Journal of Abnormal Psychology
2018, Vol. 127, No. 4, 348–358

0021-843X/18/$12.00 http://dx.doi.org/10.1037/abn0000344

Heavy Marijuana Use but Not Deprivation Is Associated With Increased Stressor Reactivity

Kathryn R. Hefner
University of Wisconsin—Madison and Uniformed Services University of the Health Sciences

Mark. J. Starr and John. J. Curtin
University of Wisconsin—Madison

Although stressors appear to motivate marijuana use, and marijuana use, in turn, is believed to induce stress system neuroadaptations, relatively little empirical work has explicitly tested for stress neuroadaptations associated with heavy marijuana use. We examined stressor reactivity to threat of unpredictable electric shock via startle potentiation among heavy marijuana users and a control group that reported minimal history of marijuana use. Heavy marijuana users were randomly assigned to 3 days of marijuana deprivation or no deprivation. This design allowed us to test contrasts for heavy (vs. minimal) use and deprivation (vs. no deprivation) on stressor reactivity. Heavy marijuana users (both deprived and nondeprived) displayed increased startle potentiation during threat of unpredictable electric shock relative to minimal use controls. In contrast, marijuana deprivation had no effect on startle potentiation. Startle potentiation was also increased among users who reported greater stress-coping motives for their marijuana use and users with cannabis use disorder diagnoses. To our knowledge, this is the 1st study to demonstrate increased reactivity to unpredictable stressors among heavy marijuana users. However, comparable increased unpredictable stressor reactivity among patients with alcohol and other substance use disorders has been previously documented. This relationship to heavy marijuana use is consistent with predictions from rodent addiction models regarding stress neuroadaptations following heavy, chronic drug use but could also represent an etiologically relevant premorbid risk characteristic. Finally, the clinical import of unpredictable stressor reactivity is reinforced by its relationships with stress-coping motives and cannabis use disorder status.

General Scientific Summary

Animal models of addiction etiology propose that heavy, chronic drug use causes changes in the brain that result in heightened stressor reactivity. Consistent with this thesis, heavy marijuana users displayed exaggerated response to the threat of unpredictable electric shock compared to individuals with minimal history of marijuana use. Furthermore, stressor reactivity was increased among users who reported greater stress-coping motives for their marijuana use and users with cannabis use disorder diagnoses.

Keywords: stress reactivity, marijuana, startle potentiation, uncertain, threat, addiction

Marijuana, the most commonly used illicit drug, has been perceived as less harmful than are other substances (Nutt, King, Saulsbury, & Blakemore, 2007). However, approximately half of those who use marijuana daily become addicted (Anthony, 2006), calling for increased efforts to elucidate causes and consequences of heavy, frequent use. In this study, we examine the relationship

Kathryn R. Hefner, Department of Psychology, University of Wisconsin—Madison, and Department of Psychiatry, Center for the Study of Traumatic Stress, Uniformed Services University of the Health Sciences; Mark. J. Starr and John. J. Curtin, Department of Psychology, University of Wisconsin—Madison.

A separate report examining behavioral indices of altered subjective reward valuation among deprived versus nondeprived marijuana users was published in the *Journal of Abnormal Psychology* (Hefner, Starr, & Curtin, 2016). An additional report in *Experimental and Clinical Psychopharmacology* (Hefner & Starr, 2017) examined sex differences in these behavioral indices of subjective reward valuation. Some of the data was presented at the 2015 American Psychological Association Convention in Toronto, Ontario, Canada, in August 2015, as well as at Kathryn R. Hefner's public dissertation defense at the University of Wisconsin—Madison in July 2014.

This work was supported by a University of Wisconsin—Madison Romnes Faculty Fellowship awarded to John. J. Curtin and National Institute on Drug Abuse Grant F31 DA032184 awarded to Kathryn R. Hefner and her sponsor, John. J. Curtin. The opinions and assertions expressed herein are those of the authors and do not necessarily reflect the official policy or position of the Uniformed Services University of the Health Sciences, the Department of Defense, or the National Institute on Drug Abuse or its affiliates.

Correspondence concerning this article should be addressed to Kathryn R. Hefner, Department of Psychiatry, Center for the Study of Traumatic Stress, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814. E-mail: kathryn.hefner.ctr@usuhs.edu

between heavy marijuana use and response to stressors, given the key role stressors play in addiction etiology. Although stressors appear to motivate marijuana use (Hyman & Sinha, 2009), and marijuana use, in turn, is believed to induce stress system neuroadaptations (Fox, Tuit, & Sinha, 2013; Koob & Le Moal, 2008), relatively little empirical work has explicitly tested for stress neuroadaptations among heavy marijuana users.

Stressors figure prominently in addiction etiology and relapse across drug types (Kaye, Bradford, Magruder, & Curtin, 2017); specifically, heavy and/or chronic drug use contributes to heightened behavioral and affective responses to stressors via central nervous system (CNS) mechanisms including corticotrophin-releasing factor (CRF) and norepinephrine (NE) in the central extended amygdala (Koob & Le Moal, 2008). These stress neuroadaptations in turn result in dysregulated emotional responses to stressors upon cessation of drug use, which provides potent motivation to resume use via craving, increasing relapse risk (Baker, Piper, McCarthy, Majeskie, & Fiore, 2004) across major classes of addictive drugs.

Of particular relevance for marijuana, the endocannabinoid system also regulates CNS response to stressors via modulation of CRF, NE, serotonin, and other stress hormones (Volkow, Hampson, & Baler, 2017). Additionally, cannabinoid receptor agonists and Δ^9-tetrahydrocannabinol (THC) activate peripheral stress mechanisms via the hypothalamic−pituitary−adrenal axis in humans (D'Souza et al., 2004) and animals (Martín-Calderón et al., 1998), during both acute administration and withdrawal (Rodríguez de Fonseca, Carrera, Navarro, Koob, & Weiss, 1997). Repeated activation of the endocannabinoid system is thought pivotal to both CNS and peripheral stress system adaptations occurring in heavy marijuana users (Fox et al., 2013; Volkow et al., 2017), but empirical evidence of stress neuroadaptations in human marijuana users has been lacking. Thus, the first aim of the present study was to test for this predicted increase in stressor reactivity among heavy marijuana users relative to minimal use controls, consistent with this stress neuroadaptation model.

Tonic alterations in mood and/or affective response have been clearly documented during withdrawal from marijuana. The robust marijuana withdrawal syndrome as observed in humans (Budney & Hughes, 2006) and animals (Lichtman & Martin, 2002) involves increased negative affective symptoms (irritability, anger or aggression, nervousness or anxiety; American Psychiatric Association, 2013; Budney & Hughes, 2006; Budney, Hughes, Moore, & Vandrey, 2004; Crippa et al., 2009). These negative affective symptoms during withdrawal are reliably reduced or eliminated by the administration of cannabinoid receptor agonist, the main psychoactive component in marijuana. Furthermore, individuals with a marijuana use disorder report this anxiety and negative affect contributes to difficulty abstaining from use and cite relief from withdrawal as a motivation for use (Bonn-Miller & Moos, 2009; Budney & Hughes, 2006; Budney et al., 2004; Coffey et al., 2002; Copersino et al., 2006). However, it remains unclear whether these affective symptoms result from stress neuroadaptation mechanisms that are enhanced during withdrawal. Therefore, a second aim was to test whether marijuana deprivation increases stressor reactivity among heavy users, consistent with a withdrawal-related impact on stress neuroadaptation mechanisms.

If heavy marijuana use produces stress neuroadaptations that contribute to the etiology and maintenance of cannabis use disorder, individuals who use marijuana to cope with stressors or negative affect may be at heighted risk. Mounting evidence has suggested that stress-coping motives are common among marijuana users (Fox et al., 2013; Green, Kavanagh, & Young, 2003; Hathaway, 2003; Hyman & Sinha, 2009) and are associated with cannabis use disorder, depression, and perceived stress according to the *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.; *DSM–5;* American Psychiatric Association, 2013; Moitra, Christopher, Anderson, & Stein, 2015). More than half of experienced users report blowing off steam, feeling less anxious, and forgetting worries as important reasons for using marijuana (Hathaway, 2003), whereas nearly three quarters of daily users report using marijuana to relax or relieve tension (Johnston & O'Malley, 1986). Moreover, heavy marijuana users endorse coping-related motives for their use more than do infrequent users, which may itself result from stress neuroadaptations (Hyman & Sinha, 2009). Therefore, a third aim of this study was to conduct exploratory analyses to determine whether marijuana use motives and cannabis use disorder diagnostic status were related to stressor reactivity.

To accomplish these three goals, we quantified stressor reactivity via startle potentiation to unpredictable shock threat, a noninvasive, psychophysiological index of stressor reactivity that is sensitive to putative stress neuroadaptations associated with drug use (Davis, Walker, Miles, & Grillon, 2010; Hogle, Kaye, & Curtin, 2010; Moberg, Bradford, Kaye, & Curtin, 2017). We chose to use unpredictable shock threat because of translational evidence that implicates CRF and NE mechanisms in the central extended amygdala specifically in response to unpredictable (vs. predictable) threats (Davis et al., 2010; Kaye et al., 2017). We assessed startle potentiation to these unpredictable threats among deprived (3 days of abstinence from marijuana) and nondeprived heavy marijuana users and a control group on individuals with minimal history of marijuana use. This allowed us to evaluate differences in mean stressor reactivity across heavy marijuana user (deprived and nondeprived heavy users vs. minimal use controls) and deprivation (deprived vs. nondeprived heavy users) contrasts. Furthermore, we assessed individual differences in characteristics regarding marijuana use to evaluate relationships between these individual differences and stressor reactivity.

Method

Study procedures were reviewed and approved by the University of Wisconsin—Madison's Institutional Review Board (protocol: SE-2008−0164). We also support emerging open science guidelines (Nosek et al., 2015) providing the data, analysis scripts, questionnaires, and other study materials associated with this report publicly via Open Science Framework (https://osf.io/sgx5c).

Participants

We used flyers and online advertisements to recruit 104 heavy marijuana users (52 female) who reported marijuana use 5 days or more per week, two or more times per day on days when used for 6 months or more (Arnone et al., 2008; Pope & Yurgelun-Todd, 1996) and 52 minimal use controls (26 female) who reported a history of lifetime marijuana use of more than one but less than 50 times and no use in the past month. Participants who reported any recreational drug use (other than alcohol, marijuana, and nicotine)

resulting in intoxication one or more times per week or with current diagnoses of alcohol or other drug dependence (other than marijuana or nicotine) were excluded from all groups. Participants reporting use of psychotropic medication(s), engagement in psychological treatment (within the last 6 months), or current or past diagnosis of psychotic disorders were also excluded from all groups.

A priori power analyses for sample size planning demonstrated that $N = 156$ provided approximately 86% power to detect a moderate sized between-subjects effect ($\eta_p^2 = .06$) with an alpha of .05 within the general linear model.

Procedure

Preliminary screening was accomplished when prospective participants called the laboratory to indicate interest in the study. Prospective participants were informed on the phone about the protections of the National Institutes of Health Certificate of Confidentiality associated with this study and provided verbal consent to assess their medical and drug use history. Those meeting preliminary eligibility criteria during the phone contact were scheduled for a subsequent formal screening session in our laboratory. During this screening session, all participants provided informed consent, and a study clinician verified inclusion–exclusion criteria by administering a standardized structured interview to assess their medical history, past and current drug use history (using the alcohol and drug use disorders components of the Mini-International Neuropsychiatric Interview [MINI] adapted to assess past use; Sheehan et al., 1998), and marijuana use patterns. All participants were asked to provide a urine sample to verify drug use. Minimal use controls were required to test negative on an immediate-result qualitative test of marijuana use using a cutoff score of 50 ng/ml (key operated drug test cup; Drug Test Systems, Dover, NH). Urine samples from heavy users were collected for quantitative comparison analysis.

Deprivation manipulation. Heavy users were randomly assigned to one of two deprivation groups (deprived or nondeprived) in this screening session. Participants assigned to the deprived group were instructed to abstain from any marijuana use (e.g., smoked, ingested) for 3 days prior to their experimental session (described later). Those assigned to the nondeprived group were instructed to maintain their typical frequency and quantity of marijuana use. However, they were instructed to refrain from using marijuana for at least 1 hr before the experimental session to avoid acute intoxication effects in that session. All participants were asked to avoid using alcohol for 24 hr and other recreational drugs (excluding marijuana) for 1 week prior to their experimental session. Those reporting use of nicotine were asked to continue their usual quantity and frequency of use. Minimal use controls were also asked to continue to avoid marijuana use. Participants scheduled their experimental session to occur within 4–10 days of the screening session.

On arrival for the experimental session, participants reported the date−time of their last marijuana use and any recent (i.e., past 24 hr) alcohol or other (1 week) drug use. Breath alcohol concentration was also assessed. Deprived participants who were noncompliant with abstinence instructions based on urine drug test or their self-report were dismissed and given the opportunity to reschedule once. Any participant indicating noncompliance with the alcohol and/or other drug use requirements was dismissed.[1]

Shock threat manipulation. We measured participants' subjective shock tolerance to a series of 200-ms electric shocks of increasing intensity (7 mA maximum) using standard procedures (Curtin, Patrick, Lang, Cacioppo, & Birbaumer, 2001; Hefner & Curtin, 2012; Hefner, Moberg, Hachiya, & Curtin, 2013). We administered electric shocks using a custom shock stimulator (Bradford, Magruder, Korhumel, & Curtin, 2014) via stainless steel electrodes across the distal phalanges of the index and ring fingers of the left hand. The procedure was discontinued once participants reached the maximum level of shock they could tolerate. We set shock intensity during the main task to each participant's subjective maximum tolerance threshold to minimize individual differences in shock tolerance.

Participants were administered unpredictable shocks in the context of a reward decision-making task[2] in which they pressed buttons to indicate preferred rewards. This task was designed to address other questions that are not a focus of the present investigation (Hefner, Starr, & Curtin, 2016). Participants completed the task in unpredictable alternating blocks of "unpredictable shock threat" or "no shock." In unpredictable-shock-threat blocks, participants were instructed that electric shocks were unpredictable and could occur at any point during the block, whereas in no-shock blocks, shocks would never be administered. To increase manipulation salience, the experimenter verbally identified block type and reiterated shock contingencies at the beginning of each block. Finally, a block label (e.g., *NO SHOCKS*) remained on the screen in the top left corner throughout the block. Participants completed four total blocks of trials (two each) in one of four between-subjects counterbalanced orders. A total of five shocks were administered throughout the main session, three in the first unpredictable-shock-threat block and two in the second. Similar procedures have been used successfully and reliably produce robust startle potentiation during unpredictable-shock-threat blocks relative to no-shock blocks (Hefner & Curtin, 2012; Hefner et al., 2013; Hogle et al., 2010; McCarthy, Gloria, & Curtin, 2009).

[1] No participants in either group were disqualified for a positive breath alcohol concentration at the experimental session. Similarly, no participants in the deprived group were disqualified because of a positive urine drug test indicating recent marijuana use prior to the experimental session. One participant was disqualified from the deprived group due to abstaining for 7 days. Two participants in the nondeprived group were disqualified for self-reported use of drugs (other than marijuana) within 1 week of the experimental session. Finally, four participants in the deprived group and seven participants in the nondeprived group were lost to attrition between the screening and experimental session.

[2] The task included 144 trials where participants were presented with a choice between a "certain" and "uncertain" monetary reward. Each certain reward was associated with a clear, single monetary value. Uncertain rewards involved the equiprobable receipt of one of two different monetary values. Each trial included a choice between one certain versus one uncertain (two possible values) reward. The values of the certain reward and the mean value and range of the two uncertain reward options were varied parametrically across trials. Participants pressed a button to indicate which of these two rewards they would prefer to receive at the end of the experiment. Participants did not receive any feedback trial-by-trial and were paid a bonus at the end of the experiment based on their preferred reward on one randomly selected trial.

Measures

Stable individual difference measures. The MINI (Sheehan et al., 1998) was used to diagnose marijuana or other drug abuse and dependence and was adapted to assess past abuse and dependence in all participants. Participants also self-reported any history of (a) anxiety, (b) depression, or (c) other psychiatric diagnoses or treatment. All participants completed the Depression Anxiety and Stress Scales-21 (DASS-21; Lovibond & Lovibond, 1995), adapted to assess symptoms over the past 30 days rather than past week. Cronbach's alphas were .65, .82, and .80 for Anxiety, Depression, and Stress subscales, respectively, in this sample. All participants completed the Marijuana Motives Measure (MMM; Simons, Correia, Carey, & Borsari, 1998) to assess motivations for using marijuana. Cronbach's alphas were .80, .92, .86, .93, and .83 for the Conformity, Stress Coping, Enhancement, Expansion, and Social subscales, respectively, in this sample. Heavy marijuana users reported their approximate quantity of weekly use.

Deprivation-related manipulation checks. All participants completed the Marijuana Withdrawal Checklist (MWC; Budney, Novy, & Hughes, 1999; Vandrey, Budney, Moore, & Hughes, 2005) and Marijuana Craving Questionnaire (MCQ; Heishman, Singleton, & Liguori, 2001) at both screening and experimental sessions to assess changes in withdrawal symptoms and craving. Cronbach's alphas were .52 and .86 for the MWC and .88 and .89 for the MCQ at screening and experimental sessions, respectively. Heavy marijuana users also reported the time of their last marijuana use.

Startle response. Electromyographic activity in the orbicularis oculi muscle was sampled at 2000 Hz with a bandpass filter (.05–500 Hz) from electrodes placed under the right eye according to published guidelines (Blumenthal et al., 2005; Van Boxtel, 1998). Eyeblink startle response was measured in response to startle-eliciting noise probes (50 ms of 102-dB white noise with near instantaneous rise time). Twenty-four noise probes were presented during a subset of trials during unpredictable- and no-shock blocks). Data reduction and processing followed published guidelines (Blumenthal et al., 2005). We performed offline processing in Matlab using EEGLab (Delorme & Makeig, 2004) and PhysBox (Curtin, 2011) plugins. This processing included high pass filtering (28 Hz, 4th-order Butterworth, zero phase shift), rectification, smoothing (30 Hz, 2nd-order Butterworth low-pass filter, zero phase shift), epoching (−50 to 250 ms surrounding probe), and baseline correction. Startle magnitude was scored as the peak response between 20- and 100-ms post probe onset. We rejected trials containing an artifact, consistent with standard practices from our laboratory (Kaye, Bradford, & Curtin, 2016). This included trials with deflections greater than ±20 μV in the 50-ms preprobe baseline (i.e., unstable baseline) and trials with mean activity ≤−10 μV between 150 and 250 ms post probe onset (i.e., baseline overcorrection due to preepoch artifact). Startle potentiation was calculated as the increase in startle magnitude during unpredictable-shock relative to no-shock blocks. We also calculated general startle reactivity as mean startle magnitude to six noise probes presented prior to the start of the task. Following previous recommendations (Bradford, Kaye, & Curtin, 2014), we used general startle reactivity as a covariate in all analyses of startle potentiation to increase power to detect focal effects.

Urinalysis of marijuana use. Urine samples from heavy users were obtained at both screening and experimental sessions for quantitative analysis to verify compliance with the abstinence instructions for the deprived users. A specimen ratio of creatinine-normalized samples at two different time points (Creatinine-Normalized Specimen 2/Creatinine-Normalized Specimen 1) was used to detect recent use among marijuana users assigned to abstain from marijuana (Huestis & Cone, 1998). Following previously established procedures and cutoff scores to detect recent drug use among abstaining individuals, we considered deprived users with specimen ratios exceeding 1.5 noncompliant with the abstinence requirement. (Huestis & Cone, 1998; Manno, Ferslew, & Manno, 1984). This specimen ratio has not been used previously to confirm continued use among regular (nonabstinent) marijuana users, and no information about its sensitivity or specificity for that purpose exists to our knowledge. Therefore, we limited its use to confirming no recent use among deprived heavy users; however, we collected and calculated specimen ratios in all heavy users to allow for quantitative comparisons across groups as a manipulation check.

Results

Data analysis and figure preparation were accomplished with R (R Core Team, 2015) within RStudio using the lmSupport (Curtin, 2015) package. We use general linear models (GLMs) for all analyses unless otherwise noted. Marijuana group effects were analyzed with two planned orthogonal contrasts: the heavy marijuana user contrast (deprived and nondeprived heavy users vs. minimal use controls) and the deprivation contrast (deprived vs. nondeprived heavy marijuana users). We report 95% confidence intervals (CIs) for raw parameter estimates (b) and also η_p^2 from the GLMs to document effect sizes.

Group Comparisons on Affect and Marijuana-Relevant Individual Differences

Means, standard deviations, and p values for the two marijuana group contrasts for the affect and marijuana-relevant individual difference measures are reported in Table 1. None of the deprivation contrasts were significant for any of the individual difference measures, as expected given that heavy users were randomly assigned to the deprived and nondeprived groups. Significant heavy marijuana user contrasts were observed for all individual difference measures. On the DASS-21, heavy marijuana users reported higher depression ($\eta_p^2 = .04$, $b = 2.6$, 95% CI [.6, 4.5]), $t(153) = 2.56$, $p = .011$; anxiety ($\eta_p^2 = .09$, $b = 3.3$, 95% CI [1.6, 5.1]), $t(153) = 3.84$, $p < .001$; and stress ($\eta_p^2 = .05$, $b = 3.2$, 95% CI [.9, 5.4]), $t(153) = 2.77$, $p = .006$, than did controls in the past month. On the MMM, heavy marijuana users endorsed greater stress coping ($\eta_p^2 = .36$, $b = 4.7$, 95% CI [3.7, 5.7]), $t(153) = 9.37$, $p < .001$; enhancement ($\eta_p^2 = .40$, $b = 5.5$, 95% CI [4.4, 6.5]), $t(153) = 10.02$, $p < .001$; expansion ($\eta_p^2 = .31$, $b = 5.9$, 95% CI [4.5, 7.4]), $t(153) = 8.28$, $p < .001$; and social motives ($\eta_p^2 = .33$, $b = 4.7$, 95% CI [3.6, 5.8]), $t(153) = 8.62$, $p < .001$, and less conformity ($\eta_p^2 = .03$, $b = -.7$, 95% CI [−1.4, −.1]), $t(153) = 2.16$, $p = .032$, than did controls. Finally, heavy marijuana users were positive for marijuana abuse and dependence diagnoses (assessed via the MINI) at higher rates than were controls, $\chi^2(4, N = 104) = 77.9$, $p < .001$.

Marijuana Deprivation Manipulation Checks

Means, standard deviations, and p values for the deprivation contrast on self-report and biological confirmation of marijuana

Table 1
Participant Characteristics by Marijuana Group

Variable	Deprived		Nondeprived		Control		Deprivation contrast (*p*)[a]	Heavy user contrast (*p*)[a]
	M (*SD*)	%	*M* (*SD*)	%	*M* (*SD*)	%		
Age	22.3 (3.6)		22.1 (3.3)		21 (2.9)		.743	.030
Sex (female)		50		50		50	1.0	1.0
Race							.524	.082
Asian		0		1.9		5.8		
Black		19.2		23.1		11.5		
White		80.8		75		82.7		
Hispanic ethnicity		7.7		3.8		5.8	.674	1.0
Depression Anxiety Stress Scales								
Anxiety	6.9 (5.2)		6.8 (5.8)		3.5 (4.3)		.939	<.001
Depression	6.2 (5.8)		6.7 (7.1)		3.8 (4.5)		.665	.011
Stress	10.4 (7.4)		9.7 (6.9)		6.9 (5.7)		.599	.006
Previous anxiety diagnosis[b]		3.8		1.9		0	1.0	.536
Previous depression diagnosis[b]		3.8		7.7		9.6		.580
Previous psychiatric diagnosis or treatment[b]		17.3		23.1		15.4		.611
Young Adult Alcohol Problems Scale	4.3 (3.0)		5.3 (3.9)		4.2 (2.7)		.138	.230
Alcoholic drinks per week	10.1 (10.7)		9.8 (12.6)		9.0 (9.4)		.883	.631
Cigarettes per week	22.9 (40.5)		19.4 (32.1)		.0 (.0)		.558	<.001
Smoker status							.290	<.001
Nonsmoker		32.7		44.2		88.5		
Former smoker		9.6		1.9		1.9		
Less than daily smoker		23.1		19.2		9.6		
Daily smoker		34.6		34.6		.0		
Marijuana Motives Measure								
Conformity	.7 (1.4)		.8 (1.7)		1.5 (2.5)		.762	.032
Enhancement	11.1 (2.6)		11.9 (2.3)		6.0 (4.3)		.190	<.001
Expansion	7.6 (4.7)		8.2 (4.8)		1.9 (3.0)		.487	<.001
Social	7.1 (3.5)		7.9 (3.6)		2.8 (2.5)		.193	<.001
Stress Coping	5.4 (3.6)		5.8 (3.1)		.9 (2.0)		.490	<.001
Cannabis use disorder diagnosis (current)							.204	<.001
No diagnosis		25.0		11.5		90.4		
Marijuana abuse		25.0		30.8		9.6		
Marijuana dependence		50.0		57.7		.0		

Note. $N = 156$.
[a] From general linear models for quantitative variables and from chi-squared tests for categorical variables. [b] Self-report.

use, marijuana withdrawal, and marijuana craving are reported in Table 2. Deprived and nondeprived heavy marijuana users reported comparable marijuana use at the screening session, as expected by random assignment ($\eta_p^2 < .01$, $b = .2$, 95% CI [−2.6, 3.1]), $t(102) = .17$, $p = .866$. Deprived marijuana users reported greater time since last marijuana use ($M = 3.7$ days, $SD = .8$; range = 3–8) relative to nondeprived users ($M = .5$, $SD = .4$; range = 0–2) at the experimental session ($\eta_p^2 = .86$, $b = 3.2$, 95% CI [3.0, 3.5]), $t(102) = 24.97$, $p < .001$, with descriptive statistics confirming that both groups adhered to instructions. Biological verification of deprivation status via urinalysis confirmed that deprived marijuana users displayed lower creatinine-normalized

Table 2
Marijuana Deprivation Manipulation Checks

Variable	Deprived	Nondeprived	Deprivation contrast (*p*)
Average marijuana use (grams/week from screening session)	7.2 (7.8)	7.0 (6.8)	.866
Days since last marijuana use (exp. session)	3.67 (.82)	.46 (.43)	<.001
Urinalysis			
Creatinine-normalized THC (exp. session)[a]	123 (158)	303 (202)	<.001
Specimen ratio (exp.−screening session)	.4 (.2)	1.3 (1.1)	<.001
Marijuana Withdrawal Checklist (exp. session)[a]			
Total score	7.4 (6.5)	3.1 (3.3)	<.001
Negative Affect subscale	1.3 (1.8)	.5 (.6)	.003
Marijuana Craving Questionnaire (exp. session)[a]	4.5 (.7)	4.2 (.6)	.024

Note. $N = 104$. Data are means, with standard deviations in parentheses. exp. = experimental; THC = tetrahydrocannabinol.
[a] Controlling for baseline scores on these measures from the screening session.

THC at the experimental session (controlling for their baseline values at screening; $\eta_p^2 = .26$, $b = -.9$, 95% CI [−1.2, −.6]), $t(102) = 6.02$, $p < .001$, and a lower specimen ratio (experimental/screening session ratio; $\eta_p^2 = .20$, $b = -179.6$, 95% CI [−251.5, −107.7]), $t(101) = 4.95$, $p < .001$, relative to nondeprived users. Furthermore, no deprived user's specimen ratio exceeded the 1.5 cutoff point advocated by Huestis and Cone (1998) for detecting recent use. Deprived users reported higher scores than did nondeprived users on the total score ($\eta_p^2 = .15$, $b = 4.2$, 95% CI [2.2, 6.3]), $t(101) = 4.19$, $p < .001$, and Negative Affect subscale ($\eta_p^2 = .09$, $b = .8$, 95% CI [.3, 1.4]), $t(101) = 3.09$, $p = .003$, of the MWC at the experimental session, controlling for their respective baseline scores at screening. Deprived users also reported higher marijuana craving than did nondeprived users at the experimental session, controlling for baseline craving at screening ($\eta_p^2 = .05$, $b = 4.9$, 95% CI [.7, 9.2]), $t(101) = 2.29$, $p = .024$.

Marijuana Group Effects on Startle Potentiation

Startle potentiation[3] was analyzed in a GLM with between-subjects regressors for the heavy marijuana user and deprivation planned orthogonal contrasts. General startle reactivity (mean-centered) and order (Order 1 vs. Order 2) were included as interactive covariates. As expected, significant nonzero startle potentiation was observed ($\eta_p^2 = .49$, $b = 37.8$, 95% CI [31.4, 44.2]), $t(141) = 11.69$, $p < .001$, such that startle response was significantly potentiated during unpredictable-shock-threat compared to no-shock blocks. The heavy marijuana user contrast was significant ($\eta_p^2 = .03$, $b = 15.0$, 95% CI [1.5, 28.5]), $t(141) = 2.20$, $p = .029$, indicating that heavy marijuana users displayed approximately 15 μV greater startle potentiation to threat of unpredictable shock compared to controls (see Figure 1). The deprivation contrast was not significant ($\eta_p^2 < .01$, $b = -1.4$, 95% CI [−17.2, 14.4]), $t(141) = .18$, $p = .861$, indicating comparable startle potentiation across deprived and nondeprived heavy users.

Supplemental robustness analyses evaluated the just-reported significant heavy marijuana user contrast while controlling for all non-marijuana-use-relevant individual differences listed in Table 1 (i.e., age; sex; race; Hispanic ethnicity; DASS scales; and self-report of anxiety, depression, or any psychiatric diagnosis or treatment, drinks per week, cigarettes per week, or smoker status) by including each of these individual differences as a covariate in separate analyses. Cannabis use disorder diagnoses and marijuana use motives were not considered as covariates in these analyses because variance on these measures is inherent to group membership in the heavy user groups (Miller & Chapman, 2001). The heavy marijuana user contrast remained significant across all models controlling for these covariates (individual p values for this contrast ranged from .022 to .047).

Marijuana-Relevant Individual Differences and Startle Potentiation

To clarify the observed significant heavy marijuana user contrast, we conducted follow-up analyses of marijuana-relevant individual differences measures (i.e., marijuana use motives, diagnostic status). Each of these individual difference variables was examined in separate GLMs that included general startle reactivity and order as interactive covariates per the primary analysis of marijuana group reported earlier. Given the exploratory nature of these follow-up analyses, we report both raw and false discovery rate (FDR) corrected p values (Benjamini & Hochberg, 1995) for these statistical tests.

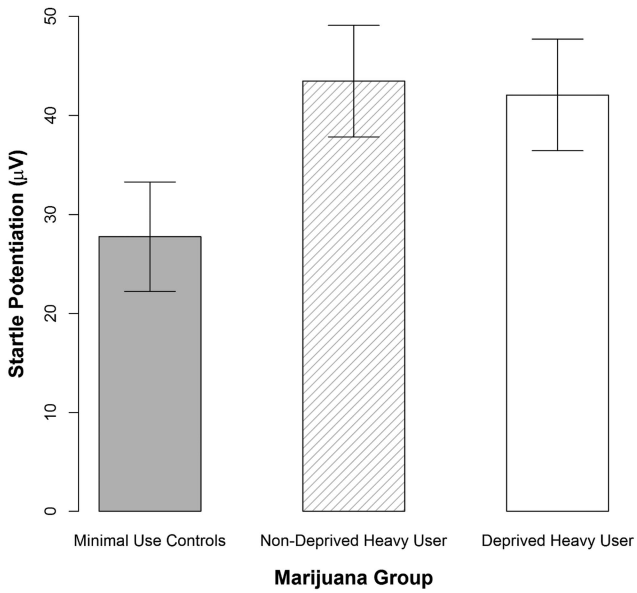

Figure 1. Startle potentiation by marijuana group. This figure displays the predicted values (i.e., means) for startle potentiation to unpredictable shock threat by marijuana group from the general linear model (GLM). Error bars represent ±1 standard error for these predicted values. This GLM is adjusted for all covariates. Figure

Participants who reported greater stress-coping motives for their marijuana use displayed significantly increased startle potentiation ($\eta_p^2 = .05$, $b = 2.4$, 95% CI [.7, 4.1]), $t(145) = 2.81$, $p = .006$, FDR-corrected $p = .031$ (see Figure 2A).[4] No significant effects were observed for conformity ($\eta_p^2 = .02$, $b = -3.5$, 95% CI [−8.1, 1.1]), $t(145) = 1.50$, $p = .137$, FDR-corrected $p = .274$; enhancement ($\eta_p^2 = .01$, $b = .8$, 95% CI [−.7, 2.3]), $t(145) = 1.07$, $p = .288$, FDR-corrected $p = .432$; expansion ($\eta_p^2 < .01$, $b = .2$, 95% CI [−1.1, 1.5]), $t(145) = .33$, $p = .744$, FDR-corrected $p = .744$; or social motives ($\eta_p^2 = .01$, $b = .8$, 95% CI [−.9, 2.4]), $t(145) = .90$, $p = .367$, FDR-corrected $p = .441$, for marijuana use. A significant linear effect for marijuana use disorder diagnosis was observed ($\eta_p^2 = .05$, $b = 18.8$, 95% CI [4.5, 33.1]), $t(141) = 2.60$, $p = .010$, FDR-corrected $p = .031$, with increasing startle potentiation observed across participants with no diagnosis versus marijuana abuse versus marijuana dependence (see Figure 2B).

[3] Three participants were identified as Bonferroni-corrected outliers ($p \leq .05$) in these analyses and were therefore removed from all analyses of startle potentiation. However, the focal heavy marijuana user contrast remains significant if these participants are retained.

[4] This effect for stress-coping motives remained significant in a supplemental model that examined its unique effect controlling for all other coping motives and participants' extent of marijuana use (i.e., grams of use/week; $\eta_p^2 = .03$, $b = 2.7$, 95% confidence interval [.2, 5.1]), $t(140) = 2.17$, $p = .032$. Also consistent with the other analyses, none of the other motives scales had significant unique effects in this model.

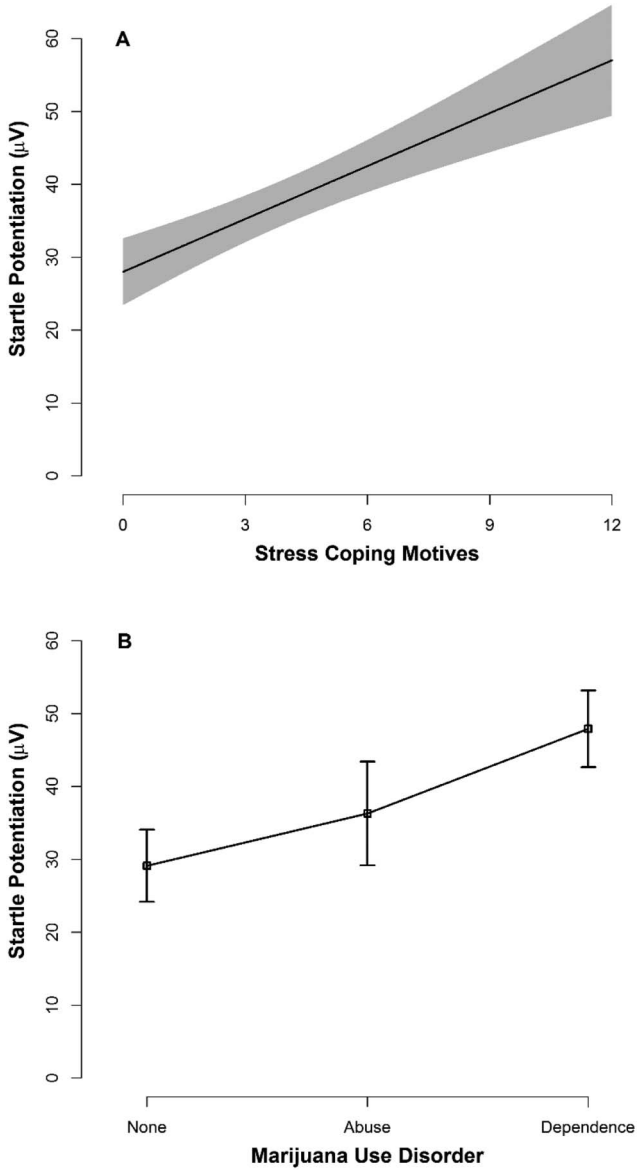

Figure 2. Startle potentiation by marijuana-relevant individual differences. Panel A: This panel displays predicted values for startle potentiation to unpredictable shock threat by stress-coping motives from the general linear model (GLM). The shaded region represents ±1 standard error for these predicted values. This GLM is adjusted for all covariates. Panel B: This panel displays predicted values for startle potentiation to unpredictable shock threat by marijuana use disorder diagnoses from the GLM. Error bars represent ±1 standard error for these predicted values. This GLM is adjusted for all covariates. Figure

Discussion

Stress Neuroadaptation in Heavy Marijuana Use

We investigated stressor reactivity among deprived and nondeprived heavy marijuana users and minimal use controls to test for predicted stress neuroadaptations that may result from heavy marijuana use. We observed that heavy marijuana users (both deprived and nondeprived) displayed increased startle potentiation during threat of unpredictable electric shock relative to minimal use controls. To our knowledge, this is the first study to demonstrate increased reactivity to unpredictable stressors among heavy marijuana users in a manner comparable to exaggerated stressor reactivity among users of other drugs. Specifically, Moberg and colleagues (2017) reported that alcohol-dependent participants in early abstinence (<8 weeks) displayed increased startle potentiation to unpredictable (vs. predictable) shock threat relative to healthy controls. Similarly, Gorka, Nelson, and Shankman (2013) demonstrated increased startle potentiation during unpredictable shock threat in participants with comorbid alcohol dependence and panic disorder relative to both participants with only panic disorder and healthy controls. Furthermore, Grillon, Avenevoli, Daurignac, and Merikangas (2007) confirmed increased startle potentiation during blocks of unpredictable but not predictable air blast cues in overnight-deprived smokers relative to nonsmokers.

Synthesis of these studies provides emerging support that is consistent with the stress neuroadaptation model in the etiology of alcohol and other drug (AOD) addiction (Kaye et al., 2017). Rodent addiction models posit that repeated homeostatic adjustments in brain stress systems to acute periods of AOD intoxication eventually lead to long-lasting, compensatory allostatic changes in CNS structures and circuits involved in behavioral and emotional response to stressors (i.e., stress neuroadaptations; Koob & Le Moal, 2008). Substantial evidence has suggested that specific adaptations in CRF- and NE-sensitive pathways in the central extended amygdala contribute importantly to increased stressor reactivity in rodents, including stress-induced reinstatement of drug use (for reviews see Mantsch, Baker, Funk, Lê, & Shaham, 2016; Silberman & Winder, 2013; Smith & Aston-Jones, 2008). It is important to note that basic affective science research with both humans and rodents have validated startle potentiation selectively to unpredictable stressors as a sensitive index of these stress mechanisms. Thus, increased startle potentiation to unpredictable threat among heavy users of marijuana, alcohol, and/or tobacco in humans is consistent with both increased stressor reactivity and the neural mechanisms proposed by the rodent stress neuroadaptation model. Future research in humans can strengthen evidence for neural mechanisms by direct pharmacologic manipulation of these neurotransmitter systems while measuring unpredictable stressor startle potentiation in AOD dependent users (e.g., National Institute on Alcohol Abuse and Alcoholism [NIAAA] R01-AA024388). Moreover, future research should explore other marijuana use characteristics (e.g., chronicity, patterns of episodic use such as binge−withdrawal) that may contribute to neuroadaptations in stressor reactivity.

Although results from recent studies have been consistent with the stress neuroadaptation model, the exclusive use of cross-sectional designs with preexisting groups across these studies does not provide definitive evidence of drug-related changes, and this allows for other plausible interpretations. In particular, increased startle potentiation to unpredictable stressors may represent a premorbid risk factor for AOD use disorders rather than merely a consequence of chronic AOD use (Gorka, Lieberman, Phan, & Shankman, 2016; Rasmussen & Kincaid, 2015). Moreover, these factors may work in tandem, such that premorbid risk involving heightened startle potentiation contrib-

utes to AOD use, which subsequently exacerbates stressor reactivity over time. Additional research including longitudinal designs to document within-subject change in stressor reactivity following heavy and/or chronic use is necessary to adjudicate between alternative but mutually tenable interpretations. Such research would also address ambiguity regarding other possible confounding variables that may differ when comparing the heavy and minimal use groups, variables that might explain differences in stressor reactivity between the two groups. Our use of covariates to control for some key individual differences reduces but cannot eliminate concern about such confounds.

Stressor Reactivity During Marijuana Deprivation

With respect to our second aim, we did not observe any impact of marijuana deprivation on startle potentiation to unpredictable shock threat among heavy users. This null result was observed despite clear evidence of a successful deprivation manipulation, wherein compliance was verified by both self-report and urinalysis. Furthermore, deprived heavy users reported significantly more intense withdrawal symptoms and negative affect symptoms, as well as greater craving, than did nondeprived heavy users. These results support that our deprived heavy users experienced a robust withdrawal syndrome including expected increases in negative affect. Nonetheless, marijuana withdrawal did not appear to influence their stressor reactivity.

The null effect of marijuana deprivation on unpredictable startle potentiation suggests potentially distinct mechanisms may be responsible for (a) the increased stressor reactivity observed among all heavy marijuana users and (b) the increased subjective negative affect displayed by deprived heavy users only, respectively. This distinction has support from rodent models; specifically, NE and CRF mechanisms proposed to experience neuroadaptations following chronic drug use support "dynamic, active response to an acute stressor" rather than tonic, persistent negative mood states (Koob & Zorrilla, 2012, p. 309; also see Heilig, Goldman, Berrettini, & O'Brien, 2011).

Thus, it appears possible that heavy marijuana users may experience two forms of affective disruption that could motivate further use or prompt relapse after cessation of use. Unpredictable stressors may elicit punctate, exaggerated reactivity that increases the reward value of further marijuana use during stressful periods. These phasic increases in drug use motivation may further be layered on top of tonically increased negative affect resulting from deprivation and also provide strong motivation for further use and/or relapse (Baker et al., 2004; Curtin, McCarthy, Piper, & Baker, 2006).

Of course, increased confidence in this dual mechanism thesis for marijuana and other drugs requires additional investigation. For example, it is possible that we did not observe a deprivation effect because reactivity to unpredictable threat may represent a stable risk marker that does not vary across periods of marijuana use and abstinence. Alternatively, this null effect for changes in stressor reactivity during marijuana deprivation could be a Type II error, though it should be acknowledged that we had adequate power to detect such an effect (86% power to detect a moderate-sized between-subjects effect). Nonetheless, confidence in this null effect would be increased with replication that used potentially more powerful within-subject manipulations of deprivation. This effect must also be scrutinized for other commonly used illicit drugs. For example, to our knowledge, short-term drug deprivation effects on unpredictable stressor reactivity have not been examined for alcohol or opiates, two drugs that produce potent withdrawal syndromes. Two studies have examined the effects of tobacco deprivation on unpredictable stressor reactivity using startle potentiation, but the results were inconsistent. Hogle and colleagues (2010) observed increased startle response to unpredictable shock threat among deprived relative to nondeprived smokers, but they did not include nonsmokers. In contrast, Grillon and colleagues (2007) reported increased startle potentiation to unpredictable air blasts among deprived smokers versus nonsmokers but no differences between deprived and nondeprived smokers. Given the paucity of evidence, we consider it premature to draw definitive conclusions about the impact of drug deprivation across drug classes on stressor reactivity (see Kaye et al., 2017, for further discussion of possible deprivation–withdrawal mechanisms).

Individual Differences in Stressor Reactivity

Our third aim was to examine relationships between individual differences in stressor reactivity and measures of marijuana use motives and marijuana use diagnostic status. As expected, heavy marijuana users reported stronger motives for marijuana use than did minimal use controls across all motives (i.e., enhancement, expansion, social, and stress coping) other than conformity. However, stress-coping motives was unique in its relationship with startle potentiation to unpredictable shock threat. Participants reporting greater stress-coping motives for marijuana use displayed increased startle potentiation during unpredictable stressor exposure. This relationship could emerge through varied etiologic pathways. If heavy marijuana use had increased stressor reactivity per the stress neuroadaptation model, users who may have experienced this neuroadaptation would be expected to use marijuana to dampen or otherwise cope with increased reactivity as it emerged over time. Support for this hypothesis would be strengthened by evidence that acute marijuana use dampened unpredictable threat startle potentiation, as has been demonstrated for other anxiolytic drugs (Baas et al., 2002; Bradford, Motschman, Starr, & Curtin, 2017; Bradford, Shapiro, & Curtin, 2013; Grillon et al., 2006; Hefner et al., 2013). Alternatively, if increased stressor reactivity were a premorbid risk factor for heavy marijuana use, use of marijuana to cope may represent an initial motive that itself leads to heavy use. This notwithstanding, the likely clinical importance of unpredictable threat startle potentiation is made salient by its unique relationship with stress coping motives. Startle potentiation to unpredictable threat also varied systematically by diagnostic status, with higher means observed for participants with marijuana dependencies versus marijuana abuse versus no diagnosis, further underscoring its clinical import.

Limitations and Future Directions

Synthesis of our results indicates that increased startle potentiation (a) was observed among heavy users versus minimal use controls, (b) was associated with increasing stress-coping motives, and (c) was associated with increased diagnostic severity for marijuana use disorder. Similar increased startle potentiation to unpredictable threat has been confirmed among individuals with AOD use disorders. This highlights heightened phasic stressor reactivity as a potential etiologically relevant mechanism across addictive drugs as proposed by the stress neuroadaptation model. Furthermore, it makes salient a potentially important negative consequence that may result from heavy use of any addictive drug,

including marijuana. However, cross-sectional designs like that of the present study are not sufficient to establish stress neuroadaptation and do not exclude the possibility that the observed effects resulted from premorbid risk-related individual differences. As noted earlier, increased confidence in these claims will require longitudinal designs to clearly document within-subject change following heavy, chronic drug use. Nonetheless, we believe that confirmation of these group differences in stressor reactivity as predicted by the stress neuroadaption model in a cross-sectional design was a necessary prerequisite to justify subsequent costlier longitudinal designs to definitively test for within-subject change.

Research with other drugs has also specifically implicated reactivity to unpredictable (vs. predictable) stressors. Unfortunately, the current study did not include a predictable stressor condition. Future research should also use methods (e.g., pharmacological challenge with neurotransmitter agonists and antagonists) and measures (e.g., functional magnetic resonance imaging, positron emission tomography) that enable more precise specification of the neural mechanisms accounting for increased stressor reactivity. For now, inference about neural mechanism rests primarily on the tight translation of both measure (startle potentiation) and methods (unpredictable shock threat) from preclinical research with rodents (Davis et al., 2010). Furthermore, it should be acknowledged that stressor reactivity was assessed in this study in the context of a reward decision-making task. Reward-related processes should not have confounded heavy user or deprivation contrasts, because all groups completed this decision-making task. However, this leaves open questions about whether these stressor reactivity effects generalize to situations without concurrent focus on rewards.

More broadly, the National Institute of Mental Health has begun to advocate for an experimental therapeutics approach in clinical research that simultaneously evaluates intervention efficacy and mechanism in clinical trials research (Insel, 2015; Insel & Gogtay, 2014). This approach requires that clinical trials explicitly measure pertinent mechanism(s) of either the disease process or the action of the intervention. In particular, the use of "surrogate endpoints"—early markers of disease process mechanisms with high predictive validity for later clinical outcomes—offer high promise to increase the pace of clinical trials research to develop and refine medications and other interventions for psychopathology broadly, including substance use disorders (Insel, 2012; Lerman et al., 2007; Litten, Falk, Ryan, & Fertig, 2016; McKee, 2009). We believe that data from this and other recent studies support the use of startle potentiation to unpredictable threat as a surrogate end point to study stress mechanisms in addiction using an experimental therapeutics approach (for review, see Kaye et al., 2017).

Finally, the focus on an etiological pathway involving increased stressor reactivity, either premorbidly or as a consequence of heavy use, highlights an important target for treatment. Clinical trials are under way to examine the use of NE alpha1 antagonist medications (e.g., prazosin, doxazosin; NIAAA R01-AA024388) to target stress-induced relapse in substance use disorders, consistent with mechanisms we propose here (for review, see Mantsch et al., 2016). More precise targeting of sources and coping strategies for unpredictable stressors may increase the efficacy of psychological interventions for addiction. Relapse prevention programs can help patients to better identify risks by explicit, personalized assessment of stressors in their lives that are both potent and characterized by a high degree of uncertainty. These programs can also help patients develop tools to reshape their environments and social interactions to reduce unpredictability (e.g., problem-solving to address uncertain financial, housing, or interpersonal stresses; practicing direct communication with others to clarify stressful but ambiguous interpersonal exchanges). This focus on reactivity to unpredictable stressors also reinforces the potential benefits offered by existing but often unavailable or underfunded harm reduction approaches that robustly reduce uncertainty for drug-dependent users regarding some of the more potent and unpredictable stressors involving housing, health, and other basic needs (Newman & Goldman, 2008). We believe that such multifaceted approaches involving medications, psychosocial interventions, and public health initiatives offer the most promise to address the high costs of AOD use disorders generally, including any increase in rates of marijuana use disorder that may result from changing perceptions and regulations of this drug.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Anthony, J. (2006). The epidemiology of cannabis dependence. In R. Roffman & R. Stephens (Eds.), *Cannabis dependence: Its nature, consequences, and treatment* (pp. 58–105). http://dx.doi.org/10.1017/CBO9780511544248.006

Arnone, D., Barrick, T., Chengappa, S., Mackay, C., Clark, C., & Abou-Saleh, M. (2008). Corpus callosum damage in heavy marijuana use: Preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. *NeuroImage, 41,* 1067–1074. http://dx.doi.org/10.1016/j.neuroimage.2008.02.064

Baas, J. M., Grillon, C., Böcker, K. B., Brack, A. A., Morgan, C. A., III, Kenemans, J. L., & Verbaten, M. N. (2002). Benzodiazepines have no effect on fear-potentiated startle in humans. *Psychopharmacology, 161,* 233–247. http://dx.doi.org/10.1007/s00213-002-1011-8

Baker, T. B., Piper, M. E., McCarthy, D. E., Majeskie, M. R., & Fiore, M. C. (2004). Addiction motivation reformulated: An affective processing model of negative reinforcement. *Psychological Review, 111,* 33–51. http://dx.doi.org/10.1037/0033-295X.111.1.33

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B, 57,* 289–300.

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & Van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology, 42,* 1–15. http://dx.doi.org/10.1111/j.1469-8986.2005.00271.x

Bonn-Miller, M. O., & Moos, R. H. (2009). Marijuana discontinuation, anxiety symptoms, and relapse to marijuana. *Addictive Behaviors, 34,* 782–785. http://dx.doi.org/10.1016/j.addbeh.2009.04.009

Bradford, D. E., Kaye, J. T., & Curtin, J. J. (2014). Not just noise: Individual differences in general startle reactivity predict startle response to uncertain and certain threat. *Psychophysiology, 51,* 407–411. http://dx.doi.org/10.1111/psyp.12193

Bradford, D. E., Magruder, K. P., Korhumel, R. A., & Curtin, J. J. (2014). Using the threat probability task to assess anxiety and fear during uncertain and certain threat. *Journal of Visualized Experiments, 12,* 51905. http://dx.doi.org/10.3791/51905

Bradford, D. E., Motschman, C. A., Starr, M. J., & Curtin, J. J. (2017). Alcohol's effects on emotionally motivated attention, defensive reactivity and subjective anxiety during uncertain threats. *Social Cognitive and Affective Neuroscience, 12,* 1823–1832. http://dx.doi.org/10.1093/scan/nsx095

Bradford, D. E., Shapiro, B. L., & Curtin, J. J. (2013). How bad could it be? Alcohol dampens stress responses to threat of uncertain intensity. *Psychological Science, 24,* 2541–2549. http://dx.doi.org/10.1177/0956797613499923

Budney, A. J., & Hughes, J. R. (2006). The cannabis withdrawal syndrome. *Current Opinion in Psychiatry, 19,* 233–238. http://dx.doi.org/10.1097/01.yco.0000218592.00689.e5

Budney, A. J., Hughes, J. R., Moore, B. A., & Vandrey, R. (2004). Review of the validity and significance of cannabis withdrawal syndrome. *American Journal of Psychiatry, 161,* 1967–1977. http://dx.doi.org/10.1176/appi.ajp.161.11.1967

Budney, A. J., Novy, P. L., & Hughes, J. R. (1999). Marijuana withdrawal among adults seeking treatment for marijuana dependence. *Addiction, 94,* 1311–1322. http://dx.doi.org/10.1046/j.1360-0443.1999.94913114.x

Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., Sanci, L., & Patton, G. C. (2002). Cannabis dependence in young adults: An Australian population study. *Addiction, 97,* 187–194. http://dx.doi.org/10.1046/j.1360-0443.2002.00029.x

Copersino, M. L., Boyd, S. J., Tashkin, D. P., Huestis, M. A., Heishman, S. J., Dermand, J. C., . . . Gorelick, D. A. (2006). Cannabis withdrawal among nontreatment-seeking adult cannabis users. *American Journal on Addictions, 15,* 8–14. http://dx.doi.org/10.1080/10550490500418997

Crippa, J. A., Zuardi, A. W., Martín-Santos, R., Bhattacharyya, S., Atakan, Z., McGuire, P., & Fusar-Poli, P. (2009). Cannabis and anxiety: A critical review of the evidence. *Human Psychopharmacology: Clinical and Experimental, 24,* 515–523. http://dx.doi.org/10.1002/hup.1048

Curtin, J. (2011). *PhysBox: The Psychophysiology Toolbox*. Retrieved from http://dionysus.psych.wisc.edu/PhysBox.htm

Curtin, J. J. (2015). lmSupport: Support for Linear Models (Version 2.9.2) [Coumputer Software]. Retrieved from. https://cran.r-project.org/web/packages/lmSupport/index.html

Curtin, J., McCarthy, D., Piper, M., & Baker, T. (2006). Implicit and explicit drug motivational processes: A model of boundary conditions. In R. Wiers & A. Stacy (Eds.), *Handbook of implicit cognition and addiction* (pp. 233–250). http://dx.doi.org/10.4135/9781412976237.n16

Curtin, J. J., Patrick, C. J., Lang, A. R., Cacioppo, J. T., & Birbaumer, N. (2001). Alcohol affects emotion through cognition. *Psychological Science, 12,* 527–531. http://dx.doi.org/10.1111/1467-9280.00397

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology, 35,* 105–135. http://dx.doi.org/10.1038/npp.2009.109

Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods, 134,* 9–21. http://dx.doi.org/10.1016/j.jneumeth.2003.10.009

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y.-T., . . . Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: Implications for psychosis. *Neuropsychopharmacology, 29,* 1558–1572. http://dx.doi.org/10.1038/sj.npp.1300496

Fox, H. C., Tuit, K. L., & Sinha, R. (2013). Stress system changes associated with marijuana dependence may increase craving for alcohol and cocaine. *Human Psychopharmacology: Clinical and Experimental, 28,* 40–53. http://dx.doi.org/10.1002/hup.2280

Gorka, S. M., Lieberman, L., Phan, K. L., & Shankman, S. A. (2016). Association between problematic alcohol use and reactivity to uncertain threat in two independent samples. *Drug and Alcohol Dependence, 164,* 89–96. http://dx.doi.org/10.1016/j.drugalcdep.2016.04.034

Gorka, S. M., Nelson, B. D., & Shankman, S. A. (2013). Startle response to unpredictable threat in comorbid panic disorder and alcohol dependence. *Drug and Alcohol Dependence, 132,* 216–222. http://dx.doi.org/10.1016/j.drugalcdep.2013.02.003

Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: A review of self-reported cannabis effects. *Drug and Alcohol Review, 22,* 453–460. http://dx.doi.org/10.1080/09595230310001613976

Grillon, C., Avenevoli, S., Daurignac, E., & Merikangas, K. R. (2007). Fear-potentiated startle to threat, and prepulse inhibition among young adult nonsmokers, abstinent smokers, and nonabstinent smokers. *Biological Psychiatry, 62,* 1155–1161. http://dx.doi.org/10.1016/j.biopsych.2006.12.027

Grillon, C., Baas, J. M., Pine, D. S., Lissek, S., Lawley, M., Ellis, V., & Levine, J. (2006). The benzodiazepine alprazolam dissociates contextual fear from cued fear in humans as assessed by fear-potentiated startle. *Biological Psychiatry, 60,* 760–766. http://dx.doi.org/10.1016/j.biopsych.2005.11.027

Hathaway, A. (2003). Cannabis effects and dependency concerns in long-term frequent users: A missing piece of the public health puzzle. *Addiction Research & Theory, 11,* 441–458. http://dx.doi.org/10.1080/1606635021000041807

Hefner, K. R., & Curtin, J. J. (2012). Alcohol stress response dampening: Selective reduction of anxiety in the face of uncertain threat. *Journal of Psychopharmacology, 26,* 232–244. http://dx.doi.org/10.1177/0269881111416691

Hefner, K. R., Moberg, C. A., Hachiya, L. Y., & Curtin, J. J. (2013). Alcohol stress response dampening during imminent versus distal, uncertain threat. *Journal of Abnormal Psychology, 122,* 756–769. http://dx.doi.org/10.1037/a0033407

Hefner, K. R., & Starr, M. J. (2017). Altered subjective reward valuation among female heavy marijuana users. *Experimental and Clinical Psychopharmacology, 25,* 1–12. http://dx.doi.org/10.1037/pha0000101

Hefner, K. R., Starr, M. J., & Curtin, J. J. (2016). Altered subjective reward valuation among drug-deprived heavy marijuana users: Aversion to uncertainty. *Journal of Abnormal Psychology, 125,* 138–150. http://dx.doi.org/10.1037/abn0000106

Heilig, M., Goldman, D., Berrettini, W., & O'Brien, C. P. (2011). Pharmacogenetic approaches to the treatment of alcohol addiction. *Nature Reviews Neuroscience, 12,* 670–684. http://dx.doi.org/10.1038/nrn3110

Heishman, S. J., Singleton, E. G., & Liguori, A. (2001). Marijuana Craving Questionnaire: Development and initial validation of a self-report instrument. *Addiction, 96,* 1023–1034. http://dx.doi.org/10.1046/j.1360-0443.2001.967102312.x

Hogle, J. M., Kaye, J. T., & Curtin, J. J. (2010). Nicotine withdrawal increases threat-induced anxiety but not fear: Neuroadaptation in human addiction. *Biological Psychiatry, 68,* 719–725. http://dx.doi.org/10.1016/j.biopsych.2010.06.003

Huestis, M. A., & Cone, E. J. (1998). Differentiating new marijuana use from residual drug excretion in occasional marijuana users. *Journal of Analytical Toxicology, 22,* 445–454. http://dx.doi.org/10.1093/jat/22.6.445

Hyman, S. M., & Sinha, R. (2009). Stress-related factors in cannabis use and misuse: Implications for prevention and treatment. *Journal of Substance Abuse Treatment, 36,* 400–413. http://dx.doi.org/10.1016/j.jsat.2008.08.005

Insel, T. R. (2012, October 10). Next-generation treatments for mental disorders. *Science Translational Medicine, 4,* 155ps19. http://dx.doi.org/10.1126/scitranslmed.3004873

Insel, T. R. (2015). The NIMH experimental medicine initiative. *World Psychiatry, 14,* 151–153. http://dx.doi.org/10.1002/wps.20227

Insel, T. R., & Gogtay, N. (2014). National Institute of Mental Health clinical trials: New opportunities, new expectations. *Journal of the American Medical Association Psychiatry, 71,* 745–746. http://dx.doi.org/10.1001/jamapsychiatry.2014.426

Johnston, L., & O'Malley, P. (1986). *Drug use among American high school students, college students, and other young adults*. Rockville, MD: Department of Health and Human Services.

Kaye, J. T., Bradford, D. E., & Curtin, J. J. (2016). Psychometric properties of startle and corrugator response in NPU, affective picture viewing, and resting state tasks. *Psychophysiology, 53,* 1241–1255. http://dx.doi.org/10.1111/psyp.12663

Kaye, J. T., Bradford, D. E., Magruder, K. P., & Curtin, J. J. (2017). Probing for neuroadaptations to unpredictable stressors in addiction: Translational methods and emerging evidence. *Journal of Studies on Alcohol and Drugs, 78,* 353–371. http://dx.doi.org/10.15288/jsad.2017.78.353

Koob, G. F., & Le Moal, M. (2008). Addiction and the brain antireward system. *Annual Review of Psychology, 59,* 29–53. http://dx.doi.org/10.1146/annurev.psych.59.103006.093548

Koob, G. F., & Zorrilla, E. P. (2012). Update on corticotropin-releasing factor pharmacotherapy for psychiatric disorders: A revisionist view. *Neuropsychopharmacology, 37,* 308–309. http://dx.doi.org/10.1038/npp.2011.213

Lerman, C., LeSage, M. G., Perkins, K. A., O'Malley, S. S., Siegel, S. J., Benowitz, N. L., & Corrigall, W. A. (2007). Translational research in medication development for nicotine dependence. *Nature Reviews Drug Discovery, 6,* 746–762. http://dx.doi.org/10.1038/nrd2361

Lichtman, A. H., & Martin, B. R. (2002). Marijuana withdrawal syndrome in the animal model. *Journal of Clinical Pharmacology, 42*(Suppl. 1), 20S–27S. http://dx.doi.org/10.1002/j.1552-4604.2002.tb05999.x

Litten, R. Z., Falk, D. E., Ryan, M. L., & Fertig, J. B. (2016). Discovery, development, and adoption of medications to treat alcohol use disorder: Goals for the phases of medications development. *Alcoholism: Clinical and Experimental Research, 40,* 1368–1379. http://dx.doi.org/10.1111/acer.13093

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour Research and Therapy, 33,* 335–343. http://dx.doi.org/10.1016/0005-7967(94)00075-U

Manno, J., Ferslew, K., & Manno, B. (1984). Urine excretion patterns of cannabinoids and the clinical application of the EMIT-d.a.u. cannabinoid urine assay for substance abuse treatment. In S. Agurell, W. Dewey, & R. Willette (Eds.), *The cannabinoids: Chemical, pharmacologic, and therapeutic aspects* (pp. 281–290). New York, NY: Academic Press.

Mantsch, J. R., Baker, D. A., Funk, D., Lê, A. D., & Shaham, Y. (2016). Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology, 41,* 335–356. http://dx.doi.org/10.1038/npp.2015.142

Martín-Calderón, J. L., Muñoz, R. M., Villanúa, M. A., del Arco, I., Moreno, J. L., Rodríguez de Fonseca, F., & Navarro, M. (1998). Characterization of the acute endocrine actions of (-)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210), a potent synthetic cannabinoid in rats. *European Journal of Pharmacology, 344,* 77–86. http://dx.doi.org/10.1016/S0014-2999(97)01560-4

McCarthy, D. E., Gloria, R., & Curtin, J. J. (2009). Attention bias in nicotine withdrawal and under stress. *Psychology of Addictive Behaviors, 23,* 77–90. http://dx.doi.org/10.1037/a0014288

McKee, S. A. (2009). Developing human laboratory models of smoking lapse behavior for medication screening. *Addiction Biology, 14,* 99–107. http://dx.doi.org/10.1111/j.1369-1600.2008.00135.x

Miller, G. A., & Chapman, J. P. (2001). Misunderstanding analysis of covariance. *Journal of Abnormal Psychology, 110,* 40–48. http://dx.doi.org/10.1037/0021-843X.110.1.40

Moberg, C. A., Bradford, D. E., Kaye, J. T., & Curtin, J. J. (2017). Increased startle potentiation to unpredictable stressors in alcohol dependence: Possible stress neuroadaptation in humans. *Journal of Abnormal Psychology, 126,* 441–453. http://dx.doi.org/10.1037/abn0000265

Moitra, E., Christopher, P. P., Anderson, B. J., & Stein, M. D. (2015). Coping-motivated marijuana use correlates with *DSM–5* cannabis use disorder and psychological distress among emerging adults. *Psychology of Addictive Behaviors, 29,* 627–632. http://dx.doi.org/10.1037/adb0000083

Newman, S., & Goldman, H. (2008). Putting housing first, making housing last: Housing policy for persons with severe mental illness. *American Journal of Psychiatry, 165,* 1242–1248. http://dx.doi.org/10.1176/appi.ajp.2008.08020279

Nosek, B. A., Alter, G., Banks, G. C., Borsboom, D., Bowman, S. D., Breckler, S. J., . . . Yarkoni, T. (2015). Promoting an open research culture. *Science, 348,* 1422–1425. http://dx.doi.org/10.1126/science.aab2374

Nutt, D., King, L. A., Saulsbury, W., & Blakemore, C. (2007). Development of a rational scale to assess the harm of drugs of potential misuse. *Lancet, 369,* 1047–1053. http://dx.doi.org/10.1016/S0140-6736(07)60464-4

Pope, H. G., Jr., & Yurgelun-Todd, D. (1996). The residual cognitive effects of heavy marijuana use in college students. *Journal of the American Medical Association, 275,* 521–527. http://dx.doi.org/10.1001/jama.1996.03530310027028

R Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Rasmussen, D. D., & Kincaid, C. L. (2015). Acoustic startle in alcohol-naïve male rats predicts subsequent voluntary alcohol intake and alcohol preference. *Alcohol and Alcoholism, 50,* 56–61. http://dx.doi.org/10.1093/alcalc/agu065

Rodríguez de Fonseca, F., Carrera, M. R., Navarro, M., Koob, G. F., & Weiss, F. (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science, 276,* 2050–2054. http://dx.doi.org/10.1126/science.276.5321.2050

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., . . . Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for *DSM–IV* and ICD-10. *Journal of Clinical Psychiatry, 59*(Suppl. 20), 22–33.

Silberman, Y., & Winder, D. G. (2013). Emerging role for corticotropin releasing factor signaling in the bed nucleus of the stria terminalis at the intersection of stress and reward. *Frontiers in Psychiatry, 4,* 42. http://dx.doi.org/10.3389/fpsyt.2013.00042

Simons, J., Correia, C., Carey, K., & Borsari, B. (1998). Validating a five-factor marijuana motives measure: Relations with use, problems, and alcohol motives. *Journal of Counseling Psychology, 45,* 265–273. http://dx.doi.org/10.1037/0022-0167.45.3.265

Smith, R. J., & Aston-Jones, G. (2008). Noradrenergic transmission in the extended amygdala: Role in increased drug-seeking and relapse during protracted drug abstinence. *Brain Structure & Function, 213,* 43–61. http://dx.doi.org/10.1007/s00429-008-0191-3

Van Boxtel, G. (1998). Computational and statistical methods for analyzing event-related potential data. *Behavior Research Methods, Instruments, & Computers, 30,* 87–102. http://dx.doi.org/10.3758/BF03209419

Vandrey, R. G., Budney, A. J., Moore, B. A., & Hughes, J. R. (2005). A cross-study comparison of cannabis and tobacco withdrawal. *American Journal on Addictions, 14,* 54–63. http://dx.doi.org/10.1080/10550490590899853

Volkow, N. D., Hampson, A. J., & Baler, R. D. (2017). Don't worry, be happy: Endocannabinoids and cannabis at the intersection of stress and reward. *Annual Review of Pharmacology and Toxicology, 57,* 285–308. http://dx.doi.org/10.1146/annurev-pharmtox-010716-104615

Received August 9, 2017
Revision received January 11, 2018
Accepted January 18, 2018 ■