The reductions in D2 receptor availability as measured by PET may be related to decreases in D2 receptor densities and/or to elevated concentrations of synaptic DA competing with the radioligand[21,27]. Consistent with these two potential mechanisms, there are several hypotheses to account for this inverse relationship between D2 receptor availability and vulnerability to cocaine reinforcement. It may be that low D2 receptor availability as measured by PET is due to elevated concentrations of DA, and there are data supporting the hypothesis that increasing concentrations of DA (by stress, for example) can increase vulnerability to stimulant reinforcement[28–30]. Conversely, it is possible that low D2 DVRs represent low D2 receptor densities, and it has been argued that this is indicative of a 'hypodopaminergic' system[31]. Thus, it is possible that low numbers of D2 receptors represent a dysregulated DA system, that when stimulated by cocaine is more sensitive to the reinforcing effects. Studies in monkeys using *in vivo* microdialysis have not shown altered basal concentrations of DA following exposure to reinforcing doses of cocaine[32], although the self-administered cocaine intakes in that study were lower than the total intakes from monkeys in the present study. *In vitro* receptor autoradiography of D2 receptor densities obtained from monkeys responding under similar experimental conditions of cocaine self-administration noted no differences in receptor densities following 1 week of exposure, but there were substantially lower D2 receptor densities after 1 month and longer cocaine exposures as compared to control tissue[33,34]. Thus, it seems that the initial effects of cocaine reinforcement result in elevated DA concentrations and that within 1 month of exposure, neuroadaptations

occur resulting in marked reductions in D2 receptor densities[34]. The rate of recovery of D2 receptor availability during abstinence is consistent with this interpretation.

The experimental protocol also allowed us to assess changes in behavior as a consequence of daily cocaine exposure. At issue is whether tolerance or sensitization develops to any of the behavioral effects of cocaine[26,35,36]. The available daily dose of cocaine was high enough that monkeys rarely self-administered the maximum dosage. As a result, changes in total session intake could be used as a dependent variable to assess tolerance (that is, increases in intake over time) or sensitization (that is, decreases in intake over time). No changes in total cocaine intake were noted across 1 year of self-administration, suggesting that neither tolerance nor sensitization to the reinforcing effects of cocaine, as measured under daily limited-access conditions, occur. A particular advantage of FI schedules is that only one response is necessary (at the end of the interval) to receive a cocaine injection. Thus, the lack of change in total cocaine intake over 1 year of cocaine self-administration is probably not due to sensitization to cocaine-induced effects on response rates. Reinforcing doses of cocaine produced robust decreases in food-reinforced responding, and there was no evidence for the development of tolerance to this behavioral effect of cocaine. Overall, the present findings do not support the hypothesis that drug addiction is a consequence of sensitization or tolerance to the behavioral effects of cocaine.

Finally, the issue of whether D2 receptor availability would recover during cocaine abstinence was addressed in a subset of monkeys. Previous human PET imaging studies indicated that lower D2 receptor DVRs were apparent for up to 4 months of abstinence compared to normal control D2 DVRs (ref. 17). One difficulty noted by these investigators was the use of a group design, in which abstinent cocaine abusers were compared to controls. The present study was able to assess, within individual subjects, the recovery of D2 receptor availability relative to each subject's precocaine D2 receptor DVR. Complete recovery was noted in three of five monkeys. However, in two monkeys, D2 receptor availability remained lower than the precocaine baseline levels for up to 1 year after the last cocaine injection. This reduction may be related to the hypothesized neuronal plasticity mediating persistent drug seeking[37]. In the present study, the lack of recovery was not related to precocaine D2 DVR, nor to the amount of cocaine self-administered. However, one variable that seemed to be associated with these two monkeys was disruption of food-reinforced responding at the beginning of each experimental session (that is, Fd1 behavior). Lower rates of food-reinforced responding may be the result of anticipating a more valued reinforcer in the next component[38], or these effects may represent a form of anhedonia that typically accompanies chronic cocaine exposure[37,39]. Irrespective of the mechanism accounting for the disruption in Fd1 responding, this behavior was predictive of insensitivity in the recovery of D2 receptor availability during abstinence from cocaine.

Individual differences in dopaminergic function can result in varying degrees of susceptibility to drug abuse[40,41]. The present study extends these findings to nonhuman primates self-administering cocaine daily for up to 1 year. The present findings also document robust decreases in D2 receptor availability consequent to cocaine reinforcement, supporting earlier human imaging studies[17,18,42]. These findings support the strategy of increasing D2 receptor availability as a means of treating cocaine addiction. This may occur pharmacologically, by chronically blocking D2 receptors[43], by indirectly reducing amounts of DA (refs. 22,44) or through environmental manipulations[16], in particular through enrichment[14]. In the present study, there was evidence for some recovery of D2 receptor function during abstinence, but this was not seen in all monkeys, suggesting that other factors may mediate recovery. For example, in human cocaine abusers, D2 DVRs are not related to the total cocaine dose used but to the duration of cocaine use[42]. Whereas the present study controlled for this variable, it seems that other factors, perhaps involving other neurotransmitter systems, mediate the rate of recovery of D2 receptor function.

METHODS

Subjects. Twelve individually housed and experimentally naïve adult male rhesus monkeys (*Macaca mulatta*) were purchased from commercial vendors (Tulane Regional Primate Research Center or Three Springs Scientific). Monkeys lived in cages (Allentown Caging) with 11.5 cubic feet of space and a water spigot. Throughout the experiment, monkeys were weighed weekly and maintained at approximately 95% of their free-feeding body weight by limited access to Purina Monkey Chow (120–150 g per day). In addition, monkeys received a multiple-vitamin tablet and fresh fruit approximately 2–3 times per week. The experimental manipulations described in this manuscript were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health, and with the approval of the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan.

Surgery. Each monkey was surgically prepared with a vascular access port (Model GPV, Access Technologies), implanted under a combination of ketamine (15 mg per kg body weight) and butorphanol (0.025 mg per kg body weight) anesthesia. A catheter was inserted into a major vein (femoral, internal or external jugular, or brachial) to the level of the vena cava. The distal end of the catheter was passed subcutaneously to a point slightly off the midline of the back, where an incision was made. The end of the catheter was attached to the vascular access port, which was placed in a pocket formed by blunt dissection. Ports were flushed with heparinized saline (100 units per ml) 5 d per week in an effort to keep the catheters patent. Antibiotic (25 mg kefzol per kg body weight, BID; Cefazolin sodium, Marsam Pharmaceuticals) was administered prophylactically for 7–10 d beginning the day of the surgery.

Food and cocaine self-administration. At approximately the same time every day, monkeys were guided into primate restraint chairs (Primate Products) and placed into ventilated, sound-attenuating chambers (1.5 m × 0.74 m × 0.76 m, Med Associates). An intelligence panel (48 cm × 69 cm) was located on one side of the chamber. The panel contained two retractable response levers (5 cm wide) with a horizontal row of three stimulus lights 14 cm above each lever. Levers were positioned to be within easy reach of the monkey seated in the primate chair. A food receptacle located between the levers was connected with a tygon tube to a feeder located on the top of the chamber for delivery of 1-g banana-flavored food pellets (PJ Noyes).

Monkeys were initially trained to respond under a multiple fixed-interval (FI) 3-min schedule of food presentation. Under this schedule, the first response after 3 min resulted in the delivery of a 1-g banana-flavored food pellet. Sessions consisted of four components, which alternated between left and right levers, and were signaled by illumination of the lights above the lever. Each component lasted for 20 min or until five food reinforcers were delivered. There was a 2-min timeout following the completion of each component. After food-reinforced responding had been established, baseline PET scans were conducted. After obtaining all baseline PET scans, each monkey was surgically prepared with an indwelling intravenous catheter as described above.

Before cocaine self-administration sessions, the back of the monkey was cleaned with 95% ethyl alcohol and betadine, and the port was connected to an infusion pump (Cole-Parmer), located outside the chamber, via a 22-gauge Huber Point Needle (Access Technologies). The pump was operated for approximately 3 s to fill the port and catheter with the concentration of cocaine available for the session. The conditions were changed to a multiple FI schedule of food and cocaine reinforcement; presentation of food or cocaine was accompanied by illumination of the red lever lights and extinction of the

white lights for 10 s. Food was available in components 1 and 3 (referred to as Fd1 and Fd2, respectively), and cocaine was available during components 2 and 4 (referred to as Coc1 and Coc2, respectively); for each drug infusion, the pump was activated for 10 s. Food reinforcement was available for 20 min or until 5 reinforcers were obtained, as described above, and cocaine was available for 60 min or until 15 reinforcers were received. The primary dependent variables were response rates (total responses divided by component time) and total number of reinforcers in each component.

PET imaging. Details regarding the PET data acquisition protocol, blood sampling procedure, and metabolite analysis have been fully described[16,20,21,23]. Monkeys were initially anesthetized with 10 mg ketamine per kg body weight, transported to the PET Center and maintained under 1.5% isoflurane anesthesia. Ketamine-induced anesthesia has been shown not to affect [^{18}F]FCP DVRs (ref. 23). An arterial catheter and a venous catheter were inserted by percutaneous stick for blood sampling and tracer injection, respectively. Monkeys were administered a paralytic (0.07 mg vecuronium bromide per kg body weight) intravenously, and ventilation was maintained by a respirator throughout the 3-h-long PET study. Supplemental doses of vecuronium (0.1 mg h^{-1}) were administered throughout the study. For 10 monkeys, four baseline PET scans were conducted: two as described above and two with 1.0 mg lorazepam per kg body weight administered 30 min before the PET study. In one study, the latter manipulation was shown to decrease amounts of extracellular DA during [^{11}C]raclopride PET studies[22]. Because there were no significant differences in these baseline DVRs, and in an effort to control for changes in extracellular DA that may result from cocaine exposure and which could affect DVRs, all remaining PET studies were conducted with 1.0 mg lorazepam per kg body weight administered before the scan. No experimental sessions were conducted on the day of the PET study, in order to further decrease the likelihood of cocaine-induced elevations in DA. The synthesis of [^{18}F]FCP was accomplished via *N*-alkylation of the corresponding des-benzyl precursor with [^{18}F]-fluorobenzyliodide, as previously described[45,46]. At the start of the PET scan, approximately 4 mCi of [^{18}F]FCP was injected, followed by 3 ml of heparinized saline. At appropriate times, arterial blood samples were withdrawn and placed into preheparinized minicentrifuge tubes for analysis, as described previously[21].

PET scans were obtained with a Siemens/CTI ECAT 951/31 PET scanner. The effective resolution (full width at half maximum) of the PET scanner was 9 mm in all axes for the reconstructed image after filtering (Hanning filter with a 0.4 cycle per pixel cutoff; resolution determined experimentally using a line source). Images were taken for the following time frames: 5 × 1 min, 5 × 2 min, 5 × 5 min, 8 × 10 min, and 3 × 20 min. The completed scan contained a total of 26 frames over 180 min. Regions of interest were drawn over the basal ganglia and cerebellum, and tissue-time activity curves were constructed by plotting the percentage of injected dose per cubic centimeter of tissue (that is, % i.d. per cm^3) versus time post intravenous injection of radiotracer. The distribution volume (DV) for the basal ganglia and cerebellum was determined using the graphic technique described previously[24]. The linear portion of the plot was used to determine the DV for each region, which in all cases included the last 80 min of the scan (five frames). The ratio of the DV for the basal ganglia and the cerebellum, the distribution volume ratio (DVR), was used as a metric of specific binding and is related to the binding potential[47] by the formula DVR − 1 = binding potential[24].

Statistical analysis. To analyze behavior, we calculated mean data (± 1 s.d.) over consecutive months for each monkey. To determine if response rates changed significantly across time, individual repeated-measures analyses of variance (ANOVAs) for each component (Fd1, Fd2, Coc1, Coc2 and mean Cocaine) were conducted with Month as a factor. To analyze imaging data from the PET experiments, we used repeated-measures ANOVA from commercially available software (GB-Stat). For all significant ANOVAs, pairwise comparisons were made using the Student's Neuman-Keuls test. Correlation coefficients were computed using the same software package. For all analyses, differences were considered statistically significant when $P < 0.05$.

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AUTHOR CONTRIBUTIONS

M.A.N., D.M., S.H.N and T.L.C. contributed to the cocaine self-administration studies; H.D.G., N.B., R.E. and R.H.M. contributed to the PET imaging studies.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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