The American Journal of Drug and Alcohol Abuse
Encompassing All Addictive Disorders

ISSN: 0095-2990 (Print) 1097-9891 (Online) Journal homepage: https://www.tandfonline.com/loi/iada20

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To cite this article: Rohan H. C. Palmer, John E. McGeary, Valerie S. Knopik, L. Cinnamon Bidwell & Jane M. Metrik (2019): *CNR1* and *FAAH* variation and affective states induced by marijuana smoking, The American Journal of Drug and Alcohol Abuse, DOI: 10.1080/00952990.2019.1614596

To link to this article: https://doi.org/10.1080/00952990.2019.1614596

Published online: 11 Jun 2019.

THE AMERICAN JOURNAL OF DRUG AND ALCOHOL ABUSE
https://doi.org/10.1080/00952990.2019.1614596

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ORIGINAL ARTICLE

CNR1 and *FAAH* variation and affective states induced by marijuana smoking

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ABSTRACT

Background: Polymorphisms in cannabinoid receptor type 1 (encoded by *CNR1*) and fatty acid amide hydrolase (encoded by *FAAH*) have been associated with cannabis dependence, but it remains unknown whether variation within these genes influences cannabis' acute effects on affect.

Objective: Conduct a secondary data analysis study to determine whether previously observed acute effects of tetrahydrocannabinol (THC) on mood was dependent upon variation in *CNR1* and *FAAH*.

Methods: A balanced placebo design was used crossing marijuana administration (i.e., 0% THC vs. 2.8% THC) with stimulus expectancy. Participants (N = 118; 64% male) provided DNA and completed the Profile of Mood States questionnaire prior to and after smoking. Haplotypes were constructed from genotyped single nucleotide polymorphisms for *CNR1* (rs1049353 and rs806368) and *FAAH* (rs4141964, rs324420, and rs11576941); rs2023239 (*CNR1*) and rs6703669 (*FAAH*) were not part of a phased haplotype block. Analyses tested both main and interaction effects for genotype across *CNR1* and *FAAH*, and drug, and expectancy effects.

Results: THC increased levels of POMS Tension-Anxiety and Confusion-Bewilderment over and above the effects of variation in *CNR1* and *FAAH*. Significant drug X genotype/haplotype and expectancy X genotype/haplotype interaction effects were observed for some but not all mood states [e.g., 'C' allele carriers of rs2023239 who received THC had higher levels of Anger-Hostility (β= 0.29 (0.12), p= .02) compared to those who received placebo].

Conclusion: These preliminary findings suggest individual differences in mood states after using marijuana depend on genetic variation. Such information might be useful in understanding either motivation for use of marijuana and/or risk for associated behaviors.

ARTICLE HISTORY
Received 16 July 2018
Revised 4 April 2019
Accepted 29 April 2019

KEYWORDS
Cannabis; THC; CNR1; FAAH; Affect; Balanced Placebo Design; marijuana; tetrahydrocannabinol

Introduction

Although cannabis use ranks highest among all forms of illicit substance use by North Americans (1,2), only about 9% of lifetime users become dependent (3–5). Results from twin studies (6) suggest that genetic influences explain between 30% and 80% of the total variance in risk for cannabis dependence. Molecular genetic studies have also provided evidence that genes that influence the action and metabolism of exogenous cannabinoids, in particular *CNR1* and *CNR2* (that encode Cannabinoid receptors CB1 and CB2, respectively) and *FAAH* (i.e., Fatty acid amide hydrolase enzyme) (3,7–11), influence cannabis-related behaviors (12–14), alcohol problems (15), and generalized drug abuse/dependence (16). Likewise, genome-wide association studies (GWAS) suggest that cannabis use and dependence are genetically complex phenotypes with only a few genome-wide significant variants identified to date (17–19), possibly due to a lack of phenotype sensitivity to biological factors that drive marijuana-related behaviors (20).

The emphasis on intermediate phenotypes that reflect sensitivity to a drug's subjective effects may be a better target in genetic association studies relative to broader diagnostic classification phenotypes related to addiction (21). Of the genes in the human genome, *CNR1* and *FAAH* are the primary candidates for vulnerability to heavy cannabis use and dependence because they are involved in the neurotransmission of marijuana's effects on the brain (22). In fact, prior studies suggest increased levels of CB1 receptor agonist binding sites in the dorsolateral prefrontal cortex and the striatum (agonist: [^{3}H] CP-55,940) (23), as well as the anterior cingulate cortex (agonist: M [3H]SR141716A) (24), which are brain regions involved in substance addiction (25,26) and emotion regulation (27). Both genes have also been associated

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with a number of mood-related phenotypes (28,29). For example, schizophrenia, which is defined by symptoms of delusions, hallucinations, and flat affect, has been the subject of several functional/neurochemical studies investigating the role of CB1 as a potential risk locus (30). CB_1 receptors are also an excellent candidate because they are expressed at high levels in limbic structures (i.e., amygdala), that are directly involved in mood regulation (31) and other regions in neural circuits known to be involved in the physiological regulation and dysregulation of mood and anxiety (32). Overall, the variation in the endocannabinoid system is apparent in neural structures (i.e., prefrontal cortex, ventral striatum) that play a role in pleasure responses and substance addiction.

Based on our review of the literature, no prior studies have involved the acute administration of THC in combination with genotyping to examine the acute affective response to cannabis administration. Previous work suggests that *CNR1* and *FAAH* gene variations are implicated in reducing negative affect due to cannabis withdrawal (11,33). Schacht et al. (11) used a sample of 40 daily marijuana smokers to study variation in the *FAAH* gene in relation to cannabis's acute effects on abstinence-related intermediate phenotypes, such as craving, withdrawal, and sensitivity (i.e., subjective effects (e.g., Happiness, Tension, Vigor, or Depression) and increased heart rate). While the study considered only one polymorphism (rs324420) in *FAAH*, was underpowered and lacked placebo control, its findings suggested that C/C carriers experienced more happiness after receiving a 1-g marijuana cigarette (3.1% Δ9-THC by weight). Haughey et al. (33) employed a 5-day abstinence paradigm in daily smokers to examine the role of the *CNR1* rs2023239 and FAAH rs324420 SNPs in changes in craving and withdrawal. While withdrawal and negative affect generally increased following abstinence, *CNR1* T/C carriers reported greater withdrawal and depressed mood, and *FAAH* C/A carriers trended towards elevated depression. While neither study provided evidence in a genome-wide context, the accumulating evidence suggests a role for genetic variation in the endocannabinoid in marijuana's behavioral effects. A question that is still largely unanswered is whether individuals with specific genotypes or haplotypes in *CNR1* and *FAAH* are more responsive to cannabis' impact on the regulation of negative affective states.

Current study

We have previously examined cannabis's acute effects on positive and negative affect (as measured by the Profile of Mood States (POMS) questionnaire) (34) and found that, relative to placebo, an administration of cannabis (i.e., delta-9-tetrahydrocannabinol [THC]) increases levels of POMS Confusion-Bewilderment and Tension-Anxiety. That study leveraged the Balanced-Placebo-Design (BPD) (35), which affords us an opportunity to examine genetic risk factors in the context of refined phenotypes (e.g., pharmacologic effect of a drug independent of the stimulus expectancy effects). BPD allows for the examination of the expectancy that marijuana was smoked independently from the pharmacological effect of THC. This 2 × 2 factorial design crosses drug administration (THC or placebo) with instructions that THC was smoked (i.e., stimulus expectancy: Told THC or Told placebo) (36). We hypothesized that both THC pharmacology (i.e., the presence or absence of 2.8% THC in the marijuana cigarette) and stimulus expectancy (i.e., instructional set about the presence or absence of THC in the marijuana cigarette) in combination with variation in the *CNR1* and *FAAH* genes would synergistically acutely impact subjective affective states post-smoking relative to pre-smoking affective state. This exploratory study capitalized on the BPD design and dense phenotyping in a laboratory experiment to understand the effects of variants within the *CNR1* and *FAAH* genes on THC's subjective effects on six negative mood states.

Methods

Study design and randomization

The current study utilized data that were drawn from an experimental study of cannabis's acute effects on impulsivity (i.e., parent study) (37). The parent study comprised a 2 × 2 randomized factorial design crossing drug administration (2.8% THC or 0% THC) with an instructional set (Told THC or Told Placebo). Participants in the parent study were randomized to one of the four experimental BPD conditions (n = 34 per condition: Told THC/Received THC, Told THC/Received Placebo, Told Placebo/Received THC, and Told Placebo/Received Placebo). The parent study comprised 136 individuals, but one participant's DNA was lost due to technical errors in order to examine the two between-subjects experimental factors: pharmacologic effect (i.e., Drug) and stimulus expectancy effect (i.e., Expect) of THC. All participants were informed of the parent study's aim and were told that they would be randomly assigned to smoke one marijuana cigarette that contained THC or one marijuana placebo cigarette with THC removed.

Sample description (parent study)

Marijuana smokers were recruited using newspapers advertisements, flyers, and social media platforms. All participants in this Brown Institutional Review Board

approved study met the following inclusion criteria: native English speakers, 18 to 30 years of age, cannabis use at least once a week in the past month and at least 10 times in the past 6 months, and self-reported ability to abstain from cannabis for 24 h without withdrawal. The exclusion criteria for this study were: history of substance abuse treatment and intent to quit or receive treatment for cannabis abuse, use of other illicit drugs and pregnancy by urine screen at each visit, nursing, past month affective disorder or history of panic attacks, psychotic or suicidal state assessed by psychiatric interview, meeting DSM-IV criteria for alcohol dependence, contraindicated medical issues by physical exam, smoking more than 20 tobacco cigarettes a day, and prior knowledge about the study procedures or contact with participants. All participants provided written consent for the study after reviewing the information that was provided.

Procedure

Full details of procedures used in the current study have been previously outlined in the parent study (Metrik et al., 2012); a brief description of the parent study follows. At baseline, study eligibility was verified and participants provided a saliva sample for DNA. Participants completed a baseline non-smoking and an experimental smoking session (average time between assessments = 14.7 (SD = 8.4) days). Participants were told to abstain from cannabis and tobacco smoking for 12 h, alcohol for 24 h, and caffeine for 1 h prior to the experimental smoking session. An alveolar carbon monoxide (CO) of <6 ppm was used to confirm no recent (past 12 h) smoking (37,38) with a Bedfont Scientific Smokelyzer®. Participants were then randomized to one of the four experimental BPD conditions. Manipulation checks revealed that the majority of participants (79–94%) believed the instructional set and that analyses excluding participants for whom the deception failed produced the same findings on all measures in the parent study; notably, the four experimental groups did not differ on a number of descriptive variables, such as age at the time of assessment, age of initiation/"regular use" of marijuana, gender, race,/ethnicity, to name a few (37).

At the experimental smoking session, participants first completed a subjective mood effects questionnaires (i.e., via the Profile of Mood States [POMS]) and were then instructed about which cigarette they were assigned to smoke (see Metrik et al., 2012, for details of the instructional set manipulation procedures). Participants were informed about the psychoactive properties of THC along with several procedures to enhance the credibility of the expectancy manipulation. The cannabis cigarettes (placebo or 2.8% THC) were provided by the National Institute on Drug Abuse. They were rolled at both ends, humidified, and smoked according to the standardized paced puffing procedure (39). Post-smoking assessment of subjective mood effects on the POMS was designed to capture intoxication effects at their peak, at 16 min after the start of the smoking (40).

The current study utilized a subsample (effective N = 118) of participants who completed all of the parent study components, provided DNA samples, and provided data on the POMS. Of the 60 individuals who received the 2.8% THC, 30 were told they received THC and the remainder were "Told they received the Placebo". Of the 58 who received the Placebo (i.e., 0% THC), 29 were told they received THC and the remainder weretold they received the Placebo.

Subjective mood effects

Subjective mood data were collected using the POMS (41), which is a 30-item measure of state affect that includes adjectives along six dimensions, including Tension-Anxiety, Anger-Hostility, Confusion-Bewilderment, Fatigue-Inertia, Depression-Dejection, and Vigor-Activity, on a 5- point Likert scale (0 = not at all to 4 = extremely). Mood was assessed at the baseline and experimental sessions. A total of N = 118 individuals who participated at baseline also completed the experimental smoking session and completed the POMS questionnaire. Analyses were limited to these 118 individuals.

CNR1 & FAAH marker information

This study utilized several markers across the *CNR1* and *FAAH* genes. Marker data was unavailable for 14 individuals who participated in the parent study (missingness rate = 9.4%). Table 1 describes the observed prevalence of the alleles for single nucleotide polymorphisms (SNPs) in *CNR1* and *FAAH*. The SNPs rs806368, rs1049353, and rs2023239 were genotyped to capture variation in *CNR1*. These SNPs have previously been identified in association studies. For instance, rs806368 has been associated with alcohol (5,42), cocaine (43,44), nicotine (45), and cannabis dependence (8,46). SNP rs2023239 has been linked to heavy cannabis consumption and dependence (10,22,33,47), generalized vulnerability to drug dependence (48), alcohol dependence (49), and cocaine dependence (44). Similarly, rs1049353 has been associated with cannabis and other indices of substance use (8,47).

The variants selected for *FAAH* have also been previously associated with cannabis use. For instance, we previously examined associations with these variants and marijuana and impulsivity (12) and found that the *FAAH* TAG haplotype was associated with a greater

Table 1. Genotype and minor allele frequencies of *CNR1* and *FAAH*.

Polymorphism	Genotypes N (%)			Alleles (%)	
CNR1					
rs806368	CC	CT/TC	TT	C	T
	4 (2.96)	44 (34.07)	83 (61.48)	0.20	0.80
rs1049353	CC	CT	TT	C	T
	85 (62.96)	44 (32.59)	6 (4.44)	0.80	0.20
rs2023239	CC	TC	TT	C	T
	6 (4.44)	25 (18.52)	99 (73.33)	0.14	0.86
FAAH					
rs6703669	CC	CT	TT	C	T
	71 (52.59)	47 (34.81)	2 (1.48)	0.79	0.21
rs6429600 [1]	GG	GA	AA	G	A
	38 (28.15)	19 (14.07)	72 (53.33)	0.37	0.63
rs4141964	CC	TC	TT	T	C
	48 (35.56)	55 (40.74)	26 (19.26)	0.42	0.59
rs324420	CC	AC	AA	C	A
	80 (59.26)	41 (30.37)	9 (6.67)	0.77	0.23
rs11576941	GG	TG	TT	T	G
	54 (40.00)	60 (44.44)	16 (11.85)	0.35	0.65

Note: Table shows the genotypes and frequencies for each marker (in order of chromosomal location) in the full sample. Notation: [1] Marker rs6429600 failed to meet the expectations of HWE and was not included in analyses.

number of marijuana problems. Similarly, *FAAH* rs4141964 and rs324420 have been positively associated with cannabis use disorder in a Mexican-American sample (50). rs324420 has also been linked to neural responses to marijuana cues (22), alcohol use and problems (4,51), chronic cannabis use and depression (52,53), and amygdala-mediated fear extinction, threat processing and stress-reactivity (54).

Marker quality control & haplotype derivation

Quality control and haplotype analyses were performed on the full sample of 135 individual with available DNA. All markers were screened for violation of Hardy Weinberg Equilibrium (HWE; p-value threshold $p < .0001$). No *CNR1* markers violated the HWE test; however, *FAAH* marker rs6429600 failed to meet the expectations of HWE, which may be due to sample selection (i.e., inadvertently selecting heavy cannabis users with certain rs6429600 genotypes) or misspecification of the mode of inheritance of the marker alleles (55). The sample call rate varied by SNP and ranged from 89.9% (rs6703669) to 100% (rs806368; see Table 1).

Linkage disequilibrium (LD) in both *CNR1* and *FAAH* was examined in order to (a) maximize the amount of information provided by the markers, and (b) circumvent loss of power due to multiple testing. All of the available SNP data were entered into Haploview (56,57) to visualize haplotype blocks amongst subjects who completed the experiment. Haplotypes on the maternal and paternal chromosomes were then confirmed and extracted using PHASE [Version 2.1 (58,59)], requiring that the probability of a haplotype be greater than or equal to 0.80. PHASE haplotypes were used to construct diplotypes (i.e., a combination of haplotypes across the pair of homologous chromosomes) that were used in the regression analyses. Because of the limited information indicating "risk" haplotypes, diplotype scores were created using a model based on haplotype dosage for each gene. Similar to our earlier report using only the baseline sample (12), we observed that *CNR1* markers rs1049353 and rs806368 formed a single haplotype block; rs2023239 was not part of a haplotype block and as such, was included in our analyses using a dichotomous measure to maximize analytical power. For *FAAH*, we observed a single haplotype block comprising *FAAH* markers rs4141964, rs324420, and rs11576941; rs6703669 was not part of a block but was still included in our analysis as a separate dichotomous predictor. Table 2 describes the haplotype frequencies of *CNR1* and *FAAH*. Given the low prevalence of individuals with two or more copies of a given haplotype, we elected to score each haplotype using a carrier versus non-carrier model (i.e., 0/1+ copy of the haplotype across the pair of homologous chromosomes) (60).

Statistical analyses

Descriptive statistics and regression analyses were executed in SPSS (61) using the 118 individuals with

Table 2. Haplotypes and frequencies of *CNR1* and *FAAH*.

CNR1 Polymorphism				Proportion N (%) of Carriers of Haplotype	
rs806368	rs1049353		Population Frequency (S.E.)	Non-Carrier	Carrier
T	C		0.59 (3.38E-3)	20 (14.81)	113 (83.70)
T	T		0.21 (2.57E-3)	85 (62.96)	48 (35.56)
C	C		0.20 (3.38E-3)	83 (61.48)	50 (37.04)
FAAH Polymorphism					
rs4141964	rs324420	rs11576941			
T	C	G	0.19 (5.08E-3)	87 (64.44)	42 (31.11)
T	A	G	0.23 (4.79E-3)	79 (58.52)	50 (37.04)
C	C	T	0.35 (5.81E-3)	54 (40.00)	75 (55.56)
C	C	G	0.23 (5.28E-3)	74 (54.81)	55 (40.74)

Note: Table shows haplotypes (derived from the full sample of participants) using PHASE. Proportions do not sum to 100 to allow for the accurate depiction of missingness in the data.

genotype and POMS data. Prior to fitting regression models, each subscale of the POMS was square-root-transformed to reduce skew and kurtosis and to better approximate a normal distribution. Our models examined the effects of stimulus expectancy (Told THC vs Told Placebo), drug manipulations (Received THC vs Received Placebo) and genotype/haplotype on the six POMS subscales. In order to understand the relative contribution of each parameter in the model, we examined the main (Model-1) and interaction effects (Model-2) to facilitate interpretation. The two models employed were:

Model-1: Mains Effects Model: $\sqrt{}(\text{Mood Score}) = \beta 0 + \beta 1(\textit{Baseline Mood Score}) + \beta 2(\textit{Race}) + \beta 3(\textit{Drug Manipulation}) + \beta 4(\textit{Stimulus Expectancy}) + \beta 5(\textit{CNR1/FAAH Genotype}[\textit{not part of the haplotype}]) + \beta 6(\textit{CNR1/FAAH Haplotype}) + \varepsilon i.$

Model-2: Interaction Effects Model: $\sqrt{}(\text{Mood Score}) = \beta 0 + \beta 1(\textit{Baseline Mood Score}) + \beta 2(\textit{Race}) + \beta 3(\textit{Drug Manipulatuion}) + \beta 4(\textit{Stimulus Expectancy}) + \beta 5(\textit{CNR1/FAAFH Genotype}[\textit{not part of the haplotype}]) + \beta 6(\textit{CNR1/FAAH Haplotype}) + \beta 7(\textit{Drug Manipulation } \chi \textit{ CNR1/FAAH Genotype}) + \beta 8(\textit{Drug Manipulatuion}) \chi (\textit{CNR1/FAAH Halpotype})) + \beta 9(\textit{Stimulus Expectancy } \chi (\textit{CNR1/FAAH Genotype})) + \beta 10(\textit{Stimulus Expectancy } \chi \textit{ CNR1/FAAH Haplotype})) + \varepsilon i.$

As shown in the equations, the main effects model (Model-1) included the pre-smoking value of the respective POMS subscale, stimulus expectancy, drug manipulation, the polymorphism genotypes were scored using a dominance model (0/1; to maximize power) and haplotype effect, and self-reported race (i.e., White/non-Hispanic (65% [n = 88]) vs. all others). The interaction effects model (Model-2) expanded Model-1 by adding the interaction of the polymorphism and haplotype with the drug and stimulus expectancy effects.

Given the size of the sample and the number of phenotypes assessed in our genetic models, we conducted a post-hoc sensitivity analysis of the drug manipulation/stimulus expectancy x genotype/haplotype interaction results (referred to as Sensitivity Analysis (SA) in the tables). Specifically, we identified potentially influential observations using the Cook's distance (D) statistic (i.e., D > 4/N = D > 4/135 = D > 0.0296) and retested each interaction model with said observations removed (62). We then compared the regression coefficients between the two models to determine if these observations had a major impact on the significance levels. These sensitivity analyses indicated the robustness of the model and the observed parameter estimates (i.e., significant findings were not driven by any identified influential values) (63). Given the degree of genetic variability and LD patterning within and around *CNR1* (https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=1268&chooseRs=all), we interpreted the effects of the SNPs as either (1) the "causal" variant or (2) reflecting the effects of other SNPs that are correlated (or in LD) with the measured variant.

Results

Effects of THC, expectancy, *FAAH*, and *CNR1* on mood states

Table 3 presents the results of the main effects of THC on each dimension of the POMS while controlling for genetic variation in *CNR1* and *FAAH*. These new findings strengthen our original report using a subset of these data (34). Specifically, these models indicate that if variation in *CNR1* and *FAAH* remains constant, THC increases levels of Tension-Anxiety and Confusion-Bewilderment; effects of THC on the remaining POMS subscales were limited. Likewise, there were limited effects of stimulus expectancy on any of the POMS mood states.

There were limited effects of variation in the *CNR1* and *FAAH* genes on affect. We observed a single association in *FAAH*, which suggested that individuals carrying the rs6703669 T allele (or a marker in high LD with rs6703669) had lower levels of Anger-Hostility, on average ($\beta = -0.17$, standard error (SE) = 0.08, $p < .05$).

Genotype dependent effects of THC on mood

Regression models that included interaction effects between *CNR1* haplotypes and the *CNR1* rs2023239 variant with drug and stimulus expectancy effects suggested a possible role for *CNR1* genetic variation in the effect of THC on affect (see Table 4). The presence of an interaction effect with rs2023239 was dependent upon the haplotype status in the same gene. For instance, relative to placebo, THC increased Anger-Hostility and Fatigue-Inertia levels among the 'C' carriers of the rs2023239 polymorphism, but only when controlling for the presence or absence of the 'TT' and 'CC' *CNR1* haplotypes. Based on the interaction models conducted using *CNR1* variants, the models that included the CC and TC haplotypes evidenced significant interaction effects. These effects were present in the models of Tension-Anxiety, Vigor-Activity, Anger-

Table 3. Main effects (*β*; standard error) of acute marijuana administration, expectancy, and *CNR1* genotype/haplotypes on mood states.

	POMS Subscales					
Parameters	Tension Anxiety	Confusion Bewilderment	Vigor Activity	Anger Hostility	Fatigue Inertia	Depression Dejection
Models including *CNR1* variants						
Main Effects treating TT as the risk CNR1 haplotype						
Expect (1 = Expect THC)	0.06 (0.08)	0.06 (0.08)	0.09 (0.07)	−0.06 (0.10)	−0.10 (0.18)	−0.07 (0.09)
Drug (1 = Got THC)	0.34 (0.08)***	0.17 (0.08)*	0.12 (0.07)	0.02 (0.10)	0.12 (0.08)	0.01 (0.09)
CNR1 rs2023239 (1 = TC/CC)	0.08 (0.08)	0.03 (0.08)	0.07 (0.07)	0.02 (0.10)	0.11 (0.08)	−0.05 (0.09)
CNR1 Haplotype (1 = TT)	0.09 (0.08)	0.10 (0.08)	0.03 (0.07)	0.08 (0.10)	−0.01 (0.08)	0.04 (0.09)
Main Effects treating CC as the risk CNR1 haplotype						
Expect (1 = Expect THC)	0.05 (0.08)	0.05 (0.09)	0.09 (0.07)	−0.08 (0.10)	−0.11 (0.08)	−0.07 (0.09)
Drug (1 = Got THC)	0.34 (0.08)***	0.17 (0.09)*	0.12 (0.07)	0.02 (0.10)	0.12 (0.08)	0.01 (0.09)
CNR1 rs2023239 (1 = TC/CC)	0.08 (0.08)	0.03 (0.09)	0.07 (0.07)	0.01 (0.10)	0.11 (0.08)	−0.05 (0.09)
CNR1 Haplotype (1 = CC)	−0.07 (0.08)	−0.04 (0.08)	−0.02 (0.07)	−0.14 (0.10)	−0.07 (0.08)	−0.08 (0.09)
Main Effects treating TC as the risk CNR1 haplotype						
Expect (1 = Expect THC)	0.06 (0.08)	0.07 (0.09)	0.09 (0.07)	−0.07 (0.10)	−0.11 (0.08)	−0.07 (0.09)
Drug (1 = Got THC)	0.33 (0.08)***	0.17 (0.08)*	0.11 (0.07)	0.01 (0.10)	0.12 (0.08)	0.01 (0.09)
CNR1 rs2023239 (1 = TC/CC)	0.09 (0.08)	0.05 (0.09)	0.07 (0.07)	0.02 (0.10)	0.11 (0.08)	−0.04 (0.09)
CNR1 Haplotype (1 = TC)	−0.03 (0.08)	−0.04 (0.09)	−0.01 (0.07)	0.05 (0.10)	0.07 (0.08)	−0.16 (0.09)
Models including *FAAH* variants						
Main Effects treating CCG as the risk FAAH haplotype						
Expect (1 = Expect THC)	0.06 (0.08)	0.06 (0.09)	0.09 (0.08)	−0.06 (0.10)	−0.09 (0.08)	−0.08 (0.09)
Drug (1 = Got THC)	0.31 (0.08)***	0.16 (0.09)	0.11 (0.08)	<0.01 (0.10)	0.10 (0.08)	0.01 (0.09)
FAAH rs6703669 (1 = CT/TT)	0.16 (0.18)	0.15 (0.21)	−0.11 (0.17)	0.14 (0.23)	0.20 (0.19)	−0.13 (0.21)
FAAH Haplotype (1 = CCG)	−0.32 (0.18)[a]	−0.16 (0.21)	0.08 (0.17)	−0.23 (0.23)	−0.31 (0.19)	<0.01 (0.21)
Main Effects treating CCT as the risk FAAH haplotype						
Expect (1 = Expect THC)	0.03 (0.08)	0.06 (0.09)	0.08 (0.08)	−0.08 (0.10)	−0.10 (0.09)	−0.07 (0.09)
Drug (1 = Got THC)	0.33 (0.08)***	0.16 (0.09)[a]	0.11 (0.08)	0.02 (0.10)	0.11 (0.08)	0.01 (0.09)
FAAH rs6703669 (1 = CT/TT)	−0.17 (0.08)*	<0.01 (0.09)	−0.06 (0.08)	−0.10 (0.10)	−0.06 (0.09)	−0.12 (0.09)
FAAH Haplotype (1 = CCT)	−0.15 (0.08[a]	0.03 (0.09)	−0.08 (0.08)	−0.11 (0.10)	0.04 (0.09)	0.03 (0.09)
Main Effects treating TAG as the risk FAAH haplotype						
Expect (1 = Expect THC)	0.04 (0.08)	0.05 (0.09)	0.09 (0.08)	−0.07 (0.10)	−0.10 (0.08)	−0.08 (0.09)
Drug (1 = Got THC)	0.32 (0.08)***	0.17 (0.09)[a]	0.11 (0.08)	0.01 (0.10)	0.11 (0.08)	0.01 (0.09)
FAAH rs6703669 (1 = CT/TT)	−0.10 (0.08)	−0.03 (0.09)	−0.02 (0.08)	−0.03 (0.10)	−0.09 (0.09)	−0.09 (0.10)
FAAH Haplotype (1 = TAG)	0.09 (0.08)	−0.10 (0.09)	0.07 (0.08)	0.14 (0.10)	−0.06 (0.09)	0.13 (0.10)
Main Effects treating TCG as the risk FAAH haplotype						
Expect (1 = Expect THC)	0.03 (0.08)	0.04 (0.09)	0.09 (0.08)	−0.07 (0.10)	−0.11 (0.08)	−0.06 (0.09)
Drug (1 = Got THC)	0.33 (0.08)***	0.17 (0.09)[a]	0.11 (0.08)	0.01 (0.10)	0.11 (0.08)	<0.01 (0.09)
FAAH rs6703669 (1 = CT/TT)	−0.09 (0.08)	0.03 (0.09)	−0.04 (0.08)	−0.07 (0.10)	−0.06 (0.09)	−0.017 (0.09)[a]
FAAH Haplotype (1 = TCG)	0.14 (0.08[a]	0.11 (0.09)	<0.01 (0.08)	0.02 (0.11)	0.08 (0.09)	−0.17 (0.09[a]

Table showing the results (*β* (standard error)) of the main effects model including *CNR1* and *FAAH* forms of genetic variation as covariates. Notations: * – p-value < 0.05; ** – p-value < 0.01, *** p-value < 0.001, [a] – 0.05 < p-value < 0.10; SE – standard error; Drug = indicates experimental drug manipulation factor (Received THC vs. Received Placebo); Expect = experimental stimulus expectancy factor (Told THC vs. Told Placebo).

Hostility, and Fatigue-Inertia. Sensitivity analyses that removed potentially influential observations showed that several of these significant interaction effects on Tension-Anxiety, Vigor-Activity, Anger-Hostility, and Fatigue-Inertia remained after the correction. For instance, the sensitivity analyses showed that the Drug–rs2023239 interaction effect on Anger-Hostility was observed to be significant (β = 0.29, SE = 0.12, p = .02), such that being a 'C' carrier and receiving THC (vs. placebo) was associated with increasingly higher Anger-Hostility ratings.

Variation in *FAAH* was related to cannabis-induced mood changes (see the lower half of Table 4). Interaction effects between *FAAH* variation and drug manipulation and stimulus expectancy effects on each mood state were limited to only four instances. In these cases, the observed *FAAH* drug/stimulus expectancy interaction effects remained after the removal of potentially influential observations in two instances (Model II, Table 4). The stimulus expectancy-TCG haplotype interaction (β = 0.33, SE = 0.13, p = .04) suggested higher levels of Confusion-Bewilderment among 'TCG' carriers Told THC relative to those Told Placebo. The other significant interactions suggested reduced levels of Fatigue-Inertia amongst carriers of the 'TAG' haplotype who received THC (β = −0.36, SE = 0.12, p = .005), relative to placebo, or who were Told THC (β = −0.43, SE = 0.12, p = .001) relative to those Told Placebo.

Discussion

The present study adds to our understanding of the role of *CNR1* and *FAAH* variation in cannabis-induced changes in mood. This is the first study to examine the effects of variation in *CNR1* and *FAAH* on THC-induced changes in affective states. Though preliminary and in need of replication, our findings suggest that certain dimensions of affective response to cannabis administration appear to be influenced by *CNR1* and

Table 4. Interaction effects (β; standard error) of acute marijuana administration and expectancy with CNR1/FAAH genotype/haplotypes.

	POMS Subscales											
Parameters	Tension Anxiety	Sensitivity Analysis	Confusion Bewilderment	Sensitivity Analysis	Vigor Activity	Sensitivity Analysis	Anger Hostility	Sensitivity Analysis	Fatigue Inertia	Sensitivity Analysis	Depression Dejection	Sensitivity Analysis
Models including *CNR1* variants												
Interaction Effects using TT CNR1 haplotype												
Drug-rs2023239 interaction	0.08 (0.11)	N/A	0.04 (0.13)	N/A	0.09 (0.10)	N/A	0.38 (0.13)**	0.14 (0.13)	0.23 (0.11)*	0.15 (0.10)	0.21 (0.13)[a]	N/A
Expect-rs2023239 interaction	−0.19 (0.12)	N/A	0.01 (0.14)	N/A	0.01 (0.12)	N/A	0.14 (0.15)	0.01 (0.16)	0.01 (0.13)	0.06 (0.13)	0.03 (0.15)	N/A
Drug-Haplotype interaction	0.11 (0.11)	N/A	0.08 (0.12)	N/A	0.10 (0.34)	N/A	0.11 (0.13)	0.01 (0.13)	−0.10 (0.11)	−0.08 (0.11)	−0.09 (0.13)	N/A
Expect-Haplotype interaction	0.21 (0.12)[a]	N/A	0.05 (0.13)	N/A	0.01 (0.95)	N/A	0.18 (0.14)	0.17 (0.15)	0.17 (0.12)	0.29 (0.12)*	−0.03 (0.14)	N/A
Interaction Effects using CC CNR1 haplotype												
Drug-rs2023239 interaction	0.10 (0.11)	N/A	0.05 (0.12)	N/A	0.10 (0.10)	0.08 (0.10)	0.41 (0.13)**	0.29 (0.12)*	0.25 (0.11)*	0.12 (0.10)	0.23 (0.13)[a]	N/A
Expect-rs2023239 interaction	−0.17 (0.12)	N/A	0.01 (0.14)	N/A	0.03 (0.11)	−0.11 (0.12)	0.16 (0.15)	−0.13 (0.16)	0.01 (0.13)	0.16 (0.15)	<0.01 (0.14)	N/A
Drug-Haplotype interaction	0.09 (0.12)	N/A	0.13 (0.14)	N/A	−0.27 (0.11)*	−0.19 (0.11)	0.06 (0.15)	−0.04 (0.15)	0.05 (0.13)	0.21 (0.14)	0.07 (0.14)	N/A
Expect-Haplotype interaction	0.14 (0.12)	N/A	0.25 (0.13)	N/A	−0.21 (0.11)*	−0.21 (0.10)*	0.11 (0.14)	0.14 (0.14)	−0.02 (0.12)	0.15 (0.13)	−0.08 (0.13)	N/A
Interaction Effects using TC CNR1 haplotype												
Drug-rs2023239 interaction	0.07 (0.11)	0.14 (0.10)	0.03 (0.12)	0.12 (0.11)	0.13 (0.10)	0.04 (0.09)	0.39 (0.13)**	0.15 (0.13)	0.22 (0.11)[a]	N/A	0.23 (0.13)[a]	N/A
Expect-rs2023239 interaction	−0.19 (0.12)	−0.13 (0.13)	−0.02 (0.14)	0.05 (0.14)	0.03 (0.12)	−0.17 (0.11)	0.13 (0.15)	−0.13 (0.18)	0.02 (0.13)	N/A	0.01 (0.14)	N/A
Drug-Haplotype interaction	−0.22 (0.17)	−0.04 (0.18)	−0.24 (0.19)	0.10 (0.20)	0.18 (0.16)	−0.08 (0.15)	−0.19 (0.21)	−0.07 (0.23)	0.07 (0.18)	N/A	0.05 (0.20)	N/A
Expect-Haplotype interaction	−0.39 (0.18)*	−0.41 (0.19)*	−0.44 (0.20)*	−0.25 (0.21)	0.41 (0.17)*	0.25 (0.16)	−0.36 (0.22)	−0.53 (0.24)*	−0.30 (0.19)	N/A	0.17 (0.21)	N/A
Models including *FAAH* variants												
Interaction Effects using CCG FAAH haplotype												
Drug-rs6703669 interaction	−0.16 (0.34)	N/A	−0.03 (0.39)	N/A	0.25 (0.33)	N/A	0.75 (0.43)[a]	N/A	<.01 (0.35)	N/A	0.02 (0.40)	N/A
Expect-rs6703669 interaction	0.51 (0.35)	N/A	0.39 (0.37)	N/A	−0.16 (0.31)	N/A	0.35 (0.41)	N/A	0.57 (0.34)[a]	N/A	0.15 (0.40)	N/A
Drug-Haplotype interaction	0.08 (0.34)	N/A	−0.02 (0.39)	N/A	−0.25 (0.33)	N/A	−0.60 (0.42)	N/A	−0.05 (0.35)	N/A	−0.03 (0.40)	N/A
Expect-Haplotype interaction	−0.60 (0.36)	N/A	−0.40 (0.38)	N/A	−0.03 (0.33)	N/A	−0.31 (0.43)	N/A	0.54 (0.35)	N/A	−0.07 (0.41)	N/A
Interaction Effects using CCT FAAH haplotype												
Drug-rs6703669 interaction	−0.06 (0.14)	N/A	−0.07 (0.16)	N/A	0.03 (0.13)	N/A	0.24 (0.17)	N/A	0.04 (0.14)	N/A	−0.03 (0.16)	N/A
Expect-rs6703669 interaction	0.02 (0.14)	N/A	0.02 (0.16)	N/A	−0.18 (0.13)	N/A	0.13 (0.17)	N/A	0.12 (0.14)	N/A	0.01 (0.16)	N/A

(*Continued*)

Table 4. (Continued).

	POMS Subscales											
Parameters	Tension Anxiety	Sensitivity Analysis	Confusion Bewilderment	Sensitivity Analysis	Vigor Activity	Sensitivity Analysis	Anger Hostility	Sensitivity Analysis	Fatigue Inertia	Sensitivity Analysis	Depression Dejection	Sensitivity Analysis
Drug-Haplotype interaction	0.08 (0.15)	N/A	−0.09 (0.17)	N/A	0.08 (0.15)	N/A	0.11 (0.19)	N/A	0.25 (0.15)	N/A	−0.11 (0.17)	N/A
Expect-Haplotype interaction	0.06 (0.14)	N/A	−0.03 (0.16)	N/A	−0.02 (0.13)	N/A	0.17 (0.17)	N/A	0.16 (0.14)	N/A	−0.17 (0.16)	N/A
Interaction Effects using TAG FAAH haplotype												
Drug-rs6703669 interaction	−0.15 (0.14)	N/A	−0.08 (0.16)	N/A	0.14 (0.13)	N/A	0.13 (0.17)	N/A	−0.11 (0.15)	−0.18 (0.13)	−0.04 (0.16)	N/A
Expect-rs6703669 interaction	−0.05 (0.13)	N/A	−0.03 (0.15)	N/A	−0.16 (0.13)	N/A	0.01 (0.17)	N/A	0.05 (0.14)	0.09 (0.12)	0.18 (0.16)	N/A
Drug-Haplotype interaction	−0.21 (0.13)	N/A	−0.16 (0.15)	N/A	0.13 (0.12)	N/A	0.22 (0.16)	N/A	−0.31 (0.14)*	−0.36 (0.12)**	−0.05 (0.15)	N/A
Expect-Haplotype interaction	−0.11 (0.14)	N/A	−0.22 (0.15)	N/A	0.11 (0.13)	N/A	−0.25 (0.17)	N/A	−0.15 (0.14)	−0.43 (0.12)**	0.23 (0.16)	N/A
Interaction Effects using TCG FAAH haplotype												
Drug-rs6703669 interaction	−0.05 (0.14)	N/A	0.03 (0.15)	−0.13 (0.13)	0.01 (0.13)	N/A	0.29 (0.17)	N/A	−0.01 (0.15)	N/A	0.05 (0.16)	N/A
Expect-rs6703669 interaction	0.02 (0.13)	N/A	0.13 (0.15)	0.05 (0.12)	−0.18 (0.13)	N/A	0.11 (0.17)	N/A	0.09 (0.14)	N/A	0.08 (0.16)	N/A
Drug-Haplotype interaction	0.06 (0.13)	N/A	0.19 (0.14)	0.18 (0.11)	−0.03 (0.12)	N/A	0.24 (0.16)	N/A	0.07 (0.13)	N/A	0.22 (0.14)	N/A
Expect-Haplotype interaction	0.13 (0.14)	N/A	0.32 (0.16)*	0.33 (0.13)*	0.06 (0.14)	N/A	0.17 (0.18)	N/A	−0.06 (0.15)	N/A	0.05 (0.16)	N/A

Table showing the results (β (standard error)) of the interaction effects model between drug, expectancy, and genetic variation across *CNR1* and *FAAH*. Notations: * – p-value < 0.05; ** – p-value < 0.01, *** p-value < 0.001, [a] – 0.05 < p-value < 0.10; SE – standard error. Abbreviations: N/A – Not Applicable to the current model as no significant interaction effects were observed. Note that sensitivity analyses were only run for models where a significant interaction effect was observed in the full dataset.

FAAH variation. Variation in *CNR1* in combination with active THC increased Anger-Hostility in rs2023239 'C' allele carriers given THC. This provides evidence to suggest that this intronic variant or a marker in LD with rs2023239 may be involved in the manifestation of affective states of anger and hostility during cannabis use. Variation in *CNR1* in combination with the expectancy of receiving THC also had varied effects across each dimension of affect, specifically – increased Fatigue-Inertia in haplotype 'TT' carriers, lower Confusion-Bewilderment in haplotype 'CC' carriers, decreased Tension-Anxiety in haplotype 'TC' carriers and decreased Anger-Hostility in haplotype 'TC' carriers. Genetic variation in *FAAH* was associated with reduced THC effects on Fatigue-Inertia, relative to placebo. Variation in *FAAH* also moderated the expectancy effect of receiving THC on fatigue-inertia and confusion-bewilderment. Carriers of the 'TAG' *FAAH* haplotype who were told they had received THC reported lower levels of Fatigue-Inertia compared to non-carriers. Carriers of the 'TCG' *FAAH* haplotype reported higher levels of Confusion-Bewilderment in comparison to non-carriers. Overall, most, but not all drug-related affective states (feelings of Depression-Dejection being the exception), exhibited some form of moderation by genotype within *CNR1* or *FAAH*, underscoring the role of variation in these two genes in mood-related responses to THC and the expectancy of receiving THC.

These findings build upon our initial investigation of the effects of THC (relative to placebo) on positive and negative affect (34) by demonstrating that the effects of both THC and the expectancy of receiving THC on mood are in part determined by an individual's genotype. As the first study of its kind, we had limited *a priori* expectations as to how variation in *CNR1* and *FAAH* would influence mood states in the presence and expectancy of THC. Since long-term use of cannabis has been shown to change brain reward circuity (22,64), we interpreted the observed main effects of THC administration as a direct pharmacologic effect on subjective mood states and the main effects of expectancy as a learned behavioral response acquired from prior experiences with cannabis (65). As such, interaction effects with genetic variants may be interpreted as sources of variation in the pharmacodynamics of cannabis-associated mood as well as the learning reenforcement processes involved in maintaining drug use. Pharmacological, genetic, and neurobiological studies have suggested a possible role for endocannabinoids in the liability of mood and anxiety states. Studies have shown that, in regards to mood, mesotelencephalic dopaminergic neurotransmission can be modulated by the endocannabinoid system (66,67). This suggests that *CNR1* plausibly modulates executive-control related mood changes and *FAAH* plausibly impacts DA related reward-based learning. Altogether, this provides a plausible explanation for the moderation effects observed in this study (insomuch as CB_1 [the major neuronal cannabinoid receptor] and CB_2, encoded by *CNR1* and *CNR2*, respectively, and endocannabinoid inactivation inhibitors (i.e., *FAAH*), influence dopaminergic neurotransmission).

Focusing on the functional consequences of the individual variants included in these analyses, rs324420 in *FAAH* is a point mutation that results in a non-synonymous base pair substitution. As a missense mutation, it is possible that 'A' allele carriers (and by extension 'TAG' haplotype carriers) of the resultant mutant form of *FAAH*, which has been shown to be associated with problem drug/alcohol use (5,15,16,68), would show greater sensitivity to the effects of THC. The current study suggests that the *FAAH* 385A/A (P129T) variant may contribute to differences in response (actual or expected) to THC. In this sample, individuals with the *FAAH* TAG haplotype reported reduced levels of fatigue. Findings from the current study also align with previous studies suggesting a role of single nucleotide variants in *CNR1* (rs806368, rs1049353, and rs2023239) in drug addiction, such as nicotine (45), cannabis dependence (8,9,46,47,69,70), and alcohol dependence (4,5), but also correlated traits, like impulsivity (71) and major depression (72), to name a few (3). Like *FAAH*, the *CNR1* haplotype indicated by rs806368 and rs1049353 moderated levels of Anger-Hostility, such that individuals who received THC and were 'CC' carriers had elevated Anger-Hostility. Overall, there is evidence to suggest a role for *CNR1* and *FAAH* variation in cannabis-induced mood.

While the current findings are novel, it is important to keep in mind that these results are preliminary and like all genetic studies, require further confirmation in the form of replication. A major strength of the current study was its use of the Balanced-Placebo-Design to provide less biased effects of THC administration on mood states. A notable limitation was our utilization of multiple polymorphisms in *CNR1* and *FAAH* to capture genetic variation across the region. While this increased the number of tests, it also provided a more accurate representation of variation in these genes. We overcame this limitation of having to correct for a large number of multiple tests by leveraging patterns of linkage disequilibrium to realize ancestral combinations of markers across these two genes. However, given no *a priori* indication of the "risk" haplotype, we present

our findings allowing for each haplotype to be the risk haplotype. In addition to modeling all possible haplotype-coding schema, we employed sensitivity analyses that corrected for extreme phenotypic values whenever there was evidence of a significant gene x drug or stimulus expectancy x drug interaction effect. These sensitivity analyses increase confidence in the observed estimates, but future replication is still needed due to the loss of power in smaller samples. Altogether, these conservative approaches provide key information that sets the stage for future studies (i.e., promising models may be adopted as *a priori* hypotheses in a replication study to reduce the number of tests being conducted). The likelihood of false discoveries remains a significant concern in genomics research, especially in the context of candidate gene and traditional genome-wide association analyses that either cannot or often fail to account for the polygenic effects on traits when evaluating the relevance of individual loci, respectively. In that regard, this study was underpowered to detect very limited marker effects at a genome-wide significant level (i.e., $p < 1 \times 10^{-8}$). These findings may also not generalize to present-day potency of recreationally available marijuana, which recent reports suggest was 12% in 2014 (73) but most recently averaging 21.2% in Washington state (74) and up to 32% in Colorado (75). Still, these preliminary findings support the role of the endocannabinoid system in affective states (32) but require additional replication using similar methods in larger samples. Lastly, our analysis of variation in *CNR1* and *FAAH* did not include an exhaustive list of other polymorphisms in the region, such as rs6454674, which is in high LD with rs2023239 and has been previously shown to also be associated with cannabis consumption and dependence (76). We caution readers to keep in mind that the variance explained by these SNPs may be a reflection of other unmeasured SNPs that may also be LD with these polymorphisms. Furthermore, additional studies are needed to understand how these SNPs are functionally linked to cannabis involvement and cannabis-induced effects on affect. Another limitation is that these findings may not generalize to individuals who have only recently started to use marijuana or may have a history of marijuana use that differs from the current sample. In order to generalize to regular users, the current participants had to have used marijuana at least once a week in the past month and at least ten times in the past 6 months. That said, the findings may not generalize to some groups of regular chronic users who have undergone neuronal regulation that could affect mood in the presence of THC.

In conclusion, the present findings add to the growing body of literature investigating the role of *CNR1* and *FAAH* variation in the acute effects of cannabis on affect. They also suggest that *CNR1* and *FAAH* genotypes/haplotypes influence the expectancy effects of THC. Future studies are needed to confirm the observed genetic effects in the context of other variants across the genome, as well as different potencies of THC.

Disclosures

The authors declare they have no conflicts of interest. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Funding

This work was supported by research grants from the National Institute on Alcohol Abuse and Alcoholism (R01AA025593) and the National Institute on Drug Abuse (DP1DA042103, R03DA027484, and R01DA044131).

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