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Reward-related Brain Response and Craving Correlates of Marijuana Cue Exposure: A Preliminary Study in Treatment-Seeking Marijuana-Dependent Subjects

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Abstract

Objective—Determining the brain substrates underlying the motivation to abuse addictive drugs is critical for understanding and treating addictive disorders. Laboratory neuroimaging studies have demonstrated differential activation of limbic and motivational circuitry [e.g., amygdala, hippocampus, ventral striatum, insula, and orbitofrontal cortex (OFC)] triggered by cocaine, heroin, nicotine, and alcohol cues. The literature on neural responses to marijuana cues is sparse. Thus, the goals of this study were to characterize the brain's response to marijuana cues, a major motivator underlying drug use and relapse, and determine whether these responses are linked to self-reported craving in a clinically relevant population of treatment-seeking marijuana-dependent subjects.

Methods—Marijuana craving was assessed in 12 marijuana-dependent subjects using the Marijuana Craving Questionnaire-Short Form. Subsequently, BOLD functional MRI data were acquired during exposure to alternating 20 second blocks of marijuana-related versus matched nondrug visual cues.

Results—Brain activation during marijuana cue exposure was significantly greater in bilateral amygdala and hippocampus. Significant positive correlations between craving scores and brain activation were found in ventral striatum, and medial and lateral OFC ($p < 0.0001$).

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Conclusions—This study presents direct evidence for a link between reward-relevant brain responses to marijuana cues and craving, and extends the current literature on marijuana cue reactivity. Further, the correlative relationship between craving and brain activity in reward-related regions was observed in a clinically relevant sample (treatment-seeking marijuana-dependent subjects). Results are consistent with prior findings in cocaine, heroin, nicotine, and alcohol cue studies, indicating that the brain substrates of cue-triggered drug motivation are shared across abused substances.

Keywords

Cannabis; Marijuana Cues; Craving; Neuroimaging; Addiction; Brain Reward Circuitry

1. INTRODUCTION

Marijuana (MJ) is the most widely used illicit drug in the United States with 3.2 million regular users and 1.5% of the US population meeting DSM-IV diagnostic criteria for abuse or dependence (Compton et al. 2004; SAMHSA 2005). Dependence is associated with negative consequences to the individual (e.g. anxiety, depression, psychosis, impaired memory and learning, lung disease, suppressed immunity, and increased cancer risk) and to society (e.g. driving accidents and related deaths, and healthcare related and economic costs) (Tashkin et al. 1987; Bolla et al. 2002; Asbridge et al. 2005; Raphael et al. 2005). Although the literature on MJ dependence remains sparse, a MJ withdrawal syndrome has been identified, and self-reported craving for MJ has been demonstrated (Singleton et al. 2002; Budney et al. 2004; Hasin et al. 2008; Bordnick et al. 2009; Gray et al. 2011).

A variety of psychosocial and pharmacological treatments have been investigated for MJ dependence with underwhelming results (Copeland et al. 2001; Tirado et al. 2008; Haney et al. 2010; Levin et al. 2011). Trials report that fewer than 50% of MJ-dependent subjects are abstinent at 4 and 6 months following treatment (Nordstrom, Levin 2007; Denis 2008). The harmful effects of this chronic, relapsing disorder to both the individual and to society underscore the importance of identifying the neurophysiological vulnerabilities that could improve treatment response.

In addiction, drug reminders or cues, such as people, places, things, and internal states previously associated with drug reward can trigger craving. Drug craving is tightly coupled to drug use (Preston et al. 2009), and can be sufficiently compelling to precipitate relapse despite the presence of significant negative consequences and effort on the patient's part to maintain abstinence (O'Brien 1978; Oslin et al. 2009).

Neuroimaging has been used to study brain changes in response to drug cues, leading to a greater understanding of the mechanisms of relapse. In line with an extensive preclinical literature, such studies have observed activation of reward-related brain circuitry during exposure to cocaine (Childress et al. 1993; Grant et al. 1996; Childress et al. 1999; Bonson et al. 2002) nicotine (Franklin et al. 2007; Janes et al. 2010), and heroin cues (Langleben et al. 2008). In addition, Janes *et al.* demonstrated heightened brain responses in reward-related circuitry during cigarette smoking cue exposure in smokers who lapsed, and reduced functional connectivity between top down cognitive control regions and emotive brain circuits that predicted time to lapse in treatment-seeking smokers (Janes et al. 2010).

There are at least two groups investigating brain responses to MJ cues (Filbey et al. 2009; Cousijn et al. 2012). Both studies focused on non-treatment seeking MJ smokers. In the first study, using tactile cues *Filbey et al.* observed activation in the ventral tegmental area, thalamus, anterior cingulate cortex (ACC), insula, and amygdala. They further showed that

activity in the reward-related orbitofrontal cortex (OFC) and ventral striatum correlated with problems associated with MJ use (Filbey et al. 2009). This work is consistent with the existing literature for other drugs of abuse. In the second study, *Cousijn et al.* observed activation (OFC, ACC, ventral and dorsal striatum) in response to visual cues in frequent users who were separated into high and low problem severity subgroups in line with *Filbey et al.* They also showed that ventral tegmental area (VTA) activation during MJ cue exposure was specific to frequent cannabis users versus sporadic users and nonusers. Although the authors tested whether craving was associated with brain responses in the frequent users group, the findings were in opposition to their hypotheses, difficult to interpret, and they concluded that further research is necessary (Cousijn et al. 2012).

The aim of the present study is to expand the sparse literature by studying MJ cue reactivity in a clinically relevant treatment-seeking group of MJ-dependent subjects, and to establish a link between reward-relevant brain responses to MJ cues and self-report of drug craving, a major relapse predictor. Using a blood oxygen level dependent (BOLD) block design, we examined the brain responses to MJ cues and the association between baseline craving and brain responses to cues. Based on the existing literature and observations in our laboratory studies of cocaine and nicotine cue reactivity (Grant et al. 1996; Childress et al. 1999; Franklin et al. 2007; Filbey et al. 2009) we hypothesized that MJ cues would activate reward-relevant regions (e.g. amygdala, ventral striatum, OFC, insula, and hippocampus) and responses would be positively correlated with baseline subjective MJ craving.

2. METHODS

2.1 Subjects

Treatment-seeking individuals were recruited through newspaper advertisements to the Center for the Studies of Addiction, a University of Pennsylvania School of Medicine-affiliated treatment center. Subjects who participated in the current study are a subset of those recruited to participate in a study examining the effectiveness of dronabinol and BRENDA for the treatment of MJ withdrawal. Subjects met DSM-IV criteria for MJ dependence, had a positive urine drug screen for MJ, and had a history of ten or more years of chronic MJ use with an average frequency of 2 or more joints per day on 5 or more days per week.

Subjects were screened, tested on study knowledge, and consented prior to psychological and physical evaluations. The *Mini-International Neuropsychiatric Interview* [M.I.N.I. (Sheehan et al. 1998)] was used to determine current DSM-IV diagnosis of psychoactive substance dependence and to diagnose current severe psychiatric symptoms. The Wechsler Abbreviated Scale of Intelligence (WASI) was used to assess intelligence (IQ) (Wechsler 1999).

Exclusion criteria included a history of head injury with loss of consciousness greater than 3 minutes; current significant medical or neurological illness; current psychiatric symptoms meeting criteria for a DSM-IV Axis I disorder; use of medications or illicit substances with psychoactive properties (other than MJ) within four weeks of starting the study; an IQ score of less than 80; and psychoactive substance dependence (other than MJ and nicotine) within 6 months of starting the study (monitored by urine drug screens). Subjects were compensated \$50.

All participants provided informed consent, and all procedures were approved and monitored by the University Of Pennsylvania School of Medicine Institutional Review Board (in accordance with the Common Rule) and adhered to the Declaration of Helsinki.

Three subjects were excluded: one due to motion artifact, one due to structural artifact, and one due to technical loss of data. Of the 12 remaining subjects 10 were male, 10 were African American and 9 were current cigarette smokers. Subjects were 37.6 ± 10.7 years of age, had 13.0 ± 2.0 years of education, and had an average IQ of 99.8 ± 14.4 . Subjects used alcohol 4.1 ± 3.8 days out of the last 30, with 2.1 ± 1.8 drinks per occasion. A 24-hour time line follow back questionnaire was used to assess the frequency and amount of MJ use at the first screening visit and two subsequent visits prior to the scanning session (See Results).

2.2 Study Design

Subjects were not instructed to alter their usual MJ smoking pattern as the present study was designed to investigate the naturalistic, baseline craving state in the absence of withdrawal. Subjects were scanned prior to randomization in a subsequent clinical trial, “Effectiveness Study of Dronabinol and BRENDA for the Treatment of Cannabis Withdrawal”. A quit date was set during the course of this subsequent clinical trial. Data from the trial are not reported here.

Behavioral Measures—The Marijuana Craving Questionnaire-Short Form (MCQ-SF), a validated, multi-dimensional assessment of self-reported MJ craving was administered immediately prior to the imaging session. The MCQ-SF is a four-factor structured scale covering a broad range of distinct behavioral experiences associated with the urge to use MJ (Heishman et al. 2009). It includes both appetitive and aversive aspects of drug motivation (i.e. compulsivity, purposefulness, expectancy and emotionality). The scores on each factor range from 3 to 21 (minimal to maximal intensity). Immediately prior to and after the imaging session, subjects were also asked to verbally rate their craving on a scale from 1 to 9. Clinical counseling was offered to subjects to aid them in coping with any residual craving induced by the cues.

Stimuli—Matching of visual cues to the individual’s conditioning history is a critical factor in creating a cue paradigm. MJ smokers recruited through our center typically smoke blunts (a cigar, emptied of its contents and filled with MJ), though other modes of administration are also used (e.g., smoking joints, pipes, and/or bongs). Thus, stimuli consisted of pictures of MJ, MJ-related paraphernalia and individuals engaged in MJ smoking through various modes of administration. Both MJ and Non-MJ cues were comparable in level of complexity, type of activity, size, brightness, and luminance (see Figure 1). Stimuli were presented using E-prime software (Psychology Software Tools Inc.) onto a rear projection screen positioned behind the MRI scanner.

Imaging session—A BOLD block design was used, consisting of six 20-second MJ cue blocks and six Non-MJ blocks. Each block consisted of 10 individual pictures. Pictures were randomly selected from among 20 for each category. Blocks were presented in a rapidly alternating (two cue blocks every minute), semi-random order using the Gellermann series (Gellermann 1933). Each picture was presented for 1.5-seconds followed by a 0.5 second fixation point. A 20-second distractor task was presented following each cue block. This task consisted of a fixation point presented every 2 seconds on the right or left side of the screen, after which subjects were asked to press a button to indicate on which side the fixation point was presented (See Figure 1). The entire cue session lasted 8 minutes.

2.3 Behavioral Data Analysis

The four MCQ-SF factor scores for compulsivity, emotionality, expectancy and purposefulness were highly inter-correlated (r values ranged from 0.74 to 0.95, $p < 0.006$). Thus, scores on each factor were combined into a single craving score, which was computed by adding the individual factor scores and dividing by four to obtain a mean.

2.4 Scanning Procedures and parameters

Whole brain MRI images were acquired using a Siemens 3T Trio MR scanner (Siemens AG, Erlangen, Germany). A 5-minute T1-weighted high-resolution scan was acquired for normalization and anatomical co-registration of the images. Acquisition parameters for the 3D High-Resolution MPRAGE structural scan in the axial plane were: FOV=250mm, TR/TE=1620/3ms, 192x256 matrix, slice thickness 1mm. A T2*-weighted gradient echo-planar-imaging (EPI) sequence was used to acquire the functional images with field of view (FOV)=192 mm, matrix 64x64, TR=2sec, TE=30msec, flip angle=90°, 33 interleaved slices with thickness of 3mm (no gap).

2.5 Data Processing

Statistical parametric mapping (SPM)-based batch scripts were used for data analyses within SPM2 software (Wellcome Department of Cognitive Neurology, London, UK) run in MATLAB R2009b. Participants' functional images were realigned, coregistered to the anatomical T1 image, normalized to the MNI standard space, and smoothed with a Gaussian kernel of FWHM 9mm³. For each subject, experimental conditions were modeled using a general linear model (GLM) with a canonical hemodynamic response function as the basis function. Contrasts between MJ and Non-MJ cues were defined in the GLM model to assess the voxel by voxel difference. The contrast maps were entered into a random effects analysis to test for a significant main effect of condition. Simple regression analyses were conducted to test for brain-behavioral correlations with the composite craving score from the MCQ-SF as a covariate of interest. A mask was generated from our *a priori* regions (bilateral amygdala, ventral striatum, OFC, ventral insula, and hippocampus), defined anatomically by the Harvard-Oxford Cortical-Sub Cortical Atlas with a probability threshold set at 25. To control for Type-I error, Monte Carlo simulation was performed using 3dClustSim (a function from AFNI Software, afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html). Parameters used with the ROI mask described above were an individual voxel p-value at $p = 0.005$ with 10,000 iterations, 2 sided and FWHM (estimated from SPM). The simulations demonstrate that a cluster extent cutoff of at least 10 contiguous voxels, exceeding a height threshold of $p < 0.005$, corresponds to a cluster corrected threshold of $p < 0.01$. To provide hypothesis generating information to the field, an interactive visual display of unmasked brain data in all 3 planes at $p = 0.01$ can be found at <http://franklinbrainimaging.com>.

3. Results

3.1 Behavioral Results

At the screening visit subjects used MJ an average of 28.3+4.2 days out of the last 30, using 12.0+9.5 joints/day, for an average of 18.7+8.2 continuous years. MJ use averaged 11.3+2 joints/day in the week prior to the scan and 10.7+2 joints/day 24 hours prior to scanning, which did not differ from use in the week prior to the scan ($p=0.47$).

Mean MCQ-SF factor scores were: Compulsivity: 9.0+4.9; Emotionality: 10.75+5.9; Expectancy: 11.5+6.0; Purposefulness: 12.2+6.9. The mean composite MCQ-SF craving score was 10.9+5.5.

Change in craving scores were generated from responses to the single-item craving question acquired prior to and following MJ cue exposure. Scores ranged from 0 to 4 (mean 1.2+1.4) and were not significantly different from pre- to post-scanning session ($p=0.26$).

3.2 Imaging Results

Two analyses were conducted (12 blocks and 6 blocks) *a priori* based on previous work in our laboratory suggesting ‘carry-over’ from repeated presentation of MJ cues would reduce contrast (See Discussion; Methodological Considerations).

Drug minus nondrug comparison—Compared with Non-MJ cues, presentation of the MJ cues elicited significantly greater BOLD activation in *a priori* regions. 12-block analysis: Increased brain activation was observed in the left amygdala ($T=4.70$, at $-24, 0, -18$). 6-block analysis: More robust effects were observed than were observed in the 12-block analysis, including bilateral activation of the amygdala and hippocampus (T values range from 4.43 to 8.65; See Table 1 and Figure 2). There were no areas wherein activation was decreased.

Correlation with baseline craving using the 6-block analysis—Baseline marijuana craving scores (MCQ-SF), that were measured an hour before the imaging session, positively correlated with brain activation to MJ cues (6 block analysis) in a large cluster containing the left ventral striatum ($r=0.87$ at $-3, 12, -3$); medial OFC ($r=0.89$ at $-3, 21, -6$); and left lateral OFC ($r=0.93$ at $-24, 21, -15$) (see Table 2 and Figure 3). Inverse correlations with MCQ-SF scores were not observed. Given that there was no significant difference in the change scores derived from the single item craving score acquired during the fMRI cue session we were unable to assess its association with brain responses.

4. Discussion

Here we demonstrate, in treatment-seeking MJ-dependent subjects, that visual MJ cues elicit greater brain activation compared to Non-MJ cues in the amygdala and hippocampus. Furthermore, we report strong correlations between baseline craving and cue-elicited responses in reward-relevant brain regions including the ventral striatum, and both medial and lateral OFC. Results are consistent with over 25 years of animal research examining the brain substrates of drug motivation (Cardinal et al. 2002) and are in close alignment with brain responses observed during exposure to cocaine, heroin and nicotine cues in human neuroimaging studies (Grant et al. 1996; Childress et al. 1999; Franklin et al. 2007; Langleben et al. 2008).

To our knowledge, only two other neuroimaging groups have studied MJ cue-reactivity. Both groups focused on non-treatment-seeking MJ users, demonstrating increased brain activation to MJ tactile cues in the ventral tegmental area, thalamus, anterior cingulate cortex, insula, and amygdala (Filbey et al. 2009) and to visual cues in the OFC, anterior cingulate cortex, and ventral and dorsal striatum (Cousijn et al. 2012). *Filbey et al.* also reported an association between the subjects’ number of MJ problems and brain responses to tactile MJ cues in the OFC and ventral striatum, and *Cousijn et al.* reported that the activation to cues in a subgroup of frequent MJ users with high problem severity scores was different from brain responses in frequent MJ users with low problem severity scores, and sporadic users and nonusers. Further, *Cousijn et al.* observed an inverse correlation between self-reported craving and putamen activity. However, these findings were in opposition to their hypotheses, and they concluded that further research is necessary. They also observed a negative relationship between craving and activity in the dorsolateral prefrontal cortex, a cognitive control region involved in decision-making, however an explanation was not provided as to why this region would be considered as a neural correlate of craving (Cousijn et al. 2012). The present study augments the sparse MJ cue literature by 1) linking a validated measure of MJ craving to enhanced activation in reward-relevant regions known to

be involved in goal-directed drug-seeking behavior, and 2) importantly, conducting the study in a clinically relevant MJ-dependent sample.

4.1 A Priori Brain Regions

Our *a priori* hypotheses are based on an extensive preclinical literature demonstrating that a network of brain regions with strong reciprocal connections, are involved in drug-seeking and drug-taking behavior. These include the lateral and medial aspects of the OFC, the amygdala, the hippocampus, the insula, and the ventral striatum.

Brain response during MJ cue exposure—We observed robust bilateral activation in the amygdala during MJ cue exposure. The amygdala exerts strong control over emotional and motivational behavior including craving and is involved in stimulus-reinforcement association learning (Di Chiara et al. 1999). We suggest that its activation here may reflect the motivational salience of the MJ stimuli. Bilateral activation of the hippocampus was also observed during MJ cue exposure. The hippocampus is important for memory consolidation. Its activation has been observed in other drug cue studies and may reflect memory of prior drug use (Franklin et al. 2007; Heinz et al. 2009). Given that the insula has been implicated in studies of drug craving (Franklin et al. 2007; Naqvi, Bechara 2010), it was included in our *a priori* ROIs. However, we did not observe either insula activation to MJ cues or correlations with insula and craving in this study. This could be related to a number of factors including gender, degree of satiation or genetic variance in our sample (Tang et al. 2012). For example, in other work in our laboratory we found and confirmed that a polymorphism in the *SLC6A3* dopamine transporter gene modulated cigarette smoking cue neuroactivity and that the involvement of the insula was strongly affected by whether smokers carried one or two copies of the 10 variable number tandem repeat allele (Franklin et al. 2009; Franklin et al. 2011). In future studies, a larger sample size will allow us to examine the effects of genetic variability on brain responses.

Correlations with baseline craving—Baseline craving (MCQ-SF) was positively correlated with activation in the OFC (both the medial and the lateral regions). Functions of the OFC include storing the reward value of sensory stimuli, and guiding actions based on reward values: while the medial OFC is implicated in reward processing (monitoring and “holding in mind” of reward values), activation of the lateral OFC has been noted in response to aversive stimuli and during suppression of a previously rewarding response (Elliott et al. 2000; Small et al. 2001). Given the opposing functions of the medial and lateral OFC, it is intriguing that in our treatment-seeking sample, self-report of MJ craving was positively correlated with brain responses to MJ cues in both the medial *and* lateral OFC. Activation of the lateral OFC may reflect attempts to overcome (inhibit) craving or the ambivalence that subjects feel about their MJ use. Although the MCQ-SF measures both the appetitive and aversive aspects of drug motivation, the aversive aspects reflect a person’s wish to continue using MJ to relieve aversive feelings and symptoms (identified by factor 2, emotionality and factor 3, expectancy). The MCQ-SF does not capture whether a person perceives MJ as aversive. As such, the MCQ-SF could not serve as a behavioral anchor to differentiate the lateral and medial OFC activation. Future studies that include tasks of craving modulation wherein subjects are instructed to attempt to either actively increase or inhibit their craving could directly test this hypothesis.

We report here that self-reported MJ craving was correlated with increased ventral striatal activity. Given that craving precipitates relapse and the ventral striatum is the final common pathway of conditioned drug responses, we demonstrate the clinically relevant link between subjective reports of drug craving and the brain substrate central for drug-seeking behavior.

The neuro-correlates of craving differ from those reported in *Cousijn et al.*, however numerous differences between *Cousijn et al.* and this study exist. For example, in *Cousijn et al.* the subjects had approximately 3 years of MJ use and smoked approximately 4 grams per week, whereas our population used MJ for approximately 19 years and smoked more than 20 grams per week. Our observation of ventral striatal and orbitofrontal neuro-correlates of craving suggest that the association between craving and reward-relevant brain activity may be contingent upon studying heavily dependent treatment-seeking MJ users. Another difference in study design that may also contribute to the differing results was that subjects in *Cousijn et al.* were abstinent from MJ use for 24 hours versus MJ use ‘as usual’ in the current study. Therefore, it is conceivable that abstinence creates a ceiling effect such that arousal in reward-relevant regions was at its maximum in the *Cousijn et al.* study prior to cue exposure eliminating variability in brain responses and thus obviating the ability to acquire associations between ventral striatal activity and subjective reports of craving. Other differences that may contribute to the opposing findings include the imaging paradigm (event related versus block fMRI), and/or the time point at which craving data were acquired (post-scan versus pre-scan).

4.2 Methodological Considerations

Rapidly alternating stimuli are susceptible to “carry over” effects, which occur when non-drug cues begin to take on the affective valence associated with drug cues (Waters et al. 2005; Wilson et al. 2007; Sharma, Money 2010). We anticipated that these “carry-over” effects would reduce our ability to detect contrast over time, however the intersection between the number of blocks necessary to acquire a brain MJ cue response and the point at which carry-over effects would emerge was uncertain. Thus, as in prior work in our laboratory (Childress et al. 2008), we compared the results from the first half of the task (6 blocks), to the results acquired over the full task (12 blocks). This examination allowed us to empirically determine that the first half of the task was less impacted by “carry-over” effects.

There were no significant differences between pre- and post cue exposure in craving as assessed with the single-item craving question. The presence of a brain response in the absence of a within session behavioral correlate is not unusual in BOLD paradigms. This is due to the inherent caveat of BOLD paradigms in which MJ and Non-MJ cues are shown repeatedly in an interleaved manner such that both brain and behavioral responses are incited (MJ cues) and diminished (Non-MJ cues). We believe that the use of a multi-dimensional, validated assessment of MJ craving (MCQ-SF), administered immediately prior to the imaging session provided an anchored and broader measure of craving (Heishman et al. 2009).

4.3 Significance of the Results

It is generally accepted in the addiction field that two overarching motivators to relapse are drug cue- and withdrawal-induced craving (Childress et al. 1993; Payne et al. 2006). In addition, a host of other factors may contribute to relapse vulnerability, including stress, menstrual cycle phase, gender, and negative affect (Cooney et al. 1997; Perkins et al. 2001; Sinha, Li 2007; Dagher et al. 2009; Franklin, Allen 2009). The advent of neuroimaging has led to an improved understanding of the impact of cues on relapse. At least two human neuroimaging studies have demonstrated that drug-related cues elicit dopamine release in the striatum (Volkow et al. 2006; Boileau et al. 2007). Further, *Janes et al.* reported that enhanced reward-related brain responses to smoking cues and reduced functional connectivity between top down cognitive control regions and emotive and motivational circuits predicted time to lapse in treatment-seeking smokers (Janes et al. 2010). These and the present study suggest that conjoining neuroimaging with clinical outcome measures will

provide a unique opportunity to explore and evaluate the impact of drug cue vulnerabilities and other relapse predictors on treatment outcome.

4.4 Strengths and Limitations

A particular strength of this study is that subjects are both MJ-dependent and treatment-seeking. Therefore, the brain responses reported here may reflect real-world treatment targets for MJ dependence. Another strength is the first demonstration of a direct link between reward-related brain responses to MJ cues and subjective reported craving.

This study is limited in that a laboratory assay of last use of MJ was not acquired. Unfortunately, there are no reliable laboratory measurements to assess MJ use in chronic daily MJ smokers. This is because tetrahydrocannabinol, the psychoactive constituent in MJ, and its metabolites, are detectable for up to 28 days in heavy MJ users, precluding accurate determination of last use (Lowe et al. 2009). Nevertheless, subjects were not instructed to alter their MJ smoking and smoked approximately 11 joints within 24 hours of scanning, suggesting that withdrawal effects did not interfere with results.

Another limitation is that sample size is small, included only a small percentage of females, and racial diversity was low, with most subjects identifying themselves as African American. Further study in a larger sample with greater diversity would provide validation of the findings and ensure the best generalizability.

Another confound arises in that 75% of the MJ dependent subjects also smoked cigarettes. Given that some MJ cues could be mistaken for cigarette cues, the inclusion of cigarette smokers precludes definitively dissociating nicotine-versus MJ-cue induced brain responses. However, we believe that the robust correlation between a measure specific to MJ craving (MCQ-SF) and *a priori* reward circuitry demonstrates specificity. These limitations should be addressed in future work by including a MJ-dependent group who do not smoke cigarettes.

5. Conclusion

The present marijuana cue fMRI study showing increased brain responses in the limbic amygdala and hippocampus, and ventral striatal and orbitofrontal craving neuro-correlates adds to a growing body of literature showing similar reward-related responses to cues for a variety of drug classes, providing additional evidence that drugs of abuse activate a final common pathway. Importantly, we observed that baseline self-report of craving for MJ correlated with cue-induced brain responses in regions demonstrated in both animal and human studies to underlie both reward processing and suppression of response to reward. A better understanding of the neural mechanisms involved in drug motivation and relapse can guide the development of specific brain-targeted treatments, can help predict the response to these much-needed agents, and can provide an opportunity to tailor interventions to the needs of individual patients.

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Figure 1. Imaging Session Cue Task

Representation of the task structure and the MJ and Non-MJ stimuli utilized during the cue reactivity task. Only the first half of the cue session is shown. Blocks are in semi-random order (Gellermann series). Vertical lines within the 20-second cue block correspond to a 2 second visual cue interval. MJ=Marijuana Cues. Non-MJ= Non-Marijuana Cues. Fix. Pt. corresponds to the 20-second cognitive task between cue blocks (See Design Section).

Figure 2. Brain response during marijuana cue exposure

Representative statistical map for the comparison of MJ cue > Non-MJ cue demonstrates significant activation in bilateral amygdala, and hippocampus at $p < 0.01$ corrected. Data are displayed neurologically (left is left). The color scale bar represents the T values.

Figure 3. Correlation between brain response during marijuana cue exposure and baseline craving

Scatter plots showing significant correlations between MCQ-SF craving scores and beta coefficients at the peak voxel for the contrast MJ cue > Non-MJ cue at a threshold of $p < 0.01$, corrected). Illustrated are statistical maps in the axial view at peak coordinates (indicated by arrows): (A) $-24\ 21\ -15$ (B) $-3\ 21\ -6$ (C) $-3\ 12\ -3$. Data are displayed neurologically (left is left). The color scale bar represents the T values.

Table 1
MJ cue vs. non-MJ cue activation for *a priori* regions

Region	X	Y	Z	T
Left Amygdala	-24	-3	-21	4.43
Right Amygdala	18	-9	-18	4.49
Left Hippocampus	-30	-18	-15	4.62
Right Hippocampus	39	-24	-12	8.65

Regions listed are corrected for multiple comparisons with a minimum extent of 10 voxels and significant at $p < 0.01$.

Table 2

MJ cue vs. non-MJ cue contrast correlated with Craving Scores

Anatomical Region	X	Y	Z	T	R* values
Left Ventral Striatum	-3	12	-3	5.65	0.87
Medial Orbitofrontal Cortex	-3	21	-6	6.27	0.893
Left Lateral Orbitofrontal Cortex	-24	21	-15	8.08	0.93

Regions listed are corrected for multiple comparisons with a minimum extent of 10 voxels and significant at $p < 0.01$.

* Correlations significant at $p < 0.0001$