



Frequency of recent cocaine and alcohol use affects drug craving and associated responses to stress and drug-related cues

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Summary Rationale: Stress is known to increase drug craving, associated physiological arousal and risk of relapse in drug dependent individuals. However, it is unclear whether these responses are altered by recent frequency of drug use. The current study examined whether frequency of cocaine and alcohol abuse alters drug craving and associated arousal with laboratory exposure to stress and to drug related cues.

Methods: Fifty-four recently abstinent treatment-seeking cocaine abusers who were part of a study on stress and drug craving were categorized into high- and low-frequency users on the basis of their recent cocaine use. The high use cocaine group also consumed significantly more alcohol than the low use cocaine group. Participants were exposed to a brief 5-min guided imagery procedure that involved imagining a recent personal stressful situation, a personal drug-related situation and a neutral-relaxing situation, one imagery session on separate days presented in random order. Subjective (craving and anxiety), cardiovascular (heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP)) and biochemical (adrenocorticotrophic hormone (ACTH), cortisol, prolactin) measures were assessed. **Results:** High-frequency abusers demonstrated a significantly greater drug craving, anxiety and associated cardiovascular and hypothalamic-pituitary-adrenal (HPA)

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response to both stress and drug-cue exposure as compared to low-frequency abusers.

Conclusions: Increased frequency of recent cocaine and alcohol use is associated with an enhanced stress and cue-induced drug craving and arousal response that appears to be similar to the effects of cocaine, and one that may increase the vulnerability to drug-seeking behavior and relapse in drug dependent individuals.

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1. Introduction

Considerable preclinical and clinical research has highlighted the ability of both stressors and drug-related environmental cues in facilitating drug craving in humans and re-instatement and/or relapse in animals (Shaham et al., 2000; Sinha, 2001). Rats have been found to significantly enhance psychostimulant and alcohol administration following both physical and social stressors (Lê and Shaham, 2001; Kosten et al., 2004; McFarland et al., 2004). Clinical studies in humans have shown that drug users and alcoholics usually cite stress and negative affect as reasons for relapse (Sinha, 2001; Goeders, 2002). Increased drug craving, anxiety and biological responses to stress and to drug cues have been documented in drug dependent individuals (Sinha et al., 1999, 2000) suggestive of interacting and overlapping neural circuitry involved in stress and putative reward circuits.

Preclinical research has shown that intensity of recent drug use is an important aspect of drug-seeking behavior in laboratory animals. Rats allowed to self-administer high compared with low cocaine doses were shown to demonstrate increased intake and a more regular pattern of dosing (Mantsch et al., 2001). Similarly, various escalating patterns of cocaine administration are known to result in a range of neuroadaptive responses associated with the transition to addiction (Koob et al., 2004). Repeated rather than single dose regimens have been shown to culminate in higher levels of ambulatory sensitization (Davidson et al., 2002; Segal and Kuczenski, 1997) as well as increased D1 receptor binding in the nucleus accumbens and olfactory tubercle (Unterwald et al., 2001). In humans, increased length of binge cocaine self-administration has been associated with higher subjective ratings of both anxiety and craving (Foltin and Fischman, 1997; Evans et al., 2002). In addition, regularity (and related increases in quantity of drug use) has also been associated with greater problematic behavioral and cognitive sequelae (Hammersley et al., 1999; Fox et al., 2001). Clinically, it is worth noting that individuals who have been using drugs at a higher

frequency have greater difficulty maintaining abstinence and achieving treatment success (Carroll et al., 1993; Dodge et al., 2005).

Whilst studies such as these indicate an integral role of both stress and frequency of drug use on relapse, there is less data on the effects of dose frequency on drug craving, stress response and its potential effects on vulnerability to further drug use. One recent preclinical study has, however, revealed that extended drug escalation is associated with increased sensitivity to the reinstating effects of stress (Ahmed et al., 2000). However, no human research has examined whether stress and drug-cue induced craving is altered by recent frequency of drug use.

In a recent comprehensive inpatient study we demonstrated that exposure to stress and to non-stress related drug cues increased cocaine craving in cocaine dependent individuals. Our findings also indicated that these drug craving states were accompanied by an increase in arousal comprising heightened peripheral HPA and cardiovascular response in response to stress and drug cue exposure when compared to neutral imagery exposure (Sinha et al., 2003). Most recently, we've documented that such increases in stress-induced cocaine craving and associated arousal responses predict cocaine relapse after inpatient cocaine treatment (Sinha et al., 2004). These findings are consistent with previous preclinical research and clinical observations suggesting that stress related drug craving and physiological alterations play a significant role in the pathophysiology of relapse in cocaine addiction (Sinha, 2001). It remains of etiological and clinical significance, however, to assess whether these associations between stress and drug craving are modified by recent drug use. Thus, we conducted secondary analyses by grouping subjects who participated in the Sinha et al. (2003) study according to their recent frequency of drug use to examine whether their stress and drug cue related responses were affected by recent levels of drug use. As both stressors and drug-related stimuli are thought to produce effects similar to that of cocaine itself, we hypothesized that the neuroadaptive changes associated with frequency of cocaine use

would significantly affect subjective and biological responses to stress and drug related stimuli in the environment, thereby affecting vulnerability to drug-seeking behavior.

2. Method

2.1. Participants

Fifty-four cocaine dependent individuals seeking inpatient treatment who participated in the [Sinha et al. \(2003\)](#) study were assessed for the current study. Participants comprised both males and females with a mean age of 37, $SD=6.5$. All met DSM-IV criteria for current cocaine dependence, with positive urine toxicology screens upon entry into the inpatient treatment and research facility at the Connecticut Mental Health Center (CMHC). Exclusion criteria included DSM-IV dependence for any drug other than cocaine, alcohol or nicotine. Participants using prescribed medications or failing to meet health requirements were also ineligible. Further details on participant recruitment and selection criteria are provided in [Sinha et al. \(2003\)](#). All participants gave both written and verbal informed consent and the study was approved by the Human Investigation Committee of the Yale University School of Medicine.

2.1.1. Dose and pattern of cocaine use

Quantity and frequency of current drug use were documented using a 90 day time-line follow-back Substance Use calendar. This was adapted from the Form-90 ([Miller and Del Boca, 1994](#)) which has been shown to be a reliable instrument for assessing

self-reported drug use in alcoholic and drug abusing populations ([Tonigan et al., 1997](#); [Fals-Stewart et al., 2000](#)). Participants were requested to provide precise details of their daily drug consumption (including alcohol) for the 90 days prior to last use of cocaine. In cases where participants were only able to recall amount of money spent on a particular day, the cash amount was converted into crack cocaine quantities using a standard scale (i.e. \$10=1 bag of crack or 1/10th of a gram).

Frequency of consumption was determined by taking a median split of the number of days in each week participants used cocaine within the previous 90 days. Use of cocaine on 3 days per week or more represented high-frequency of use and consumption of less than 3 days per week represented low-frequency of use.

Although participants were initially categorized into frequency groups on the basis of their cocaine use, post hoc analysis indicated that the high-frequency cocaine users had also reported using significantly higher amounts of alcohol compared with the low cocaine users, despite no significant group differences in current alcohol dependence ([Table 1](#)). Both animal and human studies have shown the concomitant use of both drugs to result in higher levels of sensitization and toxicity compared with the effects of either drug alone ([Lepere and Charbit, 2002](#); [Busse and Riley, 2003](#)). As such, group differences were examined between high-frequency cocaine/alcohol users compared with low-frequency cocaine/alcohol users.

2.2. Procedures

Subjects were admitted to the Clinical Neuroscience Research Unit (CNRU) of the Connecticut

Table 1 Demographics & drug use.

	High-frequency cocaine abusers ($n=24$)	Low-frequency cocaine abusers ($n=30$)	p
Age	39.8 ± 5.7	35.5 ± 6.5	0.02
Years in education	4.3 ± 1.1	4.0 ± 0.7	0.30
% Male	71%	63%	0.56
% Currently alcohol dependent	58%	37%	0.11
Years of cocaine use	9.6 ± 6.2	8.3 ± 6.3	0.43
Years of alcohol use	12.8 ± 9.6	10.3 ± 8.8	0.34
Days of cocaine use per week (over past 90 days)	5.9 ± 1.6	1.8 ± 0.8	>0.0001
Total cocaine consumed in past 90 days (g)	71.9 ± 66.7	19.4 ± 17.1	0.001
Days of alcohol use per week (over past 90 days)	3.4 ± 2.7	1.3 ± 1.6	0.002
Total drinks consumed in past 90 days (no. of drinks)	322.0 ± 409.9	90.1 ± 131.6	0.01

Mental Health Center (CMHC) for 2-4 weeks of inpatient treatment and study participation. The CNRU is a locked inpatient treatment research facility with no access to alcohol or drugs and very limited access to visitors. Drug testing was conducted regularly to ensure drug abstinence. All laboratory procedures were conducted either at the Clinical Neuroscience Research Unit (CNRU) of the CMHC or the General Clinical Research Center (GCRC) of Yale/New Haven hospital, located one block away. All laboratory testing was conducted within 2-3 weeks of inpatient treatment. Details of treatment procedures are provided in [Sinha et al., 2003](#).

2.2.1. Imagery development and training

A guided imagery induction method was used in the present study to expose individuals to stress and drug-cues. The method involves 're-living' a recent stressful and drug-related personal event through a guided imagery and recall. During the second week of inpatient stay, participants took part in the script development session and imagery training. The script development session was designed to create three personalized scripts (stress, drug-cue and neutral) required for the guided imagery induction in the subsequent laboratory sessions. The three scripts for each subject were then recorded on an audiotape for guided imagery in the laboratory sessions. These procedures have been previously shown to reliably produce drug craving and physiological arousal in drug dependent individuals ([Sinha et al., 1999, 2000](#)). Full procedures for script development are described in [Sinha et al. \(2003\)](#).

In order to minimize individual variability, all participants received imagery and relaxation training session where they were also familiarized with the self-report scales. During the habituation and training session, an IV catheter was inserted into the antecubital vein of the participant's non-preferred arm in order to allow participants to become familiarized with IV insertion. No blood was drawn during training. A structured one session progressive relaxation procedure was conducted followed by imagery training procedures as outlined in our previous studies ([Sinha et al., 1999, 2003](#)).

2.3. Laboratory sessions

The three imagery conditions (stress, drug-cue and neutral) were conducted across three consecutive days. Conditions were randomized and counter-balanced across participants. Research staff who conducted the experimental sessions was blind to the stimulus condition presented on each day.

All participants were brought to the testing room by a research nurse at 0800 h and an IV catheter was inserted by 0815 h. A blood pressure (BP) cuff was placed around the participant's preferred arm and a pulse rate sensor placed on the forefinger of the participant's non-preferred arm. Self-reports of drug craving and mood were obtained immediately following set-up. Participants were then given 1 h adjustment and relaxation time. Baseline blood draws were obtained at -20 and -5 min prior to imagery presentation and baseline BP and pulse at -5 min prior to imagery. The imagery tapes were then presented through headphones for duration of approximately 5 min. During imagery, pulse rate was monitored continuously and BP taken at four intervals. Immediately following imagery, BP and pulse rate were taken, bloods were drawn and cocaine craving and anxiety assessments administered (timepoint+5). This was repeated periodically until 75 min after imagery presentation at 1100 AM (timepoints +15, +30, +45, +60, +75).

2.4. Laboratory measures

2.4.1. Subjective measures

Cocaine craving and anxiety. Extent of cocaine craving and anxiety were measured using a visual analogue scale starting at 0 (none at all) to 10 (more than ever). Ratings were taken at baseline, immediately following imagery (0 timepoint) and recovery (+5, +15, +30, +45 and +60).

2.4.2. Cardiovascular measures

Blood pressure. An SD-700 Monitor (IBS Corp, MA) with arm cuff was used to measure systolic and diastolic blood pressure at various timepoints. These were baseline, during imagery, immediately following imagery (0 timepoint) and recovery (+5, +15, +30, +45 and +60). Multiple blood pressure measures were averaged for the baseline and imagery periods.

Heart rate. A pulse sensor was attached to the forefinger of the participant's preferred hand and connected to the SD-700 Monitor. Readings were taken at multiple timepoints during baseline and during imagery, and at the following additional timepoints: immediately following imagery (0 timepoint) and recovery (+5, +15, +30, +45 and +60). Multiple pulse measures were averaged for baseline and imagery periods.

2.4.3. Biochemical measures

Twelve ml of blood were drawn at various timepoints in order to assess blood plasma levels of cortisol, prolactin and ACTH. Bloods were taken at

baseline, immediately following imagery (0 time-point) and recovery (+15, +30, +45, +60, +75). All blood samples were stored in three heparinized tubes. Prolactin was stored and centrifuged at room temperature; cortisol and ACTH were immediately placed on regular ice and spun in a 4-degree C centrifuge. Assays were conducted at Kreek Laboratories, Rockefeller University using standard radioimmunoassay procedures.

Although the original study included catecholamine responses as well, these measures were not available on all participants and therefore were not included in the current analyses.

2.5. Design and statistical analysis

A mixed factorial design was used with frequency of cocaine/alcohol use (high/low) as the between group factor and condition (stress, drug-cue and neutral imagery) and timepoint (repeated assessment during each laboratory session) as within group factors. All statistical analyses were performed using either S-PLUS software (Everitt and Rabe-Hesketh, 2001) or SPSS for Windows (version 12). In S-PLUS, linear mixed effect (LME) models were used to analyze the data, using the within group factors of condition (stress, drug-cue and neutral imagery) and timepoint (varying intervals) and the between group factor of frequency (high/low) as the fixed effect factors. Subjects were the random effect factor. Linear mixed effect models are particularly well suited when the design calls for repeated measurements within the same individual that can lead to positive correlations between measurements. Such models are also useful when there is missing data, as it prevents exclusion of subjects with missing data points (Everitt and Rabe-Hesketh, 2001). In order to control for individual baseline variability on each testing day, change from baseline (−5 timepoint) was used in the statistical analyses to assess biological, subjective and cardiovascular responses to stress, drug cue and neutral imagery exposure.

In SPSS, demographic and baseline drug use differences were assessed using *t*-tests with frequency of use as the between group factor. Chi-Square analyses were also used in order to assess group differences in categorical variables.

3. Results

3.1. Participant characteristics

Demographics and drug use for all groups are shown in Table 1. There were no significant group

differences for either number of years spent in education or gender. However, high-frequency cocaine/alcohol users were significantly older than low-frequency users ($p=0.02$). As expected, high-frequency users consumed cocaine ($p>0.0001$) more frequently per week and at significantly higher amounts ($p=0.001$) than low-frequency users. In addition, high-frequency users had consumed alcohol significantly more frequently ($p=0.002$) and at significantly higher amounts ($p=0.01$) than low-frequency users over the past 90 days. Route of cocaine administration was predominantly freebasing, with 94% of the participants smoking crack in both groups. The groups did not differ in rates of lifetime alcohol dependence (67% in high-frequency users and 60% in low-frequency users), lifetime history of major depression (67% in high-frequency users and 60% in low-frequency users) or post traumatic stress disorder (43% in both groups).

3.2. Baseline measures

In order to compensate for variability in baseline responses for each testing day, change from baseline was used in order to assess response to stress and drug-cues across groups. Table 2 shows baseline means for all measures.

3.3. Change from baseline across stress, drug-cue and neutral conditions

All data are presented in Figs. 1 and 2. As no significant group by timepoint interactions were obtained, the data represented in Figs. 1 and 2 are collapsed across timepoints to indicate more clearly the responses to stress, drug cue and neutral conditions in the two frequency groups. As condition and time point effects were previously described in the initial study (Sinha et al., 2003), these findings are not presented in Table 2.

3.4. Subjective measures

3.4.1. Craving: (Fig. 1(a))

A main effect of frequency was observed, [$F(1, 51)=8.9, p=0.004$], indicating that high-frequency users reported greater craving ratings across conditions as compared with low-frequency users. A significant 2-way frequency \times condition interaction was also obtained, [$F(2, 874)=9.2, p<0.0001$]. Simple effects analysis indicated that

Table 2 Showing means (standard deviations) for all measures at baseline.

Baseline measures	Stress (baseline)		Neutral (baseline)		Drug (baseline)		P
	High Fr	Low Fr	High Fr	Low Fr	High Fr	Low Fr	
<i>Subjective measures</i>							
Craving	0.6 ± 0.3	0.67 ± 0.3	0.69 ± 0.3	0.33 ± 0.2	0.81 ± 0.3	0.36 ± 0.2	0.32
Anxiety	0.98 ± 0.4	1.6 ± 0.4	0.98 ± 0.3	1.17 ± 0.3	1.27 ± 0.3	1.28 ± 0.4	0.83
<i>Cardiovascular measures</i>							
Heart rate (bpm)	65.2 ± 2.3	66.8 ± 1.6	65.6 ± 2.0	66.3 ± 1.5	63.5 ± 1.9	66.9 ± 1.6	0.66
SBP (mm/Hg)	119.1 ± 2.1	116.9 ± 2.5	118.4 ± 2.1	117.1 ± 2.3	118.3 ± 2.1	114.8 ± 1.9	0.35
DBP (mm/Hg)	71.5 ± 2.0	71.1 ± 2.1	71.5 ± 1.8	72.0 ± 2.0	73.3 ± 2.2	68.9 ± 2.0	0.54
<i>HPA axis measures</i>							
Cortisol (ug/dL)	11.6 ± 0.9	10.5 ± 0.5	12.2 ± 0.8	11.0 ± 0.5	10.6 ± 0.5	11.7 ± 0.6	0.44
ACTH (pg/mL)	22.3 ± 2.7	22.1 ± 2.2	23.7 ± 3.2	26.4 ± 2.8	19.4 ± 3.0	25.8 ± 2.7	0.70
Prolactin (ug/L)	7.9 ± 3.2	7.9 ± 3.4	8.7 ± 4.9	7.3 ± 3.0	8.1 ± 3.5	8.3 ± 4.2	0.69

Note. *p* values indicate group differences between high- and low-frequency cocaine users.

high-frequency users reported higher levels of craving compared with low-frequency users in both the stress ($p < 0.0001$) and drug-cue ($p < 0.002$) conditions.

3.4.2. Anxiety (Fig. 1(b))

A significant two-way frequency × condition interaction was obtained for anxiety ratings, [$F(2, 875) = 8.4, p = 0.0002$]. Consistent with self-

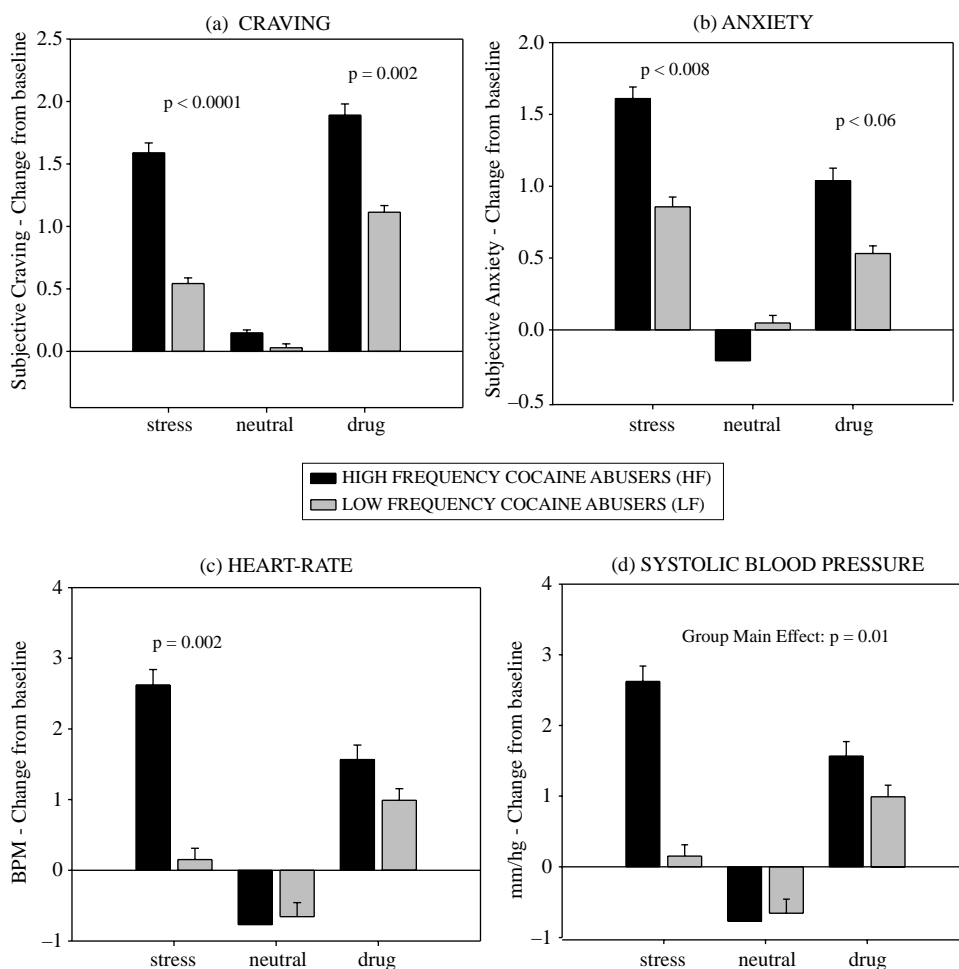


Figure 1 Group differences in subjective and cardiovascular measures across all three conditions. All data represent mean changes from baseline (standard error) and are averaged across repeated assessments.

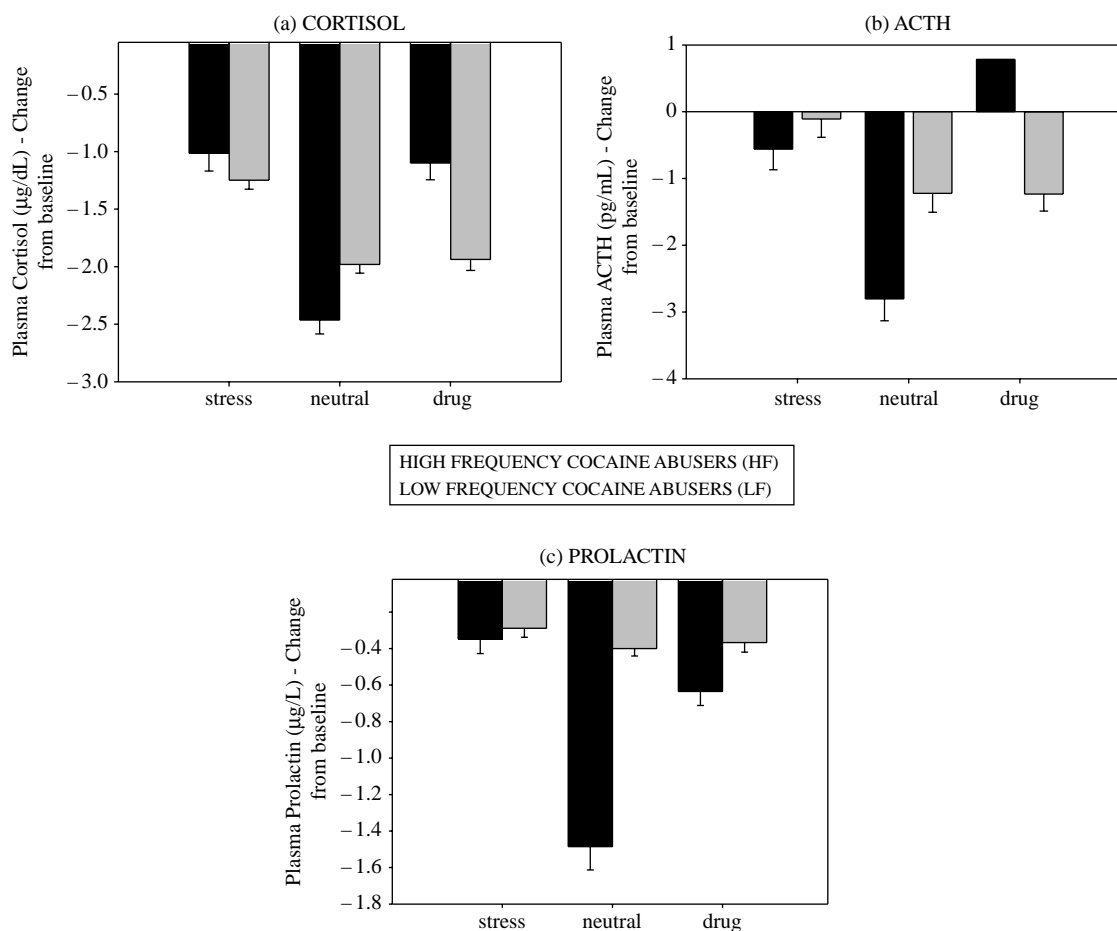


Figure 2 Group differences in HPA axis measures across all three conditions. All data represent mean changes from baseline (standard error) and are averaged across repeated assessments.

reported craving, simple effects analysis revealed that high-frequency users reported greater anxiety compared with low-frequency users in the stress condition ($p < 0.008$). Greater levels of anxiety in high compared with low-frequency users also approached statistical significance in the drug-cue condition ($p < 0.06$).

3.5. Cardiovascular measures

3.5.1. Heart-rate (Fig. 1(c))

A significant frequency \times condition interaction was observed in heart rate responses, [$F(2, 718) = 6.1, p = 0.002$]. Simple effects analysis indicated that high-frequency users demonstrated increased heart rate in the stress condition compared with low-frequency users ($p = 0.002$). Heart rate was also increased in the stress compared with the neutral condition in the high-frequency group ($p < 0.0001$), a condition effect that was not seen in the low-frequency group. Both groups showed a significant

increase in heart rate in the drug cue compared with neutral condition.

3.5.2. Blood pressure: (Fig. 1(d))

A main effect of frequency group was observed for SBP, [$F(1, 51) = 6.8, p = 0.01$] and approached significance for DBP, [$F(1, 51) = 1.9, p < 0.07$]. No significant interactions were observed for either measure. In both cases, high-frequency users showed greater blood pressure levels compared with low-frequency users.

3.6. Plasma measures

HPA axis measures of cortisol, ACTH and prolactin all showed decreased levels in the neutral imagery conditions, due to the diurnal drop in plasma levels over the course of the morning (Horrocks et al., 1990). In the high compared with low-frequency users, stressful and drug-cue stimuli attenuated the drop in plasma levels in all three markers.

3.6.1. Cortisol (Fig. 2(a))

A significant frequency \times condition interaction was observed for cortisol response, [$F(2, 812)=6.0, p<0.003$]. Simple effects analysis indicated that significantly increased levels of plasma cortisol were seen in the stress as compared to the neutral condition in both the high-frequency users ($p<0.0001$) and low-frequency users ($p<0.002$). However, in the drug-cue relative to neutral condition, increased cortisol levels were observed only in the high-frequency group ($p<0.0001$), with the between group differences in drug cue related cortisol responses only approaching significance ($p=0.07$).

3.6.2. ACTH (Fig. 2(b))

Similar to cortisol, a significant frequency \times condition interaction was also observed for ACTH responses, [$F(2, 802)=3.9, p=0.02$]. Simple effects analysis indicated that significantly increased levels of plasma ACTH were seen in the stress compared with neutral condition of both high-frequency users ($p=0.01$) and low-frequency users ($p=0.03$). However, in the drug-cue condition this elevation of ACTH was again only seen in the high-frequency users ($p<0.0001$).

3.6.3. Prolactin (Fig. 2(c))

A significant frequency \times condition interaction was seen for prolactin responses, [$F(2, 810)=9.2, p<0.0001$]. Simple effects analysis indicated that plasma prolactin levels were significantly higher in the stress ($p<0.0001$) and drug-cue ($p<0.0001$) compared with neutral conditions in high-frequency users but not in low-frequency users.

4. Discussion

Findings from the current study provide evidence that recent frequency of cocaine and alcohol use in cocaine dependent individuals is associated with significant differences in stress-induced and drug cue-induced cocaine craving, anxiety, cardiovascular and HPA responses when compared to responses with neutral imagery exposure. Notably, high-frequency abusers demonstrated significantly higher levels of self-reported craving and anxiety in both the stress and drug-cue conditions compared with low-frequency users. This increase in subjective response was also seen alongside a significantly enhanced HPA-axis response and a significantly increased cardiovascular response in the stress condition. The findings are consistent with our hypothesis that high frequency of recent

drug use enhances craving and subjective and biological arousal responses to emotional stress and to drug-related cues in the environment. Furthermore, these differences between frequency groups were not attributable to higher frequency of co-morbid psychiatric illnesses, such as PTSD or major depression, as rates of these disorders did not differ between the two severity groups.

Previous preclinical studies have shown that escalation in drug consumption compared with stable, low levels of intake increases the hedonic set-point (Ahmed and Koob, 1998, 1999) as well as the reinstating effects of stress (Ahmed et al., 2000) and drug self-administration (Mantsch et al., 2004). This enhanced motivation to administer cocaine following higher dosing regimens and in the face of stress, is similar to the current findings where higher levels of cocaine and alcohol intake increases craving following exposure to personal stressors or to drug related cues in abstinent cocaine dependent individuals. In recent findings, we have also shown that stress-induced craving predicts alcohol and cocaine relapse in dependent individuals (Breese et al., 2005; Sinha et al., 2004). These data extend these findings and suggest that higher levels of drug use may potentially increase susceptibility to relapse by enhancing vulnerability to stress and to drug cues in the environment.

Along with enhanced drug craving and subjective anxiety, high-frequency cocaine/alcohol abusers demonstrated a hypersensitivity of the HPA axis. Clear increases in ACTH, cortisol and prolactin were observed in both the stress and drug-cue conditions as compared to the neutral condition in high-frequency users. Conversely, low-frequency users did not show such enhanced responses to drug-related cues as compared to the neutral condition in any of the three measures (Fig. 2(a)-(c)). Furthermore, no significant stress-induced prolactin response was seen in the low-frequency group (Fig. 2(c)). These findings demonstrate a facilitated neuroendocrine response to stress and drug-cues in higher compared with lower-frequency cocaine/alcohol abusers.

Substantial previous research has indicated that acute cocaine and alcohol administration activates the HPA axis and increases HPA responses (Kreek and Koob, 1998; Koob and Le Moal, 2001; Sinha, 2001). As such, repeated chronic cocaine and chronic alcohol exposure has been conceptualized as a chronic stress state (Zhou et al., 1996; Sarnyai et al., 1998) that involves neuroadaptations in brain reward and stress systems such that these allostatic changes modify the susceptibility to drug seeking behavior (Koob et al., 2004). Within this

framework, our findings are consistent with pre-clinical data which has identified enhanced HPA responses to novel stressor(s) following chronic periods of stress in rats (Andres et al., 1999; Marti et al., 1994).

Current findings also indicate that significant group differences in cortisol and ACTH response were only observed in the drug-cue conditions. Whilst the drug related imagery scripts did not include stressful context information, it is still possible that any form of drug-cue exposure might be perceived as somewhat challenging and stressful to abstinent treatment-seeking cocaine users (Sinha et al., 2003). Current findings indicate that while the drug-cue condition may have been stressful enough to elicit an increased adrenocortical response in high-frequency users, it was not adequate to produce a comparable response in low-frequency users. Most importantly this may reflect a dose-related, sensitized HPA axis response in high-frequency abusers of cocaine and alcohol.

The mechanisms underlying enhanced HPA response in higher frequency drug abusers may be understood in terms of dose-related dopaminergic (DA) alterations in the limbic-striato-pallidal circuitry (Battaglia and Napier, 1998; Sizemore et al., 2000; McFarland and Kalivas, 2001). For example, prior studies have shown that either increasing cocaine dose or the number of injections administered elevates levels of extracellular DA (Maison-neuve et al., 1995; Sorg et al., 1997). Furthermore, dopamine (D1) antagonists have been shown to block enhanced behavioral sensitization to cocaine and increased levels of corticosteroid shown following binge cocaine administration in laboratory animals (Spangler et al., 1997). Findings such as these support the notion that DA systems may exert both direct and indirect effects on the functional activity of the HPA axis (Sher, 2003; Posener et al., 1994).

The interactive effects of DA transmission and HPA functioning has also been highlighted as a mechanism underpinning the cross-sensitization effects of stress and cocaine (Prasad et al., 1995; see Piazza and Le Moal, 1998 for review) as well as stress and alcohol (Phillips et al., 1997). In the current study, the increase in subjective craving alongside increased anxiety and HPA activation in high-frequency users supports the notion that the cross-sensitization of cocaine and stress responses involve common neural pathways (Piazza and Le Moal, 1997; Goeders, 1998; Sinha, 2001; Sinha et al., 2003), such that cocaine frequency-related changes in DA mesolimbic function are associated with enhanced ACTH and cortisol responses to stress and to drug-cues.

In the current sample, enhanced stress and drug cue-related HPA responses in high-frequency users may be exacerbated by concomitant alcohol consumption. In rats, ethanol administration has been shown to increase DA transmission in the NAC (Yoshimoto et al., 1992; Weiss et al., 1993) as well as increase stress-induced cortisol and ACTH hyperresponsiveness in rats prenatally exposed to alcohol (Weinberg et al., 1995, 1996; Gabriel et al., 2000). Although recent human studies have shown alcohol to suppress stress and cue-induced cortisol responses (Junghanns et al., 2005), the by-product of both cocaine and alcohol (cocaethylene) is known to have psychostimulant properties (Knackstedt et al., 2002). It is therefore possible that the combined cocaine and alcohol effects may increase stress and cue-induced HPA responsivity.

High-frequency users showed significant increases in prolactin levels in the stress and drug-cue conditions compared with neutral. These responses were not seen in the low-frequency users. Increases in cortisol-induced DA reduce prolactin via its actions on prolactin inhibiting factor (Reymond and Porter, 1985; Ben-Jonathan and Hnasko, 2001). As such, it would be expected that a depleted DA system in chronic cocaine/alcohol users might culminate in a hyperprolactinemic effect or, as found in the current study, an enhanced response during a stress and/or drug-cue induced challenge. This is supported by animal data, where repeated daily cocaine-pretreated rats demonstrate a suppressed dopamine response following systemic cocaine challenge, suggesting DA depletion (Sorg et al., 1997). Notably, withdrawal from chronic alcohol intoxication has also been associated with a reduction in DA neurotransmission (Rossetti et al., 1992, 1999; Weiss et al., 1996).

In keeping with findings from the biochemical and self-report data, cardiovascular markers of general arousal were also shown to vary with the frequency of cocaine and alcohol use. High-frequency users demonstrated significantly higher heart rate in the stress condition compared with low-frequency users. They also demonstrated significantly higher heart rate in the stress condition compared with the neutral condition. This effect of condition was not demonstrated in low-frequency users (Fig. 1(c)). High-frequency users also showed significantly increased SBP response compared with low-frequency users, however, this was observed across all three conditions (Fig. 1(d)).

Prior human research has shown that the acute and chronic effects of both cocaine and alcohol can modulate arterial pressure and heart rate (Zakhari,

1997; Kollins and Rush, 2002). Whilst low acute doses of cocaine have a tendency to increase vasoconstriction, blood pressure and heart rate, higher doses have been found to depress ventricular function (Schindler, 1996). In the case of alcohol, heavy chronic use has been associated with cardiovascular disorders including hypertension and strokes (see Beilin and Puddey, 1992 for review). As such, the general arousal response seen in high-frequency users is consistent with the direct effects of cocaine and/or alcohol on the cardiovascular system, as well as the more indirect influence of these drugs via monoaminergic brain circuitry (Tella et al., 1993). The cardiovascular hyperresponsivity seen in the high compared with low-frequency drug users may also be due to increased levels of cocaethylene, as animal studies have shown that cocaine and alcohol in combination result in greater cardiac toxicity than either substance alone (Mueller et al., 1997; Wilson et al., 2001).

The present study indicates clear stress and drug-cue related hyperarousal in high compared with low-frequency cocaine/alcohol abusers. However, the design of the present study did not allow for the direct examination of the separate and interactive effects of cocaine and alcohol. Future research may benefit from clarifying the contribution of both drugs separately on stress-induced craving and associated arousal as a comparison for co-morbid cocaine and alcohol abusers.

Overall findings indicate that high-frequency cocaine/alcohol abusers are clearly at greater risk of demonstrating enhanced stress-induced and cue-induced craving and generalized arousal response as compared with low-frequency abusers. Such sensitized responses are consistent with previously reported effects of cocaine on drug seeking behavior and may contribute to the mechanism underlying the common clinical observations that treatment seeking individuals with higher frequency of drug use show an increased vulnerability to drug-seeking behavior and relapse.

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