

Special Section on Sexual Dimorphism in Neuroimmune Cells

Effects of the α -2 Adrenergic Receptor Agonists Lofexidine and Guanfacine on Food-Cocaine Choice in Socially Housed Cynomolgus Monkeys^S

Paul W. Czoty and Michael A. Nader

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

Received February 27, 2020; accepted July 2, 2020

ABSTRACT

Although norepinephrine (NE) does not appear to play a prominent role in mediating the abuse-related effects of cocaine, studies have indicated that NE α -2 receptor agonists can attenuate reinstatement of extinguished cocaine self-administration in rats and monkeys and can decrease cocaine craving in humans. In the present studies, we examined the effects of two α -2 receptor agonists, lofexidine and guanfacine, on choice between food and cocaine (0.0–0.1 mg/kg per injection) in cynomolgus monkeys. Male and female subjects were housed in stable same-sex social groups of four; social rank did not influence the effects of lofexidine and guanfacine. When administered acutely, lofexidine (0.03–3.0 mg/kg, i.v.) significantly decreased cocaine choice in females ($n = 7$) but not males ($n = 8$). However, in males, the same lofexidine doses produced dose-dependent decreases in core body temperature ($n = 7$), and acute guanfacine (0.003–1.0 mg/kg, i.v.) significantly decreased cocaine choice ($n = 11$). When lofexidine was administered for five consecutive days to

a subset of the monkeys in whom lofexidine acutely decreased cocaine choice, tolerance to this effect developed to varying degrees of completeness in three of three males and two of four females. Taken together, these data suggest that α -2 receptor agonists can produce small decreases in the reinforcing strength of cocaine relative to food and that, even when efficacy is observed after acute administration, tolerance to the decreases in cocaine choice are apparent and more likely in males compared with females.

SIGNIFICANCE STATEMENT

Cocaine use disorder remains a significant public health problem with no US Food and Drug Administration–approved treatments. Although cocaine elevates dopamine, serotonin, and norepinephrine (NE), the latter target has received less research. The present study noted modest effects of NE agonists on the relative reinforcing strength of cocaine with greater efficacy in female compared with male monkeys.

Introduction

Cocaine use disorder remains a considerable public health problem for which there is no Food and Drug Administration–approved pharmacotherapy. Cocaine increases extracellular levels of norepinephrine (NE) in addition to dopamine and serotonin (Reith et al., 1997). Although increases in synaptic NE could theoretically play a role in the abuse-related effects of cocaine and other drugs (for review, see Fitzgerald, 2013), early behavioral studies using animal models provided little support for an important role for NE in mediating the reinforcing effects of cocaine. For example, neither NE uptake inhibitors nor NE receptor agonists are self-administered by laboratory animals under most conditions (Risner and Jones,

1976; Woolverton, 1987; Wee and Woolverton, 2004; Wee et al., 2006), with the notable exception of the NE α -2 receptor agonist clonidine (Davis and Smith 1977; Woolverton et al., 1982; Weerts and Griffiths, 1999). In contrast, models of cocaine discrimination suggested a potential role for NE in the subjective-like effects of cocaine in nonhuman primates (Spealman, 1995; Kohut et al., 2017).

Although NE does not appear to mediate cocaine reinforcement, consistent data have arisen regarding the role of α -2 receptors in models of relapse. Studies in rats and monkeys have shown that α -2 receptor agonists can block cue- and stress-induced reinstatement of extinguished responding that was previously maintained by cocaine (Erb et al., 2000; Highfield et al., 2001; Smith and Aston-Jones, 2011; Buffalari et al., 2012). Moreover, α -2 receptor antagonists can reinstate extinguished responding formerly maintained by cocaine, methamphetamine, and alcohol (Lee et al., 2004, 2005; Shepard et al., 2004). Importantly, these results in animal models of

This work was supported by National Institutes of Health National Institute on Drug Abuse [Grant R01 DA017763].
<https://doi.org/10.1124/jpet.120.266007>.

^S This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: F, female; FR, fixed-ratio; M, male; NE, norepinephrine; SAL, saline.

relapse are consistent with studies in human cocaine abusers in which α -2 receptor agonists decreased craving for cocaine (Jobes et al., 2011; Fox et al., 2012, 2014). Despite their encouraging effects in models of relapse, two studies reported a lack of effect of α -2 receptor agonists, lofexidine and UK14304, on cocaine + heroin or cocaine self-administration (respectively) under a one-response fixed-ratio (FR1) schedule of reinforcement in male rats (Highfield et al., 2001; Wee et al., 2008, respectively). In contrast to results in rodent models, the only previous study to assess the effects of an α -2 agonist in nonhuman primates found that acute lofexidine (0.1 or 0.32 mg/kg, i.m.) decreased cocaine self-administration under an FR30 schedule of reinforcement (Kohut et al., 2013). However, in another group of monkeys self-administering cocaine under a second-order FR2 (variable-ratio 16:S) schedule of reinforcement, intravenous administration of lofexidine (0.1–0.32 mg/kg per hour) for 7–10 days shifted the cocaine dose-effect curve to the left, indicating an increase in the reinforcing potency of cocaine (Kohut et al., 2013). Thus, whereas lofexidine appears effective in certain models of cocaine abuse (drug discrimination and reinstatement), it appears to lack the ability to block ongoing cocaine self-administration under FR and second-order schedules of reinforcement.

The present studies extended the preclinical examination of the effects of α -2 receptor agonists on cocaine self-administration in several ways. First, the experiments were conducted in socially housed monkeys. When housed in social groups, cynomolgus monkeys form linear dominance hierarchies, which we have conceptualized as representing a continuum ranging from environmental enrichment in socially dominant monkeys to chronic social stress in subordinate monkeys (see Nader et al., 2012). Considering the ability of NE systems to mediate stress (Wood and Valentino, 2017), we hypothesized that the effects of lofexidine might be greater, or more likely to be observed, in subordinate versus dominant monkeys. In previous studies, a differential sensitivity to dopamine receptor drugs and to various stressful and enriching environmental stimuli was observed with respect to their effects on cocaine self-administration (Czoty and Nader, 2012, 2013, 2015).

Second, whereas previous studies used ratio-based schedules (i.e., fixed- or variable-ratio) and a single operandum that provided measures of response rate or reinforcement rate as the primary dependent variable, we assessed the effects of α -2 agonists in monkeys responding under a concurrent food-cocaine choice procedure. Under this procedure, food pellets and cocaine injections are available concurrently, and the dependent variable is a measure of response allocation. Drug addiction has been described as a disorder of maladaptive choice in which obtaining and consuming drugs is overvalued compared with other activities that could function as reinforcers, resulting in adverse consequences. Thus, preclinical choice procedures have important translational relevance because they include the option for subjects to allocate their behavior toward obtaining drugs versus other environmental stimuli (e.g., Banks and Negus, 2017). This is particularly relevant for medication development because the goal of treatment with a pharmacotherapy for cocaine use disorder is to increase the proportion of times the patient will choose to pursue activities other than drug use (Perkins and Freeman, 2018).

Third, all previous preclinical studies of lofexidine in the context of models of cocaine use disorder (i.e., effects on

ongoing self-administration or reinstatement of extinguished self-administration) have been performed in males. In the present studies, we examined the effects of lofexidine on cocaine choice in both male and female socially housed monkeys. In male monkeys, we compared the effects of lofexidine to those of guanfacine, a more selective agonist for α -2 versus α -1 receptors (Summers et al., 1980a,b). In addition, because α -2 receptor agonists, including guanfacine and lofexidine, have been shown to decrease body temperature (e.g., VanDer Laan et al., 1985; Minor et al., 1989; Quan et al., 1992), we compared the effects of lofexidine on cocaine choice to its effects on body temperature in male monkeys.

Materials and Methods

Subjects. Eighteen adult cynomolgus monkeys (*Macaca fascicularis*; 11 males, indicated by an “M-” preceding their animal number; seven females, indicated by “F-”) served as subjects. All monkeys had a history of being housed in same-sex groups of three or four for more than 2 years. Monkeys 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). Social status in these established groups had previously been determined according to the outcomes of agonistic encounters, as described previously (Czoty et al., 2009); ranks did not change over the duration of the present experiments. Monkeys that were #1- and #2-ranked were considered dominant, and #3- and #4-ranked monkeys were considered subordinate. Each monkey had been fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit in a primate chair (Primate Products). Each monkey was also prepared with an indwelling venous catheter and subcutaneous vascular access port, as described previously (see Czoty and Nader, 2013). Seven of the male monkeys had previously been implanted with a subcutaneous transponder (model IPTT-300; Bio Medic Data Systems, Seaford, DE) for noninvasive measurement of body temperature, which was recorded immediately before and after selected self-administration sessions.

To prevent development of obesity and cardiovascular/metabolic problems, monkeys were not fed ad libitum. They were also not maintained at a “target weight” set to be an arbitrary percentage below free-feeding weight, because the latter can change with age and other factors, and we did not plan to remove monkeys from the study for periodic redetermination of free-feeding weights. Instead, 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. All 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 Wake Forest University Animal Care and Use Committee. Environmental enrichment was provided as outlined in the Wake Forest University Non-Human Primate Environmental Enrichment Plan.

Food-Cocaine Choice: Apparatus and Procedures. Each day, monkeys were transferred to a primate chair and placed into an operant chamber, and their port was connected to an infusion pump located outside the chamber. Two photo-optic switches (model 117-1007; Stewart Ergonomics, Inc., Furlong, PA) were located on one side of the chamber with a horizontal row of three stimulus lights positioned 14 cm above each switch. A food receptacle, above which was a single white stimulus light, was located between the switches for delivery of 1 g of banana-flavored food pellets (Bio-Serv, Frenchtown, NJ).

Monkeys were trained to self-administer cocaine under a concurrent FR schedule of food and cocaine availability in which complete cocaine dose-response curves were determined during each session (see Czoty and Nader, 2012). Responding on one switch (the “food switch”),

signaled by a yellow light, always resulted in delivery of a food pellet. Responding on the other switch (the “drug switch”) resulted in activation of the infusion pump and an injection of cocaine (0.003–0.1 mg/kg per injection). Availability of each cocaine dose was associated with illumination of a different set of stimulus lights above the drug switch; different cocaine doses were studied by varying the duration of pump activation. Assignment of food or drug to a switch was counterbalanced across monkeys.

Each daily session consisted of five components in which monkeys chose between food pellets and ascending doses of cocaine (i.e., no injection, 0.003, 0.01, 0.03, and 0.1 mg/kg per injection cocaine in components 1–5, respectively). Each component ended when 10 trials had been completed or 20 minutes had elapsed, whichever came first. Delivery of a food pellet or injection was accompanied by a 5-second illumination of the red light above the corresponding switch and a 30-second time-out; a response emitted on the alternate switch before an FR was completed reset the response requirement on the first switch. In addition, a 120-second time-out followed each component. Ratio requirements were adjusted for each monkey such that allocation of responding to the drug switch increased over the session as the available dose of cocaine increased. A monkey was considered trained when $\leq 20\%$ of reinforcers were earned on the drug switch when the alternative to food was no injection (component 1) or 0.003 mg/kg per injection cocaine (component 2) and $\geq 80\%$ of reinforcers were earned on the drug switch when the alternative to food was 0.1 mg/kg per injection cocaine (component 5). An additional criterion was observation of a dose-related increase in drug choice. Table 1 lists the FR values for food and drug self-administration for each monkey.

Effects of Drugs on Food-Cocaine Choice. When cocaine choice was stable, saline, lofexidine (0.003–3.0 mg/kg, i.v., 10 minutes before the session), or guanfacine (in males only; 0.003–1.0 mg/kg, i.v., 15 minutes before the session) was administered acutely. Typically, a drug dose was tested on Tuesday and Friday, and saline was administered on Thursday. Because monkeys showed individual differences in the potency of lofexidine and guanfacine to affect cocaine choice, a “best-dose” approach was used whereby the dose that produced the greatest effect on cocaine choice without disrupting responding (defined as a $\geq 33\%$ reduction in total reinforcers) was determined for each subject. Table 1 lists these best doses for each subject and drug.

Next, in monkeys in whom the best dose of lofexidine decreased cocaine choice, the best dose was administered prior to the self-administration session for five consecutive days. Of the monkeys in

whom lofexidine acutely decreased cocaine choice, three males and four females were tested in this experiment (M-7079, M-7082, M-6628, F-7870, F-7902, F-7833, and F-7591). The other monkeys (M-7426, M-6955, and F-7457) had been moved to another study and were no longer available for testing.

Data Analysis. The primary dependent variable was percent cocaine choice, defined as the percent of total reinforcers received as injections, calculated for each component. Because monkeys had different FR requirements (which did not differ according to social rank), percent of reinforcers was used for analysis rather than percent responses. In addition, an ED_{50} for cocaine choice (that is, the cocaine dose calculated to result in 50% choice of cocaine injections) was calculated for each condition in each monkey by interpolating the linear portion of the dose-effect curve. The total number of reinforcers, food pellets, and injections delivered was also recorded. Initially, the presence or absence of an effect of social rank was determined in each sex by subjecting ED_{50} data to a two-way repeated-measures ANOVA with social rank and treatment (saline or lofexidine) as factors. This analysis determined that rank had no significant effect in either sex; however, there was a main effect of lofexidine treatment in females, but not males (see *Results*). Subsequently, males’ and females’ data (cocaine choice, total reinforcers, food reinforcers, and injections) were analyzed separately using a two-way ANOVA with cocaine dose and treatment (saline or lofexidine) as factors. Data from the guanfacine experiment were analyzed similarly. In all cases, differences were considered statistically significant when $P < 0.05$. Additionally, to quantify the potency of lofexidine to decrease body temperature, the dose that decreased body temperature by 1°C (termed the ED_{-1}) was interpolated.

Drugs. Lofexidine HCl was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and guanfacine was purchased from Sigma-Aldrich Corporation (St. Louis, MO). Cocaine HCl was provided by the National Institute on Drug Abuse (Rockville, MD). All drugs were dissolved in sterile 0.9% saline.

Results

Effects of Acute Lofexidine on Food-Cocaine Choice and Body Temperature. Initial data analysis determined whether social rank affected cocaine choice after saline or lofexidine treatment. Analysis of ED_{50} data from male monkeys revealed no significant main effect of social rank or

TABLE 1

Social ranks and self-administration parameters for individual subjects used in the food-cocaine choice procedure

Asterisk indicates subjects that also received 5 days of treatment with lofexidine. All doses are listed in milligrams per kilogram (intravenous).

Monkey	Rank	FR Values Drug:Food	Best Dose Lofexidine	ED_{-1} Lofexidine	Best Dose Guanfacine
Males					
M-7079*	D	250:50	0.3	0.041	0.56
M-7901	D	50:200	1.0	0.079	1.0
M-7082*	D	50:100	1.0	0.144	1.0
M-6629	D	100:50	1.7	0.239	1.0
M-6628*	D	300:50	0.17	—	1.0
M-7083	D	300:50	—	—	1.0
M-6527	S	100:50	1.0	0.225	1.0
M-7426	S	50:100	3.0	—	1.0
M-6955	S	150:50	1.0	0.100	1.0
M-7478	S	250:50	—	—	0.56
M-7425	S	100:50	—	—	1.0
Females					
F-7833*	D	125:25	1.0	—	—
F-7905	D	100:50	1.7	—	—
F-7870*	D	75:10	0.03	—	—
F-7457	S	25:100	0.3	—	—
F-7591*	S	250:20	0.1	—	—
F-7558	S	200:5	1.0	—	—
F-7902*	S	100:100	0.1	—	—

D, dominant monkey; S, subordinate monkey. —, not determined

saline/lofexidine treatment, with no significant interaction between the factors. In female monkeys, there was also no main effect of social rank. However, there was a significant effect of saline/lofexidine treatment ($F_{1,5} = 7.05$, $P < 0.05$) on cocaine choice ED_{50} , with no significant interaction. In addition, a t test demonstrated that lofexidine produced a larger shift in cocaine choice ED_{50} for females compared with males ($P < 0.005$). Subsequently, data from dominant- and subordinate-ranked monkeys were combined within each sex, but data from male and female monkeys were analyzed separately.

Regarding cocaine choice in males (Fig. 1A), a two-way ANOVA revealed a significant main effect of cocaine dose ($F_{4,28} = 59.79$, $P < 0.001$), but not saline/lofexidine treatment, and no significant interaction. There was a main effect of cocaine dose ($F_{4,28} = 9.34$, $P < 0.001$), but not saline/lofexidine treatment, on total reinforcers earned across the session (Fig. 2A) and a significant interaction ($F_{4,28} = 4.25$, $P < 0.01$). Post hoc testing revealed that lofexidine significantly decreased total reinforcers only during the first component of the session when the choice was between food and 0.0 cocaine. In males, there was a main effect of cocaine dose ($F_{4,28} = 49.15$, $P < 0.001$) but not saline/lofexidine treatment on food reinforcers earned across the session (Fig. 2B) and a significant interaction ($F_{4,28} = 5.19$, $P < 0.01$). Number of food pellets delivered did not differ significantly between saline and lofexidine treatment at any cocaine dose. Regarding the number of injections delivered (Fig. 2C), there was a significant main effect of cocaine dose ($F_{4,28} = 23.40$, $P < 0.001$) and saline/lofexidine treatment ($F_{4,28} = 1.7$, $P < 0.05$), as well as a significant interaction ($F_{4,28} = 59.79$, $P < 0.001$). Lofexidine decreased the number of injections delivered when the available cocaine dose was 0.01 or 0.03 mg/kg per injection. Data for individual male subjects are shown in Supplemental Fig. 1.

Regarding cocaine choice in female monkeys (Fig. 1B), there was a significant main effect of cocaine dose ($F_{4,24} = 34.63$, $P < 0.001$) and saline/lofexidine treatment ($F_{1,6} = 14.72$, $P < 0.01$) with a significant interaction ($F_{4,24} = 10.22$, $P < 0.001$). Post hoc testing revealed that cocaine choice was decreased by lofexidine when the available cocaine dose was 0.01, 0.03, and 0.1 mg/kg per injection. For total reinforcers delivered, there was a significant main effect of cocaine dose ($F_{4,24} = 15.50$, $P < 0.001$), but not saline/lofexidine treatment, with no significant interaction (Fig. 2D). Regarding food pellets delivered (Fig. 2E), there was a main effect of cocaine dose ($F_{4,24} = 38.47$, $P < 0.001$) and saline/lofexidine treatment ($F_{1,6} = 9.79$, $P < 0.05$), as well as a significant interaction ($F_{4,24} = 10.44$, $P < 0.001$). Finally, for injections

delivered (Fig. 2F), there was a significant main effect of cocaine dose ($F_{4,24} = 14.84$, $P < 0.001$) and saline/lofexidine treatment ($F_{1,6} = 30.43$, $P = 0.001$) when the available cocaine dose was 0.01, 0.03, and 0.1 mg/kg per injection, with a significant interaction ($F_{4,24} = 10.41$, $P < 0.001$). Lofexidine increased food pellet deliveries and decreased cocaine injections when the available cocaine dose was 0.01, 0.03, and 0.1 mg/kg per injection. Data for individual female subjects are shown in Supplemental Fig. 2.

Seven of the male subjects had previously been implanted with transponders for noninvasive measurement of body temperature. Figure 3 depicts the effects of administration of saline (SAL) or a range of doses of lofexidine in these monkeys. Lofexidine decreased core body temperature in all monkeys in a manner that was dose-dependent in six of seven subjects. The dose that decreased body temperature by 1°C (i.e., the ED_{-1}) is shown in Table 1 for those six subjects.

Effects of Acute Guanfacine on Food-Cocaine Choice.

When guanfacine was administered to 11 male monkeys choosing between food and cocaine (Fig. 4A), a two-way ANOVA revealed a significant main effect of cocaine dose ($F_{4,50} = 154.90$, $P < 0.0001$) and saline/guanfacine treatment ($F_{1,50} = 8.71$, $P < 0.01$), as well as a significant interaction ($F_{4,50} = 3.45$, $P < 0.05$; Fig. 5A). Post hoc testing indicated that the effect of guanfacine differed from that of saline when the available cocaine dose was 0.01 and 0.03 mg/kg per injection. There was a main effect of cocaine dose ($F_{4,40} = 40.39$, $P < 0.001$), but not saline/guanfacine treatment, on total reinforcers delivered across the session (Fig. 4B) and no significant interaction. Regarding food reinforcers delivered, there was a main effect of cocaine dose ($F_{4,40} = 146.28$, $P < 0.001$) but not SAL/guanfacine treatment (Fig. 4C), with no significant interaction. Post hoc testing showed that guanfacine decreased food pellet deliveries when choice was between food and 0.01 and 0.03 mg/kg cocaine. Regarding the number of injections delivered (Fig. 4D), there was a significant main effect of cocaine dose ($F_{4,40} = 44.75$, $P < 0.001$) and SAL/guanfacine treatment ($F_{1,10} = 37.15$, $P < 0.001$), as well as a significant interaction ($F_{4,40} = 5.00$, $P < 0.01$). Guanfacine decreased cocaine injections when the available cocaine dose was 0.01, 0.03, or 0.1 mg/kg per injection. Data for individual male subjects are shown in Supplemental Fig. 3 Supplemental Fig. 2.

Effects of Repeated Lofexidine on Food-Cocaine Choice.

In three male and four female monkeys in whom lofexidine attenuated cocaine choice when given acutely, the effects of repeated lofexidine treatment were examined. The dose that was designated the best dose in acute experiments

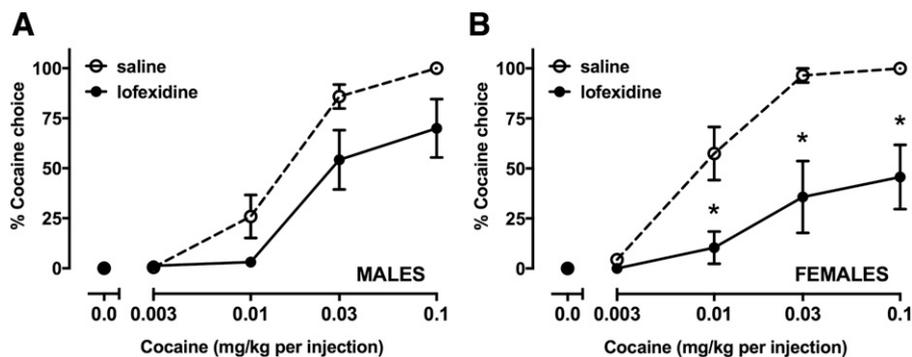


Fig. 1. Effects of lofexidine on food and cocaine self-administration under the choice procedure in male (A, $n = 8$) and female (B, $n = 7$) monkeys. Abscissae: cocaine dose available as the alternative to a food pellet. Points indicate means \pm S.E.M. Ordinates: percent cocaine choice. *Significant difference ($p < 0.05$) from data obtained after saline treatment during availability of the same cocaine dose.

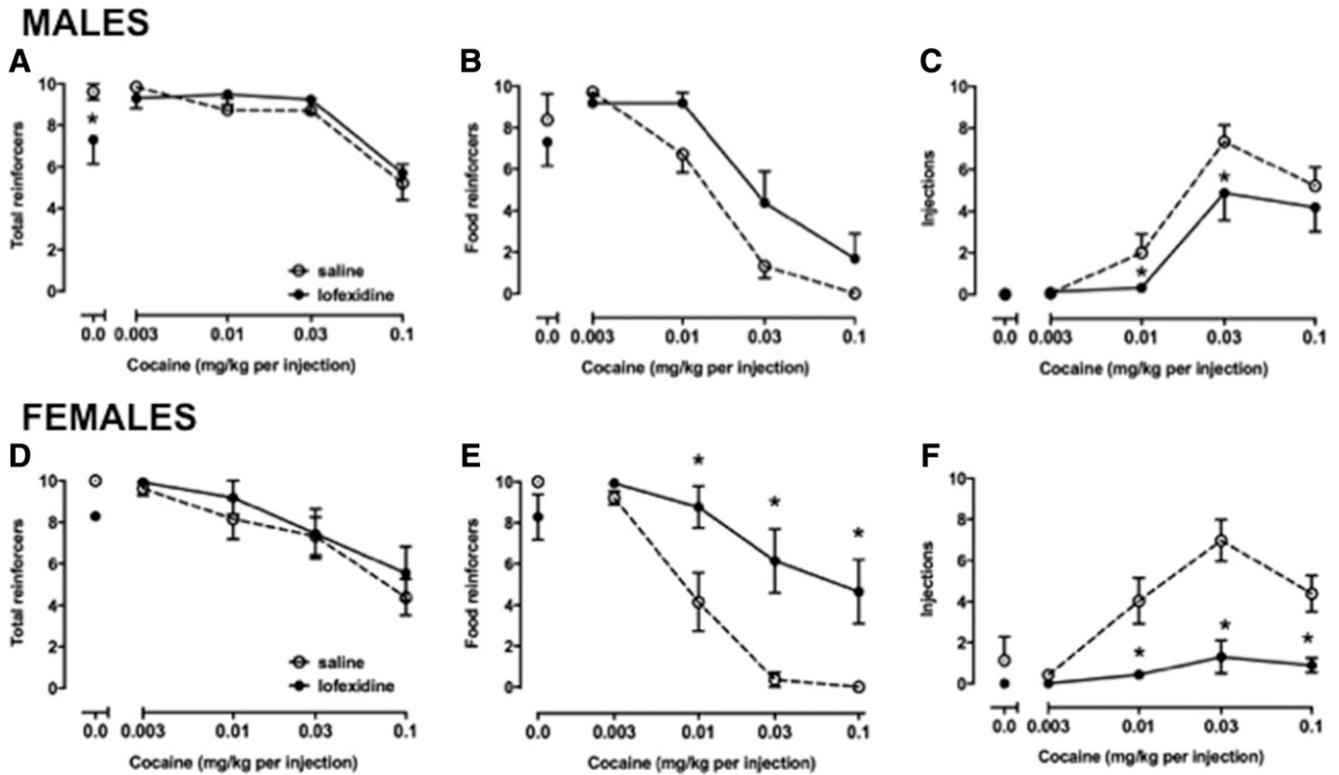


Fig. 2. Effects of lofexidine on reinforcement frequency in male (top row, $n = 8$) and female (bottom row, $n = 7$) monkeys. Abscissae: cocaine dose available as the alternative to a food pellet. Ordinates: (A and D) total reinforcers delivered, (B and E) number of food pellets delivered, and (C and F) cocaine injections delivered. Points indicate means \pm S.E.M. *Significant difference ($p < 0.05$) from data obtained after saline treatment during availability of the same cocaine dose.

was administered for five consecutive days, on which food-cocaine choice studies were conducted. In all three males (Fig. 5, top), lofexidine decreased cocaine choice on day 1, consistent with acute studies in those animals. In all monkeys, this rightward shift in the dose-effect curve was attenuated on day 5 of treatment. Tolerance was complete in M-7082 and

partial in M-7079 and M-6628. A rightward shift was also observed on day 1 in the four females (Fig. 5, bottom). By day 5, the initial effects of lofexidine were maintained or enhanced in two monkeys (F-7870 and F-7902), whereas complete tolerance was observed in two female monkeys (F-7833 and F-7591).

Discussion

The goal of the present studies was to extend the preclinical examination of the effects of the α -2 receptor agonist lofexidine by characterizing its effects on cocaine self-administration using a food-drug choice procedure in both male and female socially housed monkeys. The maximal effects of lofexidine on cocaine choice differed across monkeys of both sexes. Lofexidine decreased the reinforcing strength of cocaine relative to food (i.e., shifted the curve rightward) in 50% (four of eight) of males and 71% (five of seven) of females. In those individuals, effects of lofexidine were behaviorally selective. That is, a dose was found that increased the number of food pellets delivered and decreased injections earned without affecting the total number of reinforcers delivered in a session. On average, the cocaine choice curves of both males and females shifted to the right, but the effect was greater, and reached statistical significance, in females. The difference in statistical significance across sexes was due to a *greater proportion* of females being affected rather than a *larger effect* being observed in each individual female compared with each individual male. The observed decrease in cocaine reinforcement after acute lofexidine administration is consistent with the only other

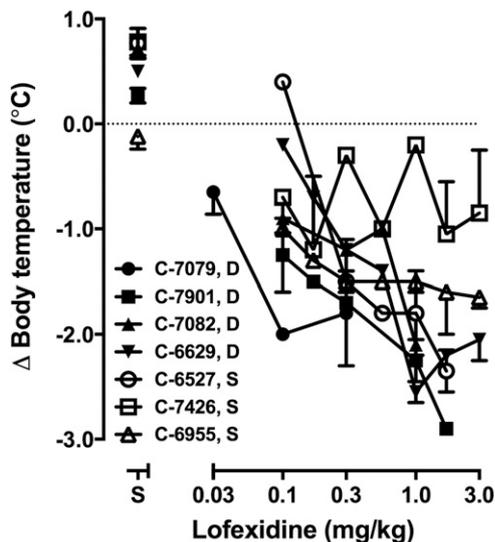


Fig. 3. Effects of lofexidine on body temperature in individual male monkeys. D, dominant monkey; S, subordinate monkey. Abscissa: lofexidine dose (milligrams per kilogram). Ordinate: change in body temperature (degrees centigrade).

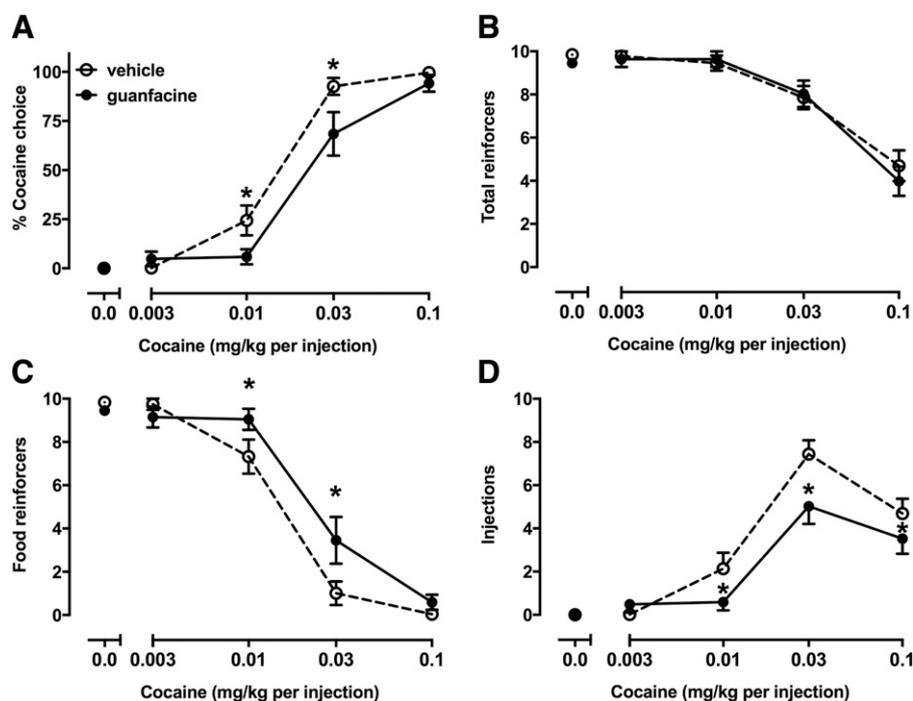


Fig. 4. Effects of guanfacine on food and cocaine self-administration under the choice procedure in male monkeys. Abscissae: cocaine dose available as the alternative to a food pellet. Ordinates: (A) percent cocaine choice, (B) total reinforcers delivered, (C) number of food pellets delivered, and (D) cocaine injections delivered. Data indicate means \pm S.E.M. ($n = 11$). *Significant difference ($p, 0.05$) from data obtained after saline treatment during availability of the same cocaine dose.

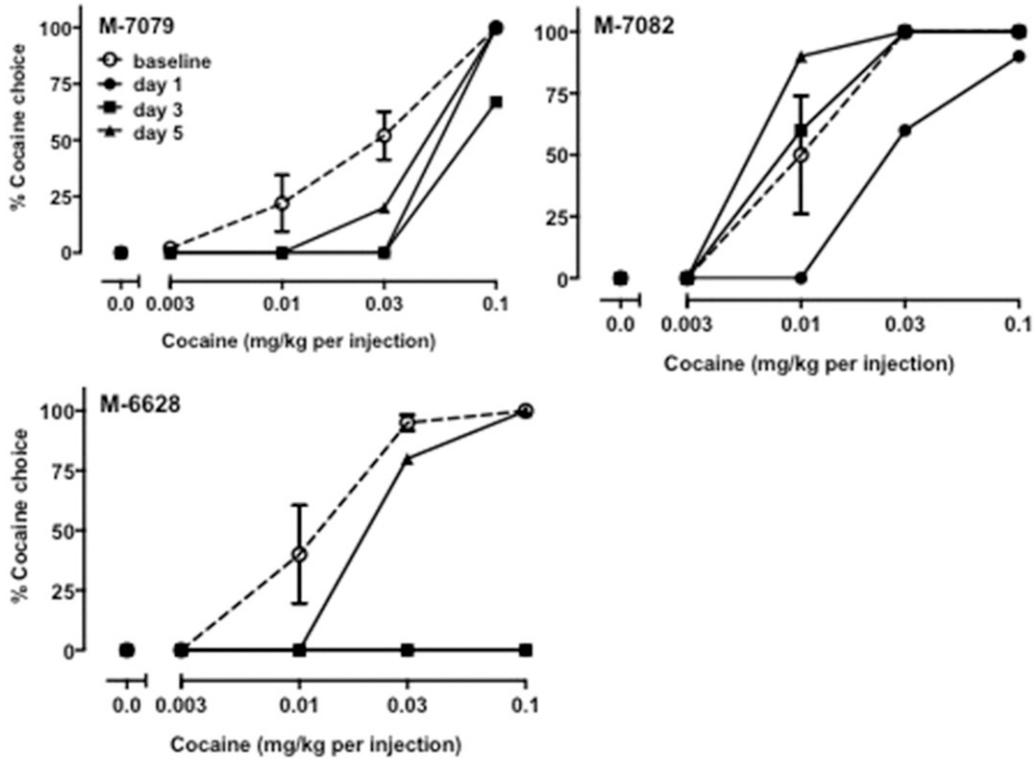
published study of the effects of lofexidine on cocaine reinforcement in monkeys (Kohut et al., 2013). The conclusion that lofexidine decreases cocaine's abuse-related effects to a greater degree in females is also consistent with a study in humans, in which the α -2 receptor agonist guanfacine decreased cocaine craving, anxiety, and negative mood in female, but not male, cocaine-dependent subjects (Fox et al., 2014). Very little is known about the potential neurobiological differences that may underlie sex differences in the potency and efficacy of α -2 receptor agonists, as the vast majority of studies have either used a single sex, pooled data, or did not report results of such a comparison. A previous autoradiographic study reported higher densities of α -2 receptors in tissue homogenates in some brain areas of adolescent female rodents (e.g., hippocampus) but not others (e.g., parietal cortex; Booze et al., 2006).

Although the small attenuation of cocaine choice by lofexidine did not reach statistical significance in male monkeys, a dose-related decrease in body temperature was observed in six of seven subjects. To compare the potency of lofexidine across the two experiments, we calculated the dose of lofexidine that decreased body temperature by 1°C (termed the ED_{-1}) and compared it with the best dose as determined in the cocaine choice experiment. The rank order of potency of lofexidine was similar in the two experiments. That is, the monkey in whom lofexidine was most potent in decreasing body temperature (M-7079) required the lowest lofexidine dose to alter cocaine choice. Similarly, the monkey with the highest ED_{-1} also had the highest best dose (M-6629). In addition, in male monkeys, the effects of the more selective agonist, guanfacine, were examined (Uhlen and Wikberg, 1991; Intengan and Smyth, 1997). Like lofexidine, the best dose of guanfacine produced a small rightward shift in the cocaine dose-effect curve, increasing the number of food reinforcers received and decreasing the number of injections delivered without reducing the total number of reinforcers earned during the session. In contrast to the lofexidine data,

however, the effect of guanfacine achieved statistical significance. It is possible that the statistical significance of the guanfacine but not lofexidine data reflects the increased pharmacological selectivity of guanfacine. Although both compounds bind α -2 receptors with relatively high affinity (K_i in rat cerebral cortical membranes is 2.3 nM for lofexidine vs. 1.9 nM for guanfacine), guanfacine has a much higher selectivity for α -2 versus α -1 receptors (3220-fold) compared with lofexidine (79-fold; Summers et al., 1980a,b). For example, despite showing a similar potency to decrease pentylenetetrazol-induced seizures in rats, guanfacine had much higher efficacy than lofexidine (Papanicolaou et al., 1982). Nonetheless, the effects of lofexidine and guanfacine in male monkeys was similar in six of the eight monkeys tested with both drugs. Taken together, these experiments demonstrate that α -2 receptor agonists are behaviorally and physiologically active and can alter food-cocaine choice in male monkeys, although males appear less sensitive than females. Future studies comparing the effects of lofexidine on body temperature in females would provide more detailed information regarding the potential sex differences in potency of these drugs.

In contrast to what has been observed with drugs that target dopamine D2-like receptors (Czoty and Nader, 2013, 2015), there was no significant effect of social rank in either sex. This result suggests that the environmental variables involved in establishment and maintenance of the nonhuman primate social hierarchy do not affect noradrenergic systems to the extent that they alter dopaminergic systems. Although subordinate monkeys undoubtedly experience chronic social stress, social rank-related differences in the effects of stress may be more prominently mediated by changes in hypothalamic-pituitary-adrenal axis function. For example, higher cortisol concentrations are observed in both male and female subordinates during initial social housing (Czoty et al., 2009; Kromrey et al., 2016). Moreover, male subordinate monkeys

MALES



FEMALES

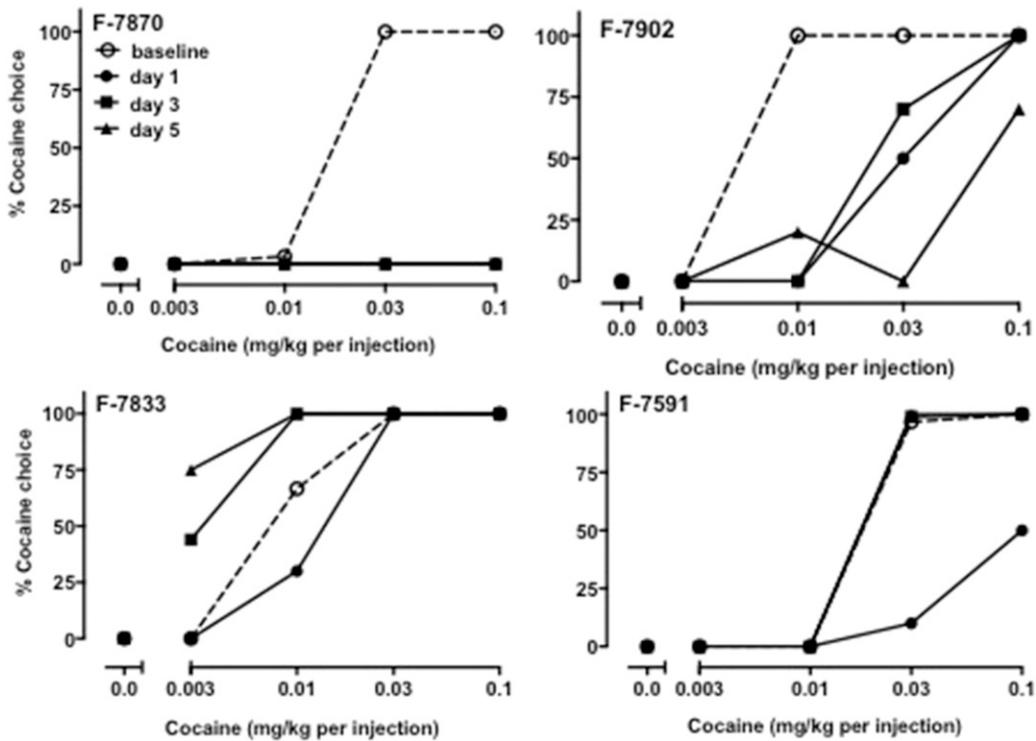


Fig. 5. Effects of five consecutive days of treatment with lofedidine in three males and four females in whom acute lofedidine decreased cocaine choice. Data termed “baseline” indicate the means (\pm S.D.) of cocaine choice over the three days prior to “day 1” of drug administration. Abscissae: cocaine dose available as the alternative to a food pellet. Ordinates: percent cocaine choice. For clarity, only data obtained on days 1, 3, and 5 are shown.

showed greater adrenal gland responsiveness to adrenocorticotropic hormone after suppression with the glucocorticoid agonist dexamethasone (Czoty et al., 2009). In these previous studies, however, there were no rank-related differences in basal cortisol concentrations in well-established social groups. Thus, it remains a possibility that more subtle effects on the noradrenergic system may contribute to formation of the hierarchy but that neuroadaptations occur that equalize NE function in dominant and subordinate monkeys. Regarding social rank and the reinforcing effects of cocaine, these findings suggest that differences between dominant and subordinate monkeys may be driven more by dopamine-mediated differences in environmental enrichment than NE-mediated differences in the experience of social stress. This hypothesis is consistent with the findings in our laboratory's original study of cocaine self-administration in socially housed male monkeys (Morgan et al., 2002). In that study, formation of the social hierarchy produced changes in dopamine D2-like receptor availability only in monkeys that became dominant. In these dominant monkeys, cocaine did not function as a reinforcer, whereas cocaine self-administration in subordinate monkeys was robust and similar to what is typically seen in individually housed monkeys.

As described above, the decrease in the reinforcing effects of cocaine observed in the present studies agrees with the only previous nonhuman primate study of the effects of α -2 agonists on cocaine self-administration (Kohut et al., 2013). Nonetheless, because medications are given chronically in the clinic, assessment of the effects of a repeated administration of a potential medication is an important part of a rigorous evaluation of therapeutic potential (Haney and Spealman, 2008; Czoty et al., 2016). Not only is it necessary to assess whether tolerance develops to therapeutic effects of a drug, but there is a precedent for drug effects on cocaine self-administration to differ after acute versus chronic administration (e.g., Thomsen et al., 2013). In fact, in the Kohut et al. (2013) study, although acute lofexidine shifted the cocaine dose-effect curve to the right, chronic lofexidine treatment (0.1–0.32 mg/kg per hour, administered via continuous intravenous infusion) increased the reinforcing potency of cocaine.

Thus, in a subset of the monkeys in whom acute lofexidine decreased cocaine choice (three males and four females), the individual monkey's best dose was administered for five consecutive days prior to a food-cocaine choice experiment. On the first day of treatment, the cocaine choice curve shifted to the right in all subjects, as had been observed during the acute study. By day 5, partial tolerance to this effect had developed in two male monkeys, and cocaine choice was slightly increased in the third male monkey. Results in females were qualitatively different. In two females, the decrease in cocaine choice observed on day 1 was sustained or larger on day 5. In one female monkey, the cocaine curve on day 5 was identical to baseline. In the fourth female monkey, cocaine choice was increased compared with baseline. Thus, unlike in males, in females, tolerance to lofexidine-induced decreases in cocaine choice only occurred in half of the monkeys. It is interesting to note that the degree of tolerance that occurred during five days of lofexidine treatment is positively related to the dose of lofexidine that was administered in both sexes. Complete tolerance occurred in the male monkey who received 1.0 mg/kg lofexidine (M-7082) but not in

the two males who received lower doses. A similar relationship was observed in female monkeys, with the exception of one subject (F-7591). On the whole, these results support the conclusion of Kohut et al. (2013), with the added observation that the tolerance and the shift from decreasing to increasing cocaine self-administration may be less likely to occur in female monkeys.

Taken together, the results of these studies demonstrate that stimulation of NE α -2 receptors can decrease choice of cocaine versus food in male and female cynomolgus monkeys. Although cocaine choice was decreased substantially in some animals, on average the effect was modest, reaching statistical significance only in females. The similar small rightward shift in the cocaine choice curve produced by another α -2 receptor agonist, guanfacine, did reach statistical significance in males. Similar to the results of a previous study, although acute lofexidine decreased cocaine self-administration, repeated administration of lofexidine resulted in tolerance in five of seven monkeys, with two monkeys demonstrating lofexidine-induced increases in cocaine choice on the 5th day of treatment. Regarding clinical relevance, data from this nonhuman primate model of cocaine abuse suggest that lofexidine would produce, at best, small reductions in ongoing cocaine use in most individuals and would likely be more effective in female cocaine abusers. The observation that tolerance develops to this effect further limits its utility as a pharmacotherapy in treatment-seeking individuals who are not abstinent from cocaine. However, preclinical data from drug discrimination and reinstatement studies suggest that this class of drugs may have greater utility in preventing relapse in abstinent cocaine users.

Acknowledgments

The authors would like to acknowledge the technical assistance of Michael Collier, Jillian Odum, and Susan Nader.

Authorship Contributions

Participated in research design: Czoty, Nader.

Conducted experiments: Czoty, Nader.

Performed data analysis: Czoty.

Wrote or contributed to the writing of the manuscript: Czoty, Nader.

References

- Banks ML and Negus SS (2017) Insights from preclinical choice models on treating drug addiction. *Trends Pharmacol Sci* **38**:181–194.
- Booze RM, Wallace DR, Silvers JM, Strupp BJ, Snow DM, and Mactutus CF (2006) Prenatal cocaine exposure alters alpha2 receptor expression in adolescent rats. *BMC Neurosci* **7**:33.
- Buffalari DM, Baldwin CK, and See RE (2012) Treatment of cocaine withdrawal anxiety with guanfacine: relationships to cocaine intake and reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)* **223**:179–190.
- Czoty PW, Gould RW, and Nader MA (2009) Relationship between social rank and cortisol and testosterone concentrations in male cynomolgus monkeys (*Macaca fascicularis*). *J Neuroendocrinol* **21**:68–76.
- Czoty PW and Nader MA (2012) Individual differences in the effects of environmental stimuli on cocaine choice in socially housed male cynomolgus monkeys. *Psychopharmacology (Berl)* **224**:69–79.
- Czoty PW and Nader MA (2013) Effects of dopamine D2/D3 receptor ligands on food-cocaine choice in socially housed male cynomolgus monkeys. *J Pharmacol Exp Ther* **344**:329–338.
- Czoty PW and Nader MA (2015) Effects of oral and intravenous administration of bupirone on food-cocaine choice in socially housed male cynomolgus monkeys. *J Pharmacol Exp Ther* **40**:1072–1083.
- Czoty PW, Stoops WW, and Rush CR (2016) Evaluation of the “pipeline” for development of medications for cocaine use disorder: a review of translational pre-clinical, human laboratory, and clinical trial research. *Pharmacol Rev* **68**:533–562.
- Davis WM and Smith SG (1977) Catecholaminergic mechanisms of reinforcement: direct assessment by drug-self-administration. *Life Sci* **20**:483–492.
- Erb S, Hitchcott PK, Rajabi H, Mueller D, Shaham Y, and Stewart J (2000) Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. *Neuropsychopharmacology* **23**:138–150.

- Fitzgerald PJ (2013) Elevated norepinephrine may be a unifying etiological factor in the abuse of a broad range of substances: alcohol, nicotine, marijuana, heroin, cocaine, and caffeine. *Subst Abuse* **7**:171–183.
- Fox HC, Morgan PT, and Sinha R (2014) Sex differences in guanfacine effects on drug craving and stress arousal in cocaine-dependent individuals. *Neuropsychopharmacology* **39**:1527–1537.
- Fox HC, Seo D, Tuit K, Hansen J, Kimmerling A, Morgan PT, and Sinha R (2012) Guanfacine effects on stress, drug craving and prefrontal activation in cocaine dependent individuals: preliminary findings. *J Psychopharmacol* **26**:958–972.
- Haney M and Spealman R (2008) Controversies in translational research: drug self-administration. *Psychopharmacology (Berl)* **199**:403–419.
- Highfield D, Yap J, Grimm JW, Shalev U, and Shaham Y (2001) Repeated lofexidine treatment attenuates stress-induced, but not drug cues-induced reinstatement of a heroin-cocaine mixture (speedball) seeking in rats. *Neuropsychopharmacology* **25**:320–331.
- Intengan HD and Smyth DD (1997) Alpha-2a/d adrenoceptor subtype stimulation by guanfacine increases osmolar clearance. *J Pharmacol Exp Ther* **281**:48–53.
- Jobs ML, Ghitza UE, Epstein DH, Phillips KA, Heishman SJ, and Preston KL (2011) Clonidine blocks stress-induced craving in cocaine users. *Psychopharmacology (Berl)* **218**:83–88.
- Kohut SJ, Fivel PA, and Mello NK (2013) Differential effects of acute and chronic treatment with the α 2-adrenergic agonist, lofexidine, on cocaine self-administration in rhesus monkeys. *Drug Alcohol Depend* **133**:593–599.
- Kohut SJ, Jacobs DS, Rothman RB, Partilla JS, Bergman J, and Blough BE (2017) Cocaine-like discriminative stimulus effects of “norepinephrine-preferring” monoamine releasers: time course and interaction studies in rhesus monkeys. *Psychopharmacology (Berl)* **234**:3455–3465.
- Kromrey SA, Czoty PW, Nader SH, Register TC, and Nader MA (2016) Preclinical laboratory assessments of predictors of social rank in female cynomolgus monkeys. *Am J Primatol* **78**:402–417.
- Lé AD, Harding S, Juzytch W, Funk D, and Shaham Y (2005) Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. *Psychopharmacology (Berl)* **179**:366–373.
- Lee B, Tiefenbacher S, Platt DM, and Spealman RD (2004) Pharmacological blockade of alpha2-adrenoceptors induces reinstatement of cocaine-seeking behavior in squirrel monkeys. *Neuropsychopharmacology* **29**:686–693.
- Minor BG, Danysz W, Jonsson G, Mohammed AK, Post C, and Archer T (1989) Adaptive changes in alpha-2 adrenoceptor mediated responses: analgesia, hypothermia and hypoactivity. *Pharmacol Toxicol* **65**:143–151.
- Morgan D, Grant KA, Gage HD, Mach RH, Kaplan JR, Prioleau O, Nader SH, Buchheimer N, Ehrenkauser RL, and Nader MA (2002) Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat Neurosci* **5**:169–174.
- Nader MA, Czoty PW, Nader SH, and Morgan D (2012) Nonhuman primate models of social behavior and cocaine abuse. *Psychopharmacology (Berl)* **224**:57–67.
- Papanicolaou J, Summers RJ, Vajda FJ, and Louis WJ (1982) The relationship between alpha 2-adrenoceptor selectivity and anticonvulsant effect in a series of clonidine-like drugs. *Brain Res* **241**:393–397.
- Perkins FN and Freeman KB (2018) Pharmacotherapies for decreasing maladaptive choice in drug addiction: targeting the behavior and the drug. *Pharmacol Biochem Behav* **164**:40–49.
- Quan N, Xin L, Ungar AL, and Blatteis CM (1992) Preoptic norepinephrine-induced hypothermia is mediated by alpha 2-adrenoceptors. *Am J Physiol* **262**:R407–R411.
- Reith ME, Li MY, and Yan QS (1997) Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. *Psychopharmacology (Berl)* **134**:309–317.
- Risner M and Jones BE (1976) Role of noradrenergic and dopaminergic processes in amphetamine self-administration. *Pharmacol Biochem Behav* **5**:477–482.
- Shepard JD, Bossert JM, Liu SY, and Shaham Y (2004) The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biol Psychiatry* **55**:1082–1089.
- Smith RJ and Aston-Jones G (2011) α (2) Adrenergic and imidazoline receptor agonists prevent cue-induced cocaine seeking. *Biol Psychiatry* **70**:712–719.
- Spealman RD (1995) Noradrenergic involvement in the discriminative stimulus effects of cocaine in squirrel monkeys. *J Pharmacol Exp Ther* **275**:53–62.
- Summers RJ, Jarrott B, and Louis WJ (1980a) Selectivity of a series of clonidine-like drugs for α 1 and α 2 adrenoceptors in rat brain. *Neurosci Lett* **20**:347–350.
- Summers RJ, Jarrott B, and Louis WJ (1980b) Displacement of [3H]clonidine binding by clonidine analogues in membranes from rat cerebral cortex. *Eur J Pharmacol* **66**:233–241.
- Thomsen M, Barrett AC, Negus SS, and Caine SB (2013) Cocaine versus food choice procedure in rats: environmental manipulations and effects of amphetamine. *J Exp Anal Behav* **99**:211–233.
- Uhlén S and Wikberg JE (1991) Delineation of rat kidney alpha 2A- and alpha 2B-adrenoceptors with [3H]RX821002 radioligand binding: computer modelling reveals that guanfacine is an alpha 2A-selective compound. *Eur J Pharmacol* **202**:235–243.
- Van der Laan JW, Van Veenendaal W, Voorthuis P, Weick G, and Hillen FC (1985) The effects of centrally acting adrenergic agonists on temperature and on explorative and motor behaviour. Relation with effects on quasi-morphine withdrawal behaviour. *Eur J Pharmacol* **107**:367–373.
- Wee S, Mandyam CD, Lecic DM, and Koob GF (2008) Alpha 1-noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. *Eur Neuropsychopharmacol* **18**:303–311.
- Wee S, Wang Z, He R, Zhou J, Kozikowski AP, and Woolverton WL (2006) Role of the increased noradrenergic neurotransmission in drug self-administration. *Drug Alcohol Depend* **82**:151–157.
- Wee S and Woolverton WL (2004) Evaluation of the reinforcing effects of atomoxetine in monkeys: comparison to methylphenidate and desipramine. *Drug Alcohol Depend* **75**:271–276.
- Weerts EM and Griffiths RR (1999) Evaluation of the intravenous reinforcing effects of clonidine in baboons. *Drug Alcohol Depend* **53**:207–214.
- Wood SK and Valentino RJ (2017) The brain norepinephrine system, stress and cardiovascular vulnerability. *Neurosci Biobehav Rev* **74** (Pt B):393–400.
- Woolverton WL (1987) Evaluation of the role of norepinephrine in the reinforcing effects of psychomotor stimulants in rhesus monkeys. *Pharmacol Biochem Behav* **26**:835–839.
- Woolverton WL, Wessinger WD, and Balster RL (1982) Reinforcing properties of clonidine in rhesus monkeys. *Psychopharmacology (Berl)* **77**:17–23.

Address correspondence to: Paul W. Czoty, Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Blvd., 546 NRC, Winston-Salem, NC 27157-1083. E-mail: pczoty@wakehealth.edu

SUPPLEMENTAL FIGURES

**Effects of the Alpha-2 Adrenergic Receptor Agonists Lofexidine and Guanfacine on
Food-Cocaine Choice in Socially Housed Cynomolgus Monkeys**

Paul W. Czoty and Michael A. Nader

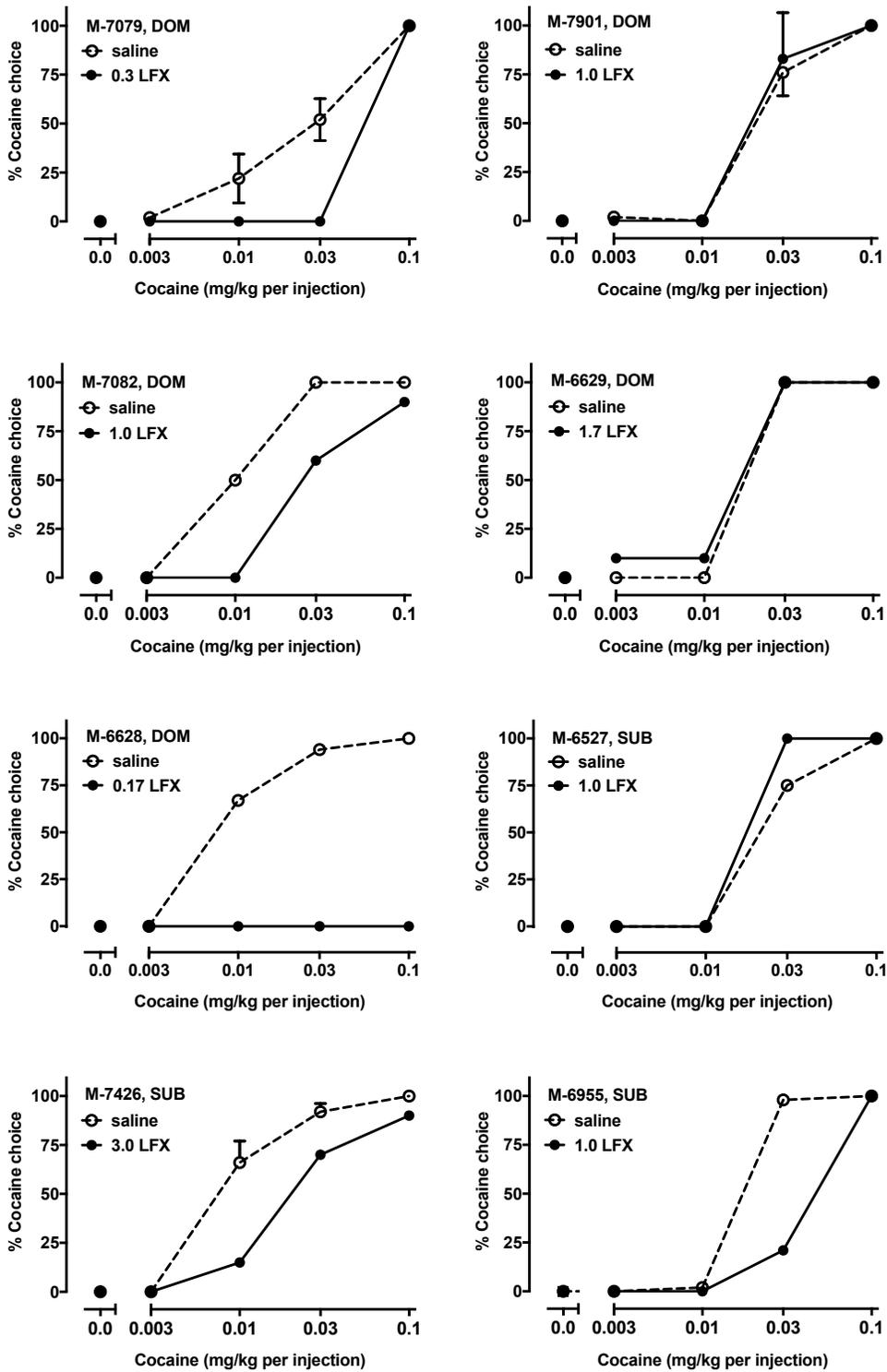
Journal of Pharmacology and Experimental Therapeutics

Supplemental Figure Legends

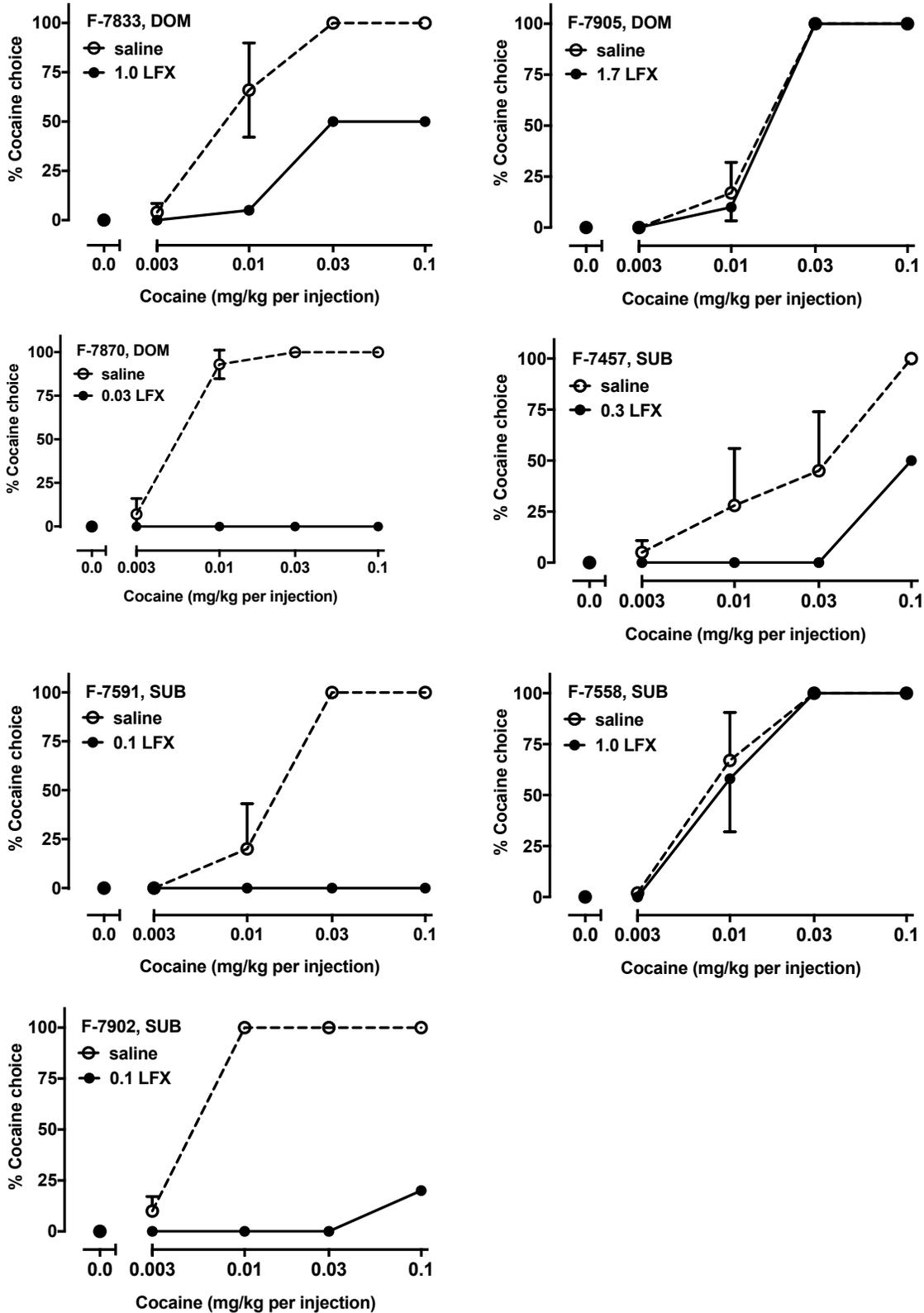
Supplemental Figure S1. Effects of the “best dose” of lofexidine (LFX) on food and cocaine self-administration under the choice procedure in individual male monkeys. Each monkey’s social rank is indicated (DOM, SUB). Abscissae: cocaine dose available as the alternative to a food pellet. Points indicate mean \pm SEM. Ordinates: cocaine choice.

Supplemental Figure S2. Effects of the “best dose” of lofexidine (LFX) on food and cocaine self-administration under the choice procedure in individual female monkeys. Each monkey’s social rank is indicated (DOM, SUB). Abscissae: cocaine dose available as the alternative to a food pellet. Points indicate mean \pm SEM. Ordinates: cocaine choice.

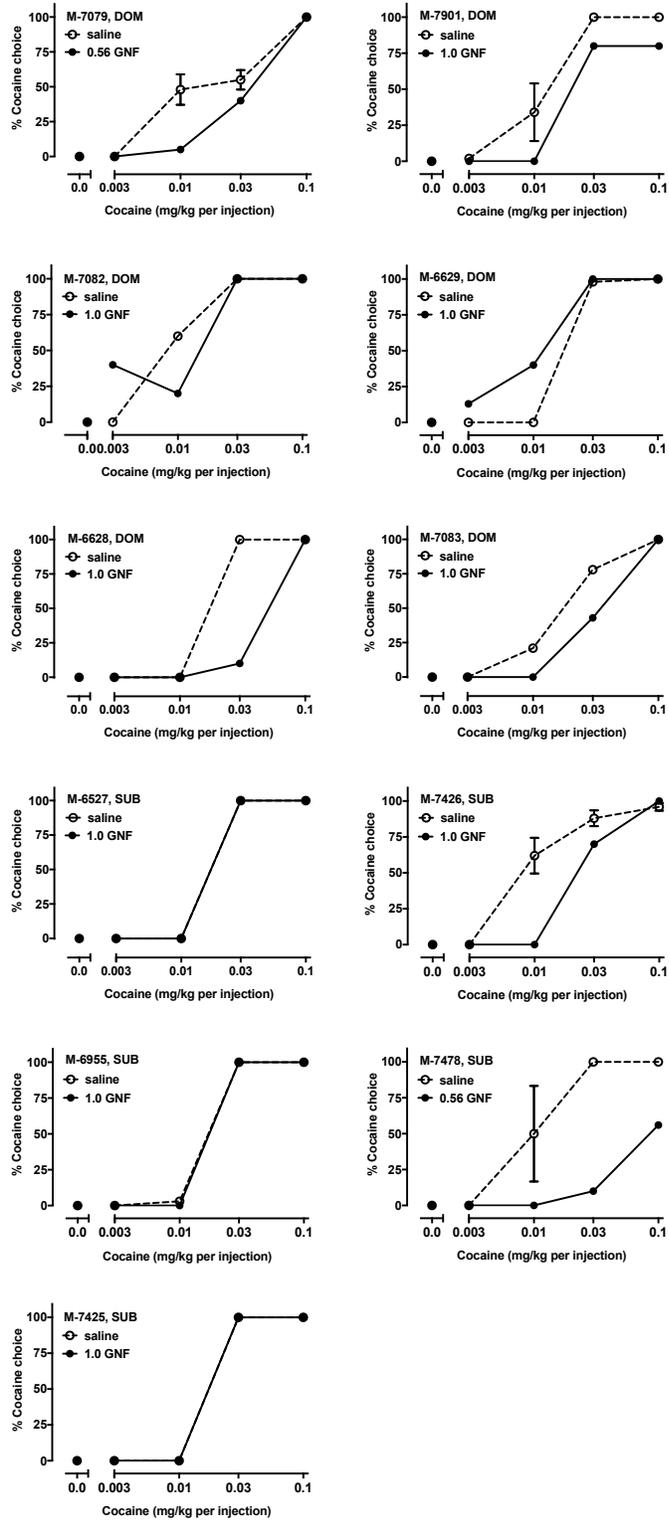
Supplemental Figure S3. Effects of the “best dose” of guanfacine (GNF) on food and cocaine self-administration under the choice procedure in individual male monkeys. Each monkey’s social rank is indicated (DOM, SUB). Abscissae: cocaine dose available as the alternative to a food pellet. Points indicate mean \pm SEM. Ordinates: cocaine choice.



SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 3

