

Acute effects of cannabinoids on addiction endophenotypes are moderated by genes encoding the CNR1 receptor and FAAH enzyme

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Word count:

Abstract: 247(250)

Main body: 4407/5000

Running Head:

Endocannabinoid genotypes moderate addiction endophenotypes

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Abstract

Understanding genetic factors that contribute to cannabis addiction is important, but to date, findings have been equivocal. Single nucleotide polymorphisms in the Cannabinoid receptor 1- gene (CNR1; rs1049353, rs806378) and the Fatty Acid Amide Hydrolase (FAAH) gene (rs324420) have been implicated in the development of addiction. Their relationship to addiction endophenotypes such as drug-cue salience, state satiety and craving after acute cannabinoid administration has not been investigated. Forty-eight cannabis users participated in a double-blind, placebo-controlled, four-way crossover study where they were administered 4 treatments in a randomised order via vaporisation: placebo, Δ^9 -tetrahydrocannabinol (THC) (8mg), THC+Cannabidiol (CBD) (8mg + 16mg), CBD (16mg). Salience of appetitive cues (cannabis, food), state satiety and cannabis cravings were assessed each day. Participants were genotyped for rs1049353, rs806378 and rs324420. Results indicated CNR1 rs1049353 GG carriers showed reduced salience to appetitive cues after THC in comparison to CBD administration. GG carriers showed reduced state satiety after THC and THC+CBD administration, in comparison to placebo; A carriers did not vary on either of these measures. CNR1 rs806378 CC carriers showed greater bias to appetitive cues in comparison to T carriers but there was no evidence for changes in state satiety. FAAH rs324420 A carriers showed greater bias to appetitive cues after THC, in comparison to CC carriers. FAAH CC carriers showed reduced bias after THC in comparison to CBD. None of the genes modulated craving. These findings show that endocannabinoid system genetics can modulate addiction endophenotypes after acute administration of cannabinoids in healthy individuals.

Keywords: addiction; cannabis; CBD; craving; THC; endophenotype; salience; addiction

Introduction

Problematic drug use is influenced by both environmental and genetic factors with genetic variation accounting for between ~40 to 60% of the variance of the total risk in vulnerable individuals (Nestler and Landsman 2001). Policies about cannabis use worldwide are becoming more liberal. Understanding the individual differences in vulnerability and resilience to the harmful effects of cannabis is a critically important aim as cannabis stands poised to join alcohol and tobacco as a legal drug across the globe (Curran et al. 2016), whereby rates of addiction to cannabis may also rise (Pacula et al. 2015). The endocannabinoid system is fundamental in drug addiction (Volkow et al. 2017). Genetic differences in the endocannabinoid system may contribute to an individual's vulnerability or resilience to cannabis addiction.

The primary psychoactive cannabinoid in cannabis, Δ^9 -tetrahydrocannabinol (THC), is a partial agonist at the endocannabinoid receptor type 1 (CB1R). THC is the primary driver of the addictive effects of cannabis (affecting 9% of those who initiate use (Lopez-Quintero et al. 2011)). The percentage of THC in cannabis has been increasing over the past two decades (ElSohly et al. 2016) which may be related to increased rates of cannabis dependence (Freeman et al. 2018). Cannabidiol (CBD), the second most abundant cannabinoid found in the cannabis plant is non-intoxicating (Hindocha et al. 2015a) and non-rewarding (Babalonis et al. 2016; Haney et al. 2015), has psychopharmacologically opposite effects to THC (Bloomfield et al. 2018; Curran et al. 2016; Parsons and Hurd 2015), but its mechanism of action has not fully been determined. Some research suggests it is a negative allosteric modulator of the CB1R (Laprairie et al. 2015); and/or increases the inhibition of Fatty Acid Amide Hydrolase (FAAH), which is an indirect mechanism of regulating the activity of the CB1R (Pertwee 2008). The ratio of THC:CBD is important for its addictive potential, as CBD protects against the addiction-related and psychotic-like effects of THC (Di Forti et al. 2016; Englund et al. 2013; Morgan et al. 2012; Morgan et al. 2010; Schubart et al. 2011; Zuardi et al. 1982).

The CNR1 gene encodes the CB1R and is located on chromosome 1 (López-Moreno et al. 2012). Meta-analyses have found that polymorphisms in CNR1 have been associated with cannabis (Agrawal et al. 2009; Benyamina et al. 2011; Hartman et al. 2009; López-Moreno et al. 2012), alcohol (Schmidt et al. 2002), nicotine (Chen et al. 2008) and cocaine dependence (Clarke et al. 2013). Polymorphisms in the CNR1 are also associated with potential endophenotypes related to cannabis dependence such as functional reward-related brain activity during exposure to cannabis cues (Filbey et al. 2010). As such, genetic influences may therefore alter mechanisms related to addiction – such as craving, satiation and the salience of drug cues.

Endocannabinoid signalling is terminated by enzymes such as FAAH which catabolises the endogenous cannabinoid, anandamide. FAAH inhibition is a mechanism that is currently being investigated as a

treatment of addiction in animals and humans (D'Souza et al. 2015; Hindocha et al. 2018; Justinova et al. 2015; Panlilio et al. 2013). The rs324420 Single Nucleotide Polymorphism (SNP) of the FAAH enzyme is a C to A polymorphism which results in a proline to a threonine substitution at codon 129 (López-Moreno et al. 2012). As such, those with the A allele have reduced FAAH expression (Chiang et al. 2004; Sipe et al. 2002). This reduction has been associated with problematic drug use (Flanagan et al. 2006; Sipe et al. 2002) and putative endophenotypes such as craving and withdrawal after short-term abstinence (Schacht et al. 2009). However, the C allele has also been associated with cannabis dependence in Genome Wide Association Studies (GWAS) (Flanagan et al. 2006) as well as other potential endophenotypes such as greater craving and withdrawal after cannabis abstinence (Haughey et al. 2008; Schacht et al. 2009). Additionally, Filbey et al. (2010) found that those who were homozygous for the C allele showed greater activation in the reward circuit (which included the orbitofrontal cortex, anterior cingulate gyrus and the nucleus accumbens) after cannabis cue reactivity, in comparison to A allele carriers. However, no studies have investigated how genes related to the endocannabinoid system predict addiction-related endophenotypes after *controlled* acute administration of cannabinoids.

Our innovative approach was to study endophenotypes of addiction after acute cannabinoid administration, which may be more valid than a single dichotomous variable commonly used in GWAS (Flint and Munafò 2007; Gottesman and Gould 2003). We focussed upon three endophenotypes of addiction. Firstly, attentional processing is an important transdiagnostic marker for depression, anxiety and addiction (Garland and Howard 2014; Hindocha et al. 2018). Indeed, THC:CBD ratio predicts attentional bias to cannabis cues when intoxicated, with those using more CBD in their cannabis strains showing reduced attentional bias (Morgan et al. 2010). The salience of appetitive stimuli (such as cannabis cues to cannabis-dependent individuals) is also related to *frequency* of cannabis use; dependence on the drug itself, and craving (Field 2005; Field and Cox 2008; Field et al. 2004). Secondly, craving, or the intense desire for a reward, is a primary behavioural component of addiction (Filbey et al. 2009) which motivates drug use and predicts cannabis use after 6 months in adults and adolescents (Cousijn et al. 2011; Cousijn et al. 2015). Thirdly, satiation after acute ingestion of a drug is a key element of addiction and is likely related to loss of control measures, such that not feeling satiety after acute drug use may be a key indicator of addiction (Sussman and Sussman 2011).

Our primary aim was to investigate if and how genetic variants in the endocannabinoid system, in particular the CB1 receptor (rs1049353 and rs806378) and the FAAH enzyme (rs324420) would modulate the acute response to cannabis, in relation to promising addiction endophenotypes: drug cue salience, satiation and craving. To this end, we carried out a randomised, double-blind, crossover study where participants were administered THC (8 mg), THC (8 mg)+CBD (16 mg), CBD (16 mg) and placebo (ethanol vehicle) across four separate sessions. We predicted that genetic variants in the CB1

receptor and FAAH enzyme would modulate acute response to THC in regards to these endophenotypes. Moreover, given research that suggests that CBD protects against the addiction-related effects of THC (Morgan et al 2010), differential effects of drug conditions were expected on addiction endophenotypes, although how this would interact with endocannabinoid genotypes was exploratory, given the paucity of research in this area.

Material and Methods

Participants

Participants were recruited on the basis of having previously volunteered in a large scale study of over 400 cannabis users (Morgan et al. 2012) where genotyping was conducted (Morgan et al. 2016). Participants were recruited based on 1) schizotypy (Schizotypal Personality Questionnaire score (top and bottom quartiles)) and 2) Frequency of cannabis use (“light” = 1-24 days per month; “heavy” = 25+ days per month). This study is a secondary analysis concerned with genetic associations across the whole sample regardless of sub-group. Additional data from this study on facial affect recognition and visual analogue scales (Hindocha et al. 2015a) and psychotomimetic symptoms and memory function have been reported elsewhere (Morgan et al. 2018).

Participants were matched for age and Spot the Word task (Baddeley et al. 1993) scores across frequency groups. Inclusion criteria were: (i) self-reported abstinence from cannabis, other drugs and alcohol use for 24h prior to each test day; (ii) English fluency, (iii) normal or corrected to normal vision. Exclusion criteria were: current self-reported (i) respiratory health problems/physical health problems, (ii) pregnancy or the risk of being pregnant, (iii) clinically diagnosed learning impairments, (iv) clinically diagnosed schizophrenia/psychosis or substance abuse problems, and (v) no illicit drug use other than cannabis more than once a week.

Design

A four session, randomised, double-blind crossover design was used to compare the acute effects of THC (8mg), CBD (16mg) and their combination (8mg THC+16mg CBD) with placebo (ethanol vehicle). Both cannabinoids were formulated in alcohol solution and were purchased from STI Pharmaceuticals (Brentwood, Essex, UK). Treatment order across the 4 sessions was determined by a balanced Latin square resulting in 12 combinations.

Drug administration

Cannabinoids and placebo (ethanol vehicle) were administered using a Volcano Medic Vaporisor (Storz & Bickel, Tuttlingen, Germany). 8mg THC dissolved in ethanol and 16mg of CBD dissolved in ethanol were administered on a 10-second inhalation cycle wherein participants was instructed to first fully exhale, next fully inhale from the balloon, hold their breath for 10 seconds and then fully exhale; this was repeated until the balloon was empty. Justification of doses and further details about

drug administration and concealment and blinding can be found in Hindocha et al. (2015a) and Morgan et al. (2018).

Genotyping

DNA was obtained from cheek swabs of all participants who completed the assessments described above. DNA extraction was performed using standard phenol–chloroform methods. Analyses were performed on two SNPs of CNR1: rs1049353, rs806378 and one single-nucleotide polymorphism of the FAAH gene (rs324420). Off the shelf Taqman assays for these polymorphisms are available as a kit (Applied Biosystems, Life Technologies, Paisley, UK). Genotype calls were discriminated on the basis of algorithmic membership of three clusters representing homozygote A/A, heterozygote A/G, and homozygote G/G genotype classes for CNR1 rs1049353, C/C, C/T and T/T for CNR1 rs806378 and CC/AC/AA for FAAH rs324420. Individuals with the minor allele of these SNPS were combined for power and due to the rarity of these alleles. For rs1049353, those with the minor allele of A, were combined with heterozygotes AG according to convention (Agrawal et al. 2012a; Domschke et al. 2008). For CNR1 rs806378, those with the minor allele T were combined with heterozygotes CT (Tiwari et al. 2010) and for FAAH rs324420, the minor allele A, was combined with the heterozygote AC (Spagnolo et al. 2016). Data was missing for 6 individuals for rs1049353, 3 individuals for rs806378 and 4 individuals for rs324420

Assessments

Before drug administration, participants completed the Beck Depression Inventory (Beck et al. 1961), Spielberger Trait Anxiety Inventory (Spielberger et al. 1970), Schizotypal Personality Questionnaire (Raine 1991), Severity Of Dependence Scale (Gossop et al. 1995) and a drug history (Curran et al. 2018; Hindocha et al. 2015a; Hindocha et al. 2017; Hindocha et al. 2015b; Morgan et al. 2018).

Dot Probe task (Fig 1)

Adapted from Morgan et al. (2010), this computer-based dot-probe paradigm was used to assess attentional bias to both cannabis- and food-related stimuli. Ten colour photographs of cannabis-related stimuli and 10 colour photographs of food-related stimuli were used, with each image simultaneously paired with a neutral photograph matched as closely as possible for visual composition and complexity. A total of 80 of the 160 total trials were critical trials of which 40 featured cannabis-related and 40 food-related stimuli, each presented twice for 250ms. Based on findings in Morgan et al. (2010), only the short exposure time was chosen to index automatic (250 ms) processing. The critical (food- or cannabis-related) images appeared once on the left and once on the right at each time interval. The side at which the probe appeared was counterbalanced across all the trials. An asterisk was used as the probe. A total of 10 neutral practice trial pairs were used as training, followed by two blocks of 80 experimental trials. Short break occurred between blocks. Versions were randomised across testing days. Each trial began with a central fixation cross shown for 1000 ms, after

which a pair of matched images would appear, one on each side of the fixation cross, for 250 ms durations. Both images then disappeared revealing the probe behind one of the two images. The task took place approximately 25 minutes after drug administration. Participants were required to respond to the probe as quickly as possible by pressing a button corresponding to the relevant side of the screen. Attentional bias was calculated as the difference in reaction time between when the probe replaced the neutral compared with the incentive (cannabis/food) stimulus [$Rt_{neutral} - Rt_{incentive}$], such that a greater difference indicated greater bias toward that stimulus.

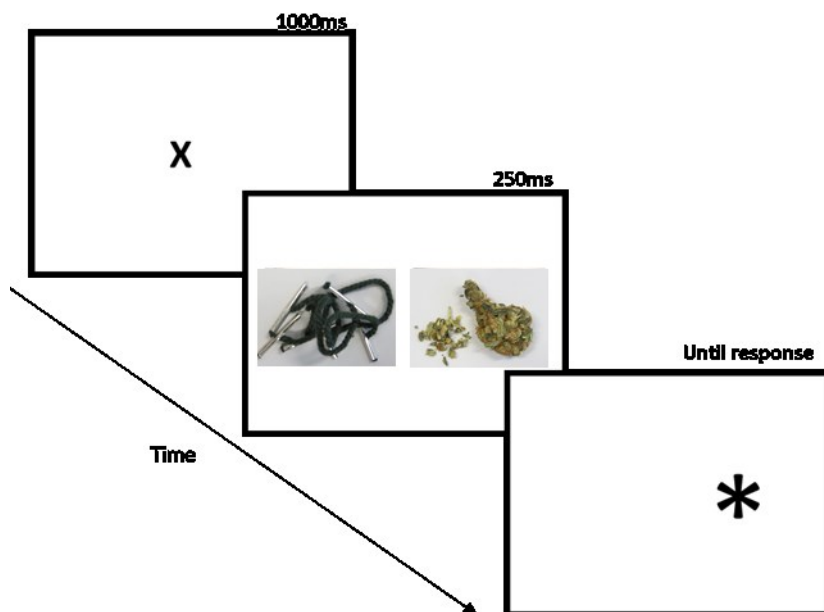


Figure 1. Trial structure for the visual probe task. Example of Cannabis (right) and matched neutral stimuli (left) provided

Bodily Symptoms Scale (BSS) (Bond and Lader 1974): “want to smoke a joint” single item.

The BSS was designed to detect physical symptoms of acute cannabinoids administration. Participants rated on scale from 0 (do not want to smoke a joint) to 10 (really want a joint), how much they wanted to smoke a joint both 10 and 70 minutes post drug administration. This measure was used to index state satiation.

Marijuana craving questionnaire (Heishman et al. 2009)

A short 12-item questionnaire was given to assess current craving for cannabis. Participants completed the MCQ immediately after the attentional bias task; approximately 35 minutes after drug administration. The MCQ is reliable for assessing craving in cannabis users not seeking treatment (Heishman et al. 2001).

Procedure

Experimental sessions occurred on four occasions each separated by a one-week washout to minimize carry-over effects (>3 times elimination half-life of THC (Hindocha et al. 2015a)). We used urine and

saliva screens to verify drug use. Participants completed assessments then two minutes after drug administration. The full test battery took approximately 1.5 hours on each test day. Participants were reimbursed £120 for their time on the last testing day and debriefed fully. All participants provided written, informed consent on each occasion and ethical approval was given by the UCL Research Ethics Committee.

Statistical analysis

All analyses were conducted with SPSS 24.0. Syntax are available from CH on request. Outliers and normality were assessed via diagnostic plots. Extreme outliers (>3 times interquartile range) were winsorized within-group to the next highest/lowest value +/- 1. Descriptive statistics based on genotype were conducted with one way analysis of variance (ANOVA) and Chi-Squared Tests. When variances were not equal between groups (Levene's test), unequal variances (Welch's) t-tests were used.

Mixed ANOVA was used for all analyses with a within-subjects factor of drug (placebo, THC, THC+CBD, CBD) and a between-subjects factor of genotype. Greenhouse Geisser corrections were applied for violations of sphericity (rounded to the nearest whole number). For attentional bias, there was an additional within-subjects factor of stimulus type (cannabis, food), for BSS "want to smoke a joint", the additional within-subject factor was time (T1, T2). Main effects were explored with a priori simple contrasts to reduce the number of comparisons (e.g. for the main effect of drug this was; placebo vs. THC, placebo vs. THC+CBD and placebo vs. CBD). Interactions were explored with Bonferroni-corrected pairwise comparisons locally within each omnibus term. η^2 was calculated as the SS_{effect}/SS_{total} .

Results

Sample Characteristics (Table 1)

CNR1 rs1049353 genotypes were in Hardy-Weinberg equilibrium (G/G=20, A/G=17, AA=5; $\chi^2(2)=0.21$, $p=0.64$), as was CNR1 rs806378 (C/C=18, C/T=24, T/T=3; $\chi^2(2)=1.04$, $p=0.17$) and FAAH rs324420 (C/C=30, A/C=10 and A/A=4; $\chi^2(2)=4.00$, $p=0.05$). As seen in table 1, participants did not differ on demographics based on genotype groupings for CNR1 rs1049353, CNR1 rs806378, or FAAH rs324420. Genotype groups differed significantly on the SDS where those who were homozygote GG for the CNR1 rs1039353 gene, had a higher cannabis dependence score than A carriers, but groups did not differ on cannabis use variables. Additionally, a significant difference was observed for FAAH rs324420, between CC homozygotes and A carriers for years of cannabis use. CC homozygotes had used cannabis more recently than A carriers.

Attentional bias

CNR1 rs1049353

There was a drug x genotype interaction ($F(3,120)=3.108$, $p=.029$, $\eta^2=.03$). Within the GG group, attentional bias was significantly lower after acute THC administration, in comparison to CBD administration (M:25.93, SE: 4.88; $p=.011$), but this was not significant for the THC+CBD ($p=.066$), or placebo ($p=.291$) conditions. A carriers show no differences in attentional bias between drug administration conditions ($p's=1.000$). No Bonferroni-corrected pairwise comparisons met significance between genotypes in each drug condition. There was no main effect of drug ($F(3,120)=2.002$, $p=.177$, $\eta^2=.20$), stimulus type ($F(1,40)=.232$, $p=.129$, $\eta^2=.005$) or genotype ($F(1,40)=.723$, $p=.40$, $\eta^2=.00$) or any other two way or three way interactions.

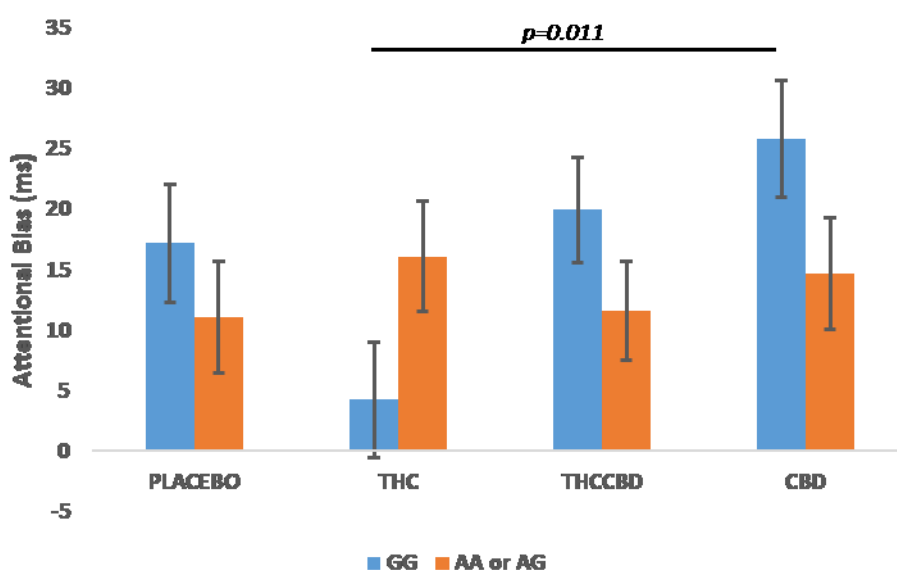


Figure 2: Mean (\pm Standard Error) attentional bias, as assessed by the dot probe task, to drug and food stimuli (ms) after drug administration for each genotype group. Bonferroni corrected p values are displayed. *CNR1 rs1049353* “A” carriers’ attentional bias remains relatively constant whilst GG homozygotes vary by cannabinoid administration.

CNR1 rs806378

A main effect of genotype emerged ($F(1,43)=5.679$, $p=.022$, $\eta^2=.047$) which showed homozygote CC carriers (M:20.21, SE:2.87) had a greater attentional bias than T carriers (M:11.38, SE:2.34), regardless of stimuli type and drug. There was no main effect of drug ($F(3,129)=1.674$, $p=.176$, $\eta^2=.002$), stimulus type ($F(1,43)=.523$, $p=.474$, $\eta^2=.00$) or other two way or three way interactions.

FAAH rs324420

A drug x genotype interaction emerged ($F(3,126)=3.385$, $p=.020$, $\eta^2=.003$). Bonferroni-corrected pairwise comparisons reveal lower attentional bias, irrespective of stimuli, between the homozygote CC group (M:5.56, SE:3.71) and A carriers (M:21.41, SE:5.42) after THC only ($p=0.02$). No differences emerged between genotype groups for placebo ($p=.518$), THC+CBD ($p=.321$) or CBD ($p=.261$). Within

the CC group, there was a significant lower attentional bias after THC in comparison to CBD (M:21.14, SE:3.81; $p=.018$). There was no main effect of drug ($F(3,126)=.418$, $p=.740$, $\eta^2=.004$) or stimulus type ($F(1,42)=1.089$, $p=.303$, $\eta^2=.002$) or genotype ($F(1,42)=.169$, $p=.683$, $\eta^2=.001$). There were no other two way or three way interactions.

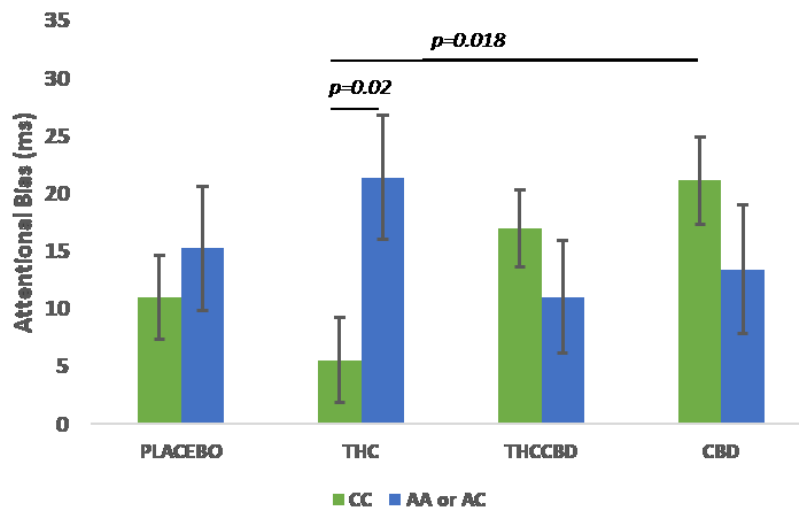


Figure 3: Mean (\pm Standard Error) attentional bias, as assessed by the dot probe task, after drug administration for each genotype group for FAAH rs324420. Bonferroni corrected p values are displayed. FAAH rs324420 “A” carriers’ attentional bias remains relatively constant whilst CC homozygotes vary by cannabinoid administration.

BSS “Want to smoke a joint”

CNR1 rs1049353

There was a drug x genotype interaction ($F(3,105)=4.192$, $p=.008$, $\eta^2=.05$). The interaction was explored with Bonferroni-corrected pairwise comparisons which showed that those with the GG genotype had decreased wanting to smoke a joint after both THC ($p=.016$) and THC+CBD ($p<.001$), but not CBD ($p=.137$) in comparison to placebo. Those with the A allele did not experience this reduction after THC/THC+CBD administration (both p 's=1.000). There was a main effect of drug ($F(3,105)=4.206$, $p=.007$, $\eta^2=.05$). Simple contrasts show lower scores for THC (M:4.73 SE:.41, $p=0.047$) and for THC+CBD (M: 4.45, SE:.42; $p=0.001$), in comparison to placebo, but not for CBD (M: 4.95, SE:.43; 0.182). There was a main effect of time ($F(1,36)=12.945$, $p=.001$, $\eta^2=.03$) which showed that wanting to smoke a joint increased across the two time-points ($p<.001$). There was no main effect of genotype ($F(1,36)=.176$, $p=.675$, $\eta^2=0.00$), there were no other two-way or three-way interactions.

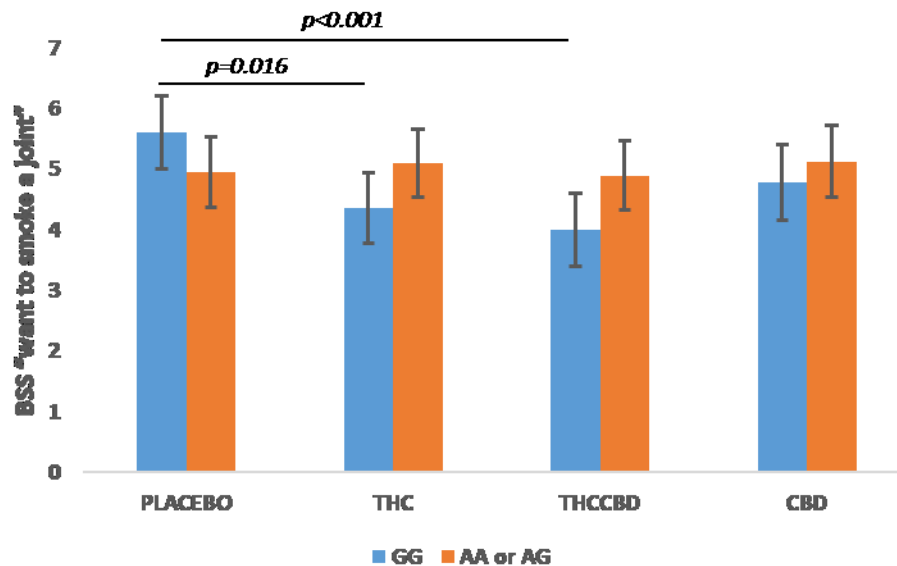


Figure 4: Mean (\pm Standard Error) of the single item of the Bodily Symptoms Scale: “want to smoke a joint” averaged across the two time-points. Bonferroni corrected p values are displayed. Homozygote GG carriers of CNR1 rs1049353 showed reduced wanting after both THC measures, but A carriers show no such reduction in state satiety.

CNR1 rs806378

There was a main effect of drug ($F(3,114)=3.784$, $p=.012$, $\eta^2=.005$). Simple contrasts show lower wanting to smoke a joint after THC (M:4.730, SE:.40; $p=.043$) and THC+CBD (M:4.55, SE:.43; $p=.004$) in comparison to placebo (M=5.36, SE:.43) but no differences emerged CBD (M:5.06, SE:.43; $p=.254$). A main effect of time emerged ($F(1,28)=16.069$, $p<.001$, $\eta^2=.04$) which showed that wanting to smoke a joint increased across the two time-points ($p<.001$). There were no main effects or interactions with genotype.

FAAH rs324420

Only a main effect of time emerged ($F(1,27)=11.738$, $p=.002$, $\eta^2=.04$) which showed that wanting to smoke a joint increased across the two time-points ($p<.001$).

Marijuana Craving Questionnaire

CNR1 rs1049353

There was no main effect of drug, genotype or drug x genotype interaction

CNR1 rs806378

There was no main effect of drug, genotype or drug x genotype interaction

FAAH rs324420

There was no main effect of drug, genotype or drug x genotype interaction

Sensitivity analysis

Because our sampling strategy aimed to include individuals with high and low scores in both schizotypy and frequency of cannabis use (Hindocha et al. 2015a; Morgan et al. 2018), we included mean-centred

days per month of cannabis use and SPQ scores as continuous covariates into each analysis. Adjusted and unadjusted main effects and interactions were generally similar and addition of covariates did not modify results. Days per month of cannabis use was seen to be a significant covariate in many of the analyses (Analyses can be requested from CH).

Discussion

This study, to our knowledge, is the first to suggest that the acute effects of different cannabinoids on addiction endophenotypes are moderated by genes encoding the CNR1 receptor and FAAH enzyme. Specifically we show that individuals who are homozygous GG on the CNR1 rs1049353 SNP show reduced wanting to smoke a joint, indicative of *increased* satiety, after THC and THC+CBD, in comparisons to placebo. They also show reduced attentional bias to appetitive stimuli after THC, in comparison to CBD. However, A carriers show no changes in attentional bias to acute THC (/THC+CBD) administration and did not appear satiated after acute administration of THC. In regards to CNR1 rs806378, homozygote CC carriers had a greater attentional bias to appetitive stimuli, regardless of cue type and drug condition. Across both CNR1 SNPs, genotype did not modulate craving.

Variations in the FAAH genotype also modulated attentional bias. Homozygous CC carriers show reduced attentional bias to incentive stimuli after THC administration, in comparison to A carriers. Homozygous CC carriers also showed reduced attentional bias after THC in comparison to CBD administration, suggesting CBD alone is not modulating drug cue salience. However, FAAH genotype did not modulate state satiation or craving. These data are very important as acute response to cannabis is thought to be a marker of the development of other risks such as addiction and psychosis from smoking the drug (Morgan et al. 2016) and may further help us understand the role of the endocannabinoid system in individual differences in risk and resilience for the abuse of cannabis.

CNR1 genes modify the binding of cannabis and endogenous cannabinoids to the CB1R, thus altering the signalling of the endocannabinoid system which is known to play a key role in addiction (Forget et al. 2009; López-Moreno et al. 2012; Maldonado et al. 2006; Parsons and Hurd 2015; Scherma et al. 2016; Sipe et al. 2002). In the brain, CB1Rs are found on GABAergic and glutamatergic interneurons in the ventral tegmental area (VTA) where they regulate the mesolimbic dopaminergic pathway leading to modulation of dopamine release in the nucleus accumbens; a key mechanism in incentive salience (Bloomfield et al. 2016; Cheer et al. 2004; Robinson and Berridge 2001). In this study, CNR1 genes seem to be modulating cannabis users' response to acute administration of cannabinoids on putative endophenotypes such as appetitive cue salience (Field and Cox 2008) and satiety (Sussman and Sussman 2011) but not craving. It may be that A carriers of the CNR1 rs1049353 are more liable for addiction because they did not feel satiety after drug administration as assessed by attentional bias and state satiety. In contrast, the GG carriers showed reductions in these endophenotypes in response

to THC administration, as expected. GG carriers had greater self-reported cannabis dependence, however, groups did not differ on other drug use measures such as frequency of use, last use of cannabis or years of cannabis use. When we adjusted for frequency of use (a strong predictor of the severity of dependence (Curran et al. 2018)), it had no effect on the results, suggesting that this effect was not explained by variation in frequency of use. CC carriers of CNR1 rs806378 showed increased bias for both cannabis and food related cues regardless of drug condition suggesting that CC carriers may be more susceptible to appetitive cues overall. This SNP has been previously related to cocaine dependence. However, no genotype specific effects were seen on state satiety or craving.

In regards to FAAH, those who are homozygote for the A allele have ~30% poorer FAAH functioning and are a minority of the population (5%) (Chiang et al. 2004; Mayo et al. 2018; Sipe et al. 2002). As a result, these individuals can be used as a human genetic model of elevations in anandamide which may be able to inform whether FAAH inhibitors would have an effect on these intermediate endophenotypes (Mayo et al. 2018). Those with the C allele, on the other hand is associated with cannabis dependence and related endophenotypes (Filbey et al. 2010; Flanagan et al. 2006; Haughey et al. 2008; Schacht et al. 2009). In this study, *A carriers* showed a greater bias towards incentive stimuli in comparison to CC carriers – which would be consistent with some previous research suggesting this polymorphism is associated with emotional-motivational reactivity (Conzelmann et al. 2012) but contradicts others (Hariri et al. 2009). However, *A carriers* also had significantly fewer years of cannabis use; but when we adjusted for cannabis use in the model, this did not change the results. In this study, low FAAH functioning may be influencing the automatic processes associated with salience of drug cues but did not influence satiation or craving after acute drug administration – which are arguably more explicit measures of addiction.

In genetic association research, there have been equivocal findings with variants in CNR1 and FAAH genotypes on cannabis dependence (Agrawal et al. 2012b). Future research should investigate the role of genetic variants in the endocannabinoid system on transdiagnostic markers for mental health found in the RDoC initiative include neuroimaging and plasma biomarkers - which may be reliable indicators (Agrawal et al. 2012b). Additionally, the CNR1 and FAAH genes noted in this study should be investigated in relation to other cannabis-related harms such as psychotic-like experiences, depression and anxiety as they have already been showed to contribute to psychiatric problems (Hillard et al. 2012). Longitudinal studies are imperative to clarify whether genetic variation influences cannabis dependence – such is the focus of the ABCD study (Lisdahl et al. 2018). Moreover, the development of polygenic risk scores for cannabis dependence that can capture a wider range of common genetic variants, should be developed and utilised.

Strengths and Limitations

Strengths of this study, include a controlled design of a four way crossover with THC, CBD and their combination on addiction-related outcomes. One criticism levied at genome wide association studies (GWAS) is that they tend to utilise a dichotomous diagnostic cut off, such as cannabis dependence (Agrawal et al. 2012b; Flint and Munafò 2007; Gottesman and Gould 2003), for which the causes are likely to be complex and involve many mechanisms and predictors (van der Pol et al. 2013). The National Institute of Mental Health (NIHM) Research Domain criteria (RDoC) initiative supports research about the biobehavioural dimensions that cut across these prescriptive diagnostics (Insel 2014). However, such intermediates or endophenotypes have remain unexplored for addictive disorders until recently (Kwako et al. 2016; Yücel et al. 2018). In this study, we took endophenotypes that might be more closely related to the disorder than diagnostic criteria which is a strength of this study. However, the sample size of this study was modest and there were unequal numbers of each genotype. The sample size calculation was based on the effects of THC, not on genetic differences. It would be important to replicate these findings with a larger sample size, oversampling for the minor allele and allowing for analysis of a dose-response relationship between genotype and risk (Di Forti et al. 2012; Morgan et al. 2016).

Conclusions

In conclusion, our experiment suggests that the genes that code for the CB1 receptor and FAAH enzyme are implicated in the acute addiction-related response to acute consumption of cannabinoids. This was found for attentional bias and state satiety, but not for craving. These results have important pharmacogenetic implications in regards to recreational users of cannabis who may be more vulnerable to addiction-forming effects of THC and who may therefore be at greater risk of transitioning into dependence.

Funding

This research was funded by a Medical Research Council (MRC) Grant (G0800268) to HVC and CJAM. CH and MAPB are supported the National Institute for Health Research University College London Hospitals Biomedical Research Centre (NIHR BRC). MAPB is also funded by a UCL Excellence Fellowship and the British Medical Association Foundation for Medical Research. TPF is currently funded by a Senior Academic Fellowship from the Society for the Study of Addiction.

Acknowledgements

We are grateful to Stork and Bickel for providing us with a Volcano vaporiser to use in this study.

Author Contributions

CJAM and VC designed the protocol. GS and CG conducted the testing assessments. CH, TPF and EB and MB conducted the statistical analysis. CH wrote the manuscript. All authors approved the final version of the manuscript.

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Table 1: Means (SD) for demographic, mental health and cannabis use variables for each of the genotype groups.

	CNR1 rs1049353			CNR1 rs806378			FAAH rs324420		
	GG	AA/AG	Test statistic	CC	CT/TT	Test Statistic	CC	AA/AC	Test statistic
N (% female)	20 (35%)	22 (27%)	$\chi^2(1)=.293$, ns	18 (33.3%)	27 (29.7%)	$\chi^2(1)=.069$, ns	30 (23%)	14 (50%)	$\chi^2(1)=3.129$, ns
Age	21.90 (1.94)	21.59 (1.94)	F(1,40)=.265, ns	21.44 (1.98)	22.00 (1.79)	F(1,43)=.953, ns	21.87 (1.92)	21.79 (1.72)	F(1,43)=.018, ns
Race/Ethnicity (self-reported)									
White British	14	17		12	20		23	8	
Other Ethnic Group	6	5	$\chi^2(1)=.28$, ns	6	7	$\chi^2(2)=.005$, ns	7	5	$\chi^2(1)=1.03$, ns
Frequency of cannabis use	19.75 (10.95)	17.72 (10.21)	F(1,40)=.394, ns	20.36 (10.15)	17.98 (10.82)	F(1,43)=.548, ns	19.53 (17.21)	17.21 (10.21)	F(1,42)=.452, ns
Severity of Dependence	4.05 (3.62)	2.09 (2.21)	F(1,40)=4.585, p=0.038 *	3.55 (3.70)	2.56 (2.47)	F(1,43)=1.187, ns	3.47 (3.26)	1.71 (2.16)	F(1,42)=3.345, ns
Last use of cannabis	3.25 (3.17)	7.81 (25.09)	F(1,40)=.652, ns	2.94 (1.98)	8.00 (23.14)	F(1,43)=.848, ns	2.63 (1.87)	13.43 (31.67)	W(1,12)=1.624, ns ^a
Years of cannabis use	6.80(2.31)	6.02 (3.05)	F(1,40)=.854, ns	6.00 (2.57)	6.31 (2.91)	F(1,43)=.138, ns	6.83 (2.64)	4.96 (2.68)	F(1,42)=3.557, p=.035*
SPQ total	19.05 (12.41)	16.55 (15.86)	F(1,40)=.320, ns	19.83 (13.43)	15.15 (14.32)	F(1,43)=1.214, ns	14.07 (9.92)	22.36 (19.46)	F(1,42)=3.542, ns
BDI	13.30 (9.42)	7.91 (8.87)	F(1,40)=3.651, ns	11.96 (10.79)	8.48 (8.25)	F(1,43)=1.485, ns	9.23 (9.16)	10.79 (10.32)	F(1,42)=.253, ns
STAI	43.50 (11.40)	40.41 (8.81)	F(1,40)=.976, ns	42.44 (11.55)	40.04 (9.63)	F(1,43)=.575, ns	40.47 (10.95)	42.14 (10.95)	F(1,42)=.239, ns

Notes: ^a - Welch's Test, ^b Includes White Other, mixed white and black Caribbean, mixed white and black African, any other mixed background, Asian/British Asian, any other Asian/British Asian background, Black/British Caribbean, Chinese and any other ethnic group, * indicated significant difference at $p \leq 0.05$