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Effects of buspirone and the dopamine D₃ receptor compound PG619 on cocaine and methamphetamine self-administration in rhesus monkeys using a food-drug choice paradigm

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Abstract

Rationale—The dopamine (DA) D₂ and D₃ receptors have been associated with cocaine abuse. A recent study with the D₃ receptor (D₃R) partial agonist PG619 found that it attenuated cocaine-induced reinstatement and the D₂-like receptor antagonist buspirone has shown positive outcomes in two studies of cocaine abuse in monkeys. However, a recent clinical trial indicated that buspirone did not improve abstinence in treatment-seeking cocaine abusers.

Objective—The objective of the study was to examine PG619 and buspirone under a food-drug choice paradigm in order to better model the clinical findings. In addition, we extended the characterization of both compounds to include methamphetamine (MA) self-administration (SA).

Methods—Six adult male rhesus monkeys were trained to respond under a concurrent food (1.0-g pellets) and drug (0.01–0.3 mg/kg/injection cocaine or MA) choice paradigm in which complete SA dose-response curves were determined each session ($N=3$ /group). Monkeys received 5 days of treatment with either PG619 (0.1–3.0 mg/kg, i.v.) or buspirone (0.01–1.0 mg/kg, i.m.). In a

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Author contributions W.S.J. and M.A.N. designed the experiments. W.S.J. performed the behavioral studies, and analyzed the data. A.K.B synthesized PG619. The manuscript was written by W.S.J., A.H.N., and M.A.N.

follow-up study, the SA doses were reduced (0.003–0.1 mg/kg/injection) to increase reinforcement frequency and buspirone was retested.

Results—PG619 did not affect cocaine or MA choice, while buspirone increased low-dose cocaine choice. Changing the SA doses increased the number of reinforcers received each session, but buspirone did not decrease drug choice.

Conclusions—Consistent with clinical findings, these results do not support the use of buspirone for psychostimulant abuse and suggest that food-drug choice paradigms may have greater predictive validity than the use of other schedules of reinforcement.

Keywords

Cocaine; Methamphetamine; Dopamine D₃ receptors; Self-administration; Monkeys

Addiction continues to be one of the most harmful and costly public health problems in the world with approximately 1.6 million people addicted to psychostimulants in the USA (SAMHSA 2013). Despite decades of research, no pharmacotherapy for cocaine or methamphetamine (MA) dependence has been successful in clinical settings, underscoring the challenge in this endeavor (Haile and Kosten 2013). The majority of pharmacotherapy development for stimulant abuse has focused on the mesolimbic dopamine (DA) system considering its prominent role in mediating reinforcement-related behaviors and most drugs of abuse (see Russo and Nestler 2013). Within the DA system, the DA D₃ receptor (D₃R), a member of the D₂-like DA receptor family, has emerged as a particularly compelling therapeutic target (Heidbreder and Newman 2010; Newman et al. 2012; Heidbreder 2013).

The initial work by Pilla et al. (1999) found that the D₃R partial agonist BP897 reduced cocaine seeking under a second-order schedule in the first component of a multiple schedule, but did not block self-administration once the animal received a cocaine injection, suggesting that this D₃R partial agonist attenuated the effects of conditioned stimuli, rather than directly blocking the reinforcing effects of cocaine. Other studies using D₃R partial agonists have reported mixed results (Martelle et al. 2007; Achat-Mendes et al. 2009; Orio et al. 2010), leaving it unclear as to the potential efficacy of a selective D₃R partial agonist strategy. Our recent study using a 4-phenylpiperazine D₃R-selective partial agonist PG619, with ~100-fold higher D₃R selectivity over the D₂R (Grundt et al. 2007) did not decrease cocaine-maintained responding under a fixed-ratio (FR) schedule of reinforcement, but did attenuate cocaine-induced reinstatement (Blaylock et al. 2011). It is possible that the negative outcome of PG619 on cocaine self-administration (SA) was due to the schedule of reinforcement (see Newman et al. 2005; Heidbreder and Newman 2010). Thus, the present study extended the investigation of PG619 on cocaine-maintained behavior to include a more complex model of drug SA using a food-drug choice paradigm.

Buspirone (Buspar[®]) binds with moderately high affinity to the D₃R, although is not selective for this target (Bergman et al. 2013) and has shown recent promise in animal models of drug abuse. For example, using a multiple schedule of food and cocaine reinforcement, buspirone decreased cocaine SA in rhesus monkeys relative to food-reinforced responding (Bergman et al. 2013; Mello et al. 2013). Unfortunately, a recent

clinical trial study reported a lack of success in maintaining abstinence in treatment-seeking cocaine abusers receiving buspirone (Winhusen et al. 2014). In fact, one group treated with buspirone (women) showed increases in cocaine use during treatment compared to subjects receiving placebo (Winhusen et al. 2014). It has been argued that concurrent food-drug choice studies have stronger predictive validity than other schedules of drug self-administration (Haney and Spealman 2008; Banks and Negus 2012), partly because a main goal of treatment is not just to decrease drug-taking behavior, but to reallocate that behavior toward alternative, non-drug reinforcers (Heyman 2009; Banks and Negus 2012). To test the hypothesis that food-drug choice conditions would have better predictive outcome than simple schedules of cocaine SA, the present study utilized a food-cocaine choice paradigm in rhesus monkeys and examined the effects of 5-day treatment with buspirone. Following evaluation of 5 days of buspirone treatment, the parameters were changed in an effort to increase the number of drug injections received in each session and then the monkeys were retested with buspirone.

In addition to assessing the effects of these compounds on cocaine SA, this study included a separate group of monkeys that self-administered methamphetamine (MA), an indirect DA agonist that shares discriminative stimulus effects with cocaine (e.g., Sasaki et al. 1995; Munzar and Goldberg 2000), but with a different mechanism of action and time course (Raeteri et al. 1977; Bennett et al. 1993; Sonders et al. 1997; Jones et al. 1998). Compared to other psychostimulants like cocaine, there is less research involving MA abuse in monkey models. For some compounds, clinical studies have reported opposite effects on cocaine and MA (Kampman et al. 2004; Johnson et al. 2007; see Winchell et al. 2012) which further warrants the evaluation of potential psychostimulant treatment drugs in animals self-administering cocaine and MA (see also Martelle et al. 2014). Thus, an additional goal of these studies was to extend this evaluation of PG619 and buspirone from cocaine to MA.

Materials and methods

Subjects

Six adult male rhesus monkeys (*Macaca mulatta*) that weighed between 8 and 13 kg served as subjects. Three monkeys were trained to self-administer cocaine and three were trained to self-administer MA (see below). All monkeys were fitted with aluminum collars and trained to sit in a primate restraint chair (Primate Products, Redwood City, CA). Monkeys were individually housed in stainless-steel cages with visual and auditory contact with each other, ad libitum access to water in their home cage, and fed sufficient standard laboratory chow (Purina LabDiet 5045, St Louis, Missouri, USA) to maintain body weights at approximately 98 % of free-feeding weights. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan. Experimental procedures as well as animal housing and handling 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.

Surgery

All monkeys were surgically prepared with a chronic indwelling intravenous catheter and subcutaneous vascular access port (VAP; Access Technologies, Skokie, IL) under aseptic conditions. An antibiotic (30 mg/kg Kefzol, i.m.; cefazolin sodium; Marsam Pharmaceuticals, Inc., Cherry Hill, NJ) was administered 1 h prior to surgery. Monkeys were initially anesthetized with ketamine (15 mg/kg, i.m.) and maintained with ketamine supplements. A catheter was inserted into a major vein (femoral or internal or external jugular) to the level of the posterior vena cava. The distal end of the catheter was passed subcutaneously to an incision made slightly off the midline of the back and attached to a VAP, which was placed in a pocket formed by blunt dissection. After surgery, an analgesic dose of Metacam (meloxicam, 0.1 mg/kg, i.m.) was administered SID for 3 days.

Apparatus

All behavioral studies were conducted in ventilated, sound-attenuating chambers (1.5×0.74×0.76 m; Med Associates, St. Albans, VT). Each chamber was equipped with an operant conditioning panel that contained two photo-optic switches (Model 117–1007; Stewart Ergonomics, Inc., Furlong, PA) located on each side of the panel with a horizontal row of three stimulus lights positioned 14 cm above each switch. The switches were positioned to be within easy reach of the monkey seated in the primate chair. A food receptacle, above which was a red stimulus light, was located between the photo-optic switches and connected with a Tygon tube to a pellet dispenser (Med Associates) located on the top of the chamber for delivery of 1-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ). A peristaltic infusion pump (Cole-Parmer Instrument Co., Niles, IL), located on top of the chamber, was used for delivering drugs at a rate of approximately 1.5 ml/10 s.

Before each session, the area on the back of the animal containing the vascular access port was cleaned with chlorhexidine and isopropyl alcohol swabs (Prevantics™, Orangeburg, NY). A 22-gauge Huber Point Needle (Access Technologies) was inserted into the port, which connected the venous catheter to the infusion pump. The pump was operated for approximately 3 s to fill the port with the concentration of drug available during the experimental session. Pretreatment drug administration was given outside the chamber at various pretreatment times (see below) and the monkey was placed into the chamber. After each session, the catheters were flushed with heparinized saline (100 U/ml) to prevent clotting.

Food-drug choice

Initially, monkeys were trained to respond in the presence of a green stimulus light by placing their finger in a photo-optic switch in which each response (FR 1) resulted in the delivery of a 1-g banana-flavored pellet. It was necessary for the monkey to completely withdraw his finger from the opening of the photo-optic switch before another response could be recorded. Over several sessions, the FR contingency was increased to 30; responding alternated from right to left switch. Next, catheters were implanted and the contingency was changed to a concurrent FR 30 schedule of food and cocaine or MA (0.03 mg/kg per injection) availability ($N=3$ /group). Food availability was signaled by

illumination of a green light above the appropriate switch while this dose of cocaine or MA was signaled by illumination of a white light above the switch and the houselight (component 3; Table 1). When choice was stable, a multiple concurrent schedule was initiated so that each daily session consisted of five components in which monkeys chose between food pellets and ascending unit doses of cocaine or MA (i.e., no injection, 0.01, 0.03, 0.1, and 0.3 mg/kg per injection) by using different discriminative stimuli (see Table 1). The drug dose was varied by manipulating the pump duration and consequently the volume delivered. Thus, a complete dose-effect curve was determined in each monkey each session, typically 5 days per week. Assignment of the food or drug-associated switch was counterbalanced across monkeys. Components were separated by a 5-min timeout (TO) period. Each component ended when 10 total reinforcers had been earned (food and drug injections) or 20 min had elapsed, whichever came first. If a response was emitted on the alternate switch before an FR was completed, the response requirement on the first switch was reset. Delivery of a drug reinforcer was accompanied by illumination of the red light in the center of the response panel, above the food receptacle, during an injection. Following the delivery of each reinforcer (food or drug), there was a 30-s TO period during which all lights remained off and responding had no scheduled consequences.

Ratio requirements for food and drug were adjusted for each monkey such that allocation of responding to the drug switch increased over the session as the available dose of cocaine/MA increased. Responding was considered stable when 20 % of reinforcers were earned on the drug switch when the alternative to food was no injection (component 1) and 0.01 mg/kg per injection cocaine/MA (component 2) and 80 % of reinforcers were earned on the drug switch when the alternative to food was 0.1 mg/kg per injection cocaine/MA (component 4) and 0.3 mg/kg per injection cocaine/MA (component 5). This criterion had to be met for three consecutive days before a drug treatment was administered.

Once responding was stable, drug treatments were begun using a 5-day treatment regimen in which the same dose of a compound was administered before five consecutive daily self-administration sessions. PG619 (0.1–3.0 mg/kg, i.v.) was administered 10 min before the self-administration session ($N=3$ /group). Due to solubility issues, the 3.0 mg/kg dose was the highest dose tested and was in the range of previous studies showing behavioral effects in monkeys (Blaylock et al. 2011). Buspirone (0.01–1.0 mg/kg, i.m.) was administered 15 min before the self-administration session ($N=3$ /group); doses and pretreatment time were based on published data in monkeys (Bergman et al. 2013; Mello et al. 2013).

Parametric manipulations

After completing the above studies, the conditions were changed because monkeys only received 1–2 injections, on average, of the high dose of cocaine and MA and it was possible that the efficacy of the drug pretreatment would be improved if more behavior could be maintained later in the session. For these studies, the cocaine and MA dose available for self-administration in components 2–5 was decreased by one-half log units to 0.003, 0.01, 0.03, and 0.1 mg/kg/injection, respectively. Ratio requirements were adjusted for each monkey under the new parameters such that choice behavior met the same criterion as before in which 20 % of reinforcers were earned on the drug switch during components 1

and 2 (when the alternative to food was no injection and 0.003 mg/kg/injection, respectively) and 80 % of reinforcers were earned on the drug switch during components 4 and 5 (when the alternative to food was 0.03 and 0.1 mg/kg/injection, respectively). Monkeys self-administered for a mean of 24 (± 4.07) sessions under the new parameters until the stability criterion was reached. When stable, buspirone was retested as described above.

Data analysis

The primary dependent variable was percent drug choice defined as (number of FRs completed on the drug-associated switch $\div$ total number of FRs completed) $\times$ 100. These variables were plotted as a function of cocaine or MA dose and the last 3 days of each experimental condition were averaged. Group mean drug choice dose-effect curves were analyzed using two-way repeated-measures ANOVA with cocaine/MA dose and treatment as the main factors. Occasionally, monkeys did not earn any food reinforcers in the first component under treatment conditions. For these cases, choice dose-effect curves were analyzed using two-way repeated-measures ANOVA in which component 1 was excluded. A significant ANOVA was followed by Bonferroni multiple comparisons post hoc tests to compare test conditions with baseline conditions. ED₅₀ values were determined using the linear portion of the cocaine and MA choice dose-effect curve that crossed 50 % choice. Paired *t* tests were used to compare average ED₅₀ values during the last three baseline days before treatment and during the last 3 days of each 5-day treatment period. Treatment-condition log ED₅₀ values were subtracted from baseline conditions to generate the magnitude of change in ED₅₀ shift of the drug choice dose-response curve. In some cases, ED₅₀ values could not be determined because cocaine or MA choice was greater than 50 % for all doses that were available during the session; these cases are indicated in Table 2 for individual monkeys. Additional dependent variables collected during each session included total reinforcers per component and drug intake per session. Values were compared by two- and one-way repeated-measures ANOVA between baseline and drug conditions. The criterion for significance was set a priori at the 95 % level of confidence ($p < 0.05$).

Drugs

(–)Cocaine HCl and (+)methamphetamine were supplied by the National Institute on Drug Abuse (Bethesda, MD). PG619 was synthesized as described in Grundt et al. (2007) in the Medicinal Chemistry Section, National Institute on Drug Abuse-Intramural Research Program (Baltimore, MD); buspirone HCl was obtained from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in sterile 0.9 % saline. Heat and sonication were used for solubility purposes and all drug solutions used for intravenous administration were passed through a sterile 0.2- μ m filter (Milipore Inc).

Results

Effects of PG619 on cocaine and MA choice

Baseline cocaine choice was characterized by monkeys exclusively choosing food when the alternative was no injection or a low dose of cocaine (0.01 mg/kg) and exclusively choosing cocaine when higher doses (0.03–0.3 mg/kg) were available (Fig. 1, top left panel, filled symbols). At the two highest cocaine doses, fewer than 10 total choices were made per

component (Fig. 1, top middle panel, filled symbols); this resulted in the less than maximal number of trials completed each session (approximately 41 out of 50; data not shown). On average, similar numbers of total trials resulted in food reinforcement and cocaine reinforcement, with an average session intake of approximately 2.2 mg/kg cocaine (Fig. 1, top right panel, filled symbol). Administration of PG619, up to 3.0 mg/kg (i.v.), for five consecutive sessions did not significantly affect any measure of cocaine-food choice, including cocaine intake (Fig. 1, top row; open symbols). There was a slight leftward shift in the cocaine choice curve in two (R-1688 and R-1692) of three monkeys although the magnitude of change was not large enough to reveal a significant effect of PG619 on ED₅₀ values for cocaine choice (Table 2).

Similar to cocaine self-administration, MA choice was characterized by monkeys exclusively choosing food when the alternative was no injection or a low dose of MA (0.01 mg/kg) and exclusively choosing MA when higher doses (0.1–0.3 mg/kg) were available (Fig. 1, lower left panel, filled symbols), although the total number of trials completed at the higher doses were substantially lower than at high cocaine doses (Fig. 1, lower middle panel, filled symbols). As a result, average session intake of approximately 1.0 mg/kg MA (Fig. 1, lower right panel, filled symbol) was also lower than total session cocaine intake. Administration of PG619, up to 3.0 mg/kg (i.v.), for five consecutive sessions did not significantly affect any measure of MA-food choice, although there was a trend ($p=0.058$) toward increased MA intake during PG619 treatment (Fig. 1, lower right panel, open symbol). While there were no significant effects of PG619 on mean ED₅₀ values for MA choice, there were more individual differences compared to the cocaine group. Two (R-1690 and R-1693) of three monkeys demonstrated a leftward shift in the MA choice curve and the third monkey demonstrated a slight rightward shift (Table 2).

Effects of buspirone on cocaine and MA choice

Five-day treatment with buspirone produced a leftward shift in the cocaine choice dose-effect curve for all subjects in which the choice of the lowest dose of cocaine was increased (Fig. 2, top panels, open symbols). In two (R-1688 and R-1689) of three monkeys, both 0.1 and 0.3 mg/kg buspirone potentiated low-dose cocaine choice. In the third monkey (R-1692), 0.1 mg/kg buspirone also potentiated low-dose cocaine choice; however, the highest buspirone dose (0.3 mg/kg) produced a one-half log unit rightward shift in the cocaine choice dose-effect curve (Fig. 2, top panels). The increases in cocaine choice at the low doses were not due to buspirone-induced reductions in trials completed in the early components (Fig. 2, lower panels). Two-way repeated-measures ANOVA on mean percent cocaine choice (Fig. 3, top left panel) demonstrated a significant main effect of cocaine dose [$F(3,6)=24.24$, $p<0.001$] and a significant cocaine dose and buspirone treatment interaction [$F(9,18)=6.01$, $p<0.001$]. The post hoc analysis revealed that both 0.1 and 0.3 mg/kg significantly increased choice of 0.01 mg/kg cocaine (Fig. 3, top left, $p<0.0001$ and $p<0.001$, respectively). For the number of reinforcers earned per component (Fig. 3, top middle panel), there was a significant main effect of cocaine dose [$F(4,8)=5.31$, $p<0.05$], buspirone treatment [$F(3,6)=7.68$, $p<0.05$], and a significant cocaine dose and buspirone treatment interaction [$F(12,24)=3.49$, $p<0.01$]. The post hoc analysis revealed that 0.1 and 0.3 mg/kg buspirone treatment significantly decreased the number of reinforcers earned in the first

component when only food was available; however, this initial decrease recovered to baseline levels by the end of the session. Total session intake was not affected by buspirone treatment (Fig. 3, top right panel) considering the increase in cocaine reinforcers occurred when the lowest cocaine dose was available.

Five-day treatment with buspirone resulted in increases in low-dose MA choice in two of three monkeys (Fig. 4, top panels). In contrast to what was observed on cocaine-food choice, buspirone decreased total trials completed, especially early in the session for all three monkeys (Fig. 4, lower panels). Two-way repeated-measures ANOVA on mean percent MA choice (Fig. 3, lower left panel) demonstrated a significant main effect of MA dose [$F(3,6)=12.20$, $p<0.001$] but no significant main effect of treatment or MA dose and buspirone treatment interaction. Notably, the potentiation of MA choice that occurred in R-1690 and R-1691 was the result of far fewer MA choices than the number of choices that produced potentiation of cocaine choice. The effects of buspirone on total reinforcers earned per component (Fig. 3, lower middle panel) were qualitatively similar to the cocaine self-administration group. Two-way repeated-measures ANOVA demonstrated a significant main effect of dose [$F(4,8)=18.12$, $p<0.0001$], buspirone treatment [$F(2,4)=15.65$, $p<0.05$], and a significant interaction [$F(8,16)=7.73$, $p<0.001$]. The post hoc analysis revealed that 0.1 mg/kg buspirone treatment significantly decreased the number of reinforcers earned in the first component when only food was available as well as in components 2 and 3 when 0.01 and 0.03 mg/kg MA, respectively, was the alternative to food (Fig. 3, lower middle panel). Buspirone treatment did not significantly alter overall session MA intake (Fig. 3, lower right panel).

Parametric changes to baseline choice: effects of buspirone

The effects of buspirone were re-evaluated under conditions in which more drug reinforcers were earned later in the session by changing the unit doses of cocaine and MA. Under the new parameters, there was a significant increase in the number of cocaine reinforcers earned at the highest dose available (component 5) when compared to the initial parameters ($t=4.12$, $p<0.05$); for MA there were significantly higher choices in components 4 ($t=7.83$, $p<0.001$) and 5 ($t=4.21$, $p<0.05$) when compared to the initial parameters (compare middle panels of Figs. 3 and 5). Although the parametric changes resulted in more behavior, no dose of buspirone significantly altered cocaine choice (Fig. 5, top left panel) or cocaine ED₅₀ values (Table 2). One monkey (R-1688) demonstrated an approximately one-half log unit rightward shift in the cocaine choice dose-effect curve following 0.03 and 0.3 mg/kg buspirone treatment under the new parameters (Table 2). Notably, these same doses either did not alter or potentiated cocaine choice when tested under the initial parameters. For MA self-administration, no dose of buspirone significantly altered MA choice following the parametric changes (Fig. 5, lower left panel) or MA ED₅₀ values (Table 2). Qualitatively, the disruptive effects of buspirone on total reinforcers earned per component were similar for cocaine and MA self-administration and similar to the effects observed when evaluated under the initial parameters (Figs. 3 and 5, middle panels). Total session intake of cocaine and MA were not significantly altered by buspirone treatment under the new parameters (Fig. 5, right panels).

Discussion

The present study extended the characterization of the D₃R partial agonist PG619 and the D₂-like receptor antagonist buspirone on cocaine SA in rhesus monkeys to the study of cocaine-food choice and to also include MA SA. PG619 doses higher than those used to attenuate cocaine-induced reinstatement (Blaylock et al. 2011) had no effect on cocaine or MA choice. Five-day treatment with buspirone increased the reinforcing effects of cocaine in which choice of lowest cocaine dose was increased. Five-day buspirone treatment also increased the choice of low MA doses although this effect did not reach statistical significance. When buspirone was retested in both groups after parametric manipulations in which the unit doses of cocaine and MA were decreased in order to maintain more drug choice behavior, no buspirone doses significantly affected percent cocaine or MA choice, although buspirone significantly decreased the number of trials completed.

One objective of the present study was to directly compare the potency and effectiveness of the low efficacy and D₃R selective agonist PG619 on cocaine and MA SA. Considering the reinforcing and cocaine-like discriminative stimulus effects of D₃R agonists (e.g., Sinnott et al. 1999; Koffarnus et al. 2012; Baladi et al. 2014), the use of low-efficacy agonists or D₃R antagonists might be the more desirable treatment strategy. D₃R partial agonists have lower intrinsic efficacy compared to full agonists and should therefore possess a lower abuse liability. In theory, a compound with low intrinsic activity would maintain a low level of DA receptor activation in order to compensate for hypodopaminergic states during withdrawal and would also function as an antagonist during hyperdopaminergic states when in the presence of cocaine or MA (Pulvirenti and Koob 1994). Thus, D₃R partial agonists could normalize dysfunctional DA neurotransmission during the various phases of psychostimulant addiction to possibly reduce craving without having abuse liability on its own.

In an earlier study (Blaylock et al. 2011), PG619 did not significantly decrease cocaine SA in monkeys responding under a fixed-ratio schedule of reinforcement, but did attenuate cocaine-induced reinstatement in the same monkeys. The present study extended this characterization to a food-drug choice paradigm and to also examine MA SA. Doses of PG619 greater than that used to attenuate reinstatement did not significantly affect cocaine or MA choice. These results are similar to a previous study in which 5-day treatment with the low-efficacy D₂/D₃ agonist, aripiprazole, during daily cocaine SA, did not decrease cocaine choice (Czoty and Nader 2013). Of note, aripiprazole has also failed in clinical trials studies with active cocaine (Haney et al. 2011) and MA abusers (Coffin et al. 2013), suggesting that the food-drug choice paradigm has strong predictive validity. The lack of effect seen with PG619 in the present study and aripiprazole in the previous study may be a result of stimulant-induced high extracellular DA levels perhaps resulting in the partial agonist being displaced from the receptor or to function more as an antagonist whereby compensatory increases in SA would occur. Czoty and Nader (2013) evaluated this hypothesis by testing 5-day aripiprazole treatment in which cocaine SA was not conducted on days 2–4 and found that aripiprazole decreased cocaine choice on day 5. It may be that chronic treatment with PG619 would be effective if cocaine or MA SA were suspended during treatment. While the present findings with PG619 are negative, other studies suggest

that chronic cocaine or MA use results in increases in D₃R availability (Boileau et al. 2012; Payer et al. 2013). The fact that the efficacy of D₃R partial agonists and antagonists are influenced by schedule conditions and stimulus events (i.e., discriminative, conditioned, reinforcing) suggests additional research is required to better determine the conditions, as well as whether the use of combination treatments that, for example, decrease DA tone to enhance blockade of D₃R would be clinically effective in treating stimulant abusers.

A second goal of the present study was to examine buspirone, an FDA-approved medication for the treatment of anxiety that was initially characterized as a serotonin (5-HT)_{1A} receptor partial agonist and a D₂-like receptor antagonist (McMillen et al. 1983; Skolnick et al. 1984). Recent radioligand binding and β -arrestin functional activity studies indicate that buspirone has ~5-fold higher affinity/potency for the D₃ and D₄ subtypes compared to the D₂ subtype (Bergman et al. 2013). Two recent preclinical studies in rhesus monkeys reported positive results on cocaine SA. Bergman et al. (2013) found that acute i.m. buspirone decreased cocaine SA to a greater degree than food-maintained responding under a multiple FR schedule of reinforcement, while Mello et al. (2013) extended this to chronic buspirone treatment (0.32 or 0.56 mg/kg/h, i.v. every 20 min, 23 h/day for 7–10 consecutive days) and to cocaine and food SA under a second-order schedule of reinforcement. As was observed by Bergman et al. (2013), Mello et al. (2013) also noted decreases in food-maintained responding, but the effects on cocaine SA were significantly greater, again, providing support for buspirone as a candidate treatment option for cocaine abuse.

In contrast to the results obtained by Bergman et al. (2013) and Mello et al. (2013), the present study found that buspirone treatment increased cocaine SA in rhesus monkeys responding under a concurrent food vs. drug choice procedure. Buspirone was ineffective in altering cocaine choice at high doses (0.03–0.3 mg/kg), but significantly increased cocaine choice at the lowest dose (0.01 mg/kg). The increases in cocaine choice occurred under conditions in which monkeys did not choose that dose (0.01 mg/kg) relative to food under baseline conditions. Perhaps consistent with this finding, Nader and Barrett (1990) reported increases in cocaine SA under a second-order schedule of reinforcement in which monkeys only received one cocaine injection per session, which occurred at the end of the session. In the present study, buspirone also produced an increase in low-dose MA choice in two of three monkeys. In addition, buspirone appeared to produce greater disruptions in responding in MA monkeys compared to monkeys self-administering cocaine. The mechanism of action for the increases in drug choice could be through D₂/D₃ receptor antagonism, as reported earlier by Woolverton and Balster (1981), Negus (2003) and Czoty and Nader (2013). Another possibility is via buspirone's agonist actions at 5-HT_{1A} receptors. Supporting this hypothesis are results from a study using the 5-HT_{1A} agonist 8-OH-DPAT, which increased the reinforcing strength of a low cocaine dose (0.003 mg/kg) that was not preferred over food under baseline conditions (Czoty et al. 2005). Similarly, 8-OH-DPAT and gepirone, an analog of buspirone that does not have D₂/D₃ receptor antagonist effects, increased cocaine-maintained responding in squirrel monkeys responding under a second-order schedule (Nader and Barrett 1990). Increases in SA may have also occurred to counteract the disruption of operant behavior induced by buspirone. Thus, cocaine and MA may have been functioning as a mutual antagonist to buspirone in order to reverse the rate-decreasing

effects evident from the reduction in food-maintained responding during the first component during treatment.

In the present study, no buspirone doses, up to doses that disrupted overall responding, decreased high-dose cocaine choice, a finding inconsistent with other reports in rhesus monkeys (Bergman et al. 2013; Mello et al. 2013). One possible reason for the disparity in results is the use of different schedules of reinforcement. The present study utilized a multiple concurrent food-cocaine schedule of reinforcement while Bergman et al. (2013) used a multiple FR schedule and Mello et al. (2013) used a multiple second-order schedule of reinforcement. This is unlikely the primary reason because in contrast to other studies with D₃R compounds showing that SA under second-order schedules of reinforcement is more sensitive to drug pretreatments, Bergman et al. (2013) reported similar findings to Mello et al. (2013). A more likely explanation is the amount of SA that was maintained under baseline conditions. The highest self-administered dose in the Bergman et al. (2013) and Mello et al. (2013) studies was 0.1 mg/kg/injection and monkeys self-administered approximately 25 and 60 injections over the course of the day, respectively. In the present study, at the two highest cocaine doses, the monkeys self-administered approximately eight injections when 0.1 mg/kg was available and five injections when 0.3 mg/kg was available. The reductions reported by Bergman et al. (2013) and Mello et al. (2013) still resulted in SA of approximately 10 and 40 injections of 0.1 mg/kg cocaine. These reductions in cocaine SA resulted in greater intakes than reported in this study under baseline (i.e., non-buspirone) conditions.

To test the hypothesis that the reinforcement frequency for cocaine was too low to observe buspirone-dependent reductions in cocaine-reinforced responding under the concurrent schedule, the dose available in each component was reduced by one-half log units. Retraining the monkeys resulted in significant increases in the number of injections delivered in the last component. However, in contrast to what was hypothesized, buspirone still did not decrease cocaine (or MA) choice relative to food. Notably, buspirone did not produce significant increases in low-dose drug choice under the new parameters in contrast to its effects when higher doses were available for SA. This may indicate that the lowest dose available under the new parameters (0.003 mg/kg) was not sufficient enough to counteract the rate-decreasing effects of buspirone. Under both concurrent conditions, buspirone continued to significantly disrupt total number of food choices. It is possible that behavior maintained under concurrent schedules are more sensitive to adverse side-effects of potential treatment drugs, which makes this model a valuable assay for pharmacotherapy assessment. A recent clinical trials study reported that buspirone was not successful in maintaining abstinence in treatment-seeking cocaine abusers and even increased cocaine use in female participants (Winhusen et al. 2014). The inability of buspirone to decrease cocaine or MA choice in the present study is consistent with the clinical trials outcome (Winhusen et al. 2014) and suggests that the food-drug choice paradigm has strong predictive validity (see Banks and Negus 2012).

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Fig. 1.

Effects of 5-day treatment with PG619 (3.0 mg/kg, i.v.) on cocaine (*top panels*; $n=3$) and MA (*lower panels*; $n=3$) choice in rhesus monkeys. *Left panel* ordines percent of reinforcers earned on drug-associated switch (*left*) and percent of reinforcers earned on food-associated switch (*right*), *Middle panel* ordines number of total reinforcers per component (maximum of 10 per component), *Abcissae* unit dose of cocaine or MA in milligrams per kilogram available as an alternative to a food pellet, *Right panel* ordines total cocaine (*top*) and MA (*bottom*) intake (mg/kg) per session. Data represent mean $\pm$ S.E.M. of three monkeys from the last three sessions of each 5-day treatment under baseline conditions (*closed symbols*) and following PG619 treatment (*open symbols*)

Fig. 2.

Individual-subject data for the effects of 5-day buspirone (0.03–0.3 mg/kg, i.m.) treatment on choice between doses of cocaine and food presentation. *Top row ordinates* percent of reinforcers earned on cocaine-associated switch (*left*) and percent of reinforcers earned on food-associated switch (*right*), *Lower row ordinates* number of total reinforcers per component (maximum of 10 per component), *Abscissae* unit dose of cocaine (mg/kg) available as an alternative to a food pellet. Data represent mean±S.D. in individual subjects from the last three sessions of each 5-day treatment under baseline conditions (*filled symbols*) and following buspirone treatment (*open symbols*)

Fig. 3.

Effects of 5-day treatment with buspirone (0.03–0.3 mg/kg, i.m.) on cocaine (*top panels*; $n=3$) and MA (*lower panels*; $n=3$) choice in rhesus monkeys. $N=2$ for the effects of 0.3 mg/kg buspirone on MA self-administration (data not included in group analysis). All other details are as described in Fig. 1. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$

Fig. 4.

Individual-subject data for the effects of 5-day buspirone (0.03–0.3 mg/kg, i.m.) treatment on choice between doses of MA and food presentation. *Top row ordinates* percent of reinforcers earned on MA-associated switch (*left*) and percent of reinforcers earned on food-associated switch (*right*), *Lower row ordinates* number of total reinforcers per component (maximum of 10 per component), *Abscissae* unit dose of MA (mg/kg) available as an alternative to a food pellet. Data represent mean $\pm$ S.D. in individual subjects from the last three sessions of each 5-day treatment under baseline conditions (*filled symbols*) and following buspirone treatment (*open symbols*)

Fig. 5.

Effects of 5-day treatment with buspirone (0.03–0.3 mg/kg, i.m.) after parametric manipulations of dose in monkeys choosing between cocaine (*top panels*) or MA (*lower panels*) and food presentation ($n=3/\text{group}$). $N=2$ for the effects of 0.03 mg/kg buspirone on cocaine self-administration (data not included in group analysis). Baseline conditions are represented with *gray symbols*; all other details are as in Fig. 1. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$

Table 1

Experimental conditions

Component	Switch 1		Switch 2		Switch 2 (parametric study)			
	Stimulus	Light	Stimulus ^a	Light(s)	Pump (s)	Stimulus ^a	Light(s)	Pump (s)
1	Food	Green	–	None	0	–	None	0
2	Food	Green	0.01	White	1	0.003	White	1
3	Food	Green	0.03	White+house	3	0.01	White+house	3
4	Food	Green	0.1	White+red	10	0.03	White+red	10
5	Food	Green	0.3	White+house+orange+red	30	0.1	White+house+orange+red	30

^a Dose of cocaine or MA (mg/kg per injection)

ED₅₀ values (mg/kg) for cocaine and MA choice at baseline and during 5-day treatment with PG619 and buspirone

Table 2

	Cocaine group				MA group			
	R-1688	R-1689	R-1692	Mean (S.E.M.)	R-1690	R-1691	R-1693	Mean (S.E.M.)
Baseline	0.017	0.017	0.017	0.017 (0)	0.041	0.040	0.023	0.035 (0.006)
+ 3.0 PG 619	0.015	0.017	0.013	0.015 (0.001)	0.005	0.058	0.017	0.027 (0.016)
Shift	-0.06	0.00	-0.12	-0.06 (0.03)	-0.87	0.16	-0.12	-0.20 (0.34)
Baseline	0.017	0.017	0.017	0.017 (0)	0.041	0.049	0.017	0.036 (0.10)
+ 0.03 BSP	0.017	0.011	0.017	0.015 (0.002)	0.023	0.055	0.016	0.031 (0.012)
Shift	0	-0.182	0	-0.06 (0.06)	-0.246	0.050	-0.026	-0.07 (0.09)
+ 0.056 BSP	N.T.	N.T.	N.T.	-	0.049	N.T.	N.T.	-
Shift	-	-	-	-	0.082	-	-	-
+ 0.1 BSP	< 0.01	0.006	0.005	-	< 0.01	< 0.01	0.017	-
Shift	-	-0.460	-0.540	-	-	-	0.000	-
+ 0.3 BSP	< 0.01	0.005	0.055	-	N.T.	0.094	< 0.01	-
Shift	-	-0.540	0.502	-	-	0.283	-	-
Baseline ^a	0.005	0.007	0.005	0.006 (0.0007)	0.017	0.016	0.006	0.013 (0.004)
+ 0.03 BSP	0.016	N.T.	0.005	-	0.017	0.017	0.005	0.013 (0.004)
Shift	0.484	-	0	-	0	0.032	-0.061	-0.01 (0.03)
+ 0.1 BSP	0.002	0.005	0.005	0.004 (0.001)	0.007	0.013	0.004	0.008 (0.003)
Shift	-0.419	0	0	-0.14 (0.14)	-0.393	-0.084	-0.158	-0.02 (0.12)
+ 0.3 BSP	0.017	0.005	0.009	0.01 (0.004)	N.T.	N.T.	< 0.003	-
Shift	0.511	0	0.216	0.24 (0.15)	-	-	-	-
+ 1.0 BSP	N.T.	0.005	N.T.	-	N.T.	N.T.	N.T.	-
Shift	-	0	-	-	-	-	-	-

All data points represent ED50 values of the last 3 days of each 5-day experimental condition. Magnitude of change in ED50 shift is expressed in log units. Drug doses (left column) are shown in mg/kg

BSP buspirone, *N.T.* not tested, - not calculated

^a Shaded area represents choice of 0.003, 0.01, 0.03, and 0.1 mg/kg/injection