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Stress system changes associated with marijuana dependence may increase craving for alcohol and cocaine

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Abstract

Objective—To date, little research exists defining bio-behavioral adaptations associated with both marijuana abuse and risk of craving and relapse to other drugs of abuse during early abstinence.

Method—Fifty-nine treatment-seeking individuals dependent on alcohol and cocaine were recruited. Thirty of these individuals were also marijuana (MJ) dependent; 29 were not. Twenty-six socially drinking healthy controls were also recruited. All participants were exposed to three 5-min guided imagery conditions (stress, alcohol/cocaine cue and relaxing), presented randomly, one per day across three consecutive days. Measures of craving, anxiety, heart rate, blood pressure, plasma adrenocorticotrophic hormone and cortisol were collected at baseline and subsequent recovery time points.

Results—The MJ-dependent group showed increased basal anxiety ratings and cardiovascular output alongside enhanced alcohol craving and cocaine craving, and dampened cardiovascular response to stress and cue. They also demonstrated elevated cue-induced anxiety and stress-induced cortisol and adrenocorticotrophic hormone levels, which were not observed in the non-MJ-dependent group or controls. Cue-related alcohol craving and anxiety were both predictive of a shorter number of days to marijuana relapse following discharge from inpatient treatment.

Conclusions—Findings provide some support for drug cross-sensitization in terms of motivational processes associated with stress-related and cue-related craving and relapse.

Keywords

marijuana; stress; drug cue; cocaine craving; alcohol craving; relapse

INTRODUCTION

In 2010, marijuana was shown to represent the illicit drug with the highest rate of past year dependence or abuse in the USA, with the number of recent adult initiates increasing from 49 000 to 247 000 between 2009 and 2010 [Substance Abuse and Mental Health Services Administration (SAMHSA), 2011]. This increase in prevalence may reflect the drugs past

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CONFLICT OF INTEREST

The authors declare that they have no competing financial interests pertaining to the aims and results of this study.

public perception of being a comparatively "soft option" in terms of medical and social consequences (Rafael *et al.*, 2005). However, marijuana has also been recognized as a "gateway" drug for more severe substance misuse (Manzanares *et al.*, 2004), which means that individuals who abuse marijuana are much more likely to be at risk for co-abusing other licit and illicit drugs (Agrawal *et al.*, 2004). This, coupled with the fact that the endocannabinoid (eCB) system may represent a neural substrate pivotal to the regulation of core stress system adaptations (Hill and Tasker, 2012; Carvalho and Van Bockstaele, 2012; Häring *et al.*, 2012) and hence the reinforcing effects of other substances (González-Cuevas *et al.*, 2007; Fox and Sinha, 2009), compounds risk of poor outcome during early marijuana abstinence.

Extensive basic science and clinical studies have shown both stimulant and alcohol dependence to reflect a chronic stress state characterized by a tonic up-regulation of autonomic markers as well as extra-hypothalamic corticotropin-releasing factor (CRF) and Norepinephrine (NE) neural circuits (Wand and Dobs, 1991; Ingjaldsson *et al.*, 2003; Thayer *et al.*, 2006; Shively *et al.*, 2007; Fox *et al.*, 2008; Sinha *et al.*, 2009). In response to stressors, including drug cues, blunted cardiovascular and Hypothalamic-Pituitary-Adrenal (HPA) axis responses are documented in alcoholics (Breese *et al.*, 2005; Fox *et al.*, 2007; Sinha *et al.*, 2011), and both dampened and sensitized HPA responses have been recorded in cocaine-dependent men and women (Waldrop *et al.*, 2010; Fox *et al.*, 2006, Fokos and Panagis, 2010). Most importantly, these stress system adaptations are robustly associated with the negative reinforcing aspects of addiction, including sensitized anxiety and negative emotion (Fox and Sinha, 2009) as well as increased craving and relapse in dependent populations (Adinoff *et al.*, 2005; Sinha *et al.*, 2006, 2011; Back *et al.*, 2010; Breese *et al.*, 2011) and risk of dependence in vulnerable populations (Sorocco *et al.*, 2006; Dai *et al.*, 2007).

As widespread support exists for the role of the eCB system in regulating HPA stress system outflow (Berrendero and Maldonado, 2002; Page *et al.*, 2007; D'Souza *et al.*, 2009; Ranganathan *et al.*, 2009), and stress system dysregulation is integral to addiction outcome, the precise nature of chronic marijuana use on stress system adaptations needs to be systematically defined in ecologically relevant polydrug-dependent individuals. In addition, eCB signaling within control regions of the brain including the prefrontal cortex, amygdala and hypothalamus (Hill and Tasker, 2012) suggests that chronic marijuana use may potentially impinge upon a range of regulatory behaviors associated with incentive salience and motivation (Chaperon and Thiébot, 1999; Spano *et al.*, 2004; McGregor *et al.*, 2005; Fattore *et al.*, 2007). Chronic marijuana use may therefore potentially increase craving for other drugs of abuse via eCB-mediated changes within core regulatory stress systems. As such, the extent to which co-dependence on marijuana can potentially induce additional stress system neuroadaptations serving to increase relapse vulnerability for other drugs of abuse also needs to be assessed.

From a general perspective, a broad range of studies from both the clinical and experimental fields have shown marijuana to impinge upon stress system function. For example, research indicates that acute administration of cannabidiol (a major component of cannabis devoid of psychotomimetic effects) can induce anxiolytic and anti-psychotic effects, as well as reduce fear conditioning and attenuate autonomic and behavioral consequences of restraint stress (Resstel *et al.*, 2008; Fusar-Poli *et al.*, 2009; Gomes *et al.*, 2011; Granjeiro *et al.*, 2011). Conversely, high doses of exogenous eCB agonists and antagonists, including Δ9-tetrahydrocannabininol [Delta (9)-THC] and SR141716 (Rimonabant) have been shown to provoke high anxiogenic effects in humans (Zuardi *et al.*, 1982; Gomes *et al.*, 2011). In terms of chronic use in humans, there also exists a wealth of clinical and anecdotal reports indicating that cannabis precipitates episodes of depression, anxiety and psychosis (Rafael *et*

al., 2005), that trauma is associated with high rates of cannabis use (Vlahov *et al.*, 2004; Bonn-Miller *et al.*, 2011) and further that unsuccessful quit attempts are associated with high levels of stress (Rooke *et al.*, 2011)

In the current study, therefore, we assess the response to stress and drug cue, also known to provoke stress system circuitry (Sinha *et al.*, 2003; Fox *et al.*, 2007, 2008) in a group of early abstinent substance abusers who meet current dependence criteria for marijuana, with substance abusers who are not dependent on marijuana and a group of healthy control volunteers. We employ an identical paradigm to our previous studies, where we have shown that exposure to both stressful personalized guided imagery and drug cue-related imagery reliably provokes dissociable stress mechanisms across a wide range of psychobiological domains within both substance abusers and healthy controls (Fox *et al.*, 2007; 2008; Hyman *et al.*, 2007; Chaplin *et al.*, 2008, 2010; Sinha *et al.*, 2009; 2011; Bergquist *et al.*, 2010). As chronic marijuana use may be associated with the dysregulation of core stress systems underlying the negative reinforcing effects of several drugs of abuse, we hypothesize that co-morbid marijuana dependence in poly-substance abusers will increase stress-induced craving for cocaine and alcohol as well as exacerbate subjective, cardiovascular and HPA axis changes that contribute to relapse vulnerability.

METHODS

Participants

All participants were recruited from the community via advertisements posted on the Internet and in local area newspapers. Fifty-nine co-morbid individuals who met DSM-IV criteria for current alcohol dependence and current cocaine dependence were admitted to the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center for 4–6 weeks of inpatient treatment and study participation. Thirty of these individuals additionally met current dependence criteria for marijuana. Twenty-six socially drinking healthy controls (HCs) were also recruited. All were light social drinkers (25 drinks or less per month) as classified by the Cahalan Quantity Frequency Variability Index (Cahalan *et al.*, 2012). A socially drinking group, rather than drug-naïve comparison group, was used in the current design to allow more thorough examination of the stress-related craving state in both a substance-dependent and non-dependent group. Previous findings using our current imagery paradigm have shown that both stress-related and cue-related imageries induce alcohol craving in light social drinkers (Chaplin *et al.*, 2008; Fox *et al.*, 2008; Sinha *et al.*, 2009). Substance-abusing individuals who met current DSM-IV criteria for dependence on another psychoactive substance other than nicotine were excluded. Healthy controls with current or past diagnoses of any substance dependence were also excluded. All participants were excluded if they were on medications for medical or psychiatric problems. All subjects underwent a thorough medical evaluation to ensure good physical health. Study procedures were approved by the Human Investigation Committee of the Yale University School of Medicine.

Design

A mixed repeated measures design was used. The Between Group factor was *Drug Group* [marijuana-dependent substance abusers (MJ), non-marijuana-dependent substance abusers (non-MJ) and HCs]. The Within Group factors were *Imagery Condition* (stress, drug cue and relaxing) and *Time point* (varying levels for each assessment).

The stress, cue and relaxing imagery conditions were presented on consecutive days with only one stimulus presentation per day. Imagery condition was assigned randomly and counterbalanced across subjects. Staff and subjects were blind to the imagery condition.

Procedures

All substance-abusing patients were admitted to the CNRU of the Connecticut Mental Health Center for study participation. The CNRU is a locked inpatient treatment research facility with no access to alcohol or drugs and a limited and monitored access to visitors. Urine and breathalyzer testing is conducted every three days to ensure drug abstinence. As subjects were treatment seeking, they participated in 4 weeks of group counseling treatment for cocaine and alcohol addiction using the standard drug counseling manual as a guide (Mercer and Woody, 1992). During the first week of inpatient stay, substance-abusing participants were administered structured baseline assessments measuring psychiatric and substance use history. In the second week, scripts for the guided imagery induction were developed as described in previous studies (Sinha *et al.*, 2003; Bergquist *et al.*, 2010). All laboratory sessions were conducted approximately 23 days after admission to allow for normalization of neurobiological changes associated with acute cocaine and alcohol abstinence.

Healthy Control participants were admitted to the Hospital Research Unit of the Yale Clinical Center of Investigation located a block away at Yale/New Haven hospital for a 4-day stay. Within that time, they were required to remain on the hospital unit, within a similar controlled environment to that of the substance-abusing participants. They were given a similar diet, and allowed limited access to visitors and limited staff-accompanied smoke breaks. Baseline demographics, psychiatric and substance use assessments as well as imagery scripts were prepared prior to their admission to the Hospital Research Unit. All social drinking controls were exposed to an alcohol-related script for the drug cue condition.

Imagery script development (for presentation in the laboratory sessions)—In the second week, scripts for the guided imagery induction were written on the basis of methods developed by Lang and colleagues (Lang *et al.*, 1980; Miller *et al.*, 1987) and further adapted in our previous studies (see Sinha *et al.*, 2003, for full details). Briefly, the stress imagery script was based on subjects' description of a recent personal stressful event that was experienced as "most stressful" (determined by ratings on a 10-point Likert scale where 1 = "not at all stressful" and 10 = "the most stress they felt recently in their life"). Only situations rated as 8 or above were accepted as appropriate for script development. The stress imagery scripts did not include scenarios either relating to or culminating in drug use. The drug cue imagery script was developed by having subjects identify a recent situation that included alcohol-related and cocaine-related stimuli and resulted in subsequent substance use (i.e., being at a bar or watching others smoke crack and drink alcohol). Drug-related scenarios did not include scenarios that involved stressful events such as being arrested. All social drinkers were required to provide alcohol-related scripts. A relaxing imagery script was developed from the subjects' description of a personal non-drug-related relaxing situation. All scripts were then recorded onto an audiotape to be played in the laboratory sessions. All scripts were recorded by the same female clinician, who was independent to the research study.

Habituation and imagery training session—On a day prior to the laboratory sessions, subjects were brought into the testing room to acclimatize themselves to specific aspects of the study procedures, including the stress of intravenous catheter insertion as well as the subjective rating forms and training in relaxation and imagery procedures. Details on the imagery script development procedures and the imagery and relaxation training procedures have been described previously (Sinha *et al.*, 2003; Sinha, 2008).

Laboratory sessions—Each subject was tested in the same room for the training and three laboratory sessions. On each day of the laboratory session, subjects abstained from

breakfast and were allowed a smoke break at 7:30 AM in order to reduce the effects of nicotine withdrawal. Subjects were then taken into the testing room at 8:00 AM. After settling into a sitting position in a hospital bed, a heparin-treated catheter was inserted by the research nurse in the antecubital region of the subject's non-preferred arm, in order to periodically obtain blood samples. A blood pressure cuff was placed on the subject's preferred arm to monitor blood pressure, and a pulse sensor was placed on the subject's forefinger to obtain a measure of pulse. This was followed by a 45-min adaptation period during which time subjects were asked to practice relaxation. Following the adaptation period, baseline blood was drawn, heart rate and blood pressure were taken and alcohol craving and anxiety rating scales were administered. At 9:00 AM, subjects were provided headphones and given the following instructions for the imagery procedure: "Close your eyes and imagine the situation being described, 'as if' it were happening right now. Let your body and mind get completely involved in the situation, doing what you would do in the real situation. Stop imagining when you hear the voice on the tape tell you to stop imaging." The length of each script was exactly 5 min. Heart rate and blood pressure were continuously monitored during the imagery period. All measures were collected immediately following imagery exposure and again at regular 15-min recovery intervals until 1 h after imagery. If the visual analog scale (VAS) ratings of anxiety remained above baseline levels following the final time point, they were taken through another series of relaxation procedures until their ratings returned to baseline levels. After the last assessment at 10:35 AM, the subject was disconnected from the apparatus and served breakfast.

All subjective, cardiac and blood measures were taken at baseline (−5), immediately following imagery (0 time point) and six recovery time points (+5, +10, +15, +30, +45 and +60 min after imagery).

Laboratory assessments—Subjective measures. Craving: The desire for using alcohol, cocaine and nicotine was assessed using three separate VASs anchored from 1 to 10, where 1 = "not at all" and 10 = "extremely high."

Anxiety: Participants were required to rate how "tense, nervous or jittery" they felt using a similar 10-point VAS anchored as above.

Physiological measures: A Critikon Dinamap 120 Patient Monitor was used to assess blood pressure. A pulse sensor was attached to the subject's finger and connected to the Dinamap Monitor to provide a continuous measure of pulse.

Blood samples (HPA markers): Twelve milliliters of blood were collected at each time point in order to assess plasma adrenocorticotrophic hormone (*ACTH*) and *Cortisol*. Blood samples were collected in heparinized tubes. All tubes were placed on ice immediately after drawing. Within 30 min of collection, all blood samples were centrifuged at 4 °C, and the plasma was pooled and aliquoted for *ACTH* and *Cortisol* assays. Blood samples for HPA axis measures were stored at −70 °C and processed at the Yale Center for Clinical Investigation Core Laboratories using standard radioimmunoassay procedures.

Statistical analysis

Linear Mixed Effect Models (Laird and Ware, 1982) were implemented to analyze the baseline and response data, using SPSS (version 19; SPSS Inc., Chicago, IL, USA). Between-subjects factor of Drug Group (MJ, non-MJ and HC) and within-subjects factors of Condition (stress, cue and relaxing) and Time point (varying levels) were the fixed effects, and Subjects was the random effect. In order to account for baseline variability across each testing day, change from baseline was used for all measures in order to assess response to the imagery exposure. Bonferroni tests were used as adjustments for all multiple

comparisons. Pearson's product moment correlational analysis and standard regression models were used for extended analysis in the MJ group, in order to ascertain relationships between marijuana use, anxiety, craving and relapse. Area under the curve response data were used for these analyses.

RESULTS

Participants

In the current sample of participants, the healthy controls were younger and spent a greater number of years in education compared with both of the substance abuse groups. Both the MJ and non-MJ substance-abusing groups were well matched in terms of drug use and demographics, with the exception of race and age. As expected, the MJ group used a significantly greater amount of marijuana in the 3 months prior to treatment entry; they were also older and comprised a higher number of African Americans compared with the non-MJ group. As such, age and race were treated as covariates for all analyses (Table 1).

Baseline findings

Subjective—Significant basal variations in Drug Group were observed with regard to *Nicotine Craving* [$F(2, 59) = 4.9$, $p = 0.01$, *without covariates;* $F(2, 54) = 3.9$, $p < 0.03$, *with covariates*], where both the MJ group and non-MJ group reported higher ratings of nicotine craving compared with the healthy controls. Following the inclusion of covariates, only the MJ group demonstrated significantly higher basal ratings of *Nicotine Craving* compared with the controls (MJ dep >HC, $p = 0.04$; non-MJ >HC, $p < 0.03$, *without covariates*; MJ dep >HC, $p = 0.04$; non-MJ >HC, $p = ns$, *with covariates*).

A significant main effect of Drug Group for *Anxiety* [$F(2, 82) = 3.7$, $p = 0.03$, *without covariates;* $F(2, 76) = 3.5$, $p < 0.04$, *with covariates*] also indicated that the MJ group reported higher ratings of baseline *Anxiety* compared with controls (MJ dep >HC, $p = 0.03$, *with and without covariates*).

Cardiovascular—At baseline, the MJ group showed enhanced heart rate and blood pressure compared with the healthy controls. This remained a trend following the inclusion of covariates. A main effect of Drug Group was observed for basal *Heart rate* [$F(2, 82) = 4.6$, $p = 0.01$, *without covariates;* $F(2, 77) = 3.1$, $p = 0.05$, *with covariates*], indicating that the MJ group demonstrated higher heart rate compared with controls ($p = 0.01$ *without covariates;* $p = 0.07$ *with covariates*).

A main effect of Drug Group was also observed for basal Systolic Blood Pressure (SBP) [$F(2, 82) = 3.0$, $p = 0.05$, *without covariates;* $F(2, 77) = 2.6$, $p = 0.07$, *with covariates*] again showing a trend for higher levels of SBP in the MJ group compared with the healthy controls ($p = 0.07$ *without covariates;* $p = 0.08$ *with covariates*). A main effect of Drug Group for Diastolic Blood Pressure (DBP) [$F(2, 82) = 4.4$, $p < 0.02$, *without covariates;* $F(2, 77) = 3.1$, $p = 0.05$, *with covariates*] again showed that the MJ group had increased basal DBP compared with controls ($p = 0.01$ *without covariates;* $p = 0.10$ *with covariates*).

Response to imagery

Subjective rating scales

Alcohol craving: A significant Drug Group × Imagery Condition interaction was observed for *Alcohol Craving* [$F(4, 1649) = 3.2$, $p = 0.01$, *without covariates;* $F(4, 1609) = 3.3$, $p = 0.01$, *with covariates*], where the MJ group reported significantly higher ratings of *Alcohol Craving* following exposure to stress ($p = 0.01$ *without covariates;* $p = 0.02$ *with covariates*)

and cue ($p = 0.05$ *without covariates;* $p < 0.08$ *with covariates*) compared with the control group (Figure 1a).

In addition, increased *Alcohol Craving* was reported following exposure to stress in both the MJ and non-MJ groups, compared with relaxing imagery ($p < 0.0001$ in all cases with and without covariates). This stress-induced increase was not observed in the control group. All three groups reported significant cue-related craving compared with the relaxing condition (C >N: $p < 0.0001$ in MJ and non-MJ; $p = 0.01$ in healthy controls *with and without covariates*).

Cocaine craving: A significant Drug Group Imagery Condition interaction was observed for *Cocaine Craving* [$F(4, 1430) = 10.0$, $p < 0.0001$, *without covariates;* $F(4, 1389) = 9.9$, $p < 0.0001$, *with covariates*], where the MJ group reported significantly higher ratings of cocaine craving following exposure to cue compared with the healthy controls ($p < 0.0001$ *with and without covariates*).

In addition, increased *Cocaine Craving* was reported following exposure to stress in both the MJ and non-MJ groups, compared with relaxing imagery ($p < 0.0001$ in all cases with and without covariates). This stress-induced increase was not observed in the control group. All three groups reported significant cue-related craving compared with the relaxing condition (C >N: $p < 0.0001$ in MJ and non-MJ; in healthy controls: $p = 0.004$ *without covariates;* $p = 0.003$ *with covariates*) (Figure 1b)

Nicotine craving: A main effect of Drug Group [$F(2, 60) = 9.9$, $p < 0.0001$, *without covariates;* $F(2, 56) = 5.5$, $p = 0.006$, *with covariates*] indicated that both the MJ group and non-MJ group reported significantly higher *Nicotine Craving* compared with the healthy controls (MJ >HC, $p = 0.007$; non-MJ >HC, $p < 0.0001$, *without covariates*. MJ >HC, $p < 0.03$; non-MJ >HC, $p < 0.02$, *with covariates*).

In addition, a significant Drug Group × Imagery Condition interaction was observed [$F(4, 1195) = 4.8$, $p = 0.001$, *without covariates;* $F(4, 1155) = 5.2$, $p\ 0.0001$, *with covariates*], where the significantly higher *Nicotine Craving* was reported in the stress compared with the cue condition in the non-MJ group ($p = 0.007$ *with and without covariates*). This cue-related difference was not observed in either the MJ group or the healthy controls.

Both the MJ group and the non-MJ group reported significantly higher *Nicotine Craving* following exposure to stress compared with the healthy controls (MJ >HC, $p = 0.002$; non-MJ >HC, $p < 0.0001$, *without covariates*. MJ >HC, $p = 0.005$; non-MJ >HC, $p = 0.002$, *with covariates*) and also following exposure to cue compared with the healthy controls (MJ >HC, $p = 0.003$; non-MJ >HC, $p = 0.001$, *without covariates*. MJ >HC, $p = 0.01$; non-MJ >HC, $p = 0.03$, *with covariates*).

The MJ group also demonstrated a stress-related and cue-related increase in *Nicotine Craving* compared with their intra-individual relaxing condition ($p < 0.0001$, in all cases *with and without covariates*). This stress-related and cue-related increase in Nicotine Craving was also observed in the non-MJ group ($p < 0.0001$, *without covariates;* $p = 0.05$, *with covariates*) but was not observed in the healthy control group.

Anxiety: A significant Drug Group × Imagery Condition × Time point interaction was observed for *Anxiety* [$F(24, 1649) = 1.7$, $p = 0.02$, *without covariates;* $F(24, 1609) = 1.6$, $p = 0.03$, *with covariates*]. This interaction reflects the fact that following exposure to cue the MJ group reported significantly higher ratings of *Anxiety* compared with the healthy control group ($p = 0.001$, *without covariates;* $p = 0.002$, *with covariates*) and the non-MJ group ($p =$

0.02, *without covariates; p* <0.03, *with covariates*) at the +82 recovery time point. This was also observed at the +90 time point (MJ >HC: *p* <0.0001; MJ >non-MJ, *p* = 0.002, *without covariates*; MJ >HC: *p* <0.0001; MJ >non-MJ, *p* = 0.004, *with covariates*) as well as the +105 time point (MJ >HC: *p* = 0.01; MJ >non-MJ, *p* = 0.02, *without covariates*; MJ >HC: *p* = 0.01; MJ non-MJ, *p* <0.04, *with covariates*).

Additionally, the MJ group reported significantly higher ratings of *Anxiety* following exposure to the cue imagery condition compared with their ratings following the intra-individual relaxing condition at the +82 time point (C >N, *p* <0.0001 *with and without covariates*). This was also the case at the +90 recovery time point (C >N, *p* = 0.002, *without covariates; p* = 0.001, *with covariates*) and the +105 recovery time point (C >N, *p* <0.04, *with and without covariates*). These cue-induced increases in anxiety during recovery were not observed in either the healthy controls or the non-MJ group.

Following exposure to stress imagery, the MJ group also reported significantly higher *Anxiety* ratings compared with the healthy control group at the +82 recovery time point (*p* = 0.001, *without covariates; p* <0.02, *with covariates*) (Figure 2a–c).

Cardiovascular measures

Heart rate: A main effect of Drug Group was observed [*F*(2, 82) = 5.7, *p* = 0.005, *without covariates; F*(2, 3334) = 12.0, *p* <0.0001, *with covariates*], indicating that the MJ group demonstrated a significantly decreased heart rate across all three imagery conditions compared with both the healthy controls (*p* <0.02 *without covariates; p* <0.0001 *with covariates*) and the non-MJ group (*p* = 0.01 *without covariates; p* <0.0001 *with covariates*) (Figure 3a).

Systolic blood pressure: A main effect of Drug Group was also observed for *SBP* [*F*(2, 82) = 3.3, *p* = 0.04, *without covariates; F*(2, 354) = 5.1, *p* = 0.006, *with covariates*], indicating that the MJ group demonstrated significantly lower SBP compared with the non-MJ group across all three imagery conditions (*p* <0.04 *without covariates; p* = 0.008 *with covariates*).

A significant Drug Group × Imagery Condition interaction was also observed [*F*(4, 1863) = 3.8, *p* = 0.004, *without covariates; F*(4, 474) = 1.4, *p* = *ns*, *with covariates*]. This indicated that the MJ group demonstrated lower *SBP* compared with the healthy control group following exposure to stress (*p* = 0.05 *without covariates; p* = 0.2 *with covariates*) and lower *SBP* compared with the non-MJ group, following exposure to cue (*p* = 0.008 *without covariates; p* = 0.01 *with covariates*) (Figure 3b).

HPA axis markers

Plasma cortisol: A significant Drug Group × Imagery Condition interaction was observed [*F*(4, 1270) = 10.0, *p* <0.0001, *with and without covariates*], indicating that the MJ group demonstrated significantly higher levels of *Cortisol* following exposure to the stress imagery condition compared with the intra-individual-relaxing control condition (S >N, *p* 0.0001, *with and without covariates*). This stress-induced increase was not observed in either the healthy controls or the non-MJ group. Both the MJ group and the non-MJ group demonstrated increases in cue-related Cortisol (MJ group; C >N: *p* = 0.001 *with and without covariates*; non-MJ group; C >N: *p* = 0.006 *without covariates; p* = 0.007 *with covariates*), which was not demonstrated in the healthy control group (Figure 4a).

Plasma ACTH: Similarly, a significant Drug Group Imagery Condition interaction was observed [*F*(4, 1272) = 3.9, *p* = 0.003, *without covariates; F*(4, 1272) = 4.0, *p* = 0.004, *with covariates*], showing that the MJ group demonstrated significantly higher levels of *ACTH*

following exposure to the stress imagery condition compared with the intra-individual relaxing control condition (S >N, $p = 0.003$, *with and without covariates*). The MJ group also demonstrated significantly higher levels of *ACTH* following exposure to the cue imagery condition compared with the intra-individual-relaxing control condition (C>N, $p < 0.0001$, *with and without covariates*). These stress-related and cue-related increases in *ACTH* were not observed in either the healthy control group or the non-MJ group (Figure 4b).

Extended analysis in the MJ group. Relationship between craving, anxiety and relapse—Mean number of days to marijuana relapse in the MJ group was 40.5 ± 36.9. Extended analysis using Pearson's product moment coefficient showed that cue-induced alcohol craving and cue-induced anxiety were both associated with the number of days to marijuana relapse following discharge from inpatient treatment (*Alcohol Craving:* $r = -0.14$, $p = 0.04$; *Anxiety:* $r = -0.21$, $p = 0.002$). The association between cue-related *Cocaine Craving* and days to marijuana relapse approached significance ($r = -0.13$, $p = 0.07$). Stress-induced *Anxiety* was also associated with the number of days to marijuana relapse ($r = -0.11$, $p = 0.03$).

Three standard regression models were subsequently conducted to assess the extent to which (i) cue-related *Alcohol Craving*, (ii) cue-related *Anxiety* and (iii) stress-related *Anxiety* predicted the number of days to marijuana relapse following discharge from inpatient treatment. Age and Race were included as covariates in all three models. Findings indicated that increased reports of both cue-induced *Alcohol Craving* and cue-induced *Anxiety* were predictive of a shorter number of days to marijuana relapse, accounting for 19% and 17% of the variance over the 90-day follow-up period, respectively (*Alcohol craving:* $\beta = -0.19$, $R^2 = 0.19$, $t = -4.1$, $p < 0.0001$; *Anxiety:* $\beta = -0.15$, $R^2 = 0.17$, $t = -3.2$, $p = 0.002$). Stress-related increases in *Anxiety* did not predict the number of days to marijuana relapse.

Relationship between craving, anxiety and previous marijuana use—As marijuana use is often associated with a high prevalence of anxiety-related disorders (Bonn-Miller *et al.*, 2011; Bujarski *et al.*, 2012), a complex reciprocal relationship may exist between both in terms of their effects on craving and motivation for drug use. Although momentary assessment studies have shown that anxiety increases the negative reinforcing effects of marijuana use (Buckner *et al.*, 2011, 2012), preclinical and clinical research has indicated that chronic marijuana use over time will serve to sensitize neural stress systems (Koob and Le Moal, 2008). We therefore conducted standard regression analyses, with marijuana use 3 months prior to treatment and baseline anxiety as predictor variables, and stress-induced and cue-induced alcohol and cocaine craving as dependent variables. Findings indicated that previous marijuana use was a significant predictor of cue-induced alcohol craving ($\beta = 0.29$, $R^2 = 0.17$, $t = 2.12$, $p < 0.04$) and cue-induced cocaine craving ($\beta = 0.43$, $R^2 = 0.22$, $t = 2.89$, $p = 0.007$) after controlling for the main effect of baseline anxiety. Neither marijuana use nor anxiety predicted stress-related craving.

DISCUSSION

Current findings indicate that MJ-dependent substance abusers who are also co-dependent for alcohol and cocaine abuse demonstrate selective tonic and phasic stress system adaptations specific to their MJ dependence. In comparison to both well-matched substance abusers who were not dependent on cannabis and healthy socially drinking controls, MJ-dependent individuals showed higher generalized anxiety as well as an up-regulated cardiovascular basal drive and enhanced alcohol craving and cocaine craving following exposure to stress and cue. Consistent with prior research, increased stress-induced and cue-induced craving in the MJ group was also accompanied by reduced cardiovascular output,

increased anxiety and enhanced HPA axis function compared with the other groups. Extended analysis also indicated that enhanced cue-related alcohol craving and anxiety were predictive of time to marijuana relapse. As such, initial findings suggest that co-morbid MJ dependence may exacerbate stress-induced and cue-induced craving for other drugs of abuse by potentially altering selective mechanisms of stress system function specific to marijuana use.

Most notably, overall findings support the existence of behavioral cross-sensitization in terms of some of the motivational processes associated with craving and relapse. For example, substance abusers who were co-morbidly dependent on marijuana additionally reported significantly higher ratings of alcohol craving following exposure to stress-related and cue-related imagery compared with both well-matched substance abusers not dependent on marijuana as well as healthy controls. They also reported significantly higher ratings of cocaine craving following exposure to cue, although no group differences were observed in terms of nicotine craving. These findings provide broad support for the possible link between the eCB receptor system and motivation to consume alcohol and cocaine (Wiskerke *et al.*, 2008).

For example, CB-sub1 receptor stimulation by Δ8-THC and WIN 55,212-2 has been shown to dose-dependently *enhance* the effects of conditioned cue on re-instatement to psychostimulants (Anggadiredja *et al.*, 2004; González-Cuevas *et al.*, 2007). Similarly, with regard to ethanol seeking, an absence of withdrawal symptoms and reductions in cue-conditioned and stress-induced drinking following alcohol cessation have both been documented in CB1 knock-out mice (Racz *et al.*, 2003; Soria *et al.*, 2005). When considered together, preclinical studies such as these broadly support current findings by highlighting the association between exogenous cannabinoid changes to the eCB system and stress-induced and cue-induced motivational aspects of cocaine and alcohol seeking.

The fact that the current MJ group did not demonstrate similar increases in nicotine craving may be related to variation in consumption expectancies compared with cocaine and alcohol. Although substance-abusing participants were kept on a locked inpatient facility with no access to cocaine or alcohol, they were allowed four regular smoke breaks per day including prior to and following the laboratory study, in order to curb nicotine withdrawal-related symptoms. As consumption expectancies are known to influence cue-related craving (Marlatt *et al.*, 1973; Berg *et al.*, 1981; Kaplan *et al.*, 1984), knowledge of a subsequent smoke break may have served to curb nicotine craving in the substance-abusing groups.

In this study, the potential for motivational cross-sensitization in terms of drug seeking is further highlighted by the fact that elevations in cue-related alcohol craving are also predictive of a shorter number of days to marijuana relapse. A trend was also observed for cue-related cocaine craving. This may be related to the fact that the negative reinforcing properties of cocaine and alcohol (Sinha *et al.*, 2003, 2011; Koob and Le Moal, 2005; Fox *et al.*, 2007; 2008) may be ameliorated by using cannabis (González-Cuevas *et al.*, 2007). In view of this, previous human studies have shown that acute cannabis administration potentiates the positive subjective effects of both alcohol and cocaine, by altering the bioavailability of both (Perez-Reyes *et al.*, 1988; Chait and Perry, 1994; Lukas *et al.*, 1994). This also corroborates preclinical studies that have highlighted the anxiolytic effects of CB1 agonists following cocaine, alcohol and stress exposure (Hayase *et al.*, 2005; Fokos and Panagis, 2010) and may provide an underlying mood-related mechanism for predicting cannabis relapse in cocaine-dependent and alcohol-dependent individuals.

In the current study, the chronic effects of marijuana following 3 weeks of abstinence were associated with significantly higher basal and phasic ratings of anxiety, which were

accompanied by enhanced alcohol and cocaine craving. In particular, the sensitized anxiety response in the MJ group was compounded following exposure to cue and remained persistently elevated up until approximately 30 min post-imagery. This is concordant with previous research linking stress-related and cue-related craving to dissociable aspects of emotional distress. Although cue-induced alcohol and cocaine craving has been associated with increases in appetitive "vigilance"-related emotions, including anxiety, fear and arousal, response to stress has been more associated with negative affect such as sadness and anger (Fox *et al.*, 2007). Furthermore, as a complex reciprocal relationship potentially exists between chronic marijuana use and anxiety disorders (Bonn-Miller *et al.*, 2011), it may be reasonable to predict persistently elevated levels of anxiety following exposure to cue in the MJ group.

Persistently elevated cue-induced anxiety was also a significant predictor of marijuana relapse. It may be the case therefore that increased anxiety symptomatology in MJ-dependent polydrug abusers enhances the negative reinforcing effects of chronic drug use (Sinha, 2001; Fox and Sinha, 2009) and promotes a greater adaptive allostatic "shift" towards a sensitized stress system (Koob, 2004; Kalivas and Volkow, 2005; Koob and Le Moal, 2008; Sinha, 2008). This in turn may increase generalized drug and alcohol craving and relapse risk (Fox *et al.*, 2007; Sinha, 2008; Sinha *et al.*, 2009; 2011). Conversely, however, it is also interesting to note that baseline marijuana use was predictive of cue-related alcohol and cocaine craving even when baseline increases in anxiety were held constant. As such, understanding more fully the relative contribution of both marijuana consumption and anxiety with regard to marijuana seeking may represent an important avenue for future treatment development research.

The current MJ group also demonstrated variations in both tonic and phasic cardiovascular output compared with the other experimental groups. At baseline, however, only a trend for higher heart rate and systolic blood pressure was recorded in the MJ group following the inclusion of covariates, suggesting that some of this variance may have been accounted for by age and race. This is consistent with research indicating that both age and African American heritage are risk factors for hypertension (Kaplan, 1994). Although baseline findings are consistent with the acute effects of marijuana (Hollister, 1988; Vandrey *et al.*, 2011), only a few studies to date have assessed the cardiovascular effects of withdrawal in cannabis-dependent individuals. These studies suggest that a general up-regulation of basal blood pressure and heart rate may be observed following 48 h of abrupt cannabis cessation as a possible rebound effect following tolerance to the repeated acute effects (Jones *et al.*, 1981; Jones, 2002; Vandrey *et al.*, 2008, 2011). As such, our findings hold some support for this; however, it is important to note that the basal up-regulation observed in the current MJ group may not be of clinical significance as blood pressure was not within the standard hypertensive range (>140 mm/Hg for SBP and >90 mm/Hg for DBP).

Although basal up-regulation of heart rate and blood pressure in the MJ group may not signify clinical hypertension following 4 weeks of abstinence, it may still be a salient factor contributing to the dampened phasic response observed following exposure to stress-related and cue-related stimuli (Sinha *et al.*, 2009), potentially reflecting a ceiling level of physiological response undermining the ability to respond effectively to stress or cue. For example, the MJ group was unable to mount an elevated SBP response appropriate for exposure to stress, exposure to cue and sensitized levels of anxiety. They also demonstrated a generalized down-regulation of heart rate and blood pressure across all three imagery conditions compared with the non-MJ-abusing polydrug group and the healthy controls. Most importantly, the inability to demonstrate an appropriate bio-physiological engagement to stress has been associated with relapse factors in alcoholics (Adinoff *et al.*, 2005; Junghanns *et al.*, 2005; Fox *et al.*, 2007; Sinha *et al.*, 2009, 2011) and co-morbid alcohol-

dependent and cocaine-dependent men (Fox *et al.*, 2009) as well as risk of dependence in vulnerable populations (Zimmerman *et al.*, 2004; Sorocco *et al.*, 2006). As this suppressed response to stress and cue was not observed in the non-MJ-dependent group, it may reflect an additional risk factor for craving, associated selectively with the co-morbid actions of marijuana on vascular function.

Current findings also showed that the MJ group demonstrated significant elevations in ACTH after stress and cue exposure, relative to their own baseline levels, as well as stress-induced elevations of cortisol that were not observed in either the non-MJ-dependent substance abusers or the healthy controls. Very broadly, this is in keeping with extensive preclinical research that has examined the role of eCB signaling in stress system regulation where the administration of exogenous cannabinoids including SR141716 (Rimonabant) and THC dose-dependently activate the HPA axis by stimulating CRH, ACTH and corticosterone secretion (Manzanares *et al.*, 1999; Brown and Dobs, 2002; Patel *et al.*, 2004; Pagotto *et al.*, 2006; Wade *et al.*, 2006; Steiner *et al.*, 2008).

Although these studies represent acute paradigms, some research in both animals and humans have also shown that tolerance develops quickly, culminating in a blunting of cortisol in response to subsequent intravenous THC exposure (Murphy *et al.*, 1998; Pagotto *et al.*, 2006; D'Souza *et al.*, 2008; Ranganathan *et al.*, 2009). As such, the elevations in stress-related and cue-related ACTH and cortisol observed in the MJ group may reflect a "rebound" up-regulation mechanism following marijuana cessation. Of the few studies that have examined HPA axis changes following cannabis cessation, stress paradigms have not been incorporated. Although one study documented a 2.5-fold increase in CRH and a 1/3 increase in corticosterone in rodents following antagonist-elicited cannabis withdrawal (de Fonseca *et al.*, 1997), this was not replicated in a similar recent human study (Goodwin *et al.*, 2012). Similarly, an early human study also found no significant change in cortisol concentrations among 30 healthy male cannabis smokers after 6 days of abstinence (Cohen, 1976). These human studies may hold some support for the unchanged basal findings in the present study, however; again, future research is warranted in order to fully elucidate cannabis-related adaptations to HPA axis function in polydrug-dependent individuals.

Interpretation of current findings is restricted by the fact that subjective craving for marijuana was not assessed in the current sample of MJ-dependent individuals. As such, it is difficult to ascertain completely the true extent of cross-sensitization with regard to motivation for drug seeking, particularly in terms of assessing the role of marijuana craving on compulsive alcohol and cocaine seeking. Additionally, although relapse-related subjective and bio-physiological stress system adaptations were observed to a greater extent in the MJ-dependent group, compared with the other experimental groups, the lack of MJ-craving ratings makes it challenging to attribute these adaptations directly to motivation for marijuana use. Despite this, findings do indicate that a higher frequency of marijuana use in the 3 months prior to inpatient treatment is predictive of greater cue-induced anxiety, alcohol craving and cocaine craving in the MJ-dependent group. Although this may potentially be attributable to greater overall substance use in the MJ group, it is important to consider that both substance-abusing groups were statistically matched on alcohol and cocaine use. Interpretation of findings are also limited to a certain extent by the fact that the healthy group did not reflect a population of regular smokers and, as such, may not have provided an optimal control group for measuring nicotine craving. However, this does not detract from the fact that there were no significant variations in both stress-induced and cue-induced nicotine craving between the two dependent groups.

Additionally, this is one of the first studies to show that MJ-dependent polydrug users may demonstrate selective tonic and phasic subjective, cardiovascular and HPA stress system

adaptations during early abstinence that may be specific to their marijuana use and associated with risk in a range of drug-abusing populations. Most notably, in the current study, these adaptations included a higher level of cue-induced anxiety and craving for alcohol, both of which were also significantly predictive of time to marijuana relapse. Increases in cue-related cocaine craving approached statistical significance. As such, findings support the need to examine motivational cross-sensitization much more thoroughly as a contributing factor to overall relapse vulnerability in polydrug-dependent individuals

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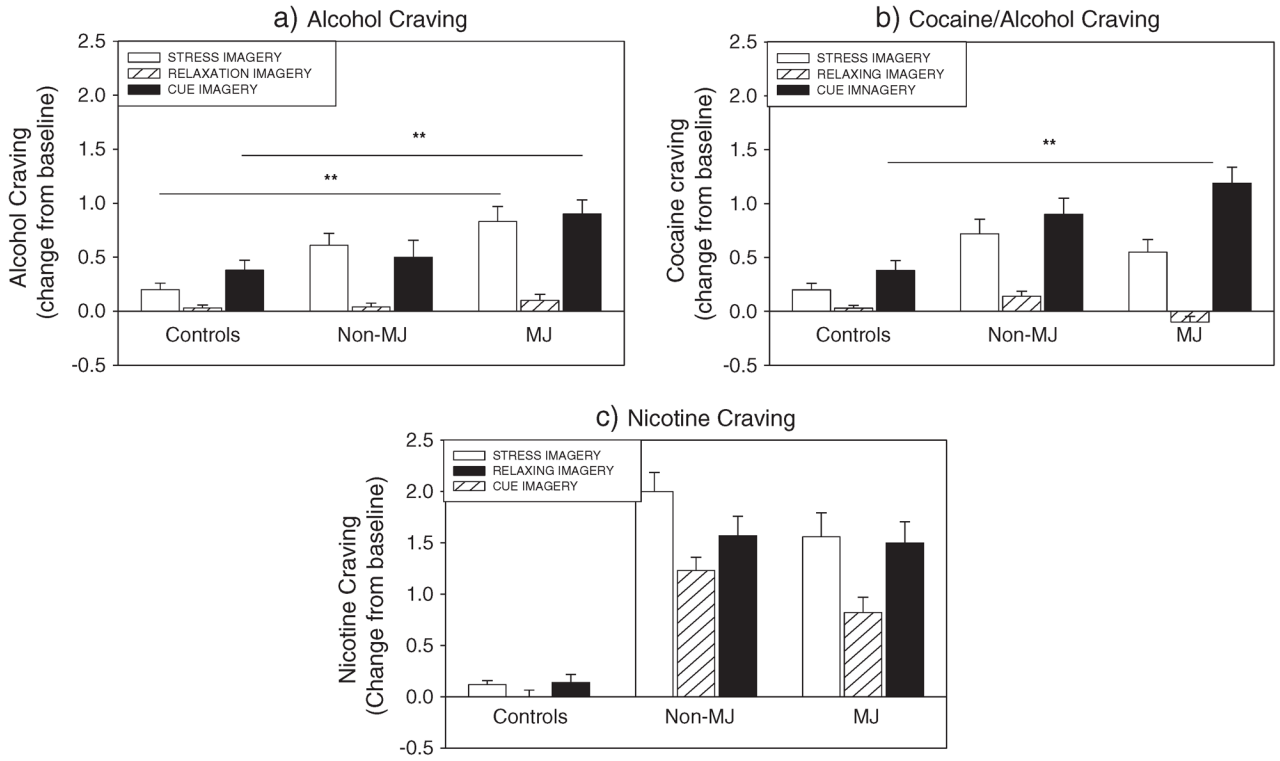

Figure 1.

Bar graphs showing alcohol craving and cocaine craving between the marijuana (MJ)-dependent group, non-MJ-dependent group and controls

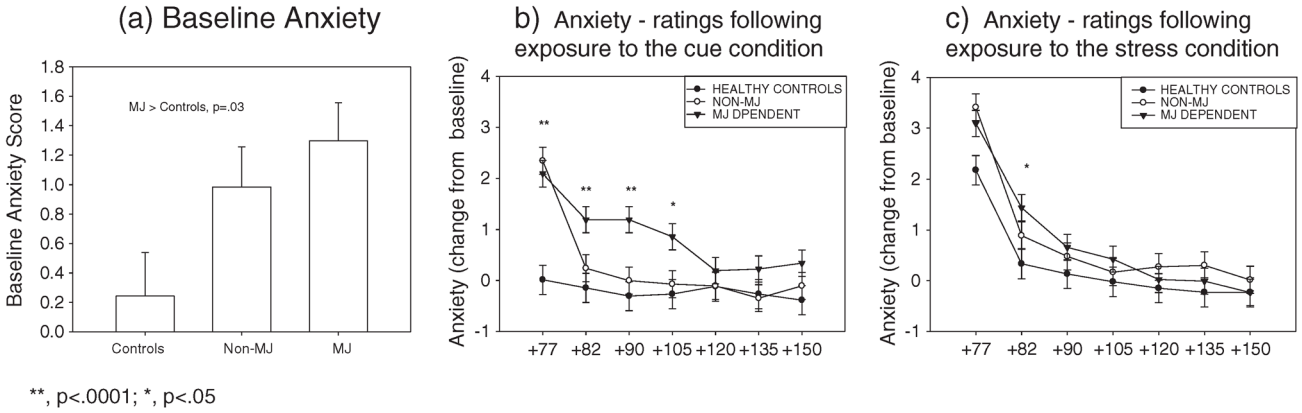

Figure 2.

Line graphs showing anxiety ratings between the marijuana (MJ)-dependent group, non-MJ-dependent group and controls at (a) baseline, (b) following cue imagery exposure and (c) following stress imagery exposure

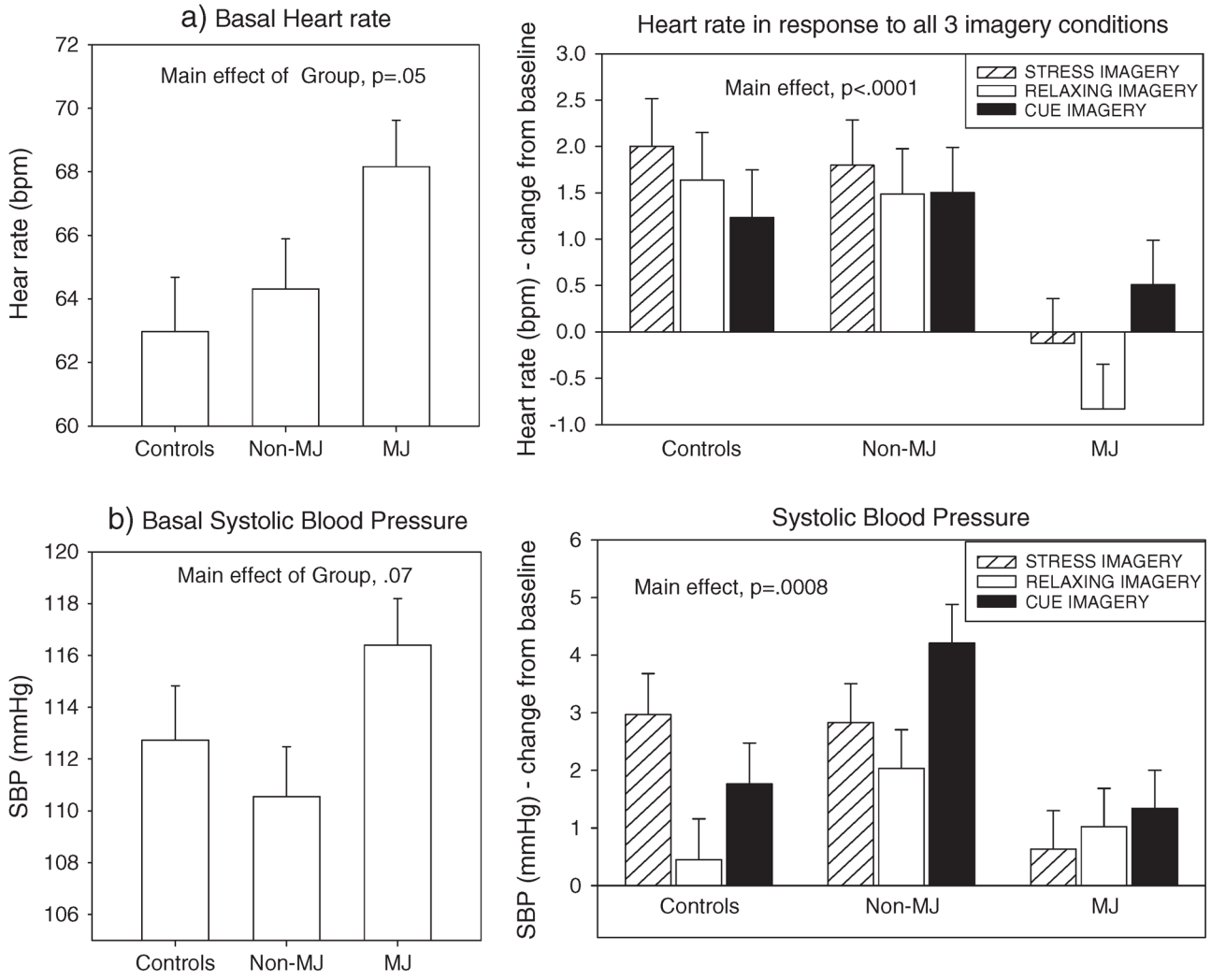

Figure 3.

Bar graphs showing basal and response heart rate and blood pressure between the marijuana (MJ)-dependent group, non-MJ-dependent group and controls

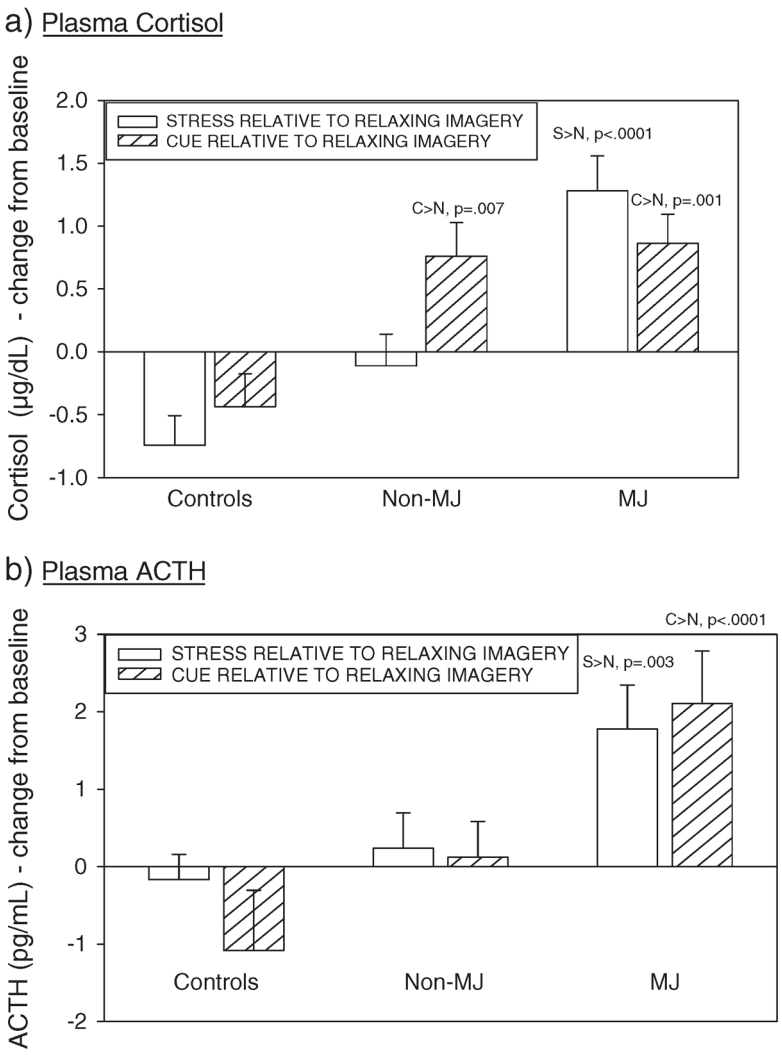

Figure 4.

Bar graphs showing differences in adrenocorticotrophic hormone (ACTH) and cortisol response to stress and cue relative to the relaxing imagery condition between the marijuana (MJ)-dependent group, non-MJ-dependent group and controls

Table 1

Participant demographic and clinical characteristics (means and standard deviations are shown)

***N* = 85**	**Substance abusers *n* = 29**	**Substance abusers with marijuana dependence *n* = 30**	**Healthy controls *N* = 26**	**p***
Gender no. male	17 (58.6%)	18 (60.0%)	10 (40%)	NS
Race				>0.02
No. Caucasian	18 (62.1%)	9 (30%)	14 (56%)	
No. African American	8 (27.6%)	20 (66.7%)	7 (28%)	
Other	3 (10.3%)	1 (3.3%)	4 (16%)	
Age	37.1 ± 6.4	33.7 ± 6.9	28.1 ± 1.4	<0.0001
Years in education	12.4 ±0.3	12.3 ± 0.4	15.1 ± 0.4	<0.0001
Smoking status no. regular smokers (%)	25 (86.2%)	26 (86.7%)	6 (24%)	<0.0001
Years of cocaine use	8.5 ± 5.4	8.2 ± 5.3	0	<0.0001
Years of alcohol use	15.7 ±8.9	11.9 ±7.3	4.8 ± 1.2	<0.0001
Years of marijuana use	5.3 ±13.2	13.2 ± 5.5	1.0 ± 0.6	<0.0001
No. of days used in the last month				
Cocaine	12.4 ±12.3	10.1 ±11.5	0	<0.0001
Alcohol	14.5 ±12.4	9.4 ± 11.7	3.8 ± 3.6	<0.0001
Marijuana	0.6 ± 2.2	9.1 ± 12.7	0	<0.0001
Amount used in the last month				
Cocaine *(grams)*	25.2 ± 23.7	36.3 ± 52.5	0	<0.0001
Alcohol *(drinks)*	203.7 ±138.4	159.8 ± 119.4	16.4 ±13.5	<0.0001
Marijuana *(joints)*	5.3 ± 10.5	96.6 ± 185.7	0	<0.0001
No. lifetime depression	8 (27.6%)	8 (26.7%)	1 (4%)	<0.0001
No. lifetime anxiety (including PTSD**)	10 (34.5%)	12 (40%)	1 (4%)	<0.0001
No. lifetime anxiety (without PTSD**)	2 (6.9%)	2 (6.7%)	0	<0.0001

* Overall statistical difference between all three groups. Shaded area represents significant differences ($p < 0.05$) between the two substance-abusing groups only.

** PTSD: Post Traumatic Stress Disorder.