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Relationships Among Ventral Striatal Dopamine Release, Cortisol Secretion, and Subjective Responses to Amphetamine

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There is evidence that stress and glucocorticoids alter drug self-administration and mesolimbic dopamine (DA) activity in preclinical models. The primary purpose of this study was to test the hypothesis that glucocorticoids are associated with psychostimulant reinforcement and DA release in humans. In total, 16 healthy adults, ages 18–27 years, underwent two consecutive 90-min PET studies with high specific activity [^{11}C]raclopride. The first scan was preceded by intravenous saline, and the second by intravenous amphetamine (AMPH 0.3 mg/kg). DA release was defined as the percent change in raclopride binding between the placebo and AMPH scans. Measures of subjective drug effects, plasma cortisol, and growth hormone (GH) were obtained. Findings showed that cortisol levels were positively associated with AMPH-induced DA release in the left ventral striatum (LVS) and the dorsal putamen. Subjects with higher cortisol responses to AMPH also reported more positive subjective drug effects than subjects with lower cortisol responses; no association was observed between cortisol levels and negative drug effects. Higher ratings of positive drug effects were also associated with greater DA release in the LVS, dorsal putamen, and dorsal caudate. A general lack of relationship was observed between GH responses to AMPH and DA release or subjective drug responses. Our findings provide evidence of interrelationships between glucocorticoid levels, subjective responses to IV AMPH, and brain DA release in humans. The results are consistent with those of preclinical studies, suggesting that individual differences in HPA axis function may influence vulnerability to alcohol and drug dependence in humans.

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INTRODUCTION

In recent years, there has been increasing interest in neurobiological mechanisms that underlie ethanol and drug reinforcement. Considerable evidence has emerged from preclinical studies suggesting that drugs of abuse act through mechanisms involving mesocorticolimbic dopamine (DA) pathways. A region at the base of the striatum, the nucleus accumbens (NA_{cc}) appears to be the key zone involved in the rewarding effects of drugs. Findings from preclinical studies have shown that psychostimulants, opioids, and alcohol all increase synaptic DA accumulation within this important brain region (Bonci *et al*, 2003; Doyon *et al*, 2003; Koob, 1992, 2003; Spanagel and Weiss, 1999; Wise, 1998). Drug reward can be reduced or attenuated by pharmacological or genetic manipulations that alter mesolimbic DA neurotransmission (Liu and Weiss, 2002; Phillips *et al*, 1998; Samson and Hodge, 1993).

Findings from numerous clinical studies have confirmed popular beliefs that stress contributes to the development, maintenance, and outcome of substance abuse disorders in humans (Brewer *et al*, 1998; Brown *et al*, 1990; Cole *et al*, 1990; Karlsgodt *et al*, 2003; King *et al*, 2003; Sussman and Dent, 2000). Findings from human laboratory studies have further shown that stress increases drug craving (Sinha *et al*, 1999, 2000, 2003) alters subjective responses to alcohol (Soderpalm and de Wit, 2002) and increases alcohol consumption (de Wit *et al*, 2003; Hull and Young, 1983). There is also evidence that genetic influences on stress reactivity may be associated with individual differences in risk for alcoholism (Bau *et al*, 2000; Dai *et al*, 2002a, b; King *et al*, 2002; Madrid *et al*, 2001; Ohannessian *et al*, 1994;

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Schuckit *et al*, 1996; Waltman *et al*, 1994; Wand *et al*, 2001, 1998, 1999a, b) and that the dynamics of the physiological stress response may be deranged in drug dependent individuals (Errico *et al*, 2002; Kemper *et al*, 1990; Kreek and Koob, 1998).

There is growing evidence that glucocorticoids are important mediators of the relationship between stress and drug-seeking behavior rodents (Marinelli and Piazza, 2002; Piazza and Le Moal, 1998). Several types of stressful experiences enhance rates of responding for drugs of abuse and facilitate the acquisition and reinstatement of psychostimulant, alcohol, and opiate self-administration in animals (Erb *et al*, 1996; Le *et al*, 1998; Piazza and Le Moal, 1997, 1998; Shaham *et al*, 2000; Tidey and Miczek, 1996). High levels of glucocorticoids have also been shown to increase rates of responding for psychostimulant drugs (Goeders and Guerin, 1996b; Piazza *et al*, 1991). Acquisition and reinstatement of cocaine-seeking behavior is reduced by glucocorticoid suppression with ketoconazole (Campbell and Carroll, 2001; Goeders and Clampitt, 2002), and alcohol consumption has been shown to be reduced by acute administration of the glucocorticoid receptor antagonist mifepristone (Koenig and Olive, 2004). Furthermore, adrenalectomy has been shown to attenuate psychostimulant self-administration (Deroche *et al*, 1997; Goeders and Guerin, 1996a) and to reduce the amount of alcohol intake in alcohol-preferring rats (Fahlke *et al*, 1994); these effects can be reversed by exogenous corticosterone replacement.

Although the behavioral and neuronal mechanisms underlying the relationship between stress and drug self-administration are not well understood, it is hypothesized that stress interacts with the rewarding properties of drugs of abuse by altering mesocorticolimbic DA neurotransmission (Marinelli and Piazza, 2002). Preclinical findings have shown that stress and glucocorticoids not only increase levels of mesolimbic DA but also interact with the effects of drugs of abuse on this neurotransmitter system (Barrot *et al*, 2001; Cho and Little, 1999; Marinelli and Piazza, 2002; Yavich and Tiihonen, 2000). Nevertheless, findings from human laboratory studies have not been consistent with those of the preclinical literature in demonstrating relationships between glucocorticoids and psychostimulant reinforcement (Alessi *et al*, 2003; Harris *et al*, 2003; Wachtel *et al*, 2001). Since it has been more difficult to study the neurochemical mechanisms that underlie behavioral effects of drugs in humans, the role of glucocorticoids in drug reinforcement and the relevance of the relationship between glucocorticoids and DA neurotransmission in humans remain ambiguous.

Fortunately, within the past decade it has become possible to examine the effects of drugs of abuse on DA neurotransmission in living human beings with the use of noninvasive PET scan technology. Importantly, since humans can describe their mood and feelings, this technology has made it possible to measure drug-induced alterations in brain DA activity as subjects verbally provide information about their immediate subjective experiences to the drug. Volkow *et al* (1994) were the first to demonstrate that stimulants induce brain DA release in humans. Findings of several subsequent studies showed that stimulant-induced DA release and D_2 receptor availability are positively associated with euphoric drug responses in healthy volunteers (Drevets *et al*, 2001; Laruelle *et al*, 1995; Leyton *et al*, 2002; Martinez *et al*, 2003; Schlaepfer *et al*, 1997; Volkow *et al*, 1999, 2002b).

In the present study, we utilized PET methodology to conduct the first examination of relationships among AMPH-induced DA release, cortisol secretion, and subjective drug responses in healthy, young adults. Findings from several prior investigations have shown that plasma levels of glucocorticoids and ACTH are increased by acute administration of AMPH in both rodents and humans (Halbreich *et al*, 1981; Jacobs *et al*, 1989; Smith *et al*, 2004; Swerdlow *et al*, 1993). Based on the observations cited above, we hypothesized that cortisol responses to AMPH would be positively correlated with drug-induced DA release, as well as with the positive subjective effects of AMPH. To evaluate whether any such effects were specific to cortisol, we conducted similar analyses for another 'stress' hormone, that is, growth hormone (GH).

MATERIALS AND METHODS

Subjects

In total, 16 healthy male and female subjects between the ages of 18 and 27 years were recruited by newspaper advertisements and posted fliers. After complete description of the study, all volunteers provided written informed consent, approved by the Johns Hopkins Medicine Institutional Review Board. Screening included a medical history and physical examination, blood chemistry profile, complete blood count, electrocardiogram, urinalysis, alcohol breathalyzer test, and urine toxicology screen. The Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz *et al*, 1994) was administered by a Master's level interviewer to screen for DSM-IV axis I psychiatric diagnoses. Exclusion criteria included: (a) serious medical conditions, (b) presence of a DSM-IV axis I disorder, including alcohol/drug dependence or abuse, (c) use of any psychoactive medications within the past 30 days, (d) treatment in the last 6 months with antidepressants, neuroleptics, sedative hypnotics, glucocorticoids, appetite suppressants, opiate, or DA medications, (e) seizure disorder or history of closed head trauma, (f) report drinking more than 30 drinks per month, (g) unable to provide clean urine drug screens at intake or during study participation, and (h) pregnancy or lack of effective nonhormonal methods of birth control in females. Demographic characteristics of the sample are shown in Table 1. All subjects were nonsmokers who reported light to moderate use of alcohol and no use of illicit drugs or psychoactive medications for at least 30 days prior to participation.

MRI Assessment and Mask Fitting

Each subject was fitted for a thermoplastic mask modeled to his/her face prior to GCRC admission. The MRIs were acquired to (1) optimize the available field of view of the PET camera; (2) customize positioning of the patient so the largest transaxial extension of the basal ganglia is in a direct slice; and (3) make possible data acquisition from the cerebellum, frontal and parietal cortices at the same time.

Table 1 Subject Characteristics[a]

Race/ethnicity (n)	
White, not Hispanic origin	11
Black, not Hispanic origin	3
Asian	1
White, Hispanic origin	1
Gender (n)	
Male	9
Female	7
Age (years)	21.1 (2.6)
BMI	24.3 (2.7)
Education (years)	14.3 (1.7)
Drinks/week (range 0.1–8.8)	2.1 (2.6)

[a]Values represent means and standard deviations or absolute numbers.

Use of the MRI allowed co-registration of the emission images so that anatomically accurate regions of interest (ROIs) could be drawn on the MRI. The MRIs were acquired with at least two sequences: an SPGR (spoiled grass sequence) with 1.5-mm thick slices, and a double echo (proton density and T2 weighted, 5-mm thick slices). The former one was used more frequently for co-registration and segmentation, the latter served as a diagnostic scan and to segment extracerebral CSF. The MRIs were rotated and resampled to match the position and resolution of the PET slices, so ROIs could be transferred easily from one modality to the other.

PET Procedures and Data Acquisition

Subjects were admitted to the Johns Hopkins Hospital General Clinical Research Center (GCRC) in-patient unit the day before the PET procedures. They were instructed not to ingest any alcohol, drugs, or over the counter medications for 48 h prior to admission. Laboratory studies upon admission included a urine toxicology screen, alcohol breathalyzer test, hematocryte, electrolyte panel, and urine pregnancy screen for females. Subjects received a calorie controlled, caffeine-free breakfast prior to the PET procedures. Beginning at 0830, subjects underwent two, consecutive 90-min PET scans with [^{11}C]raclopride (RAC). This radioligand is a low-affinity antagonist at D2 and D3 receptors that has previously been shown to be sensitive to stimulant-induced changes in DA concentration in the brain (Endres *et al*, 1997; Volkow *et al*, 1994). A high specific activity intravenous bolus injection of approximately 18 mCi [^{11}C]RAC was administered at the beginning of each scan. The first scan was preceded at −5 min by an intravenous injection of saline; the second scan was preceded at −5 min by 0.3 mg/kg AMPH, each delivered over 3 min. The scanning image protocol consisted of up to 32 scan acquisitions, starting from a 15-s duration and increasing to 6 min in length over a 90-min period in 3-D mode. All images were acquired on the 3D GE Advance whole body PET scanner and were preceded by a 10-min attenuation scan employing a rotating germanium-68 source. Subjects were under continuous cardiovascular monitoring during the scans. They were permitted to get up briefly after the first scan and were repositioned on the scanner table for the second. Following the scans, subjects were escorted back to the GCRC where they were evaluated by a physician prior to discharge.

Subjective Measures

Analog rating scales were administered 15 min before each scan and 3, 6, 10, 15, 25, 55, and 85 min after placebo and AMPH administration. Subjects were asked to verbally rate the degree to which they were experiencing each of 10 possible drug effects. Positive stimulant effects included 'high', 'rush', 'good effects', 'liking', 'desire for drug'; negative effects included 'fidgety', 'anxious', 'dizziness', 'dry mouth', and 'distrust' (Bigelow and Walsh, 1998). Each effect was rated on a 5-point scale ranging from 'least' to 'most'.

Hormone Assays

Measurements of plasma cortisol and GH were obtained at baseline (−25 and −5 min) and at scheduled intervals (+15, +35, +55, and +75 min) during the scans. Plasma cortisol concentrations were measured by Radioimmunoassay (Diagnostic Products Corporation, Inc., Los Angeles, CA). Intra- and interassay coefficients of variation were 5.2 and 8.0%, respectively. Plasma concentrations of GH were assayed by a two-site IRMA (Nichols immunoradiometric assay). The intra-assay coefficient of variation was 9.9%.

ROI Definition

Preclinical findings indicate that the NA_{cc}, a region at the base of the striatum, is the primary target for the reinforcing effects of drugs of abuse (Howell and Wilcox, 2002; Spanagel and Weiss, 1999). Nevertheless, aggregate findings from PET studies suggest that more information is needed about the relative importance and involvement of the dorsal caudate and putamen in humans (Drevets *et al*, 2001; Leyton *et al*, 2002; Martinez *et al*, 2003). Therefore, the ROIs for the present study included the left (LVS) and right (RVS) ventral striatum, the left (LDP) and right (RDP) dorsal putamen, and the left (LDCH) and right (RDCH) dorsal caudate nucleus.

To obtain regional BP values, volumes of interests (VOIs) were defined on spoiled gradient (SPGR) magnetic resonance imaging (MRI) volumes for the caudate nucleus and the putamen bilaterally, using interactive segmentation software. The program selects upper and lower MRI intensity thresholds to delineate striatal structures from surrounding structures with minimal hand drawing. The ventral striatum (VS) is automatically defined on the SPGR MRI volume reoriented so the plane containing the midline separating the left and right halves of the brain is orthogonal to the horizontal ACPC plane, which is the plane containing the points representing the anterior commissure and the posterior commissure. On each coronal slice, the VS was defined as the portion of the striatal VOIs ventral to the line crossing the ventral corner of the lateral

ventricle and perpendicular to the bisector of the internal capsule (Baumann *et al*, 1999). The MRI volume was spatially aligned to the PET volumes (averaged volumes across frames taken between 30 and 90 min after tracer-injection) using information theory advanced by Collignon *et al* (1995) and implemented in SPM2b software (Friston, 2002). The same transformation parameters were applied to transfer VOIs from MRI space to PET space.

Modeling of PET Outcome Measures

[^{11}C]RAC D2-like receptor specific binding was measured with binding potential (BP) $= B_{max}$/Kd (Wong, 2002). A simplified reference tissue models (SRTM) was used to describe voxel tracer kinetics, and cerebellum was used as a reference tissue (Lammertsma and Hume, 1996). Since the cerebellum in nearly devoid of D2 and D3 receptors, specific binding of [^{11}C]RAC is thought to be negligible in the cerebellum. Based on the SRTM model, a linear regression with spatial constraint algorithm was used to generate parametric BP images (Zhou *et al*, 2003). The VOIs defined on MRI images were transferred to BP images to obtain VOI BP values. The VOI DA release was estimated as the percent change in BP from the placebo to the AMPH scans: $((BP_{PL}-BP_{AMPH})/BP_{PL}) \times 100$; lower BP values during the AMPH scan indicate greater levels of endogenous DA. It should be noted that our use of the term 'DA release' does not convey a full description of the mechanisms by which AMPH alters DA concentration. Although the term 'DA release' has been used conventionally in the PET literature to describe AMPH-induced changes in [^{11}C]RAC BP, the increases in DA concentrations that occur following AMPH administration probably result from several different mechanisms of action. These mechanisms may include DA reuptake blockade, reverse transport of DA through the DA transporter (Schmitz *et al*, 2001), as well as possible actions on endogenous opioid systems (Schad *et al*, 2002).

Statistical Analyses

Our primary hypotheses were that (1) cortisol responses to AMPH would be positively associated with AMPH-induced DA release, (2) cortisol responses to AMPH would be positively associated with self-report ratings of positive subjective drug effects, and (3) positive subjective drug effects would be positively associated with ventral striatal DA release. Primary outcome measures included DA release, visual analog ratings of subjective drug responses, and plasma cortisol levels. DA release was defined as described above. Visual analog ratings were summarized as area under the curve time response curve (AUC), calculated by trapezoidal approximation from 3 to 85 min following drug administration. Several of the analog scores were square root transformed due to non-normality. Cortisol outcomes were evaluated as both the original plasma cortisol levels measured over time and an AUC summary measure. The AUC measure was calculated by trapezoidal approximation from 5 min before to 75 min after drug administration; baseline AUC (−25 to −5 min) was subtracted from this value. To evaluate the specificity of findings related to cortisol, GH levels were obtained at the same times as cortisol levels; summary scores were calculated identically. GH data were log transformed due to non-normality.

Longitudinal analyses with drug session (ie placebo or AMPH) and drug condition (ie pre- and postdrug) as indicator variables were used for preliminary examination of differences in plasma hormone levels across sessions. Relationships between placebo and AMPH session cortisol levels were further evaluated with Pearson correlation coefficients. Differences in BP values and in analog ratings across sessions were evaluated with paired *t*-tests or Wilcoxon signed ranks tests as indicated. The sample consisted of 16 subjects; however, two subjects completed the study before the analog and state anxiety scales were included in the protocol. Therefore, analyses involving these measures were conducted with 14 subjects.

Each of the primary hypotheses was examined with longitudinal analyses, taking into account within subject correlations among repeated measurements from individual subjects. Since our sample size was relatively small, we attempted to keep the number of covariates in the models to a minimum. Gender and drinking history (ie drinks/week) were included to adjust for the effects of demographic differences. Level of education and BMI were not controlled due to their restricted ranges; race was not included due to the limited number of nonwhite subjects. Although there is prior evidence that DA neurotransmission declines with age (Backman *et al*, 2000; van Dyck *et al*, 2002; Volkow *et al*, 2002a), findings from simple correlations showed that age was not associated with DA release, cortisol levels, or subjective drug responses in the present sample. We suspect that the lack of relationship was due to the restricted age range of the sample (all but three of the subjects were between 19 and 24 years old); age was not included as a covariate in the final models.

To examine the first hypothesis, plasma cortisol levels measured over time were used as the outcome variable. The models included indicator variables for drug session (ie placebo or AMPH) and drug condition (ie pre- and postdrug), DA release in a specific region of the striatum, interaction terms between the indicator variables and DA release as major covariates of interest, time and time^2 for both placebo and active session to adjust nonlinear time trend, and demographic covariates (ie gender and drinking history). Separate models were run for each of the striatal regions. To examine the second hypothesis, AUC scores for each of the analog scales were used as outcome variables. The models included an indicator variable for drug session (ie placebo or AMPH), cortisol AUC scores as a time-varying covariate, an interaction term between the indicator variable and the cortisol AUC scores as major covariates of interest, and demographic covariates. To examine the third hypothesis, AUC scores for each of the analog scales were used as outcome variables. The models included an indicator variable for drug session (ie placebo or AMPH), DA release in a given region, an interaction term between the indicator variable and DA release as major covariates of interest, and demographic covariates. Secondary analyses involving GH were conducted as described for those involving cortisol.

Bonferroni corrections were used to adjust for multiple comparisons. Significance levels for the first two hypotheses were based on the number of comparisons in each set of

analyses, that is, the level for the first hypothesis was set at 0.008 (0.05 divided by 6) and the level for the second hypothesis was set at 0.005 (0.05 divided by 10). Examination of the third hypothesis involved 10 analog scales and six brain regions, which would require a significance level of 0.0008 (0.05 divided by 60) with Bonferroni adjustment. However, the Bonferroni assumes that variables are independent (Wang *et al*, 2000); whereas both the regional DA release values and the scores on several of the analog scales were found to be highly intercorrelated. As we felt that the Bonferroni correction would seriously inflate the probability of Type II errors in the last set of analyses, we set a conservative, but less stringent criteria of 0.005, which was based on the number of analog scales used in the comparisons. Findings from preliminary analyses are reported as uncorrected *p*-values (0.05 level of significance). All analyses were conducted with SAS 8.1 or STATA 8.0.

RESULTS

Cortisol and DA Release

Figure 1 displays mean levels of cortisol and GH over time during the placebo and AMPH sessions. Cortisol levels prior to placebo (ie predrug baseline) did not differ from cortisol levels prior to AMPH. A significant increase in cortisol over baseline levels was observed for responses to AMPH ($z = 4.75$, $p < 0.0001$) but not for responses to placebo; AMPH-induced cortisol responses were also significantly greater than cortisol responses during the placebo session ($z = 6.22$, $p < 0.0001$). Although responses to AMPH were greater in magnitude than responses to placebo, significant correlations were found in cortisol levels between sessions; individuals with greater cortisol reactivity during the AMPH session also had greater reactivity during the placebo session (data not shown). Effects of AMPH on [^{11}C]RAC BP are shown in Table 2. Significant decreases were noted in [^{11}C]RAC binding (ie increased DA release) in all of the striatal subregions with AMPH; DA release values across most of the subregions were highly intercorrelated (data not shown). Findings from the longitudinal analyses indicated that cortisol responses to AMPH were positively associated with DA release in the LVS and the left dorsal putamen (see Table 3). Positive associations were also observed between unstimulated cortisol levels and DA release in the putamen. The relationship between LVS DA release and cortisol AUC during the AMPH session is shown in Figure 2.

To evaluate the specificity of the relationship between cortisol and DA release, GH measurements were also analyzed. Baseline levels of GH were marginally higher during the placebo session than during the AMPH session ($z = 1.89$, $p = 0.059$). Levels of GH increased marginally from baseline following placebo administration ($z = 1.94$, $p = 0.052$) and increased significantly following AMPH ($z = 3.02$, $p = 0.002$). GH responses to AMPH were significantly higher than GH responses to placebo ($z = 2.65$,

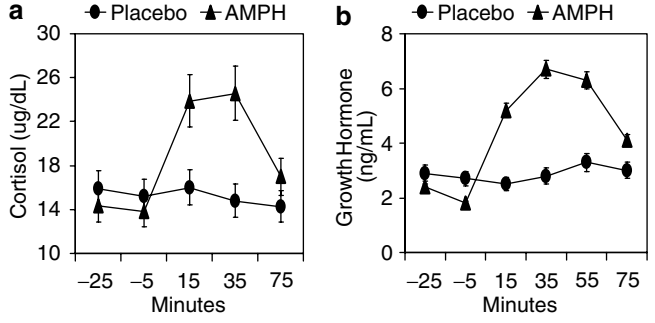

Figure 1 Plasma cortisol (a) and growth hormone (b) levels during the placebo and amphetamine PET scans. Means and standard errors are shown at each time point during each of the scans.

Table 2 Raclopride Binding Potentials during Placebo and Amphetamine PET Scans[a]

Region	Placebo	Amphetamine	Difference (%)	*p*-values
LVS	2.89 ± 0.34	2.58 ± 0.33	−10.66 ± 5.13	<0.0001
RVS	2.84 ± 0.45	2.56 ± 0.34	−9.26 ± 5.59	<0.0001
LDP	3.19 ± 0.25	2.64 ± 0.21	−16.90 ± 4.67	<0.0001
RDP	3.26 ± 0.32	2.69 ± 0.22	−17.14 ± 5.43	<0.0001
LDCH	2.49 ± 0.27	2.35 ± 0.21	−5.25 ± 4.89	<0.002
RDCH	2.51 ± 0.28	2.40 ± 0.24	−4.27 ± 3.07	<0.0001

[a]Values represent mean ± SD. LVS = left ventral striatum, RVS = right ventral striatum, LDP = left dorsal putamen, RDP = right dorsal putamen, LDCH = left dorsal caudate nucleus, RDCH = right dorsal caudate nucleus.

Table 3 Relationship between Cortisol Levels and Amphetamine-Induced Dopamine Release[a]

		LVS		RVS		LDP		RDP		LDCH		RDCH	
Session		***z***	***p***	***z***	***p***	***z***	***p***	***z***	***p***	***z***	***p***	***z***	***p***
Placebo	Pre	1.31	0.190	0.16	0.874	0.72	0.472	0.72	0.470	1.10	0.27	0.89	0.372
	Post	1.49	0.135	0.11	0.916	2.18	0.029	**2.74**	**0.006**	0.54	0.590	0.70	0.486
AMPH	Pre	2.50	0.013	0.56	0.572	**2.84**	**0.004**	0.93	0.350	1.08	0.278	0.67	0.503
	Post	**2.95**	**0.003**	0.55	0.579	**5.36**	**0.001**	2.37	0.018	0.82	0.411	0.67	0.438

[a]Values significant at or below Bonferroni corrected level of 0.008 are in bold; analyses are adjusted for gender and drinking history. LVS = left ventral striatum, RVS = right ventral striatum, LDP = left dorsal putamen, RDP = right dorsal putamen, LDCH = left dorsal caudate nucleus, RDCH = right dorsal caudate nucleus, AMPH = amphetamine, Pre = prior to drug administration, Post = following drug administration.

$p = 0.008$). In contrast to the findings for cortisol, no significant relationships were observed between GH response to AMPH and DA release in any of the defined regions of the striatum. However, significant negative associations were found between placebo session GH levels and DA release in all regions except the LVS and LDCH (p-values < 0.008).

Cortisol and Subjective Drug Effects

No differences were observed in baseline analog ratings between sessions except for 'fidgety' ratings, which were higher before the AMPH session than before the placebo session ($t = -2.22$, $p = 0.045$). Drug effects were noted on analog measures of 'high', 'rush', 'good effects', 'liking', 'desire for drug'; ratings on each of these positive effect scales were higher during the AMPH session than during the placebo session ($p < 0.05$ in all cases). Higher cortisol levels during the PET scans were associated with higher AMPH session self-report ratings of 'high' ($t_{11} = 5.12$, $p = 0.0003$), 'rush' ($t_{11} = 5.70$, $p = 0.0001$), 'good effects' ($t_{11} = 3.83$, $p = 0.0028$), 'like' ($t_{11} = 4.41$, $p = 0.0010$), and 'desire for drug' ($t_{11} = 4.30$, $p = 0.0013$). These relationships all met the Bonferroni adjusted criterion of $p < 0.005$. Cortisol levels were not associated with ratings of negative drug effects during the AMPH session or with scores on any of the analog scales during the placebo session. Relationships between cortisol AUC and two of the positive subjective responses to AMPH are shown in Figure 3. GH levels were not associated with any of the positive subjective effects of AMPH or with any subjective drug effects during the placebo scan, but were positively associated with AMPH session 'dry mouth' ($t_{11} = 3.14$, $p = 0.0013$).

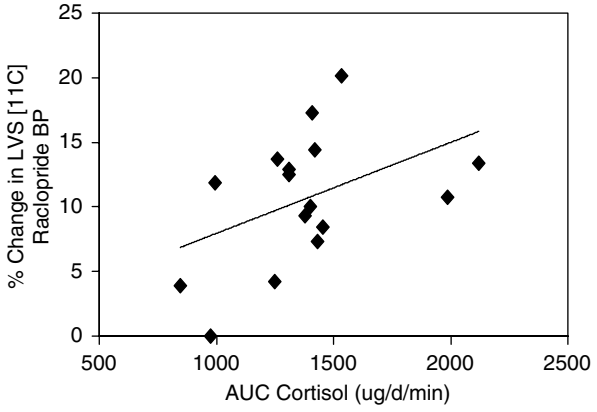

Figure 2 Unadjusted relationship between amphetamine-induced left ventral striatal (LVS) dopamine release and cortisol responses (AUC) to intravenous amphetamine.

DA Release and Subjective Drug Effects

Findings showed some positive associations between the subjective effects of AMPH and DA release in each of the defined subregions of the striatum with the exception of the RVS (see Table 4). Relationships between LVS DA release and scores on two of the analog scales are shown in Figure 4. No associations were noted between ratings of

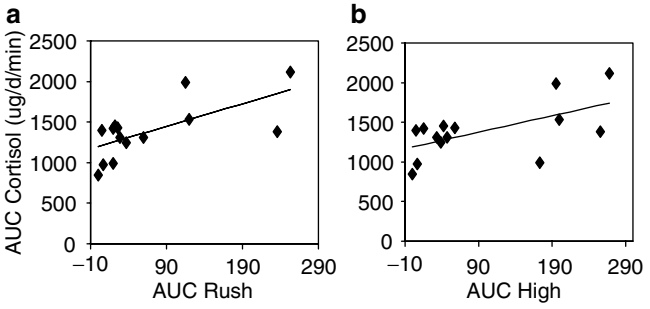

Figure 3 Unadjusted relationships between cortisol responses (AUC) to intravenous amphetamine and analog ratings (AUC) of rush (a) and high (b).

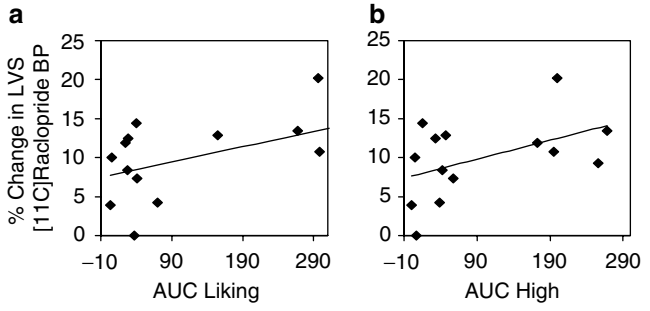

Figure 4 Unadjusted relationships between amphetamine-induced left ventral striatal (LVS) dopamine release and analog ratings (AUC) of liking (a) and high (b).

Table 4 Relationship between Positive Subjective Responses to Amphetamine and Dopamine Release[a]

	LVS		RVS		LDP		RDP		LDCH		RDCH	
Analog measure	***z***	***p***	***z***	***p***	***z***	***p***	***z***	***p***	***z***	***p***	***z***	***p***
HIGH	**4.03**	**0.000**	0.42	0.676	2.32	0.020	**3.85**	**0.000**	1.34	0.181	1.05	0.295
RUSH	**3.18**	**0.001**	1.17	0.242	2.68	0.007	2.81	0.005	2.21	0.027	2.44	0.015
GOOD	**3.02**	**0.003**	0.78	0.435	1.99	0.047	2.58	0.010	1.32	0.187	1.57	0.116
LIKE	**3.94**	**0.000**	1.23	0.218	2.63	0.008	**2.97**	**0.003**	2.21	0.027	2.02	0.043
DESIRE	**2.86**	**0.004**	0.52	0.605	**3.30**	**0.001**	2.42	0.015	3.26	0.001	2.83	0.005

[a]Values significant at or below Bonferroni corrected level of 0.005 are in bold; analyses are adjusted for gender and drinking history. LVS = left ventral striatum, RVS = right ventral striatum, LDP = left dorsal putamen, RDP = right dorsal putamen, LDCH = left dorsal caudate nucleus, RDCH = right dorsal caudate nucleus.

positive drug effects during the placebo session or negative drug effects during the AMPH session and AMPH-induced DA release. However, placebo session 'fidgety' ratings were negatively associated with DA release in all regions with the exception of the LVS and the RDCH ($p < 0.003$ in all cases).

DISCUSSION

This investigation was designed to examine relationships between cortisol levels, mesolimbic DA release, and subjective drug responses to AMPH in healthy human volunteers. Three important observations emerged from the data. First, higher cortisol levels were associated with greater AMPH-induced DA release in several regions of the striatum, including the LVS, LDP, and RDP. Second, subjects with higher cortisol levels reported more positive subjective effects with AMPH (ie 'high', 'rush', 'drug liking', 'good effects', and 'desire for drug') than subjects with lower cortisol levels. Third, higher ratings of positive subjective responses to AMPH were also associated with greater DA release in the several subregions of the striatum, including the LVS, LDP, RDP, LDCH, and RDCH. In contrast to the findings for cortisol, a general lack of relationship was observed between AMPH-induced GH and subjective drug responses or DA release, suggesting that the findings were specific to cortisol and cannot be generalized to all 'stress' hormones.

Findings from *in vivo* microdialysis studies indicate that a variety of laboratory stressors increase mesocorticolimbic DA activity in animals (Cadoni *et al*, 2003; Cuadra *et al*, 2001; Piazza and Le Moal, 1998; Tidey and Miczek, 1996). Stress has been reported to cross-sensitize to both psychostimulants (Kosten *et al*, 2003; Sorg, 1992) and alcohol (Yavich and Tiihonen, 2000), leading to greater increases in striatal DA concentrations following drug administration. Moreover, innate differences in reactivity to mild stressors are associated with differences in vulnerability to acquire amphetamine (AMPH) administration, as well as with differences in basal levels of DA in the NA_{cc}, and mesolimbic DA release in response to stress or drug challenges (Kosten and Ambrosio, 2002; Rouge-Pont *et al*, 1993). Chronic stress has also been noted to produce many of the same biochemical adaptations in the mesolimbic DA system as long-term drug administration (Ortiz *et al*, 1996).

Pruessner *et al* (2004) recently conducted a landmark PET study showing that ventral striatal [^{11}C]RAC binding was decreased (ie DA concentration increased) in response to a psychosocial stressor in humans who reported low early life maternal care. Importantly, the change in [^{11}C]RAC binding was positively associated with salivary cortisol responses to the stressor in the whole sample, providing the first evidence of a relationship between glucocorticoid levels and DA release in humans. Our data now extend these findings by showing that the relationship between glucocorticoids and DA is manifested in response to psychostimulant administration, as well as in response to a psychological challenge and can be detected by measuring either plasma or salivary cortisol levels. These parallels across studies suggest that psychological stressors and rewarding pharmacological agents may share the same physiological mechanisms in simultaneously elevating DA and cortisol levels.

Our findings are consistent with evidence from preclinical studies suggesting that glucocorticoids alter sensitivity to the reinforcing effects of alcohol and other drugs of abuse through their interactions with the mesolimbic DA system. Glucocorticoid receptors have been identified in the NA_{cc} or VTA and there is evidence that high physiological levels of glucocorticoids facilitate DA release, modulate the functional activity of postsynaptic DA receptors, and increase synaptic strength in midbrain DA neurons (Barrot *et al*, 2000; Cho and Little, 1999; Deroche *et al*, 1995; Piazza *et al*, 1996; Saal *et al*, 2003). High levels of glucocorticoids increase rates of responding for psychostimulant drugs (Goeders and Guerin, 1996b; Piazza *et al*, 1991) and facilitate locomotor responses to psychostimulants injected into the NA_{cc} in a DA-dependent manner (Marinelli and Piazza, 2002). Several methods of glucocorticoid suppression, such as synthesis blockade with ketoconazole, receptor antagonism with mifepristone, as well as adrenalectomy inhibit drug-seeking behavior in animal studies. Adrenalectomy has also been shown to reduce extracellular DA concentration in the NA_{cc} shell in response to both psychostimulants and stress (Barrot *et al*, 2001).

Given these findings, it is surprising that data from most human studies have not been consistent with the preclinical literature in showing a relationship between glucocorticoids and psychostimulant reinforcement. Although findings from one human laboratory study indicated that intravenous cortisol administration increased reports of craving in cocaine abusers (Elman *et al*, 2003), the majority of findings in this area of research have been negative. For example, pharmacological blockade of drug-induced increases in cortisol did not alter the subjective responses to psychostimulants in humans in several studies (Harris *et al*, 2003; Ward *et al*, 1998, 1999), nor did it modify patterns of cocaine self-administration in non-human primates (Broadbear *et al*, 1999). Furthermore, treatment with cortisol synthesis inhibitors has been reported to increase cocaine use in methadone maintained patients with a history of cocaine abuse (Kosten *et al*, 2002). Negative findings have also been reported in studies examining the influence of hydrocortisone on physiological, behavioral, and/or subjective responses to AMPH in human volunteers (Hearn *et al*, 2004; Wachtel *et al*, 2001), as well as in one study that examined relationships between salivary cortisol levels and drug reinforcement following oral AMPH administration (Alessi *et al*, 2003).

How does one reconcile our findings showing strong relationships among cortisol secretion, AMPH-induced mesolimbic DA release, and positive subjective responses to AMPH with the prior human literature showing negative relationships between cortisol activity and subjective drug responses? Several methodological issues may account for the negative findings in the prior human literature. First, findings related to glucocorticoid synthesis inhibitors need to be interpreted cautiously since these agents not only alter glucocorticoid levels, but also modify the bioactivity of other steroids (Marinelli and Piazza, 2002) and dramatically increase secretion of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). Thus, the interpretation of findings from studies using these

agents is not straightforward. Second, high or sustained levels of corticosterone are generally needed to modify drug self-administration behavior in rodents; these levels are achieved by administering stress-level doses of corticosterone or, more often, by repeated or prolonged stress exposure. It is possible that the changes produced by single or subchronic doses of hydrocortisone in humans are not comparable to those that facilitate drug self-administration in animals.

We propose a model in which frequent bouts of stress are associated with intermittent, but chronic exposure to high glucocorticoid levels that produces sensitization of the mesolimbic DA system. In this hypothetical model, sensitization could lead to amplification of DA release following psychostimulant administration and, consequently, increased drug reinforcement. Although our findings support the notion that individual differences in cortisol responsivity underlie differences in vulnerability for addiction, we do not believe that it is the acute drug-induced rise in cortisol itself that mediates the subjective effects of the drug. In the present study, high cortisol producers in response to AMPH were also high cortisol producers in response to placebo. These individuals appear to be high stress responders who may have chronically elevated glucocorticoid levels as a function of either genetic and/or environmental factors. In this regard, the rise in cortisol during the scans may signal a mesolimbic system that has already been sensitized by repeated HPA axis activations. This interpretation is consistent with prior observations that stimulant-induced increases in glucocorticoids are not themselves critical to drug self-administration in animals (Marinelli and Piazza, 2002).

A limitation of the present study is that firm conclusions about the causal nature of the relationship between glucocorticoids and drug reinforcement cannot be established due to the correlational nature of the design, nor can we rule out the possibility that there may be another factor associated with stress that accounts for the changes in both glucocorticoid levels and subjective drug responses. There is considerable evidence that both extrahypothalamic and hypothalamic CRH mediate the actions of drugs of abuse (Sarnyai *et al*, 2001). Thus, activation of CRH pathways may be the primary mediator of stress-induced sensitization to drugs and glucocorticoids merely the surrogates of this relationship. Mendelson *et al* (2002) recently showed a temporal concordance between cocaine-induced stimulation of ACTH and subjective euphoria, suggesting that events mediating the relationship between HPA axis activity and drug reinforcement may occur at or above the level of the adrenal gland. Additional research is needed to further clarify these relationships.

Consistent with findings of several prior PET and SPECT studies (Drevets *et al*, 2001; Laruelle *et al*, 1995; Leyton *et al*, 2002; Martinez *et al*, 2003), our data indicate that differences in subjective effects of AMPH can be least partially accounted for by differences in AMPH-induced DA release in healthy volunteers. AMPH administration decreased [^{11}C]RAC binding in all of the defined subregions of the striatum. Nevertheless, inspection of Figure 4 suggests that the associations between DA release and positive subjective effects of AMPH may have been driven by the scores of a few outliers and may actually be the weakest findings of the study. The fact that this relationship has been observed in several other PET studies attests to its validity (Drevets *et al*, 2001; Laruelle *et al*, 1995; Leyton *et al*, 2002; Martinez *et al*, 2003; Schlaepfer *et al*, 1997; Volkow *et al*, 1999, 2002b). The apparently weaker findings in our study may reflect differences in definitions of the ROIs or may be the result of relatively modest DA release scores in the sample as a whole. Nevertheless, DA release and subjective response scores of all subjects were within normal limits and appear to reflect 'real' measures of individual differences in biological and subjective responses.

Multiple intercorrelations were found between DA release values across regions indicating that subjects who had higher release in one area had higher release in another. Therefore, it is not surprising that the findings showing relationships between DA release and positive subjective drug effects generalized across regions. Although findings from animal studies suggest that the NA_{cc} is the structure that is most important in drug reinforcement, findings from human PET studies with [^{11}C]RAC and AMPH have been somewhat variable. Inconsistencies have been reported with respect to the relative amount of DA that is released across striatal subregions with AMPH, the presence or absence of correlations between regional DA release values, and the specific subregions in which DA release has been linked to positive subjective drug effects (Drevets *et al*, 2001; Leyton *et al*, 2002; Martinez *et al*, 2003). Differences in methods, differences in the delineation of striatal subregions, and limitations associated with resolution may account for some of these inconsistencies.

An unexpected finding was that both positive subjective drug effects and cortisol responses to AMPH were positively correlated with LVS, but not RVS DA release. Since separate analyses were conducted for each of the defined subregions of the striatum in the present investigation, caution is warranted in interpreting the meaning of these apparent differences across subregions. However, it should be noted that lateralization differences have been reported in glucose metabolism in the orbitofrontal cortex in humans following methylphenidate (Volkow *et al*, 2003), as well as in cerebral blood flow in the prefrontal (Tiihonen *et al*, 1994) and posterior (Wendt *et al*, 1994) cortex following alcohol. There is also evidence that specific binding of DA D2/D3 receptors is decreased in the left temporal brain (Kuikka *et al*, 2000) and that presynaptic DA function is diminished in the left caudate of type 1 alcoholics (Tiihonen *et al*, 1998).

One caveat of the present study is that the placebo scan was always conducted before the AMPH scan due to the potential carryover effects of AMPH. Although there is currently no evidence of diurnal variation in basal levels of endogenous DA, it is possible that other extraneous variables that may be associated with order, such as anticipatory anxiety, could have an influence on endogenous DA levels and response to AMPH. Baseline anxiety was positively associated with DA release following methylphenidate in one study (Volkow *et al*, 1994) and change in anxiety following AMPH was negatively correlated with DA release in another (Drevets *et al*, 2001). No differences in baseline anxiety were noted on the analog scales between sessions and analog ratings of anxiety were not associated with DA release in the present study. Future studies with

larger sample sizes will allow further investigation of these and other covariates. An alternative to the present design would have been to counterbalance the order of the two drug conditions by administering them on different days. With the latter alternative, however, the design would have been compromised by less experimental control over confounding variables between sessions, as well as greater likelihood that some subjects might not complete both scans. A possible advantage of the fixed order of the scans was that the AMPH scan was always conducted as the second scan of the day during the mid-morning hours when cortisol levels would normally be falling, giving added credence to conclusions that the increases in hormone levels that were observed during this period of time were drug-induced effects.

In summary, our findings provide support for notions that glucocorticoids play a role in psychostimulant reinforcement and that interactions between glucocorticoids and DA neurotransmission underlie this role. The findings have implications for the development of new pharmacotherapies for alcohol and drug dependence, suggesting that efforts to develop therapeutic agents that target the HPA axis hold promise for the future. The data also support hypotheses that individual differences in stress reactivity may be a factor that influences vulnerability for alcohol and drug dependence.

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