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Title Page:

Further Characterization of Quinpirole-Elicited Yawning As a Model of Dopamine D3 Receptor Activation in Male and Female Monkeys

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Running Title Page:

a) Running title: Quinpirole and DRD3 in Monkeys

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d) Nonstandard Abbreviations: Cb, cerebellum; Cd, caudate nucleus; DA, dopamine; DRD2, dopamine D_2 receptor; DRD3, dopamine D_3 receptor; GP, globus pallidus; HIP, hippocampus; Pt, putamen; SN, substantia nigra; Thal, thalamus; VP, ventral pallidum; VS, ventral striatum

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ABSTRACT

The dopamine (DA) D_3 receptor (DRD3) has been associated with impulsivity, pathological gambling and drug addiction making it a potential target for pharmacotherapy development. Positron emission tomography (PET) studies using the DRD3-preferring radioligand [^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin ([^{11}C]PHNO) have shown higher binding potentials in drug abusers compared to control subjects. Preclinical studies have examined DRD3 receptor activation using the DA agonist quinpirole and the unconditioned behavior of yawning. However, the relationship between quinpirole-elicited yawning and DRD3 receptor availability has not been determined. In Experiment 1, eight drug-naïve male rhesus monkeys were scanned with [^{11}C]PHNO and the ability of quinpirole (0.01-0.3 mg/kg, i.m.) to elicit yawning was examined. Significant positive (globus pallidus) and negative (caudate nucleus, putamen, ventral pallidum, and hippocampus) relationships between DRD3 receptor availability and quinpirole-induced yawns were noted. Experiment 2 replicated earlier findings that a history of cocaine self-administration (N=11) did not affect quinpirole-induced yawning and extended this to examine monkeys (N=3) with a history of methamphetamine (MA) self-administration and found that monkeys with experience self-administering MA showed greater potency and significantly higher quinpirole-elicited yawning compared to controls. Finally, quinpirole-elicited yawning was studied in drug-naïve female monkeys (N=6) and compared to drug-naïve male monkeys. Sex differences were noted, with quinpirole being more potent and eliciting significantly more yawns in males compared to females. Taken together these findings support the use of quinpirole-elicited yawning as a behavioral tool for examining DRD3 activation in monkeys and that both drug history and sex may influence individual sensitivity to the behavioral effects of DRD3 compounds.

INTRODUCTION

Psychostimulant drug use is a global problem. In 2011, the United Nations Office on Drugs and Crime estimated 14 to 21 million people worldwide used cocaine at least once in 2009, with 20% of users in the United States meeting the criteria of drug dependence (Degenhardt and Hall, 2012). Despite the array of negative health and societal consequences, a successful pharmacotherapy for psychostimulant addiction has remained elusive (Newman et al., 2005, 2012). There is evidence that dopamine (DA) D_3 receptors (DRD3), a subtype of the D2-like family of DA receptors, mediate many of the effects of psychostimulants associated with high abuse potential (Heidbreder and Newman, 2010; Heidbreder, 2012), including the role of conditioned stimuli (Achat-Mendes et al., 2010; Neisewander et al., 2004; Orio et al., 2010; Yan et al., 2013), discriminative stimulus effects (Achat-Mendes et al., 2010; Collins and Woods, 2009; Martelle et al., 2007), and cue conditioning (Le Foll et al., 2002). In addition, DRD3 partial agonists and antagonists have been shown to reduce self-administration of methamphetamine (MA) in a rodent model of compulsive drug intake (Orio et al., 2010). Postmortem studies indicate higher DRD3 densities in cocaine overdose victims compared to age-matched controls (Staley and Mash, 1996; Segal et al., 1997), supporting a role for this receptor subtype in drug addiction. Finally, recent brain imaging studies in humans using the positron emission tomography (PET) DRD3-preferring ligand [^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin ([^{11}C]PHNO) revealed that DRD3 availability was higher in MA polydrug users (Boileau et al., 2012) and cocaine-dependent individuals (Payer et al., 2014) compared to age-matched controls; an outcome opposite to the decreases in D2-like receptor availability following chronic stimulant exposure (e.g., Volkow et al., 1990, 2001; Nader et al., 2006).

A behavioral assay that has been frequently used to characterize DRD3 agonists is the unconditioned behavior of drug-elicited yawning using the D_3/D_2 receptor agonist quinpirole in rodents (e.g., Collins et al., 2005, 2007; Baladi and France, 2009) and nonhuman primates (Martelle et al., 2007; Hamilton et al., 2010; Blaylock et al., 2011). The quinpirole-induced

yawning dose-response curve is an inverted-U shaped function of dose. Collins, Woods and colleagues concluded that the ascending limb of the quinpirole dose-response curve is mediated by DRD3, while the descending limb is primarily D_2 receptor (DRD2)-mediated and coincides with an onset of hypothermia (Collins et al., 2005, 2007). Despite the differences noted in D2-like and DRD3 availability following cocaine exposure, quinpirole-elicited yawning was not different in cocaine-naïve vs. cocaine-experienced monkeys (Blaylock et al., 2011). However, while DRD3 partial agonists do not elicit yawning when administered to cocaine-naïve monkeys (Martelle et al., 2007), they do elicit yawning in monkeys with an extensive cocaine history (Blaylock et al., 2011). This finding suggests that drug-elicited yawning may be an effective baseline on which to assess functional consequences of chronic cocaine exposure.

In the present study, we utilized quinpirole-elicited yawning to examine the relationship between behavioral sensitivity and measures of DRD3 receptor availability using PET imaging with [^{11}C]PHNO in drug-naïve male rhesus monkeys. Secondly, we extended previous findings that a cocaine history does not influence sensitivity to quinpirole-elicited yawning (Blaylock et al., 2011; Collins et al., 2011) to monkeys with an extensive MA self-administration history. Finally, recent data investigating DA D_2/D_3 receptor availability in cocaine abuse (Nader et al., 2012) suggested possible sex differences in the relationship between DA receptor function and cocaine abuse. Thus, we examined the effects of quinpirole-elicited yawning in a group of drug-naïve female rhesus monkeys and compared them to drug-naïve male rhesus monkeys.

METHODS

***Subjects*:** Adult rhesus monkeys (*Macaca mulatta*; 22 male, 7 female) served as subjects. At the start of the study, 15 monkeys were drug naïve (8 male and 7 females), three male monkeys had a lifetime intake of methamphetamine ranging from 143 – 200 mg/kg and 11 male monkeys had varying cocaine histories (minimum of 225 mg/kg). Drug-experienced monkeys were

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previously implanted with a chronic indwelling catheter and associated vascular access port (Access Technologies, Skokie, IL) as described elsewhere (Czoty et al., 2006) and excluding days of quinpirole-elicited yawning, underwent experimental sessions involving drug self-administration daily (Mon-Fri). All monkeys were fitted with aluminum collars and trained to sit calmly 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 healthy body weights (determined by veterinary staff and somewhat less than 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 Laboratory Animals* and were approved by the Animal Care and Use Committee of Wake Forest University.

Experiment 1: Relationship between DRD3 availability and quinpirole-elicited yawning

***Imaging Procedure*:** A magnetic resonance imaging (MRI) scan was acquired in seven of eight experimentally naïve male monkeys; monkey #1711 had been previously implanted with a non-MR compatible telemetry device. Monkeys were anesthetized with ketamine (10-15 mg/kg, i.m.) approximately 20 min prior to scanning, transported to the MRI facility, and T1-weighted images of the entire brain were acquired with a 3.0-T Siemens SKYRA scanner. PET data were co-registered to individual MRIs using PMOD Biomedical Image Quantification Software (version 3.1; PMOD Technologies, Zurich, Switzerland). Spherical regions of interest (ROIs) were drawn on individual MR images for the caudate nucleus (Cd; 2.5 mm radii), putamen (Pt; 2.5 mm radii), globus pallidus (GP; 2.5 mm radii), ventral striatum (VS; 2.0 mm radii), ventral pallidum (VP; 2.0

mm radii), thalamus (Thal; 3.0 mm radii), substantia nigra (SN; 2.0 mm radii), hippocampus (HIP; 2.4 mm radii), and cerebellum (Cb; 4.0 mm radii). The GP, VP, Thal and SN were chosen because they are rich in DRD3 (Tziortzi et al., 2011), the Cd, Pt and VS were chosen because of their involvement in addiction (Koob and Volkow, 2010) and the HIP because of its role in yawning (Argiolas and Melis, 1998). For monkey #1711, the PET data were co-registered to the MRI obtained from #1710, based on the similar weight and age of the two subjects.

PET studies were performed on a GE 64 slice PET/CT Discovery VCT scanner (GE Medical Systems, Milwaukee, WI, United States) with an approximate 5-6 mm resolution, using the DRD3 radiotracer [^{11}C]PHNO, synthesized as described in Wilson et al. (2005). On the day of the PET study, monkeys (n=8) were initially anesthetized with a 10–15 mg/kg (i.m.) ketamine HCl and transported to the PET center where they were maintained on inhaled 1.5% isoflurane anesthesia for the duration of the 90 min PET scan. Arterial and venous catheters were placed by percutaneous stick for blood pressure and tracer injection, respectively. Due to an irregular respiratory rate and hypotension following injection of the radiotracer, two monkeys were put on a ventilator for the remainder of the scan. This effect following tracer doses of PHNO has previously been reported (Gallazot et al., 2012). At the start of the PET scan, 4-6 mCi of [^{11}C]-(+)-PHNO was injected, followed by a 90 min 3D emission scan consisting of 36 sequential frames: 10 x 6 s (0-1 min), 5 x 1 min (1-6 min), 7 x 2 min (6-20 min), 14 x 5 min (20-90 min). Initiation of each PET scan coincided with intravenous injection of [^{11}C]-PHNO. The 3D data were attenuation corrected and reconstructed transaxially using OSEM VUE point (28 subsets; 2 iterations) with a 3-mm FWHM filter resulting in a 128 x 128 matrix. PET studies were obtained prior to examining cumulative quinpirole-elicited yawning dose-response curves.

***Quinpirole-elicited Yawning*:** On the day of testing, monkeys were not fed before the experiment. Prior to the start of the session, monkeys were taken from the home cage, placed in a primate chair, and transferred to a quiet procedure room that contained a camera for video

recording. They were allowed to habituate to the room for five minutes. The session was divided into five 30-min components in which saline was administered before the first component followed by ascending doses of quinpirole (cumulative doses of 0.01, 0.03, 0.1, 0.3 mg/kg) injected intramuscularly in the upper quadriceps. Beginning immediately after each injection, the total number of yawns observed during each 30-min bin was recorded. Yawning was defined as a full extension of the jaws, withdrawal of lips, and exposure of teeth (Code and Tang, 1991). Temperature was taken by rectal thermometer immediately after the monkey habituated to the room and following completion of each 30-min bin. It was difficult to obtain body temperature measures in some monkeys following the highest dose of quinpirole. Only data from monkeys with temperature obtained from the entire dose response curve were used for hypothermia analyses (N=4). Videos were scored by two observers blind to the experimental conditions; the inter-observer variability was <5%.

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Experiment 2: Effects of sex and drug history on quinpirole-elicited yawning

***Procedure*:** Separate groups of monkeys were used in this study and compared to the drug-naïve male monkeys used in Experiment 1. First, we replicated an earlier study using 11 male rhesus monkeys with an extensive history of cocaine self-administration and compared quinpirole-elicited yawning with data obtained from the drug-naïve male monkeys from Experiment 1. Next, we extended this characterization to three male monkeys with an extensive history of MA self-administration (N=3) and finally, to drug-naïve female rhesus monkeys (N=7). For both the cocaine and MA group, 24 hours elapsed between drug self-administration sessions and quinpirole-elicited yawning. Quinpirole-elicited yawning and body temperature (for drug-naïve females) measures were obtained as described in Experiment 1. Following completion of the quinpirole dose-effect curve for the MA group, the lowest dose of quinpirole (0.01 mg/kg) elicited the most yawns. Therefore, the MA group underwent an additional acute

session consisting of a saline injection followed 30 minutes later with 0.003 mg/kg quinpirole; this dose was not included in statistical analyses comparing groups.

Data analysis**:** *Experiment 1***.** One-way repeated-measures ANOVAs and Bonferoni post-hoc analyses were used to analyze quinpirole-induced yawning and hypothermia. For analysis of PET data, a binding potential (BP_{ND}) was calculated for each of the eight ROIs (left hemisphere, right hemisphere, and average) by implementing the simplified reference tissue method (Lammertsma and Hume, 1996) in PMOD using the cerebellum as the reference region, resulting in 24 separate measures of $BP_{ND.}$ Due to the longitudinal nature of these data, repeated-measures mixed models were conducted, in order to account for within-monkey correlations. For this analyses, the total number of yawns at each of the five 30-minute bins was the dependent variable and average BP_{ND} as the independent variable, adjusting for dose of quinpirole. The mixed model analysis has been used in other PHNO studies (Gallezot et al., 2014) and was necessary because of the covariance between structures in the same individuals (Littell and Ammerman, 1998). These analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC). Because we could obtain body temperatures from only four monkeys following the highest dose of quinpirole, correlative data of quinpirole-induced hypothermia and BP_{ND} could not be obtained. *Experiment 2*. The effects of sex and drug (cocaine and MA) history on quinpirole-elicited yawning were analyzed with separate two-way repeated-measures ANOVAs using group (drug vs. naive and male vs. female) and quinpirole dose, followed by post-hoc Bonferroni *t* tests (significance level, $p<0.05$). Female rhesus quinpirole-induced yawning and hypothermia were analyzed using one-way repeated measures ANOVAs.

RESULTS

Experiment 1: Relationship between DRD3 availability and quinpirole-elicited yawning

In experimentally naïve male rhesus monkeys, quinpirole-elicited yawning varied as a function of dose ($F(4,39) = 3.901$, $P = 0.012$) and was characterized as an inverted U-shaped function of dose, with peak yawns following 0.1 mg/kg (Figure 1). Post-hoc Bonferroni analysis showed that quinpirole elicited significantly more yawns at 0.1 mg/kg ($t = 3.59$, $P < 0.01$) and 0.3 mg/kg ($t = 2.89$, $P < 0.05$) than saline. Body temperature significantly decreased with increasing doses of quinpirole ($F(4,19) = 13.33$, $P = 0.0002$) (Figure 1). Post-hoc Bonferroni analysis revealed temperatures taken after 0.03, 0.1, and 0.3 mg/kg quinpirole were significantly different than temperatures following saline administration ($t = 3.05$, 4.90, 5.86 respectively, $P < 0.05$).

Each monkey was also studied with [^{11}C]PHNO (Table 1). There were no significant differences between BP_{ND} in left and right regions for any ROI (data not shown), thus average values for each BP_{ND} were used for analyses. The highest binding potential was in the putamen, followed by the caudate nucleus and the ventral striatum. In those regions, the between-subject variability was between 5-8%. Table 2 summarizes the results of the mixed model analysis for the relationship between quinpirole-elicited yawning and [^{11}C]PHNO BP_{ND}. Using the mean binding potential for the caudate nucleus as an example, the Beta value of -3.95 means that there are approximately 4 fewer yawns on average for every 1-unit increase in binding potential. There were significant negative relationships between yawns and BP_{ND} (Table 2) in the Cd ($\beta = -3.95$, $P < 0.0001$), Pt ($\beta = -2.02$, $P < 0.0001$), VP ($\beta = -2.13$, $P = 0.0219$) and HIP ($\beta = -4.68$, $P = 0.0289$) and a significant positive relationship in the GP ($\beta = 4.63$, $P = 0.0002$).

Experiment 2: Effects of sex and drug history on quinpirole-elicited yawning

There was a significant effect of quinpirole dose on the frequency of yawns ($F(4,68) = 4.82$, $P = 0.002$), but no significant group differences or interactions between drug-naïve and cocaine-

experienced monkeys (Fig. 2, filled circles vs. open circles). There was a significant effect of quinpirole dose ($F(4,36) = 5.18$, $P = 0.002$) and a significant interaction ($F(4,36) = 4.55$, $P = 0.005$) between drug-naïve and MA-experienced monkeys (Fig. 2, filled circles vs. triangles). Post-hoc Bonferroni comparisons revealed monkeys with a history of MA self-administration yawned significantly more times than drug-naïve monkeys ($t = 3.25$, $P < 0.05$) at 0.01 mg/kg quinpirole.

There were no significant effects of quinpirole dose on yawning in drug-naïve female monkeys (Figure 3, filled symbols); the increasing trend at 0.3 mg/kg was due to one monkey yawning 36 times, while 5 animals did not yawn following this dose. Body temperature (Figure 3, open symbols) decreased as a function of quinpirole dose ($F(4,34) = 42.14$, $P < 0.0001$). Post-hoc Bonferroni analysis revealed temperatures taken after 0.01, 0.03, 0.1, and 0.3 mg/kg quinpirole were significantly different than temperatures obtained following saline ($t = 2.81$, 5.89, 9.19, and 11.3 respectively, $P < 0.05$). The results of a two-way ANOVA comparing drug-naïve males (from Experiment 1) and females found significant effects of dose ($F(4,52) = 3.28$, $P = 0.0181$) and sex ($F(4,52) = 6.17$, $P = 0.027$) on quinpirole-elicited yawning. For quinpirole-induced hypothermia, there was a significant effect of quinpirole dose ($F(4,40) = 37.57$, $P < 0.0001$) but no significant effect of sex or interaction.

DISCUSSION

The purpose of the present study was three-fold. The first objective was to ascertain the relationship between DRD3 binding potentials and the behavioral effects of the D3-preferring agonist quinpirole in monkeys. PET imaging using the D3-preferring radioligand [^{11}C]PHNO revealed a significant correlation between DRD3 binding potential and quinpirole-induced yawning in several regions of the brain including the caudate nucleus, putamen, globus pallidus, ventral pallidum and hippocampus. The second objective was to test whether a history of MA self-administration differentially affected sensitivity to the unconditioned behavioral effects of

quinpirole. Using a cumulative quinpirole dosing procedure, differences between MA-experienced monkeys and drug-naïve monkeys were noted, with the MA group showing greater sensitivity to quinpirole. We also replicated an earlier study (Blaylock et al., 2011) showing no differences in quinpirole-elicited yawning between drug-naïve and cocaine-experienced monkeys. Lastly, the comparison of drug-naïve male and female monkeys revealed sex differences, with quinpirole showing greater efficacy and greater potency in eliciting yawning in males compared to females. These findings suggest that the unconditioned behavior of quinpirole-elicited yawning reflects DRD3 function in monkeys and that both sex and drug history are determinants of individual sensitivity.

Receptor autoradiography studies have indicated similar DRD3 receptor distribution in nonhuman primates compared to humans (Levant, 1998; Morisette et al., 1998; Sun et al., 2012). In the present study, the highest uptake and binding potentials were in the caudate nucleus, putamen and ventral striatum. Previous studies in humans have found a similar pattern of distribution of PHNO binding, with highest binding potentials in the putamen, globus pallidus and substantia nigra (Searle et al., 2010; Boileau et al., 2012, 2013; Gallezot et al., 2014). Tziortzi and colleagues (2011) utilized the DRD3 antagonist GSK598809 to better determine the contribution of DRD3 and DRD2 to the PHNO signal in various brain regions. These investigators found that 100% of PHNO signal in the substantia nigra and hypothalamus and the majority of the signal in the ventral pallidum and globus pallidus were DRD3 mediated. In contrast, only 20% of the signal in the ventral striatum was attributed to DRD3 whereas 100% of the signal in the caudate nucleus was a result of DRD2 binding (Tziortzi et al., 2011). Since the ascending limb of the quinpirole-elicited yawning dose-response curve is thought to be DRD3 mediated (Collins et al*.,* 2005, 2007), the results of the mixed model which showed approximately 5 greater yawns for every 1-unit increase in binding potential in the globus pallidus, supports this conclusion. Furthermore, in the present study, yawning was negatively correlated with binding in the caudate nucleus and putamen, consistent with predominant

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DRD2-mediated signal associated with [^{11}C]PHNO binding potential and pharmacological studies suggesting that the descending limb of the quinpirole-elicited yawning dose-response curve is DRD2 mediated (Collins et al., 2005, 2007).

Recent PET studies of chronic cocaine (Payer et al., 2014) and poly-drug MA (Boileau et al., 2012) users reported that both drugs were associated with elevated levels of DRD3 availability in the substantia nigra. Furthermore, the use of cocaine and MA has previously been shown to be associated with decreased D2-like receptor availability (Volkow et al., 1990, 2001; Nader et al., 2006; Payer et al., 2014). In the present study, quinpirole-elicited yawning was used to assess group differences in DRD3 activity in monkeys with a cocaine or MA history and drug-naïve male monkeys. In drug-naïve monkeys, the quinpirole dose-response curve was an inverted U-shaped function of dose, as has been reported previously in monkeys (Martelle et al., 2007; Hamilton et al., 2010; Blaylock et al., 2011). Similarly, quinpirole-elicited yawning was not different between drug-naïve and cocaine-exposed monkeys (Blaylock et al., 2011), although there was substantial individual-subject variability in the quinpirole dose-response curves. The reasons for these individual differences are not yet determined, but may be due to differences in cocaine intake. Consistent with the present findings, a recent study found substantial individual subject differences in the reinforcing effects of DRD3 agonists, quinpirole, pramipexole, and ropinirole (Koffarnus et al., 2012), which may be due to differences in cocaine self-administration histories. Additional studies are needed to better understand how different cocaine exposures affect DRD3 compounds.

In contrast to the lack of differences between drug-naïve and cocaine-exposed monkeys, MA-exposed monkeys were more sensitive to the behavioral effects of quinpirole compared to control monkeys. Changes in sensitivity on the ascending limb of the quinpirole dose-response curve have been hypothesized to be due to upregulation of DRD3 and/or downregulation of DRD2 (Collins et al., 2011); PET and receptor autoradiography studies support this mechanism following long-term MA exposure (Kuczenski et al., 2009; Boileau et al., 2012). It also remains

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possible that changes in DA transporter (DAT) and subsequent changes in extracellular DA may interact with quinpirole's effects at DRD3 and DRD2. Consistent with this hypothesis is that MA exposure has been shown to decrease DAT (Chu et al., 2008; Groman et al., 2013) while chronic cocaine exposure has been reported to increase DAT density (Letchworth et al., 2001). Irrespective of the mechanisms, the present findings suggest that despite both stimulants resulting in increases in [^{11}C]PHNO binding (Boileau et al., 2012; Payer et al., 2014), the behavioral effects of DRD3 compounds may be different in subjects with a MA history compared to a cocaine history.

There is accumulating evidence of sex differences in cocaine abuse (O'Brien and Anthony, 2005), including greater vulnerability in initiating drug use, progressing to dependence faster, and more adverse physical, mental, and social consequences of abuse in women compared to men (Zilberman et al., 2003; Greenfield et al., 2010). Several animal models using female subjects have supported these observations (e.g., Lynch and Carroll, 2002; Mello et al., 2007; Mello, 2010; Nader et al., 2012). As it relates to DRD3 function, sex differences have not been reported. In the present study, we found significant differences between male and female monkeys at lower quinpirole doses, with females being less sensitive to the behavioral effects of quinpirole. It is possible that the entire quinpirole dose-response curve for females is shifted to the right of males and that if higher doses of quinpirole were tested, even greater sex differences would have been observed. However, it is important to note that quinpirole-induced hypothermia was not different in drug-naïve females and males suggesting that baseline DRD2 availability is not different. PET data using DRD2-like radiotracers in monkeys have confirmed no significant differences between male and female monkeys (Hamilton et al., 2010). Although DRD2-availability may not be different at baseline, our data suggest that sex differences in pharmacodynamics may be influencing the behavioral results. Consistent with this hypothesis, an investigation of healthy human subjects reported that drug-elicited DA release in the ventral striatum, caudate nucleus and putamen was greater in male subjects compared to females

(Munro et al., 2012). Additionally, a recent study showed that female smokers have significantly higher D2-like receptor availability than male smokers (Brown et al., 2012), further supporting the growing evidence of gender differences in dopaminergic system dynamics (Becker, 1999; Dreher et al., 2007; Festa et al., 2010; Hedges et al., 2010). On the other hand, the quinpirole-elicited yawning dose-response curve has been shown to be malleable to changes in food content and body weight (Baladi and France, 2009). Although males and females in this study ate the same high-protein diet, the heaviest female weighed less than the lightest male. Whether changes in diet would similarly affect the quinpirole dose-response curve in males and females remains to be investigated.

There is evidence supporting the use of DRD3 antagonists and partial agonists for the treatment of cocaine and MA abuse as well as accompanying symptoms such as cognitive dysfunction (Newman et al., 2012; Mugnaini et al., 2013; Nakajima et al., 2013). Considering female rhesus monkeys in the current study were less sensitive to the behavioral effects of quinpirole, it is likely that drug potency will be a critical variable in future studies investigating DRD3 compounds as a treatment option. As there are known gender differences in drug pharmacokinetics and pharmacodynamics (see Gandhi et al., 2004 for review), and also that the fields of Neuroscience and Pharmacology publish predominately male subject-based studies (Beery and Zucker, 2011), the present findings support the consideration of sex differences as a critical variable in the development of treatment strategies for drug abuse (Zilberman et al., 2003; see Becker and Hu, 2008). Finally, this and other studies (Bouileou et al., 2013; Payer et al., 2014) have associated DRD3 availability and a history of psychostimulant abuse but have yet to establish the nature of this relationship. Future studies will need to focus on whether high or low DRD3 availability is a risk factor in psychostimulant abuse or a result of exposure to the drug.

There are some limitations to the present study. The relationship between [^{11}C]PHNO binding potential and quinpirole-elicited yawning is not consistent with DRD3 and DRD2 signal

in every brain region examined. For example, monkeys experienced fewer yawns with increasing DRD3 binding potential in the ventral pallidum. This relationship is not consistent with the high DRD3 signal reported in that brain region (Tziortzi et al., 2011), suggesting possible alternative mechanism. Past studies have implicated specific brain areas in dopamine agonist-elicited yawning including the nigrostriatal pathway, hypothalamus, and the paraventricular nucleus (Dourish et al., 1985; Melis et al., 1987), thus it is expected that yawning does not necessarily have to correlate with DRD3 and DRD2 signal in every brain region. Furthermore, yawning is not limited to dopaminergic modulation and several other neurotransmitters and neurohormones are involved (Argiolas and Melis, 1998) creating a complex neural circuit (Sanna et al., 2012) that requires additional testing.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Martelle, S.H. Nader, Czoty, John, Duke, P.K. Garg, Newman, M.A. Nader

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Wrote or contributed to the writing of the manuscript: Martelle, S.H. Nader, Czoty, John, Duke, P.K. Garg, S. Garg, Newman, M.A. Nader

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FOOTNOTE

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LEGENDS FOR FIGURES

FIGURE 1. Quinpirole-elicited yawning (filled symbols) and hypothermia (open symbols) in experimentally naïve male monkeys. Cumulative quinpirole dose-response curves (saline, 0.01-0.3 mg/kg) for yawing are the mean (± SEM) of 8 monkeys and hypothermia the mean (± SEM) of 4 monkeys; each point was obtained over a 30 min period. * $p < 0.05$ vs. saline.

FIGURE 2. Differential effects of drug history on quinpirole-elicited yawning in male rhesus monkeys. Each point represents the mean (± SEM) of monkeys with a cocaine history (n=11; open circles) and methamphetamine history (n=3; triangles) and drug-naïve controls (n=8; open circles). * $p < 0.05$ vs. drug-naïve controls.

FIGURE 3. Quinpirole-elicited yawning and hypothermia in drug-naïve female monkeys. Each point represents the mean (± SEM) of 7 monkeys. All other details are as in Figure 1.
* $p < 0.05$ vs saline.

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TABLE 1. Binding potentials for [^{11}C]PHNO in drug-naïve male rhesus monkeys

	Cd	Pt	VS	VP	GP	SN	HIP	Thal	Yawns¶
1710	3.39	4.19	2.15	1.37	1.12	0.42	0.39	0.20	27
1711	3.93	4.58	3.12	1.99	1.55	1.66	0.28	0.13	20
1712	2.99	3.11	2.35	1.49	1.00	0.35	0.48	0.30	34
1713	2.55	2.67	1.79	1.25	1.19	0.49	0.14	0.50	39
1763	3.47	3.43	2.38	1.32	2.31	-0.04	0.30	0.12	21
1765	2.56	2.54	1.69	1.36	1.15	0.75	0.25	0.03	44
1706	3.56	3.94	2.82	2.18	1.54	0.67	0.41	0.64	47
1681	3.30	3.67	1.60	1.44	1.24	0.43	0.54	0.62	10
AVG	3.22	3.52	2.24	1.55	1.39	0.59	0.35	0.32	30.25
SEM	0.17	0.25	0.19	0.12	0.15	0.17	0.05	0.09	4.89

¶ Total yawns across the entire quinpirole dose-response curve (0.01-0.3 mg/kg)

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TABLE 2. Results of mixed models of total yawns adjusting for dose of quinpirole and accounting for within-monkey correlation

	Beta	p-value
Caudate	-3.95	<.0001
Putamen	-2.02	<.0001
Ventral Striatum	-0.02	0.9703
Ventral Pallidum	-2.13	0.0219
Hippocampus	-4.68	0.0289
Thalamus	0.81	0.4812
Substantia Nigra	-1.02	0.0867
Globus Pallidus	4.63	0.0002
Cerebellum	-2.24	0.2155

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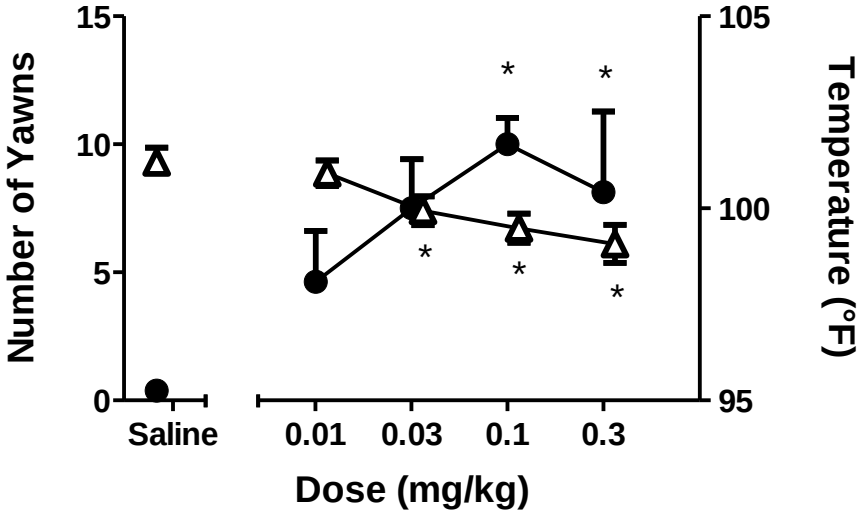

Figure 1

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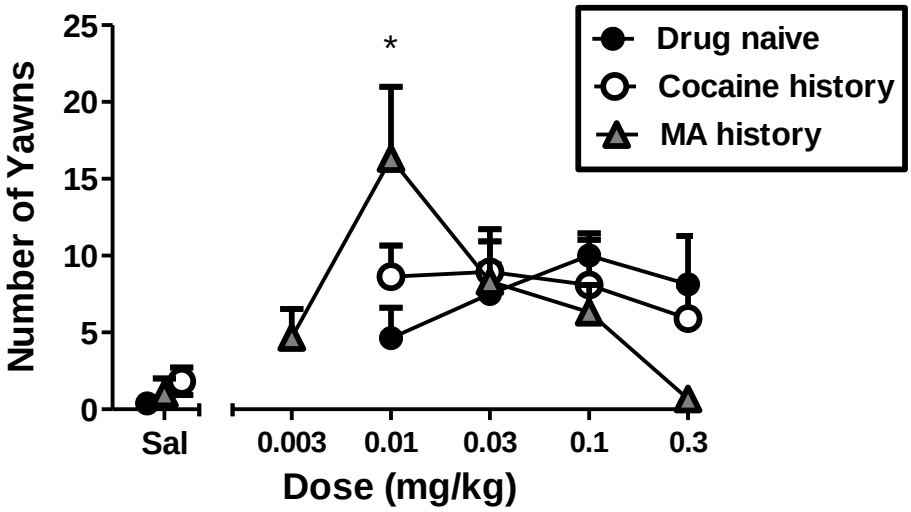

Figure 2

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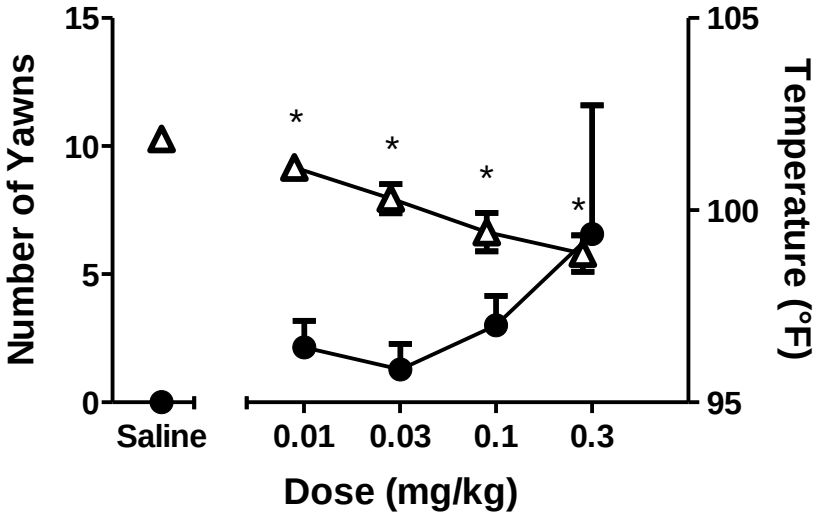

Figure 3