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The Contributions of the Endocannabinoid System and Stress on the Neural Processing of Reward Stimuli

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Abstract

The brain endocannabinoid system plays a crucial role in reward processes by mediating appetitive learning and encoding the reinforcing properties of substances. Evidence also suggests that endocannabinoids are an important constituent of neuronal substrates involved in emotional responses to stress. Thus, it is critical to understand how the endocannabinoid system and stress may affect reward processes given their importance in substance use disorders. We examined the relationship between factors that regulate endocannabinoid system signaling (i.e., cannabinoid receptor genes and prolonged cannabis exposure) and stress on fMRI BOLD response to reward cues using a multivariate statistical technique. We found that proxies for endocannabinoid system signaling (i.e., endocannabinoid genes and chronic exposure to cannabis) and stress have differential effects on neural response to cannabis cues. Specifically, a single nucleotide polymorphism (SNP) variant in the cannabinoid receptor 1 (CNR1) gene, early life stress, and current perceived stress modulated reward responsivity in long-term, heavy cannabis users, while a variant in the fatty acid amide hydrolase (FAAH) gene and current perceived stress modulated cue-elicited response in non-using controls. These associations were related to distinct neural responses to cannabis-related cues compared to natural reward cues. Understanding the contributions of endocannabinoid system factors and stress that lead to downstream effects on neural mechanisms underlying sensitivity to rewards, such as cannabis, will contribute towards a better understanding of endocannabinoid-targeted therapies as well as individuals risks for cannabis use disorder.

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Ethical Statement

The Institutional Review Board of the University of Texas at Dallas and University of Texas Southwestern Medical Center approved these study procedures. All experiments were conducted according to the principles expressed in the Declaration of Helsinki.

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1. Introduction

The current literature suggests that the endocannabinoid system interacts with the dopaminergic system to signal motivation for natural rewards and modulates the rewarding effects of addictive substances [1]. Endocannabinoids mediate retrograde signaling in neuronal tissues and are involved in the regulation of synaptic transmission to modulate neurotransmitter release by presynaptic cannabinoid receptors. A reduction in the catabolic enzyme fatty acid amide hydrolase (FAAH) that metabolizes anandamide (N-arachidonoyl-ethanolamine) - an endogenous agonist for cannabinoid 1 receptors (CB1Rs) [2] – has been associated with increased dopamine D3 receptors, suggesting a link between the endocannabinoid and dopaminergic systems that may underlie risk for substance use disorders [3]. Potential mechanisms by which cannabinoids modulate dopamine transmission include (1) location of CB1Rs in the ventral tegmental area, where mesocorticolimbic dopaminergic efferent projections originate, (2) CB1R expression in dopamine neuronal targets, and (3) location of CB1Rs on pre-synaptic GABAergic and glutaminergic neurons that directly interact with dopaminergic neurons [4, 5] [1]. Thus, activation of CB1Rs can, to some degree, modulate dopaminergic function in regions related to reward processing. Taken together, prolonged activation of CB1Rs by agonists, via chronic exposure to delta (9)-tetrahydrocannabinol (THC), may therefore stimulate dopaminergic neurotransmission associated with the rewarding effects of substances.

The modulatory action of endocannabinoids on synaptic transmission also has significant functional implications on synaptic plasticity in stress response. During acute stress, FAAH acts to degrade anandamide, which then leads to increased amygdala excitability [6]. Preclinical models have suggested that targeting the endocannabinoid system by increasing deficient levels of anandamide through FAAH inhibition can alleviate effects of stress [7]. Gray and colleagues (2016) indicated that increased corticotropin-releasing hormone in mice is common following increased stress-induced changes in endocannabinoid signaling, where increased FAAH led to reductions in anandamide in the amygdala and prefrontal cortex [8]. Rossi *et al.* (2008) reported that social defeat stress altered neural transmission in the CB1Rs within the striatum and that this effect was due to corticosteroids as the alterations were prevented by blockade of glucocorticoid receptors [9].

Genes that modulate endocannabinoid signaling are therefore likely to have downstream effects on reward processing and stress response, and impact risk for substance use disorders. Variability in the cannabinoid receptor 1 (CNR1) single nucleotide polymorphism (SNP) rs2023239 that codes for CB1 protein causes alternative splicing of CNR1 [10] and has been associated with craving and withdrawal [11] [12] as well as proclivity toward cannabis use disorder [13]. The C allele of the CNR1 rs2023239 SNP has been indicated to have enhanced cue-elicited activation in mesocorticolimbic areas associated with increased reward sensitivity [1]. Similarly, the FAAH gene regulates the expression of anandamide, and has been associated with anti-stress effects (e.g., anxiolytic effects during high stress conditions) [14]. In clinical models, a specific SNP, FAAH C385A, increases reward-related neural response in the ventral striatum in healthy controls, suggesting a role of FAAH in susceptibility to substance abuse [15] [16]. A recent study in female mice by Burgdorf et al., [17] found that this FAAH SNP variant leads to densely populated CB1Rs on GABAergic

interneurons associated with dopamine increases in the ventral tegmental area [11, 17]. The degradation of endocannabinoid signaling in the nucleus accumbens after chronic stress in mice [18] has also been translated in human studies demonstrating modulated subcortical response associated with CNR1 gene variability in those with depression and anxiety [19]. Interestingly, stress-induced synaptic deficiencies were relieved by natural rewards, such as access to a running wheel or sucrose, in addition to a cocaine injection, suggesting a potential mechanism by which the interaction between the endocannabinoid system and stress contributes to the development of reward dysfunction [10]. Preclinical studies on alcohol dependence have shown that the interaction between CNR1 variants and stress was associated with increased alcohol consumption in $CNR1^{+/+}$ mice, but not $CNR1^{-/-}$ mice [20].

Taken together, dysregulation within the endocannabinoid system through genetic [11] and environmental (e.g., prolonged cannabis exposure, stress) [21] [22] factors appear to disrupt response to rewards [23]. Despite clear links between stress and endocannabinoid signaling, including apparent vulnerability for cannabis use disorder, the neurobiological mechanisms that underlie this link have not yet been directly examined in humans. The goal of this study was to determine the contribution of endocannabinoid system factors (endocannabinoid genes, prolonged exposure to cannabis) and stress (early trauma, current perceived stress) in neural response to reward. We hypothesized that there would be contributions of CNR1 and FAAH SNP variants and stress in cannabis users that would be associated with neural response to cannabis cues. Based on the existing literature, we expected that the presence of CNR1 rs2023239 G and FAAH rs324420 C alleles, perceived and early life stress and greater neural response to cannabis cues (vs. control cues) will be associated in cannabis users but not in non-using controls.

2. Materials and Methods

The Institutional Review Board of the University of Texas at Dallas and University of Texas Southwestern Medical Center approved these study procedures. All experiments were conducted according to the principles expressed in the Declaration of Helsinki.

2.1 Participants

One hundred and thirty-seven participants were recruited for this study aimed to examine the contributions of genes and neurobiological mechanisms in cannabis use disorder [11, 21, 24]. Participants were included if they were: right-handed, spoke English as their primary language, and had no current or history of psychosis, traumatic brain injury, and MRI contraindications (e.g., pregnancy, non-removal metallic implants, claustrophobia). All participants were screened via urinalysis for drugs of abuse and were excluded if drugs were detected (except cannabis for the cannabis group). Participants were excluded for regular tobacco use (i.e., more than one pack of cigarettes per month) or current alcohol dependence based on the Structured Clinical Interview for DSM-IV (SCID) [25]. We defined participants as regular cannabis users based on self-reported history of cannabis use with a minimum of 5,000 lifetime occasions and daily use over the preceding 60 days. Cannabis use was verified via quantification of THC metabolites as ng/ml (over creatinine) via gas chromatography/

mass spectroscopy (GC/MS). We defined participants as non-using controls based on the absence of daily cannabis use at any period in their lifetime and no current illicit drug use in the past 60 days.

Of the 137 participants who provided consent, five were excluded for excessive motion during the fMRI scans (>3 mm or >3 degrees) and four had incomplete data. Of the resulting 128 participants with complete imaging, genetic, and behavioral data, 72 were non-using controls [CON; mean (SD) age = 30.5 (10.4) years] and 56 were prolonged heavy cannabis users [MJ; 20 qualified as dependent; mean (SD) age = 29.7 (8.1) years]. See Table 1 for demographic and group information.

2.2 Behavioral Measures

We measured stress with the Perceived Stress Scale [26] and the Early Trauma Inventory [27]. The perceived stress scale measures current perception of stress and the early trauma inventory measures early (i.e., developmental) physical, emotional, and sexual abuse traumas. See Table 1 for summary scores for the perceived stress scale and early trauma inventory. There was no difference in perceived stress scale ($t(102.4) = -0.42$, $p = 0.68$), but there was a significant difference in early trauma inventory ($t(88) = -2.9$, $p = 0.0053$) between the cannabis users and non-using controls.

We assessed cannabis use behaviors as age of first cannabis use, number of years of cannabis use, self-reported craving via the Marijuana Craving Questionnaire [28] before and after the cannabis cue-exposure task, and withdrawal via the Marijuana Withdrawal Checklist [29].

2.3 DNA Collection and Genotyping of Candidate Loci

Two SNPs, rs2023239 (CNR1) and rs324420 (FAAH), have previously been reported to be associated with neural response to cannabis cues [11] were included in the analysis. See Supplemental Methods for details of genetic data acquisition and processing. Table 2 provides a distribution of genotypes across race and ethnicity. While the minor allele frequency for both SNPs was suitable (> 5%), there were two clear distributional effects: (1) the minor homozygotes for both genes were relatively infrequent (FAAH = 11%, CNR1 = 7%), where in the “other” group the minor homozygote was non-existent and (2) there was an overrepresentation of the heterozygote in those that identified as Hispanic or African American. We used the dominant model (i.e., AA vs.{Aa + aa}) for both SNPs. There was no distributional effect between groups and genotypes in FAAH ($\chi^2 = 0.29$, $p_{perm} = 0.72$), but there was a distributional effect between groups and genotypes for CNR1 ($\chi^2 = 5.4$, $p_{perm} = 0.027$). See Section 2.6 Data Analyses below for additional preprocessing details.

2.4 MRI Procedure

Similar to our published studies [22, 24], MJ were scanned following a 72-hour abstinence from cannabis use to capture peak craving following last cannabis use. According to Budney et al. (2001), craving for marijuana is significantly increased within 72 hours of abstinence from ad libitum use among heavy users [30]. We measured THC metabolites as ng/ml (over creatinine) (via GC/MS) from the participants before and after the ~72-hour period to detect

reductions in THC metabolites in addition to self-report. All participants were asked to abstain from alcohol for 24 hours and from caffeine and cigarettes for the 2 hours before their scheduled scan. Breath alcohol level was collected to confirm blood alcohol content of 0.0 at the beginning of the scan. All participants were asked to eat a meal before their scan appointment to reduce confounding effects of hunger.

MRI scan acquisition took place in the Advanced Imaging Research Center (AIRC) at the University of Texas Southwestern Medical Center (UTSW). MRI images were collected using a 3T Philips whole body scanner equipped with Quasar gradient subsystem (40 mT/m amplitude, a slew rate of 220 mT/m/ms). Structural MRI scans were collected with a MPRAGE sequence with the following parameters: TR/TE/TI = 8.2/3.70/1100 ms, flip angle = 12°, FOV = 256×256 mm, slab thickness = 160 mm (along left-right direction), voxel size =1×1×1 mm, total scan time = 3 min 57 sec. fMRI scans were collected using a gradient echo, echo-planar sequence with the intercomissural line (AC-PC) as a reference (TR: 2.0 s, TE: 29ms, flip angle: 75 degrees, matrix size: 64 × 64, 39 slices, voxel size: 3.44 × 3.44 × 3.5 mm3).

2.5 fMRI cue-reactivity task

Participants completed a previously described cannabis cue-reactivity task designed to examine BOLD response to cannabis, neutral, and appetitive cues to measure cue-reactivity [24]. This task was presented in two separate EPI runs of 18 pseudorandom tactile presentations of cannabis paraphernalia (MJ cue × 6 trials), a pencil (neutral cue × 6 trials), or the participants' preferred fruit (appetitive cue × 6 trials). Each trial started with a cue-exposure period when participants were presented with both tactile exposure (cue placed in the participant's left hand) and visual (images of themselves holding the) cues. Participants then had a 5-second urge rating period in which they were asked to "Please rate your level of urge to use marijuana right now" on a scale of 1 (no urge) to 10 (high urge) via button presses. There was a washout period with a fixation cross in between each trial. The number of repetitions per run was 405 with a task duration of 27 minutes for the entire experiment. The task was presented using a back-projection to a mirror system mounted on the head coil. Stimulus presentation was delivered using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA) and was synchronized with trigger pulses from the scanner to ensure precise temporal integration of stimulus presentation and fMRI data acquisition.

2.6 Data analyses

2.6.1 First-level analyses of imaging data—First-level analyses of the imaging data were the same as those described in Filbey *et al.,* 2016 [24] and outlined here briefly. Time series for each participant were analyzed with FSL's FILM. Regressors were generated using FEAT by convolving the regressors (Cue exposure, Craving Rating, Washout) for each cue type (MJ, neutral, and appetitive) with a double gamma hemodynamic response function. Activation maps were then registered to their own MRI T1 weighted MPRAGE structural images and co-registered to the MNI 152 template space with FLIRT. Individual level estimates for MJ cue > appetitive (fruit) cue were then extracted for use in our primary statistical analysis (partial least squares correlation; PLSC). Estimates were extracted from a common mask and then vectorized. Each vector was normalized so that results are not

simply because of very large magnitudes of activation in one or a few individuals [31]. Although there are many possible contrasts for the experimental design, the focus in this study was the MJ cue > fruit cue contrast to investigate neural response to a cannabis-related cue beyond a response to a natural reward cue.

2.6.2 Partial least squares correlation analysis—Partial least squares correlation (PLSC) is a multivariate statistical technique that identifies orthogonal components (i.e., latent variables; LVs) that explain maximal variance from the cross-covariance between two data sets [32]. The two data sets here were: 1) fMRI scores - BOLD response during the cannabis cue-exposure task and 2) explanatory variables - genetics, group, and behavioral data. LVs are rank-ordered by explained variance. Bootstrap resampling was used to generate confidence intervals and "bootstrap ratios" (BSRs; are akin to *t*- or *Z*-like tests) for the voxels and explanatory variables. We used a lower threshold for multiple comparison corrections (https://neuroimage.usc.edu/brainstorm/Tutorials/PLS). For more detailed explanations see [33–35]. PLSC and resampling were performed in R with the TExPosition 2.6.10 package [36] and additional custom code.

Group and genetic measures were coded as presence (i.e., 1) vs. absence (i.e., 0) for their respective variables. FAAH and CNR1 were each coded under a dominant model: major homozygote vs. {heterozygote + minor homozygote} because minor homozygotes were rare. The Early Trauma Inventory and Perceived Stress Scale scores were included as continuous data. The explanatory variables of group, gene, and stress measures were then combined, and each variable was normalized to a sum of squares equal to 1. Explanatory variables were corrected for effects of race and ethnicity.

Bootstrap resampling was performed 1,000 times in order to identify explanatory variables and voxels that stably contributed to each component. A large BSR means that an item has a high loading with small variance. BSRs at a cut-off magnitude $> \pm 3$ ($\alpha \approx 0.001$) identified which explanatory variables and voxels stably contributed to the components [37]. We used Multi-Image Analysis GUI (Mango 4.0.1; http://ric.uthscsa.edu/mango/) with no minimum cluster-size and no cluster-wise correction to identify voxel clusters; all voxels within a cluster had a BSR $> \pm 3$. Finally, LV scores were correlated with cannabis use measures and cue-related responses within the cannabis group.

3. Results

The first two LVs were included in the interpretation because they explained the majority of the variance (i.e., 59.8%; Figure 1A). The expected variance component scores for the first two LVs are shown in Figure 1B. For the explanatory variables, both BSRs are provided and their corresponding confidence intervals for each LV are shown (see Figure 2).

3.1 Latent variable 1 (LV1)

LV1 explained 34.1% of the variance. Figures 2A and 2B show the LV1 scores for participants and how each participant contributed to the explanatory variables and fMRI scores. Group, CNR1, and both stress measures (i.e., Early Trauma Inventory and Perceived Stress Scale) significantly contributed to LV1 (Figure 2B). LV1 separated the data by group

and CNR1 genotype, showing that CON and CNR1 TT (major homozygote) were associated, while MJ, CNR1 CC+CT (presence of minor allele), and increasing scores on the perceived stress scale and early trauma inventory were associated.

We described the three largest brain activation clusters in response to {MJ > Fruit} that were related to LV1 in Figure 3A-F and Table 3A-F (see Supplemental Tables 1 and 2 for all clusters). The top clusters associated with {CON and CNR1 TT} were found in the middle and inferior occipital gyri, superior and middle temporal gyri, and superior temporal gyrus and insula. Thus, the CON group and CNR1 TT are positively correlated with {MJ > Fruit} responses in these brain regions. The top three clusters associated with {MJ, CNR1 CC +CT}, increasing scores on the Perceived Stress Scale and Early Trauma Inventory} were located in the cingulate gyrus, medial frontal gyrus, and precentral gyrus. These results suggest a negative correlation between these variables such that MJ group, CNR1 CC+CT, and increasing stress scores are related to reduced response to {MJ > Fruit} in these brain regions.

3.2 Latent variable 2 (LV2)

LV2 explained 25.7% of the variance. Figures 2C and 2D show the LV2 scores for the participants and how each participant contributed to the explanatory variables and fMRI scores. Group and increasing scores on the Perceived Stress Scale significantly contributed to LV2 whereas FAAH had a moderately stable contribution (BSR ≈ ± 2.78; Figure 2D). Specifically, LV2 showed the following: (1) CON and increasing scores on the Perceived Stress Scale were associated [with a lesser contribution by FAAH CC (major homozygote)], and (2) MJ, and to a lesser extent FAAH AA+AC (presence of minor allele) were associated.

As with LV1, we focused on the three largest clusters associated with LV2 (shown in Figure 3G-L and Table 3G-L; see Supplemental Tables 3 and 4 for all clusters). The top three clusters associated with {CON, increasing scores on the Perceived Stress Scale, and FAAH CC} were positively correlated with {MJ>fruit} response located in the superior temporal gyrus, insula, middle temporal gyrus, and cuneus. Those associated with the {MJ and FAAH AA+AC} were generally negatively correlated with {MJ>fruit} response located in the cuneus and precuneus, caudate, and cuneus.

3.3 Post-hoc Correlations

The correlation analyses between fMRI LV scores and cannabis use measures showed a significant positive correlation between scores on the Marijuana Withdrawal Scale and both LVs. This suggests that the greater the withdrawal symptoms in the cannabis users, the greater the strength of associations between regions active during response to cues. There were no significant correlations between the fMRI LV scores and other cannabis use variables.

4. Discussion

The aim of this study was to determine how endocannabinoid system factors, specifically, endocannabinoid genes and chronic exposure to cannabis, interact with stress to influence neural response to salient reward cues. We predicted that the presence of CNR1 rs2023239

G and FAAH rs324420 C alleles, perceived and early life stress, and neural response to cannabis cues (vs. control cues) will be associated in cannabis users but not in non-using controls. Contrary to our predictions, PLSC analyses showed that these variables contributed differentially between the groups: current perceived stress, early life stress, and presence of the CNR1 C allele were associated with increased cue-elicited response in heavy cannabis users, while current perceived stress and the FAAH CC was associated with greater cue-elicited response in controls. Additionally, cannabis withdrawal symptoms were associated with the strength of the association among the regions active during response to cues.

In general, the explanatory variables (group, genes, stress) were associated with neural response to cues in widespread brain areas. Cue-elicited response in the anterior cingulate gyrus [41] [42] [43] has been reported frequently in cannabis users, especially as they relate to problems related to cannabis use [24] [44]. A study recently demonstrated that altered activation and connectivity of the anterior cingulate may underlie the impact of early life stress on the reward network. The dysfunctional connectivity between the anterior cingulate gyrus regions, implicated in decision-making, suggest aberrant regulatory mechanisms conferring risk for affective disorders [45].

The cannabis group exhibited patterns of responsivity predominantly in the precuneus, cuneus, and caudate; each of which also play important roles in the reward circuit. While the caudate has been shown to play a role in responsivity in chronic cannabis use, van Hell and colleagues (2010) reported that caudate activity decreased with respect to reward anticipation [46]. The cuneus was a region that exhibited activation in both the control and cannabis groups; however, LV2 revealed that the cuneus responded in opposite ways in the cue-exposure task. In the control group the cuneus exhibited greater activity in response to the fruit cue whereas in the cannabis group the cuneus exhibited greater activity in response to the cannabis cue. Increased activation in the cuneus in cannabis users compared to controls has been consistently found [24, 46]; however, the differential brain responsivity to cannabis and fruit cues in these groups suggests an effect of exogenous cannabis beyond that of a natural reward. Similar differential responsivity in reward regions has been previously observed in a number of tasks and populations including substance using adolescents [47] and unmedicated major depressive disorder [48].

These regions span across several brain networks including the salience network, the cognitive control network, and to a lesser degree, midline regions often associated with the default mode network. Recent work has shown that these networks, specifically the “canonical regions” of these networks, are not necessarily distinct in that they share structural and functional connections [49] and tend to be dynamic across a wide range of cognitive processes [50], including reward processing and visual attention [51]. For example, the posterior medial frontal gyrus, cingulate gyrus, caudate, and anterior insula have been postulated to play a role in the visual processing as well as the encoding of reward [52]. THC administration has been found to alter functional connectivity in these networks [53] that has been associated with impairment in reward-based decision making [54]. Overall, these networks, through neuromodulatory inputs from the dopaminergic system, contribute towards maintaining homeostasis through the integration of autonomic signals with environmental demands [55]. Aberrant connections and activation in these three brain

networks have been associated with multiple psychiatric disorders and affective states, referred to as the “triple network model of psychopathology” [56], including substance use disorder [57]. Notably, while the preclinical literature demonstrates that CB1R modulation of the dopamine system involves the amygdala, we did not find associations with cue response in this region. Because it has been suggested that coupling between the amygdala and anterior cingulate cortex more effectively enhances emotional control [58], it is possible that an absence of association between the explanatory variables (group, genes, stress) and amygdala response to cues reflects a dysfunction in connectivity in regions underlying control and affective processing.

4.1 CNR1 and both current perceived and early life stress are associated with reward sensitivity in chronic, heavy cannabis users

Our findings indicated that the CNR1 G allele and higher levels of early life stress and current perceived stress were present in cannabis users. These contributions were associated with response to cannabis cues in midline areas and extends our previous findings of cue-responsivity in cannabis users with CNR1 G allele [24] to demonstrate positive associations with stress. This increased neural response to cues may be attributed to the impact of stress on regulatory and affective networks that influence reward response. For instance, studies have shown disruptions in amygdala-cingulate inhibitory circuitry during failure to regulate emotional conflict [59]. This disruption has been associated with attenuated reward sensitivity that has been related to risk for psychopathology, such as substance use disorders. Alternatively, exposure to cannabis particularly during adolescent development may exacerbate response to stress. Animal models demonstrate that adolescent rats exposed to CB1R agonists had higher levels of corticosterone and increased stress responsivity [60] and that these effects may be long-term on multiple neurotransmitter systems including those in mesolimbic brain structures. Notably, despite low stress scores in both groups, scores on the perceived stress scale and early trauma inventory were more related to the cannabis group but not the control group. This could be interpreted as potentiating effects of CNR1 CC+CT on reward system signaling such that even relatively low levels of stress have an effect in cannabis users.

4.2 Current perceived stress and FAAH are associated with reward sensitivity in controls

Latent variable 2 showed contributions of FAAH and stress that were orthogonal to that of CNR1 and stress (i.e., latent variable 1 discussed above). Specifically, we found associations between perceived stress scores and a variant in the FAAH SNP in the non-using controls, which was associated with greater response to the appetitive (fruit) cues in temporal regions and insula. Response in the insula in the control group supports previous reports of the role of the insula in interoception and response to natural rewards (i.e., the appetitive cue in this context) [61–64]. The interaction between FAAH and stress is well established in the literature and describes how chronic stress exacerbates disruptions of FAAH (and anandamide) as in the case of FAAH CC carriers. For example, rodents exposed to chronic stress show long-term reductions in amygdala anandamide levels resulting in dendritic branching and heightened intrinsic excitability related to enhanced anxiety. Inversely, FAAH inhibition led to recovery of signaling and amygdala function [7].

Latent variable 2 also showed that the cannabis users were associated with FAAH A allele, which supports recent findings suggesting that reduced FAAH is associated with increased D3 receptor density in the amygdala that demonstrates how the endocannabinoid system modulates the dopaminergic system to increase risk for substance use disorders [3]. It is interesting to note that while this allele was associated with cannabis users, it was not associated with elevated self-reported stress. This is consistent with findings demonstrating reduced stress-induced negative affect in FAAH A allele carriers along with attenuated stress-induced anandamide levels reduction [65].

4.3 Differential genes-stress interaction on prolonged cannabis exposure

Taken together, the differentiation between the effect of CNR1 and FAAH variants on cannabis users and controls poses an interesting question regarding how these variants interact with stress differently in the presence of prolonged cannabis exposure. These significant group effects on both latent variables underlines the large group effect contributing to higher overall variance than any of the other measures. Results from the current study indicate that the presence of CNR1 C allele (CC+CT) and stress resulted in a greater neural response to cannabis cues in cannabis users, while FAAH CC resulted in a greater neural response to natural reward cues in non-using controls. This suggests that while both CNR1 and FAAH SNP variants have been considered risk factors for cannabis use disorder, particularly as it relates to increasing response cue-elicited craving [11, 12] [66], the mechanisms by which these two genetic factors influence response to cues differ. Additionally, while previous studies have linked both CNR1 and FAAH variants to cannabis use [11, 12], they are limited in that they only included cannabis users. Our findings suggest that CNR1 and stress contribute to this relationship and are associated with greater neural response to cannabis cues whereas FAAH does not interact with stress factors related to cannabis cue response. The differential effect in cannabis users and non-using controls found in the current study advances our understanding and suggests a more nuanced interaction with both exogenous cannabis and stress.

Previous studies have reported differential effects of CNR1 and FAAH variants on affective states. For example, Palmer and colleagues (2019) reported an interaction between acute THC exposure and CNR1 such that those with the CNR1 rs2023239 C allele had higher anger-hostility scores (from Profile of Mood States questionnaire) following THC administration relative to placebo. This supports our finding of greater stress in CNR1 C allele carriers that is associated with greater neural response to cues. Lazary et al's (2009) study reported that CNR1 rs2023239 T allele carriers have significantly greater CB1R binding site density in the prefrontal cortex, which likely mediate the hypothalamic-pituitary-adrenal axis' negative feedback response to stress [67]. The same study by Palmer and colleagues (2019) also found that FAAH A allele carriers reported lower fatigue-inertia scores following exposure to placebo (vs. THC) [68]. This finding also overlaps with the absence of association between stress factors and FAAH A allele in cannabis users in the current study. Dincheva et al. (2015) reported that there was selectively enhanced fronto-amygdala connectivity in FAAH A allele carriers that may explain the control of stress response observed in mice and humans [69] [70]. An anti-stress effect has previously been demonstrated in preclinical studies where acute stress via foot-shock enhanced THC and a

FAAH inhibitor's (URB597) anti-stress effects but did not affect reward response (via conditioned place preference paradigm) [71]. This is consistent with preclinical studies reporting that inhibiting FAAH increases anandamide levels in the basolateral amygdala and alleviated effects of stress [7]. N-Arachidonoylserotonin (AA-5-HT), a dual blocker at FAAH, also generates anti-stress effects in preclinical models [14], supporting the role of the endocannabinoid pathway as a potential target for anti-stress therapeutics. It is also worth noting the possibility of opposing effects of the two endocannabinoids in mediating the stress response. Specifically, stress-related increases in 2-Arachidonoylglycerol or 2-AG in the amygdala has been thought to have restorative effects that counteract the effects of stress-induced anandamide depletion. For example, chronic administration of 2-AG inhibitors appear to prevent stress-related increases in anxiety and amygdala impairments [7]. In this context, it is possible that stress-related 2-AG related to the presence of THC may have a role in off-setting the expected correlations between FAAH CC and stress in cannabis users.

4.4 Limitations

One of the challenges of our complex study design is the feasibility of acquiring a sample size sufficiently large for typical genetic studies, despite being comparatively large for neuroimaging studies. Despite this limitation, our findings replicate and extend previous work and put forth a testable framework on the interactions between endocannabinoid system, genetic and environmental factors on cue-reactivity.

4.5 Conclusions

This study applied a multivariate statistical approach to integrate data from various modalities and indicated that both endocannabinoid system factors and stress (i.e., prolonged cannabis use, current stress, and early life stress) underlie differential neural mechanisms of reward cue responsivity. Specifically, PLSC identified two latent variables that revealed that CNR1, early stress, and current perceived stress play an important role in reward responsivity to cannabis cues in cannabis users and that current perceived stress, and to a lesser degree FAAH, play an important role in reward responsivity to natural reward cues in non-using controls. These results suggest that, together, endocannabinoid genes and stress contribute to a differential reward cue response in multiple regulatory networks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The interaction between cannabinoid receptor 1 (CNR1) CC+CT, early life stress, and current perceived stress modulate the brain's reward responsivity in long-term, heavy cannabis users
- Long-term cannabis using adults with CNR1 CC+CT genotype, perceived current stress and early life stress had brain response to cannabis cues in midline areas such as post- and precentral gyrus and medial frontal regions with the largest effects in the cingulate gyrus.
- The interaction between fatty acid amide hydrolase gene (FAAH) CC and current perceived stress modulate the brain's reward response in healthy controls.
- Healthy controls with CNR1 TT genotype had largest brain response to cannabis cues in the middle and inferior occipital gyrus.

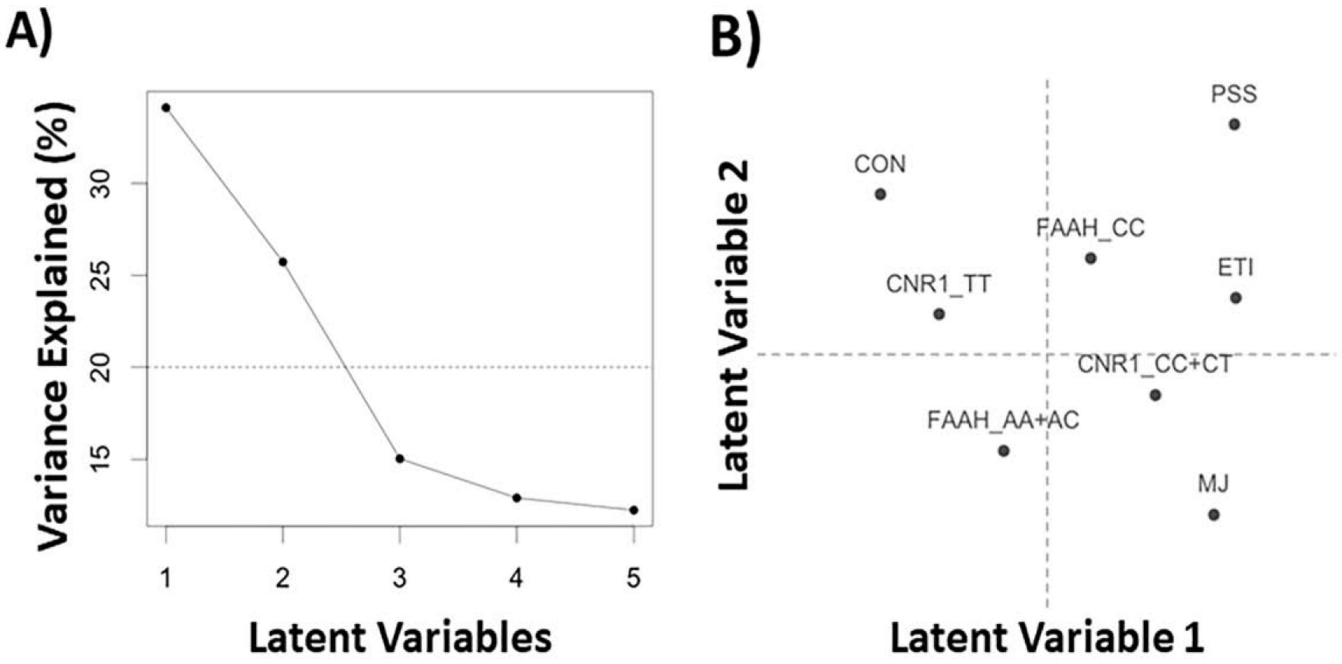

Figure 1:

Scree plot and latent variable (LV) scores for the explanatory variables (i.e., groups, genes, and stress). (A) The scree plot shows the explained variance per LV with a horizontal line as a cutoff for average (expected) variance. (B) The LV scores plot show how each explanatory variable contributes to the first two LVs. ETI=early trauma inventory; PSS=perceived stress scale; MJ=cannabis users; CON=controls

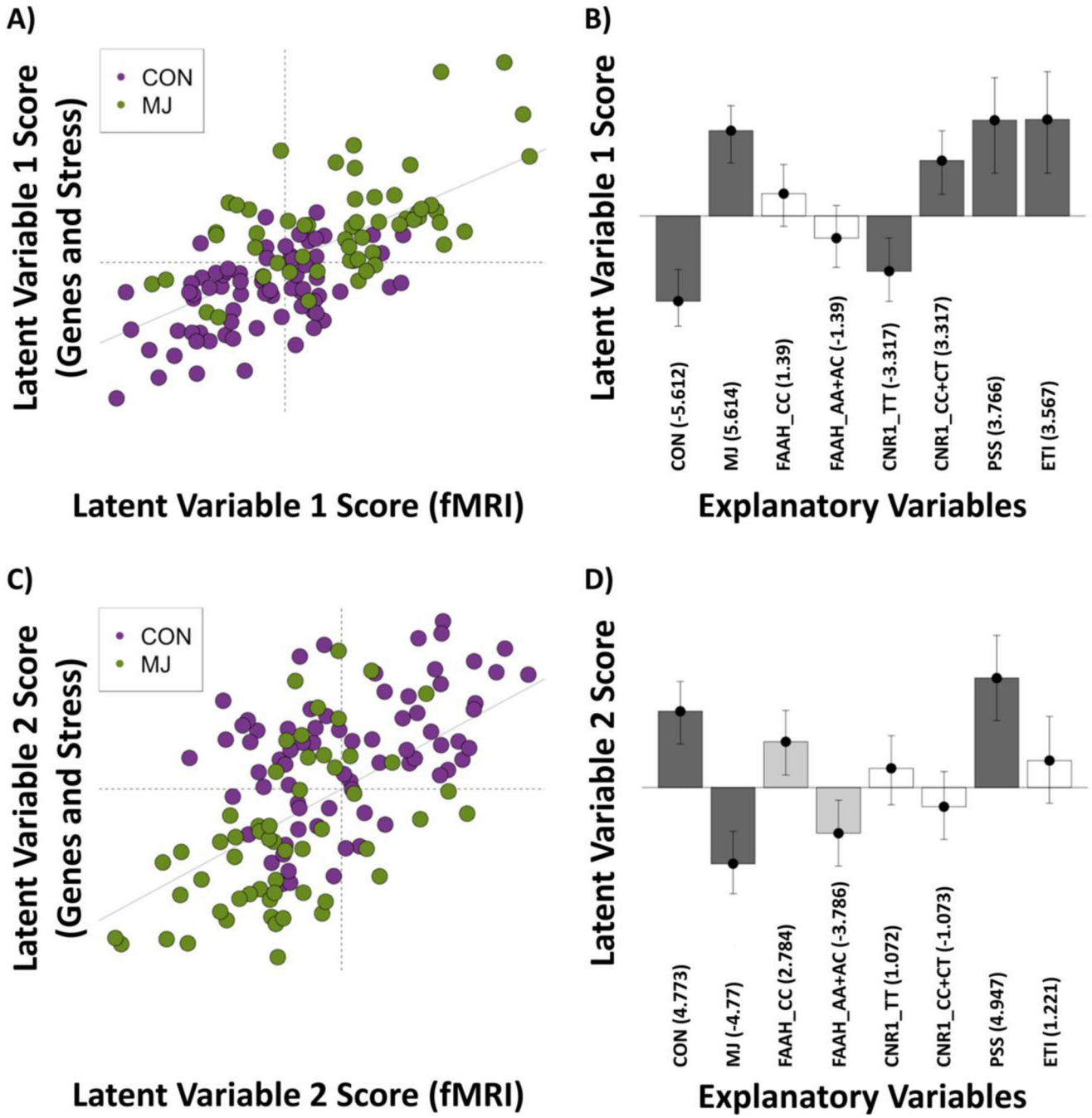

Figure 2:

Latent variable 1 (LV1) and 2 (LV2) scores and loadings. (A) LV1: fMRI scores vs. [38] scores and (B) Loadings for expected variance with bootstrap ratios (BSRs) denoted in parentheses; 95% confidence intervals around loadings shown. The LV plot shows that in general there is a separation of cannabis users (MJ) from controls (CON). The BSRs for the explanatory variables show that the effect is driven by [39] vs. {MJ CNR1 CC/CT, Early Trauma Inventory (ETI), Perceived Stress Scale (PSS)}. (C) LV2: fMRI scores vs. [38] scores and (D) Loadings for explanatory variables with BSRs denoted in parentheses; 95%

confidence intervals around loadings shown. The LV plot shows that in general there is a separation of MJ from CON. The BSRs for the explanatory variables show that the effect is driven by {Controls (CON), PSS} vs. [40], with a likely (albeit not significant at BSR > 3) effect of FAAH.

Note: Explanatory variables that load in the same direction are positively correlated.

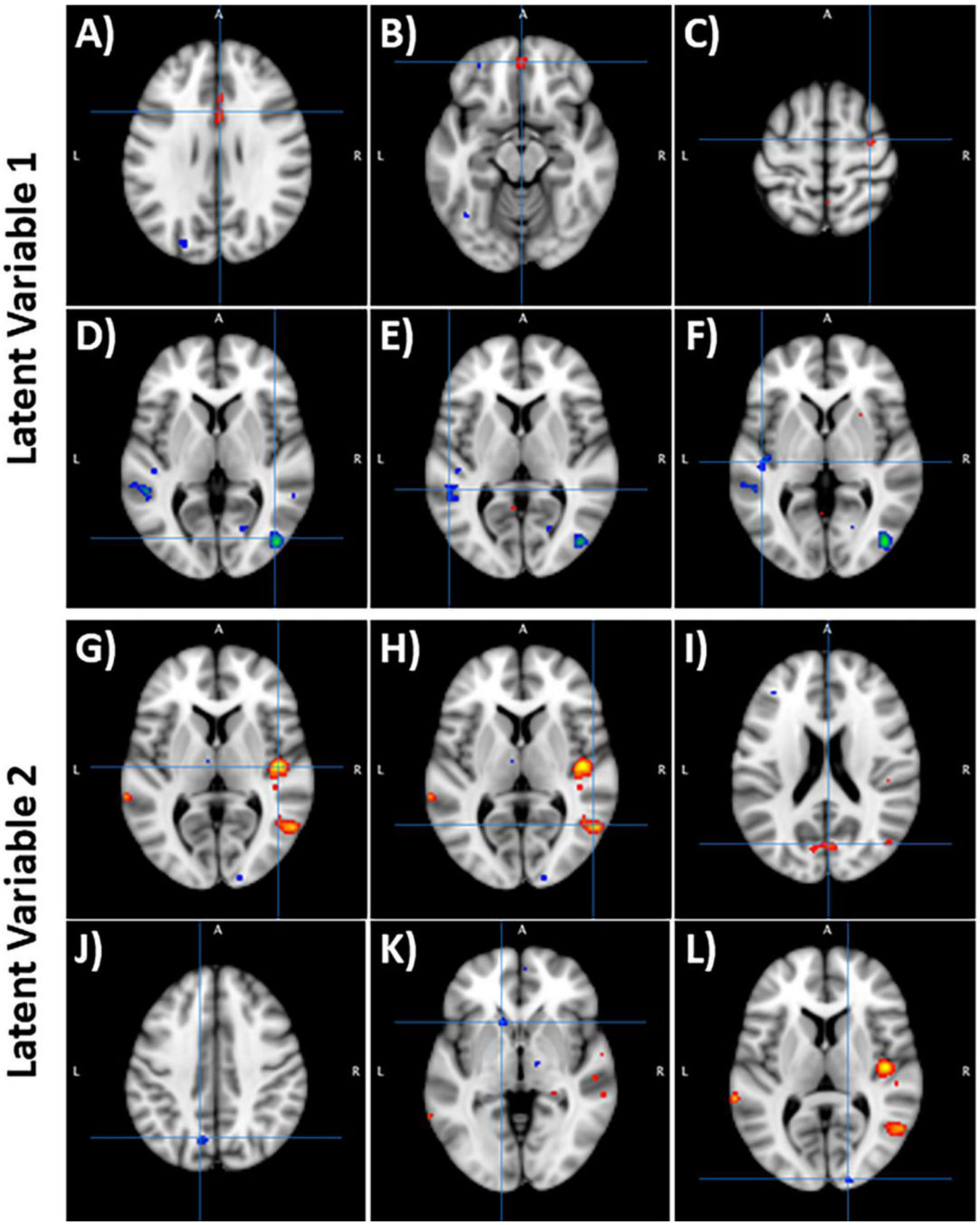

Figure 3:

Clusters associated with latent variables 1 (LV1) and 2 (LV2). Hot colors reflect positive correlations (+ BSR scores), whereas cool colors reflect negative correlations (- BSR scores). A–C (top row) – Activation clusters of LV1 that were associated with the {cannabis users, CNR1 CC/CT, early trauma inventory, perceived stress scale} effects. The top three clusters were in (A) Cingulate Gyrus, (B) Medial Frontal Gyrus, and (C) Precentral Gyrus. D–F (second row) – Activation clusters of LV1 that were associated with the {controls, CNR1 TT} effects. The top three clusters were in (D) Middle and Inferior Occipital Gyrus,

(E) Middle and Superior Temporal Gyrus, and (F) Superior Temporal Gyrus and Insula. G–I (third row) - The clusters of LV2 that were associated with {controls, perceived stress scale} effects. The top three clusters were in (G) Superior Temporal Gyrus and Insula, (H) Middle Temporal Gyrus, and (I) Cuneus. J–L (bottom row) - The clusters of LV2 that were associated with the {cannabis users} effects. The top three clusters were in (J) Precuneus and cuneus, (K) Caudate, and (L) Cuneus.

Table 1:

Demographic, substance use, stress, and genetics measures of participants across the control (CON) and cannabis (MJ) groups. Permutation was performed for χ^2, with 5000 permutations. Welch's two-sample *t*-test was used to test for group differences, which adjusts degrees of freedom when variance is unequal.

	CON (N = 72) Mean (SD)	MJ (N = 56) Mean (SD)	Statistic
Age (years)	30.5 (10.4)	29.7 (8.1)	$t(126) = 0.47$, $p = 0.64$
Sex (M/F)	39/33	36/20	$\chi^2 = 1.3$, $p_{perm} = 0.29$
IQ	111.2 (12.5)	107.6 (10.9)	$t(124.2) = 1.7$, $p = 0.087$
Years of education	16.5 (2.6)	13.4 (2.6)	$t(119.5) = 6.8$, $p < 0.001$
Ethnicity			$\chi^2 = 0.0008$, $p_{perm} = 1$
Hispanic/Latino	13	10	
Non-Hispanic/Latino	59	46	
Race			
Caucasian/White	32	35	
African American/Black	15	11	
Asian	21	1	
Other	4	9	
Substance use variables			
Age of first cannabis use (years)	n/a	15 (3.2)	
Duration of regular cannabis use (years)	n/a	11.4 (8.1)	
# Cannabis use / last 90 days	n/a	58.4 (8.2)	
# Cigarette smoking / last 90 days	0.3 (2.6)	0.9 (2.7)	$t(117.1) = -1.1$, $p = 0.28$
# Alcohol drinking / last 90 days	7.9 (14.1)	11.6 (14.6)	$t(116.5) = -1.4$, $p = 0.15$
Stress measures			
Perceived Stress Scale	35.7 (7.2)	36.4 (9.1)	$t(102.4) = -0.42$, $p = 0.68$
Early Trauma Inventory	4.1 (3.3)	6.3 (5.2)	$t(88) = -2.9$, $p = 0.0053$
Genetics			
FAAH (rs324420)			$\chi2 = 0.29$, $p_{perm} = 0.72$
CC	42	30	
AC/AA	30	26	
CNR1 (rs2023239)			$\chi2 = 5.4$, $p_{perm} = 0.027$
TT	54	31	
CT/CC	18	25	

Table 2:

Distribution of genotypes by ethnicity and race.

	FAAH (rs324420)			*CNR1* (rs2023239)		
	CC (N= 72)	**AC (N = 42)**	**AA (N = 14)**	**TT (N= 85)**	**CT (N = 39)**	**CC (N = 9)**
Hispanic/Latino (N = 23)	9	11	3	17	5	1
Non-Hispanic/Latino (N = 105)	63	31	11	68	29	8
Caucasian/White (N = 67)	37	23	7	47	16	4
African American/Black (N = 26)	8	13	5	10	12	4
Asian (N = 22)	18	2	2	18	3	1
Other (N = 13)	9	4	0	10	3	0

Table 3:

Three largest clusters associated with latent variable 1 and latent variable 2. (A-F) Largest clusters for LV1 associations, respectively and (G-L) Largest for LV2. BSR = bootstrap ratio. LV = latent variable.

	BSR	x	y	z	Region	Brodmann Area
(A) LV1{MJ, CNR1 CC+CT, increasing scores on the Perceived Stress Scale and Early Trauma Inventory}, Cluster 1 (Voxels = 118)						
Max	3.6	0	18	22	Cingulate Gyrus	BA24
Min	3.0	−2	18	26	Cingulate Gyrus	BA24
Centroid	3.1	2	14	28	Cingulate Gyrus	BA24
(B) LV1{MJ, CNR1 CC+CT, increasing scores on the Perceived Stress Scale and Early Trauma Inventory}, Cluster 2 (Voxels = 34)						
Max	3.4	0	50	−14	Medial Frontal Gyrus	BA10
Min	3.0	−2	52	−14	Medial Frontal Gyrus	BA10
Centroid	3.4	0	50	−14	Medial Frontal Gyrus	BA10
(C) LV1{MJ, CNR1 CC+CT, increasing scores on the Perceived Stress Scale and Early Trauma Inventory}, Cluster 3 (Voxels = 22)						
Max	3.6	34	−8	58	Precentral Gyrus	BA6
Min	3.0	34	−6	56	Precentral Gyrus	BA6
Centroid	2.9	32	−6	60	Precentral Gyrus	BA6
(D) LV1{CON and CNR1 TT}, Cluster 1 (Voxels = 158)						
Max	−3.0	40	−78	10	Medial Occipital Gyrus	BA19
Min	−4.4	44	−78	2	Inferior Occipital Gyrus	BA19
Centroid	−4.0	42	−76	4	Medial Occipital Gyrus	BA19
(E) LV1{CON and CNR1 TT}, Cluster 2 (Voxels = 82)						
Max	−3.0	−46	−48	6	Superior Temporal Gyrus	BA39
Min	−3.9	−50	−42	4	Medial Temporal Gyrus	BA22
Centroid	−3.1	−52	−40	6	Medial Temporal Gyrus	BA22
(F) LV1{CON and CNR1 TT}, Cluster 3 (Voxels = 40)						
Max	−3.0	−46	−22	2	Superior Temporal Gyrus	BA22
Min	−3.6	−42	−16	0	Insula	BA13
Centroid	−3.1	−46	−20	2	Superior Temporal Gyrus	BA22
(G) LV2{CON, increasing scores on the Perceived Stress Scale, and FAAH CC}, Cluster 1 (Voxels = 276)						
Max	4.5	44	−16	6	Insula	BA13
Min	3.0	52	−20	2	Superior Temporal Gyrus	BA22
Centroid	4.5	44	−16	6	Insula	BA13
(H) LV2{CON, increasing scores on the Perceived Stress Scale, and FAAH CC}, Cluster 2 (Voxels = 141)						
Max	4.1	54	−60	6	Medial Temporal Gyrus	BA37
Min	3.0	44	−58	8	Medial Temporal Gyrus	BA39
Centroid	3.8	52	−58	6	Medial Temporal Gyrus	BA37
(I) LV2{CON, increasing scores on the Perceived Stress Scale, and FAAH CC}, Cluster 3 (Voxels = 83)						
Max	3.7	4	−72	18	Cuneus	BA18

	BSR	x	y	z	Region	Brodmann Area
Min	3.0	8	−74	18	Cuneus	BA18
Centroid	3.2	2	−72	20	Cuneus	BA18
(J) LV2 {MJ and FAAH AA+AC}, Cluster 1 (Voxels = 23)						
Max	−3.0	−14	−68	38	Precuneus	BA7
Min	−3.5	−10	−68	38	Cuneus	BA7
Centroid	−2.9	−12	−66	40	Precuneus	BA7
(K) LV2 {MJ and FAAH AA+AC}, Cluster 2 (Voxels = 19)						
Max	−3.0	−16	20	−6	Caudate	Caudate Head
Min	−3.4	−12	16	−4	Caudate	Caudate Head
Centroid	−3.1	−14	18	−2	Caudate	Caudate Head
(L) LV2{MJ and FAAH AA+AC}, Cluster 3 (Voxels = 10)						
Max	−3.0	20	−98	10	Cuneus	BA17
Min	−3.3	18	−98	8	Cuneus	BA17
Centroid	−3.0	16	−96	8	Cuneus	BA17

Table 4:

Post-hoc correlations between the fMRI LV scores and cannabis use measures. LV = latent variable

Measure	Statistic	
	LV1	LV2
Age of onset of cannabis use	$r(54) = -0.047$, $p = 0.73$	$r(54) = -0.18$, $p = 0.18$
Duration of regular cannabis use	$r(54) = -0.018$, $p = 0.90$	$r(54) = -0.18$, $p = 0.19$
Marijuana craving score (pre-scan minus post-scan)	$r(52) = -0.15$, $p = 0.28$	$r(52) = -0.08$, $p = 0.57$
Average marijuana cue rating	$r(53) = 0.26$, $p = 0.056$	$r(53) = 0.22$, $p = 0.11$
Average fruit cue rating	$r(53) = 0.090$, $p = 0.52$	$r(53) = 0.22$, $p = 0.11$
Marijuana Withdrawal Scale score	$r(53) = 0.37$, $p = 0.006$*	$r(53) = 0.30$, $p = 0.025$*