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Social Status in Monkeys: Effects of Social Confrontation on Brain Function and Cocaine Self-Administration

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Individual differences in response to social stress and environmental enrichment may contribute to variability in response to behavioral and pharmacological treatments for drug addiction. In monkeys, social status influences the reinforcing effects of cocaine and the effects of some drugs on cocaine self-administration. In this study, we used male cynomolgus macaques ($n = 15$) living in established social groups to examine the effects of social confrontation on the reinforcing effects of cocaine using a food-drug choice procedure. On the test day, a dominant or subordinate monkey was removed from his homecage and placed into another social pen; 30 min later he was studied in a cocaine-food choice paradigm. For the group, following social confrontation, sensitivity to cocaine reinforcement was significantly greater in subordinate monkeys compared with dominant animals. Examining individual-subject data revealed that for the majority of monkeys (9/15), serving as an intruder in another social group affected cocaine self-administration and these effects were dependent on the social rank of the monkey. For subordinate monkeys, sensitivity to the reinforcing effects of cocaine increased while sensitivity decreased in dominant monkeys. To investigate potential mechanisms mediating these effects, brain glucose metabolism was studied in a subset of monkeys ($n = 8$) using [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) with positron emission tomography. Dominant and subordinate monkeys displayed distinctly different patterns of brain glucose metabolism in their homecage, including areas associated with vigilance and stress/anxiety, respectively, and during social confrontation. These data demonstrate that, depending on an individual's social status, the same social experience can have divergent effects on brain function and cocaine self-administration. These phenotypic differences in response to social conditions support a personalized treatment approach to cocaine addiction.
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INTRODUCTION

Stimulant-use disorder continues to be a worldwide problem (Degenhardt *et al*, 2014) with no clinically effective therapy (Verrico *et al*, 2013). There has been a growing appreciation that environmental variables such as social stress and enrichment can modulate the acute and long-lasting reinforcing effects of abused drugs (Miczek *et al*, 2008; Stairs and Bardo, 2009; Nader *et al*, 2012a). Altogether, environmental and physiological, as well as genetic factors, likely contribute to the heterogeneity common in human studies stressing the importance of a personalized treatment approach for addiction, similar to other CNS disorders (Van Der Stel, 2015). In monkeys, social hierarchy and in humans, socioeconomic status (Patrick *et al*, 2012; Kendler *et al*, 2014) can profoundly influence physiological health (Sapolsky, 2005). Studies in animals and humans have begun to elucidate the brain substrates and mechanisms that mediate these environmental influences, supporting a relationship between social hierarchy, the dopamine (DA) neurotransmitter system and abuse-related effects of cocaine (Volkow *et al*, 1999; Morgan *et al*, 2002; Czoty *et al*, 2010; Martinez *et al*, 2010; Nader *et al*, 2012b).

Male cynomolgus monkeys living in social groups form hierarchies that are linear and transitive based primarily on outcomes of agonistic interactions (Kaplan *et al*, 1982). Previous studies have shown that the formation of these hierarchies influences DA D2/D3 receptor (D2/D3R) availability (Morgan *et al*, 2002; Nader *et al*, 2012b), which has been strongly implicated in mediating the abuse-related effects of cocaine (Volkow *et al*, 1999; Dalley *et al*, 2007; Nader *et al*, 2012a). This relationship between social hierarchy and brain D2/D3R availability has been extended to humans (Martinez *et al*, 2010), making this animal model highly translational. We have shown rank-related differences in cocaine self-administration, such that subordinate monkeys self-administer cocaine at lower doses compared with

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dominant monkeys under several conditions (Morgan *et al*, 2002; Czoty *et al*, 2005).

Most recently, we have utilized a food-cocaine choice procedure in which complete cocaine dose–response curves are determined in each session (Czoty and Nader, 2012). For these studies, the parameters are adjusted so that there are no baseline differences between dominant and subordinate monkeys in cocaine-food choice. This allows for the study of the influence of social hierarchy on cocaine reinforcement following exposure to environmental and pharmacological variables that are not confounded by differences in baseline cocaine self-administration. Under these conditions, we have found social rank-related differences in the effects of drugs on cocaine self-administration (Czoty and Nader, 2013, 2015).

Acute exposure to a stressor has been shown to increase (Miczek *et al*, 2004), whereas exposure to environmental enrichment has been shown to decrease (Stairs and Bardo, 2009; Lynch *et al*, 2013) sensitivity to the reinforcing effects of psychostimulants. We have extended these studies to include social rank-related differences in the effects of environmental stressors (eg, toy snake) and enrichers (eg, larger housing) on cocaine self-administration in monkeys (Czoty and Nader, 2012). One goal of the present study was to extend research on environmental variables in socially dominant and subordinate monkeys to include a resident/intruder confrontation paradigm, a widely used model of acute social stress in animal studies (Yap *et al*, 2015). In rats, acute social stress increases rate of acquisition to cocaine self-administration and increases sensitivity to the reinforcing effects of low and moderate doses of cocaine (Miczek and Mutschler, 1996). Further, in rats trained to discriminate saline from the anxiogenic drug pentylenetretrazol (PTZ), social defeat occasions PTZ responding (Vivian *et al*, 1994). In the present study, if the resident/intruder confrontation paradigm was a stressful event, we hypothesize that the sensitivity to the reinforcing effects of cocaine would be increased and the cocaine dose–response curve would shift to the left. However, if this environmental event were enriching, we hypothesize that the reinforcing effects of cocaine would be decreased and the cocaine dose–response curve would be shifted to the right. Unlike the relationship between plasma cortisol concentrations and stress, there are no peripheral biomarkers associated with environmental enrichment, thus we have utilized behavioral consequences as an indicator of enrichment (Nader *et al*, 2012a).

Individual differences in response to environmental and pharmacological challenges are a hallmark of addiction and have been observed in our studies involving dominant and subordinate monkeys (eg, Czoty and Nader, 2015). Thus, in addition to providing group data, we also examine individual-subject data to better understand those that are 'affected' by the manipulation, defined as a change in the ED_{50} for cocaine by 0.25 log-units. In the present study, the social confrontation paradigm affected the cocaine dose–response curve in a subset of monkeys. Importantly, in all cases, when a subordinate monkey was affected the cocaine dose–response curve was shifted to the left, while for all affected dominant monkeys, the dose–response curve was shifted to the right.

At present, it is not clear what neurobiological mechanisms mediate these social rank-related differences in cocaine self-administration following acute exposure to environmental stimuli. While D2/D3R availability is associated with vulnerability to cocaine abuse (Morgan *et al*, 2002), with continued exposure to cocaine, the initial social rank-related differences in D2/D3R availability dissipate, although the influence of the social environment on cocaine self-administration persists (Czoty *et al*, 2004, 2005). Thus, a second goal of this study was to extend beyond D2/D3R and explore patterns of brain activation as a function of social rank by measuring rates of regional cerebral glucose metabolism (CMR_{glc}) using [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) and positron emission tomography (PET) imaging (eg, Henry *et al*, 2010; Gould *et al*, 2012; Porrino *et al*, 2016).

MATERIALS AND METHODS

Subjects

Seventeen adult male cynomolgus monkeys (*Macaca fascicularis*) served as subjects. Of these, 15 completed experiment 1 and 8 completed experiment 2; 6 monkeys completed both sets of studies. All monkeys had a history of being housed in groups of 3 or 4 for more than 2 years. At the outset of the present experiments, monkeys were housed in groups of four; not all monkeys from a social group participated in these experiments. Each monkey was prepared with an indwelling venous catheter and subcutaneous vascular access port (Supplementary Methods). All had self-administered cocaine for more than 2 years before the start of this study; cocaine histories were not significantly different between dominant and subordinate animals (Supplementary Material and Supplementary Table S1). Each monkey had been fitted with an aluminum collar (Primate Products, Redwood City, CA, USA) and trained to sit in a primate chair (Primate Products). Monkeys were weighed weekly and fed enough food daily (Purina LabDiet 5045, St Louis, MO, USA and fresh fruit and vegetables) to maintain healthy body weights. Water was available *ad libitum* in the homecage. 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.

Monkeys lived in stainless steel cages ($0.71 \times 1.73 \times 1.83$ m; Allentown Caging Equipment, Allentown, NJ, USA) with removable wire mesh partitions that separated monkeys into quadrants ($0.71 \times 0.84 \times 0.84$ m). Social status in these established groups had previously been determined according to the outcomes of agonistic encounters as described previously (Morgan *et al*, 2000; Czoty *et al*, 2009). In experiment 1, #1- and #2-ranked monkeys were considered dominant and #3- and #4-ranked monkeys were considered subordinate. We have shown that in these stable group-housed cohorts, $>90\%$ aggression is directed at, and $>90\%$ submissive behaviors are exhibited by the #3 and #4-ranked monkeys (Czoty *et al*, 2009; Table 2). Only #1 and #4-ranked monkeys were compared for PET imaging studies in experiment 2, similar to previously employed study designs (eg Morgan *et al*, 2002; Riddick *et al*, 2009). Social hierarchy was confirmed by an experienced observer each month. These observations occurred over several years and the

individual conducting the observations was not aware of the hypotheses being tested in the present study.

Cocaine Self-Administration: Apparatus and Procedures

Nine dominant and six subordinate monkeys were studied in self-administration experiments. Each day, monkeys were transferred to a primate chair, placed into an operant chamber, and their port connected to an infusion pump located outside the chamber (Supplementary Methods). Two photo-optic switches (Model 117–1007; Stewart Ergonomics, Furlong, PA, USA) 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 banana-flavored food pellets (Bio-Serv, Frenchtown, NJ, USA).

Monkeys were trained to self-administer cocaine under a concurrent fixed-ratio (FR) schedule of food and cocaine availability in which complete cocaine dose–response curves were determined during each session (Czoty and Nader, 2012). Responding on one 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/injection). Availability of each cocaine dose was associated with illumination of a different set of stimulus lights above the 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 5 components in which monkeys chose between food pellets and ascending doses of cocaine (ie, no injection, 0.003, 0.01, 0.03 and 0.1 mg/kg/injection cocaine in components 1–5, respectively). Each component ended when 10 total reinforcers had been earned or 20 min had elapsed, whichever came first. Delivery of each reinforcer was accompanied by a 5-sec illumination of the red light above the corresponding switch and a 30-s time out (TO); a response emitted on the alternate switch before an FR was completed reset the response requirement on the first switch. In addition, a 120-s TO 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 (Table 1). A monkey was considered trained when $\leqslant 20\%$ of reinforcers were earned on the drug switch when the alternative to food was no injection (component 1) or 0.003 mg/kg/injection cocaine (component 2) and $\geqslant 80\%$ of reinforcers were earned on the drug switch when the alternative to food was 0.1 mg/kg/injection cocaine (component 5). An additional criterion was observation of a dose-related increase in drug choice.

Table 1 Social Ranks and Self-Administration Parameters for Individual Subjects Used in the Food-Cocaine Choice Procedure

		Ratio of FR values			
Monkey	**Rank**	**Food FR**	**Cocaine FR**	**Fd:Coc**	**Affected[a]**
C-6526	1	100	60	1.7	Y
C-5386	1	50	50	1.0	N
C-7079[b]	1	100	50	2.0	Y
C-6628[b]	1	50	100	0.5	Y
C-6625	2	50	50	1.0	N
C-7081	2	125	60	2.1	Y
C-7426[b]	2	125	75	1.7	Y
C-6629	2	50	75	0.7	N
C-6529[b]	2	50	175	0.3	Y
C-7084	3	75	50	1.5	Y
C-6527	3	50	60	0.8	Y
C-6216	3	75	50	1.5	N
C-7424[b]	4	175	50	3.5	Y
C-6530[b]	4	150	50	3.0	N
C-7425	4	100	75	1.3	N

Abbreviations: Y, affected; N, unaffected.
[a]Denotes whether each individual response to the intruder condition produced a shift in the subsequent food-cocaine choice procedure.
[b]Subjects used in experiments 1 and 2.

Resident-Intruder Paradigm

When food-cocaine choice was deemed stable (three consecutive days on which the above mentioned criteria were met), a resident-intruder session was conducted on the following day. Two partitions were placed into a pen of 'resident' monkeys such that the lower left quadrant of the cage was empty and the four residents occupied the three remaining quadrants. Next, one monkey from a different pen (the 'intruder') was transferred to the open quadrant. The intruder was protected from physical harm, but visual and auditory contact could take place between the monkeys. The intruder remained in the pen for 30 min (experiment 1) or 40 min (experiment 2), during which behavior of all monkeys was videotaped and later scored for aggressive, anxious, and submissive behavior based on an ethogram modified from Kaplan *et al* (1982) utilizing Noldus Observer software (Noldus Information Technology; Wageningen, The Netherlands; see Supplementary Methods). In addition, for FDG-PET studies, immediately before ($\text{cortisol}_{\text{pre}}$, $T=0$) and after ($\text{cortisol}_{\text{post}}$, $T=45$) the homecage and intruder sessions a blood sample was obtained to determine cortisol concentrations and radioactive decay (Supplementary Methods).

FDG Administration and PET Imaging

Eight monkeys ($n=4$/social rank) were studied in brain imaging experiments under two conditions. For both conditions, a #1- or #4-ranked monkey was placed in a primate chair, a blood sample ($T=0$) was collected followed by a 30-s intravenous infusion of 5–7 mCi of [^{18}F]FDG followed by 5 ml sterile 0.9% NaCl and the monkey was either returned to his cage (homecage condition) or transferred to the bottom left quadrant of a pen of four unfamiliar monkeys (intruder condition). After 40 min, the monkey was returned to the primate chair and anesthetized with ketamine HCl (10 mg/kg i.v.). After a second blood sample was collected ($T=45$), the monkey was transported to the PET imaging facility and PET scan data were acquired

using a General Electric Advance NXi PET scanner (GE Medical Systems, Milwaukee, WI, USA) with 4-mm resolution (Supplementary Methods).

Data Analysis

Self-administration studies. The primary dependent variable was percent cocaine choice, defined as the percent of total reinforcers received as injections and calculated for each component. Because monkeys had different FR requirements, percent of reinforcers was used for analysis rather than percent responses. The total number of reinforcers, food pellets earned and injections received were also recorded. Each of these four dependent variables was analyzed separately for baseline and the intruder condition using a two-way analysis of variance (ANOVA) with cocaine dose and social rank (dominant *vs* subordinate) as factors, followed by Holm–Sidak multiple comparisons testing. To examine individual monkey data, a monkey was considered to be 'affected' by the resident/intruder paradigm if the ED_{50} value, calculated from a linear portion of the food-cocaine choice curve, shifted >0.25 log units from baseline; monkeys in which the intruder condition altered the ED_{50} value by <0.25 log units were considered to be 'unaffected' (Czoty and Nader, 2012). Data from 'affected' monkeys were initially analyzed with a three-way analysis of variance (ANOVA) with cocaine dose, social rank and condition (homecage *vs* intruder) as factors. When a significant effect of rank was found, dependent variables were then analyzed separately for dominant and subordinate monkeys using a two-way analysis of variance (ANOVA) with cocaine dose and condition as factors, followed by Holm–Sidak multiple comparisons testing.

Imaging studies. (1) CMR_{glc}: PET data were analyzed using the Statistical Parametric Mapping (SPM12) software (University College London, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) in conjunction with MATLAB (MathWorks, Natick, MA, USA). Effects at each voxel were estimated according to the general linear model using the multi-subject conditions and covariates option in SPM with a minimum threshold of $p<0.01$. Correction for multiple comparisons was accomplished at the cluster level. Contrasts across homecage and intruder challenge conditions using unpaired *t*-tests were used to evaluate differences between dominant and subordinate subjects. Results were displayed as color-coded maps of statistical significance projected onto the MRI template. Because a $T=45$ blood sample could not be collected from one subordinate monkey before transportation to the PET imaging facility, group data were analyzed using unpaired *t*-tests. (2) Social behavior: Intra-group analyses were performed using two-tailed paired Student's *t*-tests comparing time points across conditions. For analysis of cortisol and social behaviors, unpaired *t*-tests were conducted for time points across rank. Because $T=0$ samples ($cortisol_{pre}$) were collected while monkeys were awake and $T=45$ ($cortisol_{post}$) samples were collected when monkeys were anesthetized for transport to the PET facility, within-subject analyses were not performed. In all cases, differences were considered statistically significant when $p<0.05$.

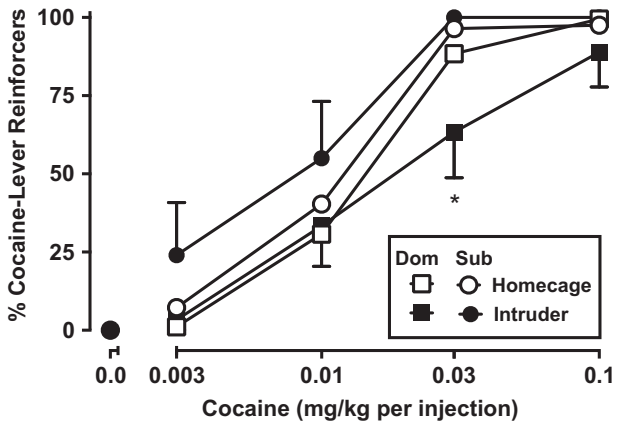

Figure 1 Cocaine-food choice under homecage conditions (open symbols) and after serving as an intruder (filled symbols) in dominant (squares, $n=9$) and subordinate (circles, $n=6$) monkeys. Ordinate: percent of total reinforcers earned as cocaine injections; abscissa: available cocaine dose as an alternative to a food pellet. Asterisk indicates significant ($p<0.05$) difference between dominant and subordinate monkeys after serving as an intruder.

RESULTS

Experiment 1. Effects of Intruder Paradigm on Food-Cocaine Choice

Under baseline (homecage) conditions, the frequency of cocaine choice increased with cocaine dose, such that cocaine was preferred ($>80\%$) when 0.03 and 0.1 mg/kg/injection cocaine were the alternatives to food. By design, baseline dose-effect curves were not significantly different between dominant and subordinate monkeys (Figure 1, open symbols). The food and cocaine FR values needed to generate similar cocaine dose–response curves were not significantly different between dominant and subordinate monkeys (Table 1). Following 30 min of the intruder condition, cocaine self-administration was studied and the cocaine dose–response curve for subordinate monkeys was situated to the left of the cocaine dose–response curve for dominant monkeys, with the main effect of rank approaching significance ($p=0.07$; Figure 1, closed symbols). *Post-hoc* comparisons revealed significantly higher cocaine choice in subordinate monkeys when 0.03 mg/kg was available concurrently with food reinforcement ($p<0.05$).

Examining individual-subject data, the cocaine dose–response curve was shifted in 9 monkeys following the intruder condition (Figure 2, left panel, filled symbols). For the 6 dominant and 3 subordinate monkeys affected, a three-way ANOVA indicated significant interactions between cocaine dose and rank ($F_{4,70}=2.55$, $p<0.05$) and between condition and rank ($F_{1,70}=6.43$, $p<0.05$). In dominant monkeys there was a main effect of cocaine dose on choice ($F_{4,20}=39.15$, $p<0.001$), and post-hoc tests indicated that dominant monkeys chose the 0.03 mg/kg dose of cocaine significantly less often during the intruder *vs* homecage condition ($p<0.05$). In subordinates, there were significant main effects of cocaine dose ($F_{4,8}=22.77$, $p<0.001$) and condition ($F_{1,2}=21.8$, $p<0.05$); post-hoc tests revealed that subordinate monkeys chose 0.003 and 0.01 mg/kg cocaine more frequently during the intruder *vs* homecage condition.

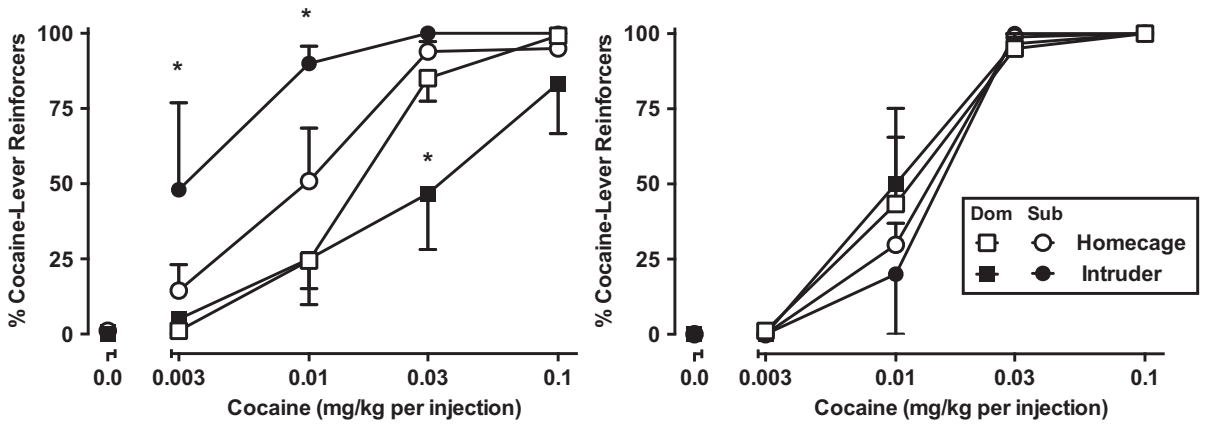

Figure 2 Cocaine-food choice under homecage conditions (open symbols) and after serving as an intruder (filled symbols) in dominant (squares) and subordinate (circles) monkeys. Left panel: Monkeys in which cocaine choice was affected by the intruder condition on cocaine choice ($n=6$ dominant, $n=3$ subordinate). Right panel: Monkeys in which cocaine choice was unaffected by the intruder condition ($n=3$/group). Ordinate: percent of total reinforcers earned as cocaine injections; abscissa: available cocaine dose as an alternative to a food pellet. Asterisk indicates significant ($p<0.05$) difference between homecage and intruder conditions.

The cocaine dose–response curve for the 6 monkeys unaffected by the intruder condition are shown in the right panel of Figure 2.

To further examine the interaction of social rank and the intruder condition on behavior, we examined the total number and allocation of reinforcers in the 9 monkeys ($n=6$, dominant; $n=3$, subordinate) in which the food-cocaine choice curve was affected. The total number of reinforcers earned in each component was not different between baseline and intruder conditions in either dominant or subordinate monkeys (Figure 3, top panels); no significant main effects or interactions were revealed by the 3-way ANOVA other than the expected main effect of cocaine dose ($F_{4,70}=18.90$, $p<0.001$). Three-way ANOVAs did show, however, the distribution of reinforcers was affected by the monkeys' social rank. Significant interactions between cocaine dose and rank were observed for food pellets delivered ($F_{4,70}=2.67$, $p<0.05$) and injections delivered ($F_{4,70}=2.65$, $p<0.05$). Significant interactions between condition and rank was also observed for food pellets delivered ($F_{1,70}=5.72$, $p<0.05$) and injections delivered ($F_{1,70}=6.96$, $p=0.01$). More specifically, with increases in cocaine dose available as the alternative to food reinforcement, there were dose-dependent decreases in number of food reinforcers delivered (Figure 3, middle panels; dominant monkeys: $F_{4,20}=41.66$; subordinates: $F_{4,8}=24.36$; both $p<0.001$) and increases in number of cocaine injections delivered (Figure 3, bottom panels; dominant monkeys: $F_{4,20}=15.51$; subordinates: $F_{4,8}=12.95$; both $p<0.001$). However, after serving as an intruder, allocation of responding was changed in an opposite manner in dominant and subordinate monkeys. Dominant monkeys received significantly more food pellets and fewer injections ($p<0.05$) when 0.03 mg/kg/injection cocaine was available in the intruder condition compared with the homecage condition (Figure 3, middle and bottom panels). In contrast, compared with the homecage condition, following the intruder condition subordinate monkeys received significantly more drug injections and fewer food reinforcers ($p<0.05$) when 0.003 and 0.01 mg/kg cocaine were available.

Experiment 2. Effects of Intruder Paradigm on CMR_{glc}

During the homecage condition, dominant monkeys showed significantly higher levels of CMR_{glc} relative to subordinate monkeys in the precuneus and visual cortex (Figure 4a, b and Table 2). In contrast, subordinate monkeys showed greater activation relative to the dominant monkeys in the amygdala, hippocampus and cingulate gyrus (Figure 4c). These differences in CMR_{glc} were apparent in the absence of differences in basal cortisol concentrations (Table 3), and despite the fact that the well-established hierarchy did not result in any overt forms of aggression. In fact, during the 40-min period in which monkeys remained in the homecage, cortisol levels decreased in all monkeys (Table 3).

The intruder confrontation showed significant effects on CMR_{glc} across all monkeys relative to the homecage condition in several brain regions (Table 2) including the medial prefrontal cortex, the ventral striatum, ventral midbrain, hypothalamus and cerebellum (Figure 5a–c). Directly comparing CMR_{glc} between dominant and subordinate monkeys during the intruder session revealed that subordinate monkeys, relative to dominant monkeys, had significantly greater CMR_{glc} in the posterior cingulate cortex (PCC; Table 2). Dominant monkeys did not show greater activity compared with subordinate monkeys in any brain region. Due to the complex nature regarding study design and implementation, relatively small sample sizes precluded analysis of #1 and #4-ranked monkeys alone.

When these same monkeys served as intruders into pens of unfamiliar monkeys, the average numbers of aggressive behaviors and yawns were significantly ($p<0.05$) different in dominant and subordinate monkeys (Table 3). Frequency of aggression and yawning were significantly higher in dominant monkeys whereas subordinate monkeys showed a significant reduction in yawning frequency between the homecage and intruder condition ($p<0.05$); the frequency of submissive behaviors was not significantly different between ranks. There was a positive correlation between aggression initiated and aggression received from surrounding residents ($r^2=0.63$, $p=0.018$). Cortisol concentrations increased

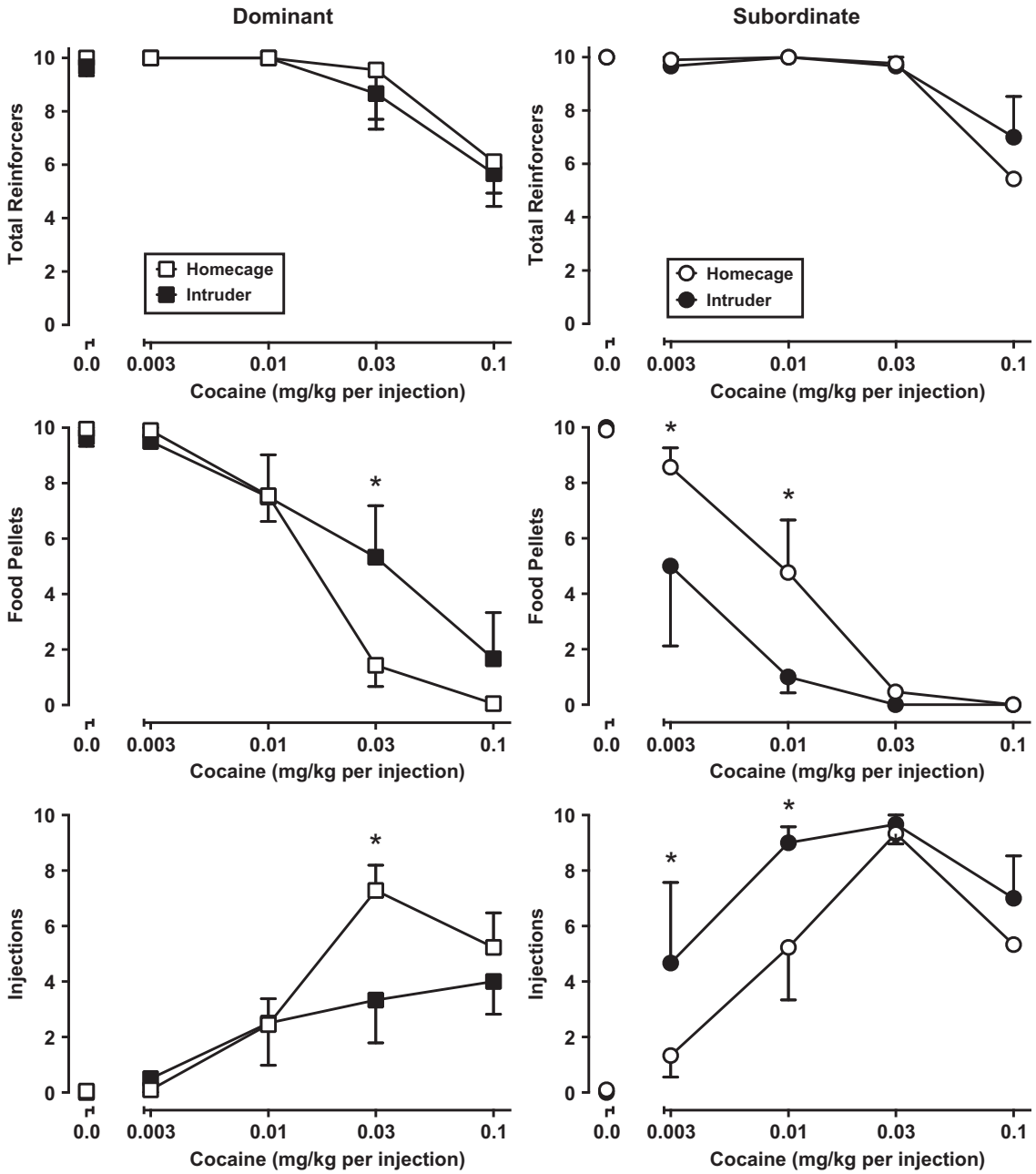

Figure 3 Interactions of social rank during homecage (open symbols) and intruder (filled symbols) conditions on food-cocaine choice in the dominant (left, $n=6$) and subordinate (right, $n=3$) monkeys affected by the intruder condition. Top panels: total reinforcers earned; middle panels: food pellets earned; bottom panels: injections received. Asterisk indicates significant ($p<0.05$) difference between homecage and intruder conditions.

significantly in both groups such that mean concentrations were higher following the intruder ($cortisol_{post}$) compared with the homecage condition (both $p<0.05$; Table 3).

DISCUSSION

The main goals of the present study were to examine the effects of the resident/intruder confrontation paradigm on cocaine self-administration in socially housed male monkeys and to identify brain regions that may be involved in these social rank-related differences. The intruder paradigm is hypothesized to model social stress, but effects in the present study were different depending on the monkey's social rank. The resident/intruder confrontation affected the cocaine dose–response curve in a majority of monkeys. Importantly, of those 'affected,' when subordinate animals served as intruders, low-dose cocaine self-administration increased and the cocaine dose–response curve shifted to the left. In contrast, the same environmental manipulation resulted in decreases in cocaine self-administration and rightward shifts in the cocaine dose–response curve in dominant monkeys. Measures of brain glucose metabolism indicated several differences between dominant and subordinate monkeys that may underlie these diametrically opposite behavioral effects.

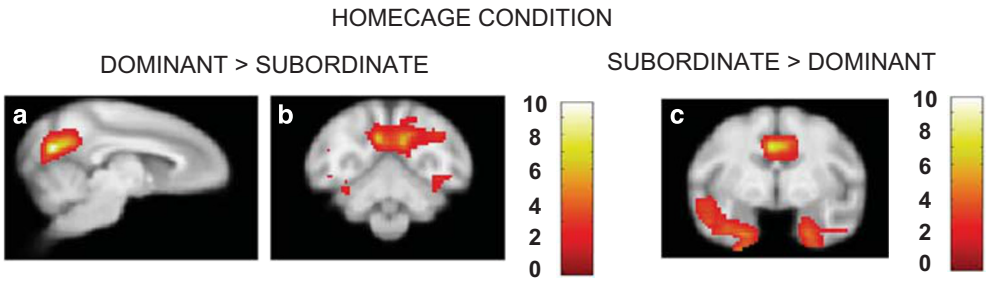

Figure 4 Areas of significant difference (threshold $p<0.01$, corrected at the cluster level) between dominant and subordinate monkeys ($n=4$/group) during the homecage condition. Dominant monkeys showed *greater* activity relative to subordinate monkeys in the (a) precuneus and (b) visual cortex, while subordinate monkeys showed *greater* activity compared with dominant monkeys in amygdala, hippocampus, and cingulate gyrus (c). Scale bars represent extent of activation, corresponding with *t*-values reported in Table 2.

Table 2 Significant Differences in Regional Cerebral Glucose Metabolism

				Coordinates			
Analysis	**Area**	**Hemisphere**	**Cluster**	***x***	**y**	**z**	**Maximum voxel *t*-value**
Between-group comparison							
Homecage condition							
Dominant > subordinate	Precuneus; visual cortex	L, R	4733	4	−30	10	10.37
Subordinate > dominant	Amygdala; hippocampus	L	1574	−10	−10	−10	9.81
	Amygdala; hippocampus	R	1687	22	−10	−12	9.38
	Anterior cingulate	L, R	2956	6	−8	14	8.01
Intruder condition							
Dominant > subordinate	No significant clusters						
Subordinate > dominant	Posterior cingulate	L,R	2183	0	−10	24	5.77
Within-group comparison							
Intruder > homecage (all 8 monkeys)							
	Cerebellum	L, R	2168	−14	−24	−10	5.84
	Ventral striatum; hypothalamus; ventral midbrain	L, R	1014	3	0	−5	5.56
	Medial prefrontal cortex	L, R	279	2	12	8	4.90

Table 3 Comparison of Behaviors[a] and Cortisol Concentrations[b] Between Dominant and Subordinate Monkeys in the Homecage

Condition	**Aggression**	**Submission**	**Yawning**	**Cortisol$_{pre}$**	**Cortisol$_{post}$**
Homecage					
Dominant[c]	0 (0)	0 (0)	4.3 (3.1)	27.1 (6.5)	18.4 (4.4)
Subordinate[d]	0 (0)	0 (0)	7.3 (1.6)	21.9 (3.1)	17.4 (3.6)
Intruder					
Dominant[c]	76.0 (15.9)[e]	1.8 (0.6)	17.0 (2.1)[e]	28.3 (8.0)	33.1 (6.0)[f]
Subordinate[d]	18.3 (12.1)	10.0 (6.3)	2.3 (1.8)[g]	19.3 (1.5)	25.6 (3.2)[f]

[a]Values are frequency measures over 40 min. [b]Values are μg/dl. [c]Values are means (±SEM) for four dominant monkeys. [d]Values for behavior are means (±SEM) for four subordinate monkeys; values for cortisol are means (±SEM) for three subordinate monkeys. [e]Denotes significant behavioral differences between dominant and subordinate monkeys during the intruder condition ($p<0.05$). [f]Denotes significant difference between cortisol$_{post}$ concentrations between homecage and intruder conditions. [g]Denotes significant behavioral differences between homecage and intruder condition in subordinate monkeys only ($p<0.05$).

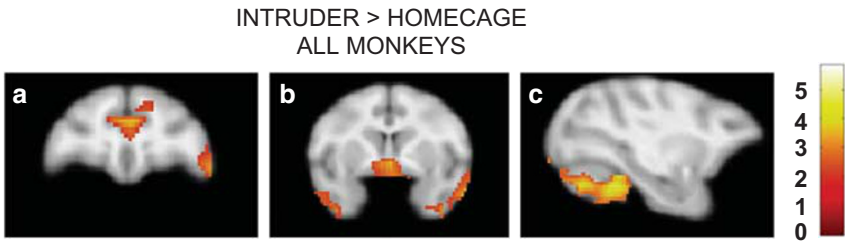

Figure 5 Areas of significant difference (cluster threshold $p < 0.05$, corrected for search volume) between intruder challenge and homecage condition in all monkeys (a–c; $n = 8$). All monkeys showed *greater* activity during the intruder challenge in the medial prefrontal cortex, including anterior cingulate cortex (a), ventral striatum and hypothalamus (b), and cerebellum (c). Scale bars represent extent of activation, corresponding with *t*-values reported in Table 2.

These findings, in monkeys living in stable social groups and with similar baseline cocaine self-administration behavior, provide insight into variables mediating differential responses to environmental events.

Our study employed a resident-intruder paradigm similar to what has been used extensively in rodents to understand how social stress contributes to drug abuse. Previous studies in rodents have demonstrated that acute social stress increases cocaine self-administration (Miczek and Mutschler, 1996). More recent studies have also reported variability in the behavioral response to social stress (Wood *et al*, 2010), and in subsequent patterns of cocaine self-administration with recent efforts attempting to understand these individual responses (Boyson *et al*, 2016). Extending earlier studies in rats in which serving as an intruder constituted a stressor and subsequently increased cocaine self-administration (Miczek *et al*, 2004), monkeys in different positions in the social hierarchy experienced social confrontation in fundamentally different ways. These results in 'affected' subordinate monkeys are consistent with rodent studies in which social defeat increased cocaine self-administration at low and moderate unit doses of cocaine (Miczek and Mutschler, 1996). However, the rightward shift in the cocaine dose–response curve in "affected" dominant monkeys was unexpected. Cortisol concentrations increased in dominant and subordinate monkeys following the intruder condition and were not different between social ranks, consistent with rodent studies in which corticosterone is elevated in both resident and intruder (Schuurman, 1980). Moreover, the lack of social rank differences in cortisol concentrations is consistent with the clinical literature demonstrating an inconsistent relationship between cortisol and socioeconomic status (Dowd and Goldman, 2006). The present findings suggest that the acute stress response (<45 min), as quantified via cortisol concentrations, is similar across rank, although further investigation may be warranted regarding downstream HPA-axis function and catecholamine levels on behavior at later time points (>45 min post-stress; Hermans *et al*, 2014). Following social confrontation, the average dose-effect curve of 'affected' dominant monkeys was situated to the right of the average dose-effect curve of 'affected' subordinate monkeys, consistent with the intruder condition representing a form of environmental enrichment in dominant monkeys (Czoty and Nader, 2012; Nader *et al*, 2012a; Smith and Lynch, 2012). Data from these studies involving an environmental manipulation extend earlier work showing social rank-related differences in the behavioral effects of dopaminergic compounds (Morgan *et al*, 2002; Czoty *et al*, 2005; Czoty and Nader, 2013, 2015). These findings highlight the importance of social context in mediating behavior and the behavioral effects of drugs and provided rationale for the second part of our study examining brain areas that may mediate these differential effects.

Although monkeys in these stable social groups rarely aggressed in the homecage, distinct differences in CMR_{glc} were observed that were related to social rank. Under homecage conditions, dominant monkeys, who are hypothesized to be living in an enriched environment (Nader *et al*, 2012a), showed significantly higher CMR_{glc} in regions involved in visual processing, attentional control and vigilance (Niv *et al*, 2015). In contrast, subordinate monkeys, who are chronically exposed to social stress, showed higher CMR_{glc} in brain regions associated with emotional processing, fear and anxiety (Kalin *et al*, 2008; McEwen, 2007). In response to the intruder challenge, all monkeys showed effects of CMR_{glc} in regions associated with the mesocorticolimbic system and HPA-axis, with subordinate monkeys showing even greater activation in the PCC. In one study, degree of activation of the PCC directly correlated with risk of relapse to cocaine use (Kosten *et al*, 2006), and may also contribute to the increased cocaine self-administration in subordinate monkeys, especially at low doses. In the present study, six of the eight monkeys used in the imaging experiments were also studied in experiment 1 and five of these monkeys represent 'affected' animals. Thus, these imaging data may provide insight into the interactions of social status with environmental challenges that lead to individual differences in drug self-administration.

Dominant, but not subordinate monkeys demonstrated significant increases in aggression during the social confrontation condition. Previous studies in humans and other animal species have demonstrated that the opportunity to aggress can serve as a reinforcer (Van Hemel, 1972; Fish *et al*, 2005); such an outcome is consistent with the intruder condition being an environmental enricher and decreasing subsequent cocaine self-administration in dominant monkeys. In addition, an unconditioned behavior associated with aggression, yawning, was significantly elevated in dominant monkeys, but not in subordinate animals. Yawning has been shown to be mediated by activation of dopamine D3 receptors (Collins *et al*, 2005) and may suggest a possible mechanism for further decreasing cocaine self-administration in dominant monkeys (Heidbreder and Newman, 2010). Consistent with this hypothesis, we recently found that a purported D3 receptor antagonist effectively

decreased cocaine self-administration in dominant, but not subordinate monkeys (Czoty and Nader, 2015).

Our group has previously shown different effects of pharmacological treatments on cocaine self-administration based on social rank (Czoty and Nader, 2013, 2015). In the present study, dominant and subordinate monkeys demonstrated distinctly different behavioral and brain responses to the same social event, reiterating the influence of environment on brain function and behavior. Consistent with observations in the clinical population in which a heterogeneous response to environmental stimuli is observed (Stoops and Rush, 2013), it is equally important to note that the food-cocaine choice curve was unaffected in a subset of monkeys (30% of dominant, 50% of subordinate monkeys). Preclinical studies have identified predisposing behaviors, neurobiological, neurochemical, or genetic factors that contribute to susceptible *vs* resilient phenotypes related to cocaine self-administration (Morgan *et al*, 2002; Economidou *et al*, 2009; Shimamoto *et al*, 2015). Identifying translational biomarkers using PET or MRI methods to aid stratification of a heterogeneous population into subgroups that may be more susceptible to different treatment approaches or likely to relapse is warranted (Volkow *et al*, 2015). For example, an fMRI study in cocaine-dependent individuals correlated the BOLD response to cocaine-related cues (including the PCC) with risk of relapse (Kosten *et al*, 2006). Similarly, an fMRI study in smokers treated with varenicline identified different patterns of brain function in participants that quit smoking *vs* relapsed (Wang *et al*, 2016). The present PET data suggest that some individuals may be more resilient in coping with stressors (eg, socioeconomic, social) with less risk of relapse, while others demonstrate less resilience and may be at higher risk for relapse. Identifying resilient *vs* susceptible phenotypes may aid behavioral and pharmacological treatment approaches. For example, employment-based abstinence reinforcement might work for those more resilient to stress, whereas stress management or behavioral therapy might be more advantageous in an individual more susceptible to stress.

Studying unaffected subordinate monkeys may provide insight into understanding the behavioral, physiological, and neural differences mediating some protective mechanisms during environmental challenge. In contrast, studying differences between unaffected and affected dominant monkeys may enhance our understanding of mechanisms mediating 'treatment resistance'. Understanding individuals' perceptions and the underlying brain response to similar environmental experiences and the effect of those experiences on cocaine use will enhance our ability to develop personalized treatments for cocaine use disorders.

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