Cannabis and Psychosis: Biological and Environmental Risk Factors



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Cannabis and Psychosis: Biological and Environmental Risk Factors

DISSERTATION

to obtain the degree of Doctor at Maastricht University, on the authority of the Rector Magnificus, Prof. Dr. Jan Smits, in accordance with the decision of the Board of Deans, to be defended in public on Friday 27 November 2026,

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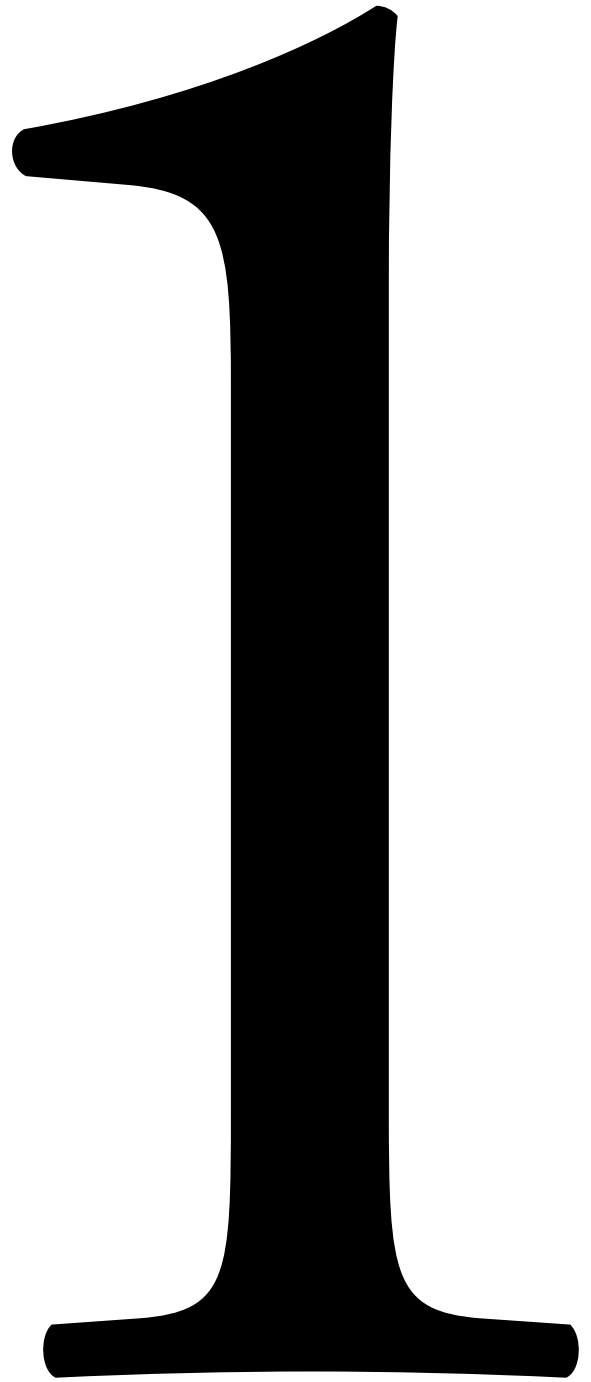


Chapter 1

Introduction

Prevalence of Cannabis Use is Increasing

The prevalence of cannabis use has been steadily increasing around the world over the past decade. Per the World Health Organization (WHO), about 147 million people, 2.5% of the world population, consume cannabis (annual prevalence) compared with 0.2% consuming cocaine and 0.2% consuming opiates. Cannabis abuse has grown more rapidly than cocaine and opiate abuse in the past decade. The past decade has also witnessed societal changes in the legal status of cannabis. This was notable in the phenomenon of legalization of "medical cannabis" (i.e. legislation allowing the possession and use of cannabis for medical indications) that has emerged in many countries, including Canada, United States and even the Asian subcontinent (in Thailand and South Korea as of 2019). Many states in the USA and Canada have further legalized recreational use of cannabis. Not surprisingly, the perception of harm related to cannabis use appears to be decreasing among adolescents and young adults (Monitoring the Future 2022). There is hence an urgent need to fully understand the implications of early and long-term cannabis use on mental health outcomes.

The association between cannabis use and psychosis has long been recognized. The population attributable risk of cannabis use in the incidence of psychosis has been estimated to be 20%. However, it is less clear as to why certain individuals develop psychosis following cannabis exposure. The ability to identify vulnerable individuals based on epidemiological and biological risk factors has significant public health implications in preventing the emergence of psychosis.

Epidemiology of cannabis-related psychotic disorder.

Epidemiological studies show an increase in the incidence and prevalence of cannabis-related psychotic disorder and schizophrenia, with approximately 10-20% cases attributable to cannabis use. Meta-analysis shows a dose–response association with the highest odds of psychotic disorder in those with the heaviest cannabis use (Marconi et al, 2016).

In a Danish nationwide, register-based cohort study, the population-attributable risk fraction (PARF) for cannabis use in schizophrenia increased from approximately 2% in the period before 1995 to approximately 6% to 8% since 2010 (Hjorthoj et al, 2021). In another nationwide Danish register-based cohort study including individuals aged 16-49 at some point during 1972-2021, comprising 6 million individuals and 45,327 cases of incident schizophrenia during follow-up across 129 521 260 person- years, the overall risk (adjusted Hazard Ratio (aHR)) for cannabis use disorder (CUD) on schizophrenia was slightly higher among males (aHR = 2.42, 95% CI 2.33-2.52) than

females (aHR = 2.02, 95% CI 1.89-2.17) (Hjorthoj et al, 2023). Interestingly, the risk for males aged 16-20-year-olds (adjusted Incidence Risk Ratio (aIRR)) was more than twice that for females (males: aIRR = 3.84, 95% CI 3.43-4.29; females: aIRR = 1.81, 95% CI 1.53-2.15). The annual average percentage change in PARFs for CUD in schizophrenia incidence during 1972-2021 was higher among males (4.8 (95% CI 4.3-5.3; $p < 0.0001$) vs 3.2 among females (95% CI 2.5-3.8; $p < 0.0001$)). In 2021, the PARF among males was 15% vs. 4% among females.

Similarly, the population-attributable risk fraction of high-potency cannabis was 12.1% in the EU-GEI (EUropean network of national schizophrenia networks studying Gene-Environment Interactions) multicenter case-control study of first-episode psychosis including 11 sites across Europe and Brazil (Di Forti et al, 2019). The study included data from 901 patients with first-episode psychosis across 11 sites and 1237 population controls between 2010 and 2015. Daily cannabis use was associated with increased odds of psychotic disorder compared with never users (adjusted odds ratio [OR] 3·2, 95% CI 2·2–4·1), with approximately 5 times increased odds for daily use of high-potency cannabis (4·8, 2·5–6·3). The PAF was 12.2% indicating that if high-potency cannabis were no longer available, 12·2% (95% CI 3·0–16·1) of cases of first-episode psychosis could be prevented across the 11 sites, rising to 30·3% (15·2–40·0) in London and 50·3% (27·4–66·0) in Amsterdam.

In a population-based cohort study conducted in Ontario, Canada, from 2006 to 2022 that included 13 million people aged 14 to 65 years without a history of schizophrenia, 91,106 individuals (0.7%) were diagnosed with schizophrenia in the general population without cannabis use disorder (CUD) (Myran et al, 2025). Notably 8.9% of those with CUD were diagnosed with schizophrenia, representing a tripling of population-attributable risk fraction (PARF) from 3.7% (95% CI, 2.7%-4.7%) during the period before cannabis legalization to 10.3% (95% CI, 8.9%-11.7%) during the legalization period. Male individuals aged 19-24 years were noted to be at higher risk with the PARF in the post-legalization period of 18.9% (95% CI, 16.8%-21.0%) vs 1.8% (95% CI, 1.1%-2.6%) among females aged 45 to 65 years. The annual incidence of schizophrenia was stable over time, while the incidence of psychosis not-otherwise-specified (psychosis NOS) increased from 30.0 in the pre-legalization period to 55.1 per 100,000 individuals (83.7%) in the post-legalization period. The PARF for CUD associated with schizophrenia increased steadily over the study with no accelerations after cannabis policy changes, while increases in the PARF for CUD associated with psychosis NOS accelerated after medical cannabis legalization.

In the largest online drug survey world-wide, the Global Drug Survey (GDS), 0.47% (95%CI 0.42; 0.52) cannabis users reported lifetime occurrence of psychotic symptoms, defined as the occurrence of hallucinations and/or paranoia requiring emergency

medical treatment following the use of cannabis (Schoeler et al, 2022). Risk factors for psychotic symptoms in the past year included age < 21 years [risk ratio (RR) 2.66, compared to individuals], residence in Denmark (RR 3.01, compared to other countries), use of high-potency resin (RR 2.11, compared to herbal cannabis), mixing cannabis with tobacco (RR 2.15, compared to not mixing with tobacco) and those with a diagnosis of psychosis (RR 14.01), bipolar (RR 4.30), anxiety (RR 2.92) and depression (RR 2.68) (compared to no mental health diagnosis). Taken together, acute self-limiting psychotic symptoms in the context of cannabis use may occur in about 1 in 200 cannabis users lifetime. Some individuals could be particularly sensitive to the adverse psychological effects of cannabis, such as young individuals or those with pre-existing mental health vulnerabilities.

Defining the cannabis-psychosis risk: Evidence from epidemiological studies

An important question that arises from the evidence for an association between cannabis and psychosis, is whether those who are particularly sensitive to the adverse psychological effects of cannabis can be identified before they develop adverse consequences. This cannabis-psychosis risk state can be defined in terms of epidemiological risk factors, psychological risk factors, biological risk factors including genetic risk, environmental exposure risk factors and experimental laboratory studies to identify risk eg. tetrahydrocannabinol (THC)- challenge studies.

Chapter 2 reviews the history of the concepts of psychosis proneness, including schizotaxia, schizotypy, psychoticism, clinical-high risk (CHR), attenuated psychosis and genetic risk for psychosis. The review found that the literature on schizotypy and CHR showed that there was a relationship between cannabis use and higher rates of conversion to schizophrenia, but also lower rates of negative schizotypy, social anhedonia and better social functioning. This may partially account for the counter-intuitive observation of higher rates of cannabis use among the CHR population.

Other epidemiological risk factors associated with cannabis-psychosis risk include young age (especially adolescence), family history of psychosis, family history of affective liability, childhood trauma, urbanicity, affective dysregulation, and chronic, heavy use of high-potency cannabis.

In the Finnish birth cohort (comprising 6552 individuals) (Denissoff et al. 2022), 3 distinct trajectories of cannabis use were identified during adolescence. Of the cannabis use trajectories (i.e. early-onset persistent, late-onset, and experimental), those with

early-onset persistent use, had the highest risk for psychosis-like experiences (OR = 3.2, 95 % CI: 2.4–4.3).

Adolescence has been recognized as a period of increased vulnerability to psychosis-like experiences with cannabis use (McDonald et al, 2024; Hjorthoj et al, 2023; Mustonen et al, 2018). McDonald et al, 2024 found that cannabis use was associated with over 11 times (95% CI 4.6–27.3) greater risk of psychotic disorder at any point during adolescence (ages 12–19 years), compared to no cannabis use.

The quantity and type of cannabis has also been shown to impact psychosis risk. Regular use of high-potency cannabis (tetrahydrocannabinol/ THC >10 %) was associated with significantly higher rates of incident psychosis-like experiences (adjusted OR = 2.8, 95 % CI: 1.9–4.1) compared to users of traditional cannabis (Hines et al, 2024). Similar increased risk with higher potency cannabis has also been shown in the EU-GEI study (DiForti et al, 2019).

Mood dysregulation, in terms of anxiety and depression have also been identified as risk factors for psychosis related to cannabis use. Bernusky et al, 2024, found that anxiety symptoms significantly mediated the relationship between cannabis use and psychosis-like experiences (indirect effect = 0.15, 95 % CI: 0.08–0.23), particularly in frequent users. Notably, this mediation effect was consistent across sexes. In another study, focused on depressive symptoms in a sample of 204 women (Branas et al, 2017), depressive symptoms significantly moderated the relationship between stress exposure and intensity of psychosis-like experiences (interaction effect: $\beta = 0.41$, $p < 0.001$).

Biological factors associated with cannabis-psychosis risk:

Biological vulnerability of cannabis-psychosis risk has been identified in terms of genetic factors and, sensitivity of dopaminergic, GABAergic and endocannabinoid systems.

While early genetic studies identified polymorphisms of the catechol-O-methyltransferase (COMT) and AKT1 genes as a risk factor (Henquet et al, 2006; Morgan et al, 2016), these have not been robustly replicated.

A mendelian randomization analysis of a genome-wide association study for lifetime cannabis use, found a causal positive influence of schizophrenia risk on cannabis use (Passman et al, 2018).

A twin and sibling study (N = 4830) found that shared genetic factors explained 69.2 %–84.1 % of the association between cannabis use and psychosis-like experiences

and frequent and problematic cannabis use also contributed independently to psychosis-like experiences (Karcher et al, 2019).

PRS-Sz has been consistently associated with varying levels of cannabis use across numerous cohorts (Carey et al, 2016; Guloksuz et al, 2019; Jones et al, 2020). These studies suggests that the relationship between PRS-Sz and cannabis use may represent a pathway from genetic risk for schizophrenia to cannabis use (Jones et al, 2020), or that PRS-Sz moderates the cannabis-psychosis risk (Guloksuz et al, 2019). However, other studies have failed to find an association between PRS-Sz and cannabis use disorder, suggesting that the association between cannabis use and schizophrenia is not explained by common genetic risk (Hjorthøj et al, 2021).

A recent study from the All of Us Research Program using multimodal genetic and phenotypic data, examined associations between polygenic scores for cannabis use disorder (PRS-CUD) and schizophrenia (SZ) with both heavy cannabis use and SZ diagnosis. Results showed that CUD and SZ PRS were independently associated with heavy cannabis use (with a modest SZ PRS effect) and with SZ status, including a significant CUD PRS effect even among individuals without documented cannabis use, indicating widespread genetic pleiotropy (Trotta et al, 2026).

A cross-disorder GWAS meta-analysis of schizophrenia and bipolar I disorder identified 553 independent genome-wide significant loci for combined psychosis liability, including 122 novel associations. Mendelian randomization supported bidirectional causal effects between psychosis and cannabis use disorder (CUD), with MR-clustering revealing three distinct pathway clusters linking CUD to psychosis (intracellular signaling, synaptic function, and neuronal development). Exploratory analyses further showed that glutamate pathway-specific polygenic score consistently predicted psychosis status in the EU-GEI first-episode sample, including among both cannabis users and nonusers (Austin-Zimmerman et al, 2026). The recent and best-powered genome-wide association study (GWAS) of cannabis use disorder (CUD) showed that the association between cannabis use disorder and schizophrenia was bidirectional using Mendelian randomization. The recent and best-powered genome-wide association study (GWAS) of cannabis use disorder (CUD) showed that the association between cannabis use disorder and schizophrenia was bidirectional using Mendelian randomization (Levey et al, 2023).

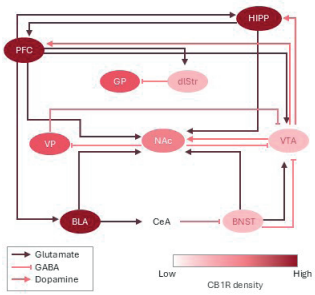
A key limitation is the still relatively low-to-moderate statistical power of the available CUD and lifetime cannabis use GWAS compared with the much larger SZ GWAS, which may have reduced the ability to detect weaker genetic associations or more subtle shared pathways.

Of note, lifetime cannabis use (lCU) and cannabis use disorder (CUD) represent distinct but partially overlapping phenotypes with different genetic architectures, which has important implications for interpreting associations with schizophrenia (SZ) or psychosis.

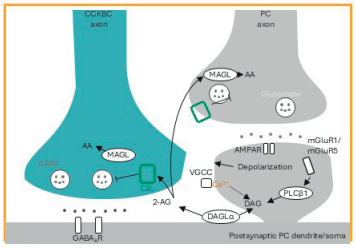
Lifetime cannabis use (lCU) is typically a binary measure of ever having used cannabis and is strongly shaped by environmental factors, whereas cannabis use disorder (CUD) is a clinical diagnosis of problematic, dependent use that is less common and more severe. Although the two phenotypes share moderate genetic correlation ($r_g \approx 0.50$–0.58) (Johnson et al, 2020, 2021), their genetic architectures are partially distinct, with lCU GWAS highlighting loci linked to risk-taking and showing only modest overlap with schizophrenia ($r_g \approx 0.25$) (Pasman et al, 2018; Johnson et al, 2021), while CUD GWAS display stronger genetic correlations with psychopathology including schizophrenia ($r_g \approx 0.35$–0.37) (Johnson et al, 2021) and different associations with educational and physical-health traits. Consequently, Mendelian-randomization and polygenic-score findings diverge: lCU primarily supports a causal effect of schizophrenia liability on cannabis initiation, whereas CUD more consistently indicates bidirectional causality with a clearer signal from CUD toward psychosis risk; thus, using CUD better captures shared psychiatric liability and clinically relevant pathways, while relying on lCU can attenuate signals of true comorbidity and lead to incomplete inferences about the cannabis–schizophrenia relationship.

Another strategy that has been used to identify biological risk factors for cannabis-psychosis risk is to examine the effects of cannabis (or it's psychoactive ingredient delta-9 tetrahydrocannabinol (THC) on biological abnormalities seen in schizophrenia as a way to explore whether the psychosis-like effects of cannabis are exaggerated. For example, since dopaminergic abnormalities such as increased presynaptic dopamine has been seen in schizophrenia, studies have shown that THC also increases presynaptic dopamine (Bossong et al, 2015, Stokes et al, 2010).

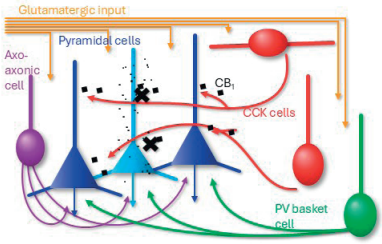
Since abnormalities in GABAergic system such as reduced GAD-67 protein level has been demonstrated in schizophrenia, one hypothesis is that GABA-deficit may contribute to increased risk of psychosis-like experiences with cannabis use. The figure (below) depicts CB1 receptor density and GABA-cannabinoid interaction. **Chapter 3** examines this hypothesis using an experimental, laboratory challenge-paradigm in healthy individuals. Using Iomazenil, a GABA inverse-agonist, I showed that pharmacologically-induced GABA deficit increased the psychosis-like effects of delta-9 tetrahyrocannabinol (THC), the main psychoactive ingredient in cannabis. This challenge paradigm provides the opportunity to test cannabis-psychosis risk at the individual participant level.



A. Expression of CB_1 receptors within the reward circuit. HIPP, hippocampus; PFC, prefrontal cortex; GP, globus pallidus; dlStr, dorsolateral striatum; VP, ventral pallidum; NAc, nucleus accumbens; VTA, ventral tegmental area; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; BNST, bed nucleus of the stria terminalis.



B. Pathways leading to CB_1 receptor-mediated suppression of GABA release from CCKBCs. Phasic inhibition produces eCB-mediated short-term depression (eCB-STD), which can be calcium-driven (including Depolarization induced Suppression of Inhibition DSI), receptor-driven ($G_{q/11}$-PLCβ1), or synaptically driven; each route leads to 2-AG production and presynaptic CB_1 activation.



C. Pyramidal cells receive GABAergic input from PV-expressing basket cells (green), axo-axonic cells (purple), and several types of CCK-expressing cell (red).

Adapted from:
1) Malhotra S, Donneger F, Farrell JS, Dudok B, Losonczy A, Soltesz I. Integrating endocannabinoid signaling, CCK interneurons, and hippocampal circuit dynamics in behaving animals. Neuron, 2025; 113, 1862-1885. PMID:40267911 PMCID:PMC12410895 2) Klausberger T, Marton LF, O'Neill J, Huck JH, Dalezios Y, Fuentealba P, Suen WY, Papp E, Kaneko T, Watanabe M, Csicsvari J, Somogyi P. Complementary roles of cholecystokinin- and parvalbumin-expressing GABAergic neurons in hippocampal network oscillations. J Neurosci. 2005 Oct 19;25(42):9782-93. PMID:16237182; PMCID:PMC6725722.

While most of the literature on identifying cannabis-psychosis risk factors have focused on the positive psychosis-like experiences, it is noteworthy that cognitive deficits are thought to be the earliest and most significant change in schizophrenia. Cannabis (and THC) has also found to have a significant effect on verbal memory (Ranganathan et al, 2006). In **Chapter 4**, I examined whether THC impairs verbal memory similar to that in schizophrenia and may hence serve as a laboratory challenge paradigm that identifies risk. I found that THC impairs encoding of information but not retrieval, at variance to that seen in schizophrenia whether both encoding and retrieval are impaired.

Since the effects of THC are mediated via the cannabinoid CB1 receptor, in **Chapter 5**, I examined the effects of age, sex and body-mass index on CB1 receptor availability. CB1 receptors were found to be higher at younger age, providing a biological mechanism that explains why adolescents may be more vulnerable to the effects of cannabis. While the published chapter refers to "gender", this is more accurately about sex as a biological variable rather than gender as a social construct. The sample-size for this analysis is also relatively modest and these findings need to be interpreted within this context.

Both schizophrenia and cannabis use have been associated with brain changes including reduction in gray matter volume and decreased cortical thickness (French et al, 2015; Frissen et al, 2018; Penzel et al, 2021). Given these effects of cannabis on brain structure, I examined the effects of cannabis on brain synaptic density using [11C]UCB-J PET imaging in **Chapter 6**. I found that chronic, heavy cannabis use was associated with lower hippocampal synaptic density and this correlated with lower verbal memory performance. Lower hippocampal synaptic density is also seen in schizophrenia. This brain region may hence serve as a point of convergence of cannabis-psychosis risk.

These results should also be interpreted as preliminary given the small sample-size of this study.

Interaction between environmental risk factors and biological factors in the development of cannabis-related psychosis:

While individual environmental and biological risk factors have been identified as noted earlier, the interaction between these factors in moderating or mediating cannabis-psychosis risk is less well understood.

In the EU-GEI cohort, examining the interaction between polygenic and polyenvironmental risk factors (Rodriguez V et al, 2024), PRS-SZ was the strongest genetic predictor for schizophrenia-spectrum, having a nominally larger effect in those not exposed to strong environmental factors such as frequent cannabis use (unexposed vs exposed OR 2.43 and 1.35, respectively) and childhood adversity (3.04 vs 1.74). There was no evidence of interaction between PRS and polyenvironmental score.

In **Chapter 7**, I examined the interaction between environmental risk factors and familial affective liability in influencing cannabis-psychosis risk in the NEMESIS cohort. I found that childhood adversity and cannabis use, and to a lesser extent urbanicity, displayed greater-than-additive risk if there was also evidence of familial affective liability; and the risk was progressively greater across increasing levels of psychosis severity (none, attenuated psychosis, clinical psychosis) complicating affective dysregulation.

Is the relationship between cannabis use and psychosis causal?

An important question that arises is whether the association between cannabis use and psychosis is causal. Sir Bradford Hill, the English epidemiologist, conceptualized criteria for deciding whether a causal relationship is present between risk factors and a disease state. The criteria include temporal relationship, biological gradient, biological plausibility, coherence, consistency, and experimental evidence. Cannabis fulfills many but not all the criteria for causality and has been considered a component cause for psychosis (Radhakrishnan et al, 2014).

Genetic studies using mendelian randomization techniques have yielded mixed findings with studies showing that the association between cannabis use and psychosis is causal (Vaucher et al, 2018), is bidirectional (Passman et al, 2018) or that the evidence for a causal relationship is mixed (Johnson et al, 2021).

In **Chapter 8** I examined the mediational relationship between cannabis use, anxiety/depressive symptoms and psychotic experience, and showed that there is a bidirectional relationship between cannabis use, anxiety/depressive symptoms and psychotic experiences.

Thesis Outline

The overall aim of this thesis was to investigate the association between cannabis use and psychosis. In order to understand this complex association, this broad topic was examined using several strategies, ranging from epidemiological studies to laboratory challenge paradigm and PET imaging.

Chapter 2 reviews the literature on cannabis-psychosis risk syndrome, including concepts of schizotypy and clinical-high risk (CHR) and their association with cannabis use. The review identified that while cannabis use increases the risk of conversion to psychosis in schizotypy and CHR, it also resulted in lower negative schizotypy, lower social anhedonia, and better social functioning.

In **Chapter 3,** I examined whether cannabis-psychosis risk can be identified by experimental, laboratory challenge-studies. Using Iomazenil, a GABA inverse-agonist, I showed that pharmacologically-induced GABA deficit increased the psychosis-like effects of delta-9 tetrahyrocannabinol (THC), the main psychoactive ingredient in cannabis. This challenge paradigm offers the opportunity to test cannabis-psychosis risk at the individual participant level.

While cannabis-psychosis risk has predominantly been defined in terms of positive psychotic symptoms, cognitive deficits, especially those related to verbal memory have been shown to be significantly impacted in schizophrenia.

In **Chapter 4**, I examined whether THC impairs verbal memory similar to that in schizophrenia and may hence serve as a laboratory challenge paradigm that identifies risk. I found that THC impairs encoding of information but not retrieval, at variance to that seen in schizophrenia.

Since the effects of THC are mediated via the cannabinoid CB1 receptor, in **Chapter 5**, I examined the effects of age, sex and body-mass index on CB1 receptor availability. CB1 receptors were found to be higher at younger age, providing a biological mechanism that explains why adolescents may be more vulnerable to the effects of cannabis.

Given the cognitive effects of cannabis, I examined the effects of cannabis on brain synaptic density using [11C]UCB-J PET imaging in **Chapter 6**. I found that chronic, heavy cannabis use was associated with lower hippocampal synaptic density and this correlated with lower verbal memory performance. Given the presence of lower hippocampal synaptic density in schizophrenia as well, this brain region may hence serve as a point of convergence of cannabis-psychosis risk.

In **Chapter 7**, I examined the interaction between environmental risk factors and familial affective liability in influencing cannabis-psychosis risk in the NEMESIS cohort. I found that childhood adversity and cannabis use, and to a lesser extent urbanicity, dis- played greater-than-additive risk if there was also evidence of familial affective liability; and the risk was progressively greater across increasing levels of psychosis severity (none, attenuated psychosis, clinical psychosis) complicating affective dysregulation.

Finally, in **Chapter 8** I examined the mediational relationship between cannabis use, anxiety/depressive symptoms and psychotic experience, and showed that there is a bidirectional relationship between cannabis use, anxiety/depressive symptoms and psychotic experiences. However, the contribution of anxiety/depressive symptoms as a mediator was greater than that of cannabis.

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Chapter 2

Cannabis and Psychosis Proneness

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Abstract

Cannabis use is associated with the development of psychosis in multiple studies. With increasing cannabis use worldwide, it is important to identify individuals who are at risk of developing psychosis following cannabis use. The concept of psychosis proneness has evolved over the past century from schizotypy, to denote people at clinical high risk for psychosis (CHR), those at genetic risk of schizophrenia (defined by polygenic risk score for schizophrenia) and those who experience attenuated psychotic symptoms in the general population. Across these definitions of psychosis proneness, cannabis use has been shown to increase the risk of psychosis expression. General population samples show that cannabis interacts with other environmental risk factors, such as childhood trauma and urbanicity, as well as with genetic liability in an additive manner, such that the risk of psychosis expression is greater with increasing number of risk factors. The role of affective dysregulation in increasing the risk of psychosis expression is also recognized. While biological and psychological studies suggest the role of striatal dopamine release, reasoning bias, impairment in face processing and working memory, the precise biological underpinnings of the effects of cannabis use on psychosis proneness remain to be elucidated.

Introduction

Cannabis is one of the most commonly used drugs worldwide (2021 World Drug report). With increase in legalization of medical and recreational use of cannabis, and decrease in the perception of harm, adolescents and young adults are more likely to use cannabis. Converging lines of evidence from epidemiological studies (Marconi et al., 2016; Linscott and van Os, 2013; Di Forti et al., 2019), human laboratory studies (Hindley et al., 2020; Ganesh et al., 2020), and genetic studies (Johnson et al., 2021; Quattrone et al., 2021) suggest that cannabis use is a risk factor for the later development of psychosis (Radhakrishnan, 2012; Radhakrishnan et al., 2014). Hence, there is a pressing need to identify individuals who are at risk of developing psychosis following cannabis use.

The concept of psychosis proneness has undergone many transformations since its initial conceptualization in the early 20th century. These include early taxonomic concepts such as schizotypy (Rado), schizotaxia (Meehl), and dimensional constructs such as psychoticism (Eysenck) and the fully dimensional model of Claridge. These made way for the concepts of endophenotype, familial high-risk for schizophrenia, prodromal states, basic symptoms, clinical high-risk for psychosis (CHR)/ultra high-risk for psychosis (UHR) and attenuated psychosis syndrome. These conceptualizations of psychosis proneness are based on the idea that psychotic disorders such as schizophrenia emerge on the backdrop of less intense symptoms resembling psychosis. The presence of less intense symptoms resembling psychosis may hence identify a group that is at increased risk i.e psychosis proneness. Of note, these conceptualization have focused largely on the positive symptoms of psychosis. With the advent of large, genome-wide association studies (GWAS) in schizophrenia, psychosis proneness has been defined in terms of genetic risk independent of the presence of less intense symptoms resembling psychosis i.e. highest tertile of polygenic risk score for schizophrenia. More recent epidemiological studies have also identified symptom clusters that increase the risk for psychotic experiences in the general population.

While there are conceptual differences in how psychosis proneness has been defined over the years, research on the effects of cannabis on different constructs of psychosis proneness point to some consistent threads of evidence. In this chapter, we focus primarily on the effects of cannabis on symptom based conceptualizations of psychosis-proneness such as schizotypy, CHR/UHR, and subclinical psychotic symptoms in the general population.

Psychosis Proneness: Historical Perspective

The concept of psychosis proneness has its roots in early efforts to identify individuals who are at risk of developing schizophrenia. The initial observation that psychiatric disorders ran in families, resulted in longitudinal follow-up of children of people diagnosed with schizophrenia to identify those who are at increased risk of developing the disorder (Pearson and Kley, 1957). Concurrently, cross-sectional studies also noted the presence of schizophrenia-like traits (eg. social isolation, suspiciousness, irritability, eccentricity) among their family members, as observed by Bleuler (1911), Kraepelin (1919) and others. These cross-sectional studies were referred to as investigations of psychosis-proneness (Claridge, 1994)

Enshrined in a psychodynamic framework, Rado introduced the term "schizotype" (schizophrenic phenotype) to denote an ensemble of psychodynamic traits (i.e. anhedonia, emotional imbalance and interpersonal impairment) that are present throughout life and undergo "a sequence of schizotypal changes" with time; this became known as schizotypal personality organization (Rado, 1953). Meehl expanded on this concept to define four core features (thought disorder; associative dyscontrol or "cognitive slippage"; interpersonal aversiveness; anhedonia; and ambivalence). He hypothesized that was the result of a genetically determined neurophysiological "neural integrative defect", called schizotaxia (Meehl, 1962). It was noted that only a small proportion of people with schizotypy develop schizophrenia and this allowed for interaction with environmental risk factors.

This concept, i.e. that there was a continuity of traits even in non-clinical populations in the absence of symptoms of schizophrenia, was further refined by Eysenck and Claridge (Claridge, 1994; Eysenck, 1992). During a period when the Kraepelin-Bleuler dichotomy of schizophrenia and bipolar disorder was challenged and the existence of a single unitary psychosis was proposed, Eysenck suggested that if schizotypy existed as a dimension that resulted in schizophrenia at one extreme, there ought to be another entity that resulted in bipolar disorder since "..we cannot have a single dimension with 'psychosis' at both ends" (Eysenck, 1992). In order to resolve this, he proposed the existence of 3 personality dimensions: Psychoticism, Extraversion, and Neuroticism. According to Eysenck's model, psychotic disorders were considered as extreme points along quantitative dimensions (ie, extreme values of Psychoticism combined with individual expressions of varying values of Extraversion and Neuroticism) resulting in different clinical syndromes eg. schizoid personality, schizotypy and schizophrenia (Eysenck, 1992). "Psychoticism" was hence conceptualized as the underlying dimensional liability for all psychotic disorders, in keeping with the concept of Einheitspsychose (unitary psychosis).

Several scales were developed to measure schizotypy in non-clinical populations, including the Chapman scales, Schizotypal Personality Questionnaire (SPQ) (Raine, 1991), Schizotypal Trait Questionnaire (STQ) (Claridge and Broks, 1984) Oxford–Liverpool Inventory of Feelings and Experiences (O-LIFE) (Mason and Claridge, 2006), Structured Interview for Schizotypy (SIS) (Kendler et al., 1989) or specific schizotypal dimensions: positive (e.g. Magical Ideation (Eckblad and Chapman, 1983), Perceptual Aberation Scale (scale by Chapman and Chapman, unpublished)) or negative (e.g. Scales for Physical Anhedonia and Social Anhedonia (Chapman et al., 1976)). Claridge proposed a fully dimensional model of schizotypy and schizophrenia based on factor analysis of the different scales developed to measure schizotypy. They identified a 3 factor model, comprising i) cognitive–perceptual or positive schizotypy dimension, ii) interpersonal deficit or negative schizotypy dimension, and iii) disorganization dimension; this appeared to recapitulate the factor structure of schizophrenia (Wuthrich and Bates, 2006; Raine, 2006). Eysenck's psychoticism scale appears to map onto a personality dimension more closely related to nonconformity and antisocial traits. The Community Assessment of Psychic Experiences (CAPE) (Stefanis et al., 2002) provides measures of positive and negative dimensions (as well as an affective-depressive dimension) in the general population.

Effect of cannabis use on schizotypy

Does schizotypy increase sensitivity to the psychotomimetic effects of cannabis?

Schizotypy did not moderate psychotomimetic effects of delta-9 tetrahydrocannabinol (THC) in a pooled analysis of 128 cannabis users who received THC in double-blind, randomized, placebo-controlled, crossover design laboratory challenge studies (Freeman et al., 2021). An online survey (n = 129, 64% women, predominantly students) who had used cannabis at least once in their lifetime, however, found that those with schizotypy (measured using O-LIFE scale) was the only significant predictor of unusual experiences and this did not correlate with frequency of cannabis use. Schizotypy was positively correlated with cognitive impairment and negatively correlated with age (Airey et al., 2020). A meta-analysis of 29 cross-sectional studies found that 'ever' using cannabis was associated with higher schizotypy scores with moderate effect-sizes (0.3-0.4 range) on domains of positive schizotypy and disorganization; and small effect-sizes (0.15-0.2 range) on the domain of negative schizotypy (Szoke et al., 2014).

Among those with high schizotypy, cannabis use was not associated with divergent thinking, a measure of creativity. However, cannabis use was associated with divergent thinking in the non-schizotypy group (Minor et al., 2014). In another study, cannabis

use increased verbal fluency in those with low divergent thinking to levels similar to those with high divergent thinking. Although the group with higher divergent thinking had significantly higher trait schizotypy, they did not show a change in verbal fluency with cannabis use (Schafer et al., 2012).

In an interesting study from the Netherlands and Flanders employed sibling-control and cross-sibling comparison among patients with a psychotic disorder (n = 1120), their siblings (n = 1057), and community controls (n = 590). Compared to controls, siblings displayed greater sensitivity to the effects of current cannabis use on positive schizotypy and negative schizotypy. The analyses suggested that familial risk increased sensitivity to cannabis (i.e. moderation) rather than familial risk increasing use of cannabis (mediation) (Genetic and Outcome in Psychosis, 2011).

Does cannabis use increase rates of schizotypy?

In a study on a non-clinical population comprising university students (n=910), higher frequency of cannabis use was associated with higher schizotypy as measured by the positive- and disorganization subscales of the SPQ. The SPQ subscales 'odd beliefs', 'ideas of reference', 'unusual perceptual experiences', 'odd speech', and total SPQ scores were found to be mediated by measures of aberrant salience (measured using the aberrant salience inventory (ASI))(O'Tuathaigh et al., 2020)

The effect of age on the relationship between cannabis use and schizotypy has been examined in a number of studies. In a study of 155 cannabis users, aged 15-24 years, who were assessed at 6 month intervals, younger age was associated with increased negative schizotypy over time among frequent cannabis users. Contrarily, younger age was associated with decreasing negative schizotypy over time among occasional cannabis users (Albertella et al., 2018).

Does cannabis impact the rate of conversion from schizotypy to schizophrenia?

Rates of transition from schizotypal disorder to schizophrenia has been estimated to be approximately 25%-50% (Albert et al., 2017; Hjorthoj et al., 2018; Nordentoft et al., 2006; Parnas et al., 2011). In the Danish OPUS trial, the rate of conversion to a psychotic disorder at 2-year follow-up was 25% for those randomized to integrated treatment vs. 48.3% for those who received standard treatment (Nordentoft et al., 2006). In the OPUS-II study, at 3.5-year follow-up, the rate of conversion from schizotypal disorder to schizophrenia was 32% (Albert et al., 2017). The Copenhagen Prodromal Study found that the rate of conversion from schizotypal personality disorder to schizophrenia was 25% at 5-year follow-up (Parnas et al., 2011). In a large Danish nationwide prospective cohort study including all individuals born between January 1,

1981, through August 10, 2014, with an incident diagnosis of schizotypal disorder and without a previous diagnosis of schizophrenia, 2539 participants with schizotypal disorder were identified (57.0% male) (Hjorthoj et al., 2018). The rate of conversion to schizophrenia was 16.3% (95% CI, 14.8%-17.8%) after 2 years, and 33.1% (95% CI, 29.3%-37.3%) after 20 years. Among those with cannabis use disorder, the rate of conversion at 20 years was 58.2% (95% CI, 44.8%-72.2%). Adjusting for covariates, cannabis use disorder, in addition to amphetamine-, and opioid-use disorder, indicated that the greater risk of conversion to schizophrenia remained significant (HR, 1.34; 95% CI, 1.11-1.63).

Effect of cannabis use on clinical-high risk for psychosis (CHR)/ ultra-high risk for psychosis (UHR)

Does CHR/UHR increase sensitivity to the psychosis-like effects of cannabis?

The question of whether CHR/UHR individuals show increased sensitivity to the psychosis-like effects of cannabis has not been well studied in experimental paradigms. CHR/UHR individuals who use cannabis, were found to experience transient increase in paranoia and anxiety, and decrease in neurocognitive performance following cannabis intoxication in a small laboratory study (Vadhan et al., 2017). This is consistent with the clinical finding that CHR/UHR individuals who use cannabis have higher rates of unusual thought content and suspiciousness than non-users (Carney et al., 2017).

Does cannabis use increase rates of CHR/UHR?

CHR/UHR has been shown to be associated with higher rates of current and lifetime cannabis use eg. 36.5% vs 11.1% in healthy controls in one study (Auther et al., 2012). In a meta-analysis of 30 studies UHR individuals (n = 4205 vs n = 667 controls) were found to have high rates of current (26.7%) and lifetime (52.8%) cannabis use, and cannabis-use disorder (CUD) (12.8%); and increased odds of lifetime cannabis use (OR=2.09) and CUD (OR=5.49) compared to controls (Carney et al., 2017). UHR cannabis users were observed to have had higher rates of unusual thought content and suspiciousness than non-users. Intriguingly, CHR with a lifetime history of cannabis use have been found to have less social anhedonia and better social functioning compared to CHR who did not use cannabis (Auther et al., 2012).

Does cannabis impact the rate of conversion from CHR/UHR to schizophrenia?

Studies suggest that current cannabis abuse/dependence, but not lifetime cannabis use, is associated with higher risk of transition to psychosis. For example, in a study of 182

CHR individuals, risk factors for transition to psychosis included cannabis use before age 15 years and frequent use of cannabis. However, within the whole sample, current cannabis users were not more likely to develop psychosis than those who had never used cannabis. Of note in this study was that although lifetime prevalence of cannabis use was 73.6%, the majority of individuals (n=98, 73.1%) had stopped using cannabis before clinical presentation (n=98, 73.1%) (Valmaggia et al., 2014). In the North American Prodrome Longitudinal Study Phase 1 project, CHR individuals (n=340) were followed up for > 2 years. In order to examine the relationship between cannabis use and risk of transition to psychosis, individuals were categorized in to three groups: No Use (n= 211); Cannabis Use without impairment (n = 63); Cannabis Abuse/Dependence (n = 67). The cannabis abuse/dependence group had higher rates of conversion to psychosis than those without any use and those with cannabis use without impairment. Adjusting for alcohol use weakened this relationship (HR = 1.875, CI 0.963–3.651, P = 0.064). While the authors interpreted this finding as alcohol use confounding the association between cannabis use and risk to transition, it is also possible that a true relationship exists but that the study was underpowered to adjust for multiple confounders (Auther et al., 2015). Participants (N = 283) were followed for ≥2 years to determine psychosis conversion.

The risk of transition to psychosis may be greater among those who experience cannabis-induced attenuated psychotic symptoms. In a study of 190 UHR individuals (76 males), those who reported a history of cannabis-induced attenuated psychotic symptoms (APS) were found to be at 4.90 (95% CI 1.93-12.44) times greater risk of transition to a psychotic disorder at mean 5-year follow-up. Greater severity of cannabis use was also found to increase the risk of transition to psychosis. However, this relationship was mediated by greater severity of cannabis use among those with a history of cannabis-induced APS (McHugh et al., 2017).

Kraan and colleagues conducted a meta-analysis of seven studies published between 1996 to 2015, identifying seven prospective studies reporting on lifetime cannabis use in UHR subjects (n = 1171); and five that also examined current cannabis abuse or dependence. While lifetime cannabis use was not significantly associated with transition to psychosis, current cannabis abuse or dependence was associated with higher odds of transition to psychosis 1.75 (95% CI 1.14–2.71) (Kraan et al., 2016).

Effect of cannabis use on psychosis expression in general population samples

As outlined above, there is increasing recognition that psychosis exists as a continuum in the general population, with approximately 5-15% of the population self- or interview-based reporting one or more symptoms of psychosis (van Os et al., 2000; Johns et al., 2004). The ability to measure psychosis expression in the general population paved the

way for a deeper understanding of the factors that lead to the evolution and persistence of psychosis. General population samples are also of larger sample size and provide an opportunity to examine the interactive role of environmental risk factors.

Longitudinal studies such as The Netherlands Mental Health Survey and Incidence Study (NEMESIS) and the Christchurch Health and Development Study (CHDS) (in New Zealand) have consistently shown that cannabis use is associated with increased rates of psychosis expression (Fergusson et al., 2003; Verdoux et al., 2003). In the first wave of the NEMESIS longitudinal study (1997-1999), baseline cannabis use predicted the presence of psychotic symptoms (adjusted odds ratio (OR) = 2.76, 95% CI= 1.18 - 6.47), the severity of psychotic symptoms (OR = 24.17, 95% CI=5.44 -107.46), and the need for treatment for psychotic symptoms as assessed by a clinician (OR = 12.01, 95% CI=2.24 - 64.34), at follow-up (van Os et al., 2002). The CHDS is a longitudinal birth cohort comprising 1,265 children followed-up from birth to age 21. Those with cannabis dependence (DSM-IV criteria) had significantly higher rates of psychotic symptoms at age 18 years (rate ratio = 3.7; 95% CI =2.8-5) and 21 years (rate ratio = 2.3; 95% CI= 1.7-3.2). This relationship was consistent even after adjusting for confounders (Fergusson et al., 2003).

The picture that emerges from general population samples suggests that cannabis use interacts with other environmental risk factors such as childhood trauma and urbanicity; and genetic risk to lead to the expression of psychosis. There is evidence to suggest that the contribution of these risk factors is additive i.e. the greater the number of risk factors, the greater is the odds of psychosis expression (Pries et al., 2018).

Emerging evidence also suggests the role of affective dysregulation in contributing to the risk of psychosis emerging from cannabis use and other environmental risk factors- the so-called "affective pathway to psychosis" (Myin-Germeys et al., 2003). In data from three waves of the second Netherlands Mental Health Survey and Incidence Study (NEMESIS-2), cannabis use and childhood trauma, and to a lesser extent urbanicity, showed greater-than-additive risk when there was a family history of affective dysregulation. In addition, the interaction contrast ratio grew progressively greater across severity levels of psychosis (none, attenuated psychosis, clinical psychosis) in the presence of affective dysregulation (Radhakrishnan et al., 2019). Even when other symptom dimensions are included in the analysis, after controlling for age, sex, and education, only affective dysregulation was found to be a significant moderator of psychosis expression (Pries et al., 2018). The role of affective dysregulation was also noted in an experience sampling study in a general population sample of 514 women. This study examined the moment-to-moment dynamics between negative affect and paranoia in daily life. Using time-lagged analysis, the study found that moments of increased negative affect resulted in a significant increase in paranoia over the subsequent 180 minutes, and psychotic

symptoms at follow-up. The transfer from negative effect to paranoia was moderated by stress-sensitivity, while persistence of paranoia was moderated by depressive symptoms (Myin-Germeys et al., 2003).

More recently, Guloksuz and colleagues (Guloksuz et al., 2019), examined the independent and interactive effects of cannabis use and genetic risk for schizophrenia (captured by polygenic risk score for schizophrenia (PRS-SCZ)) in data from the European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EUGEI) and the Genetic Risk and Outcome of Psychosis (GROUP) study within the EUGEI. Using PRS-SCZ defined by the 75th percentile (PRS-SCZ75) in Caucasian patients with schizophrenia (n= 1,699) and healthy, unrelated controls (n=1,542), they compared the risk of schizophrenia among regular cannabis users i.e those who used cannabis once or more per week. The analysis revealed that while both regular cannabis use (OR=3.96, 95% CI=3.16-4.97) and genetic risk (PRS-SCZ75) (OR=2.85, 95% CI=2.43-3.35) independently increased the risk of schizophrenia, there was also an additive interaction between PRS-SCZ75 and regular cannabis use (relative excess risk due to interaction (RERI)=5.60; 95% CI: 0.88-10.33; p=0.020).

Is the relationship between cannabis use and psychosis expression "causal"?

Whether cannabis is causally related to psychosis expression has been widely debated in the field (see Chapter 17). In a study using the Swedish national registry database, cannabis use was strongly associated with later schizophrenia (OR=10.44, 95% CI= 8.99-12.11). While this association was reduced when controlling for increasing degrees of familial confounding, it continued to be significant (Giordano et al., 2015). Mendelian randomization studies, a type of instrumental variable analysis where single-nucleotide polymorphisms (SNPs) are used to enable randomization to experimental and control conditions, have been performed to interrogate the question of causality. While one study found that genetic liability for lifetime cannabis use was associated with a significant 37% increase in the risk of schizophrenia (Vaucher et al., 2018), two other studies found that genetic liability for schizophrenia was associated with increased risk of lifetime cannabis use (Gage et al., 2017; Pasman et al., 2018), suggesting that the relationship is bidirectional. Of note, these studies relied on lifetime cannabis use and not the frequency/severity of cannabis use.

Linkage disequilibrium score regression (LDSR) and polygenic risk scores (PRSs) studies have also demonstrated significant genome-wide genetic correlation between lifetime cannabis use/misuse and schizophrenia (genetic r range, 0.22-0.25) (Verweij et al., 2017; Pasman et al., 2018), and that high PRSs for schizophrenia significantly predicted greater cannabis use (Verweij et al., 2017), respectively.

van Os et al examined the question of causality in a within-subject study - which obviates confounded by genetic risk - from two general population cohorts (Early Developmental Stages of Psychopathology Study, n = 1395; Netherlands Mental Health Survey and Incidence Study-2, n = 6603). In an adjusted fixed-effects model, prior cannabis use was associated with psychotic experiences (adjusted OR = 7.03, 95% CI= 2.39 - 20.69), whereas prior psychotic experiences were not associated with cannabis use (adjusted OR = 0.59, 95% CI= 0.21 -1.71). This study suggests that the relationship between cannabis use and subsequent psychosis expression is likely causal (van Os et al., 2021).

The TRacking Adolescents' Individual Lives Survey (TRAILS) study, a large prospective population study of Dutch adolescents (n=2120), examined the direction of association between vulnerability for psychosis and cannabis use throughout adolescence using cross-lagged path analysis, while controlling for sex, family psychopathology, alcohol use and tobacco use. Cannabis use in the past year was documented according to self-report at all three waves of the study. The study found significant associations (r = 0.12-0.23) between psychosis vulnerability and cannabis use. Cannabis use at age 16 predicted psychosis vulnerability at age 19. Furthermore, psychosis vulnerability at ages 13 and 16 predicted cannabis use at ages 16 and 19year, respectively. This study suggests that the association between cannabis use and psychosis is bidirectional causal (Griffith-Lendering et al., 2013). This conclusion of bidirectionality gains general support from the literature.

Biological Mechanisms underlying the effects of cannabis on psychosis proneness

The precise biological mechanisms underlying the effects of cannabis use on psychosis proneness remain to be elucidated (see Chapters 10, 16). A small study that included relatives of patients with schizophrenia found an increase in striatal dopamine release in schizophrenia patients and their relatives following administration of delta-9-tetrahydrocannabinol, the main psychoactive ingredient in cannabis (Kuepper et al., 2013). This suggested that striatal dopamine release following exposure to cannabis may reflect an underlying genetic risk for psychosis. Other studies have suggested that constructs such as aberrant salience (O'Tuathaigh et al., 2020), jumping-to-conclusions and deficits in working memory (Reininghaus et al., 2019) may underlie the effects of cannabis on psychosis expression. Interestingly, cannabis use and schizotypy have been shown to be associated with abnormalities in face processing. In a study that measured evoked response (ERPs) during a visual face perception task among 97 individuals, those with high cannabis use and high schizotypy had significantly reduced N170 ERPs compared

to the control group. Cannabis use (measured using Cannabis Experience Questionnaire (CEQ)) was also found to be correlated with schizotypy scores (measured using SPQ) (Brooks and Brenner, 2018). Recently, people with cannabis use disorder (D'Souza et al., 2021) and schizophrenia (Radhakrishnan et al., 2021) have also been shown to have lower brain synaptic density, consistent with the finding of the deficits in information processing.

Conclusions

Although the definition of psychosis proneness has undergone change over time, the association with cannabis use and psychosis has been consistent across definitions i.e. schizotypy, CHR/UHR, and genetic high risk. Psychosis proneness is also associated with higher rates of cannabis use. Studies from general population samples show that cannabis use interacts with other environmental and genetic risk factors in an additive fashion to increase the risk of psychosis expression and that affective dysregulation plays an important role in mediating this risk. Whether the association between cannabis use and psychosis is causal remains debated, with studies suggesting that the relationship may be bidirectional with genetic risk for psychosis also increasing the risk of cannabis use. Finally, the biological mechanisms underlying the association between cannabis use and psychosis remain to be elucidated.

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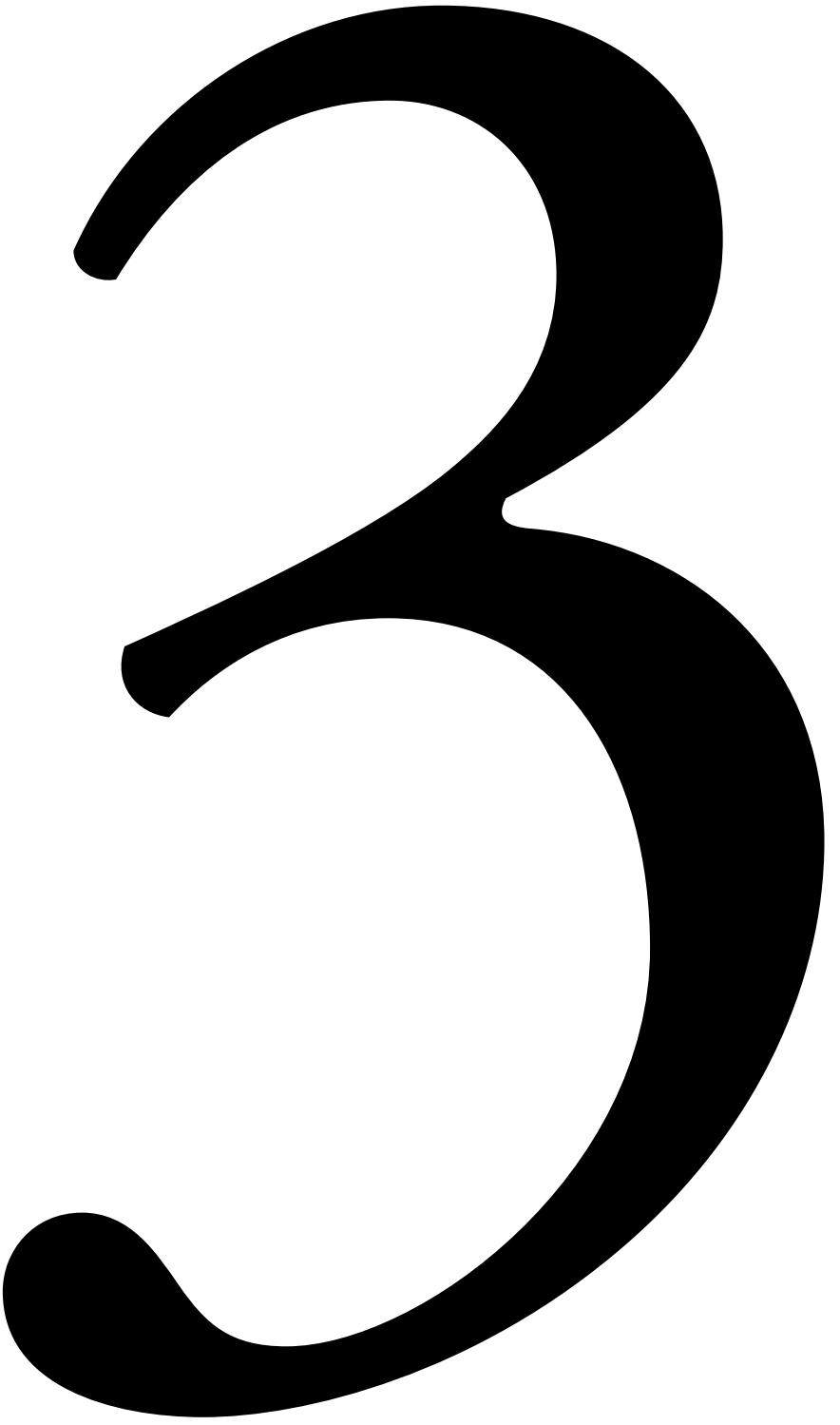

Chapter 3

GABA Deficits Enhance the Psychotomimetic Effects of Δ^9-THC

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Abstract

Background: Cannabis and Δ9-tetrahydrocannabinol (THC) can produce psychosis-like symptoms, yet individuals differ in their vulnerability to these effects. Because γ-aminobutyric acid (GABA) dysfunction is implicated in schizophrenia and interacts with the endocannabinoid system, this study examined whether reducing GABAergic function would enhance the psychotomimetic effects of THC in healthy adults.

Methods: In this randomized, double-blind, placebo-controlled, counterbalanced, within-subjects study, 27 male volunteers completed up to four test sessions involving intravenous iomazenil (3.7 μg/kg), used to induce a net reduction in GABA-A receptor function, and intravenous THC (0.015 mg/kg), administered alone and in combination, or placebos. Psychosis-relevant symptoms were assessed with the Positive and Negative Syndrome Scale (PANSS); dissociative and perceptual alterations with the Clinician-Administered Dissociative States Scale (CADSS); and anxiety and intoxication with visual analogue scales. Auditory event-related potentials, including P300 and N100 components, were measured using electroencephalography. Twenty-one participants completed all four sessions, and EEG data were available for 23 participants.

Results: Iomazenil pretreatment significantly enhanced the effects of THC. Compared with THC alone, the combination produced greater increases in total PANSS scores and anxiety ratings; perceptual alterations on the patient-rated CADSS were also significantly increased relative to placebo. Clinician-rated dissociative symptoms increased significantly under the combined condition relative to placebo. The combination additionally reduced target P300b amplitude, an effect not observed with either drug alone. Iomazenil did not significantly alter plasma THC or THC-COOH concentrations, and the combination did not increase subjective "high" ratings beyond THC alone, supporting a pharmacodynamic rather than pharmacokinetic interaction.

Conclusions: These findings indicate that reduced GABAergic function can amplify THC-induced psychosis-relevant, perceptual, affective, and electrophysiological effects. They support a role for GABA deficits in individual vulnerability to cannabinoid effects and may help explain heightened cannabis sensitivity in schizophrenia. Interpretation is limited by the small, all-male sample and intravenous THC administration; replication in larger, more diverse samples is warranted.

Introduction

Emerging evidence supports a number of associations between cannabis and psychosis/psychotic disorders, including schizophrenia. Acute exposure to both cannabis and synthetic cannabinoids (CBs) (including Spice and K2) can produce a range of transient psychotomimetic symptoms, cognitive deficits, and psychophysiological abnormalities that bear a striking resemblance to symptoms of schizophrenia (D'Souza et al, 2004; Radhakrishnan et al, 2014). Furthermore, epidemiologic studies suggest that early and heavy exposure to cannabis confers a higher risk for developing a psychotic disorder such as schizophrenia (Moore et al, 2007). However, only a minority of individuals exposed to CBs appear to be vulnerable to CB-related acute or persistent psychosis outcomes.

In individuals with schizophrenia, CBs have been shown to transiently exacerbate symptoms (D'Souza et al, 2005), trigger relapse, and have negative consequences on the course of the illness (Linszen and van Amelsvoort, 2007). A number of lines of evidence suggest that schizophrenia patients are more vulnerable to some of the effects of CBs. In an experience sampling study, schizophrenia patients were more sensitive to the psychosis-inducing effects of cannabis than controls (Henquet et al, 2010). Epidemiologic studies also show that cannabis use is associated with greater negative consequences on the course and expression of schizophrenia (van Os et al, 2002). In an experimental study, despite receiving stable doses of antipsychotic medications and being clinically stable, 80% of schizophrenic patients, but only 25% of controls, experienced clinically significant psychosis (43 points on the Positive and Negative Syndrome Scale (PANSS) positive subscale) with a low dose of delta-9-tetrahydrocannabinol (THC) (D'Souza et al, 2005). Finally, individuals who are psychosis prone as determined either psychometrically or by family history are more sensitive to the psychosis-inducing effects of cannabis (Arendt et al, 2008; GROUP, 2011). However, the basis of the enhanced vulnerability to the psychosis-inducing effects of CBs in schizophrenia patients is not clear. Several other mechanisms might explain vulnerability to THC effects including polymorphisms of genes for COMT (Henquet et al, 2006), AKT1, and DAT1 (Bhattacharyya et al, 2012, 2014), and γ-aminobutyric acid (GABA) deficits. Further- more, it is conceivable that combinations of these factors may coexist and have additive or synergistic effects on increasing vulnerability to THC effects.

GABA deficits have been observed in the dorsolateral prefrontal cortex in schizophrenia (Lewis et al, 2005), and furthermore, there is important interplay between the CB and GABA systems (Eggan et al, 2010). Indeed, converging lines of evidence, including post-mortem (Lewis et al, 2005), genetic (reviewed by Charych et al, 2009), and brain imaging studies (Busatto et al, 1997; Verhoeff et al, 1999) suggest that dysfunction of the GABA system contributes to the pathophysiology of schizophrenia. Although there

is strong support for the existence of a GABA deficit in schizophrenia, the proportion of schizophrenia patients with this deficit is not known. The limited data available suggest that only 50% of schizophrenia patients have lower GABA levels compared with the lowest level found in healthy normal controls (Yoon et al, 2010).

In several brain regions, particularly the cerebral cortex and hippocampus, CB1 receptors (CB1-Rs) are present on the axon terminals of cholecystokinin (CCK) containing GABA interneurons that target the perisomatic region of pyramidal cells (PCs) (Eggan et al, 2010). CB1-Rs are activated by endocannabinoids released postsynaptically by depolarized PCs (Wilson and Nicoll, 2002). The activation of CB1Rs inhibits the release of GABA by CCK-basket cells, leading to a disinhibition of postsynaptic PCs (Bartos and Elgueta, 2012; Klausberger et al, 2005). Thus, a CB1-R-mediated braking mechanism regulates the timing and release of GABA, and subsequently the overall inhibitory/ excitatory balance in cortical networks (Farkas et al, 2010). This interplay between GABA and CB1-R systems provides a mechanism that could explain the higher vulnerability to CBs in schizophrenia. For instance, if CB1-R activation occurred in the presence of a pre-existing GABA deficit (as might be the case in schizophrenia), this could lead to further disinhibition and desynchronization of PC activity, leading to perturbations in gating, associative functions, and neurocognition, which could culminate in psychotic symptoms.

This study tested the hypothesis that if, among other mechanisms, GABA deficits contribute to the increased vulnerability of schizophrenia patients to the psychosis-exacerbating effects of CBs, then inducing a GABA deficit in healthy subjects will increase the psychosis-inducing effects of THC. As described below, a GABA deficit was pharmacologically modeled by the administration of the $GABA_A$ inverse agonist, iomazenil.

Materials and methods

In a four test day double-blind, placebo-controlled, randomized and counterbalanced study, healthy volunteers received iomazenil followed by THC, placebo iomazenil followed by THC, iomazenil followed by placebo THC, and placebo iomazenil followed by placebo THC. The study was conducted in the Neurobiological Studies Unit (VA Connecticut Healthcare System (VACHS), West Haven, CT). Subjects were recruited by advertisements and by word of mouth, and were paid for their participation.

Approvals

The study was approved by the institutional review boards of the VACHS and Yale University School of Medicine and was carried out in accordance with the Declaration

of Helsinki (1975). The study was carried out under Investigational New Drug applications (51 671 and 75 099). Subjects were informed about the potential for adverse effects of THC, iomazenil, and the combination.

Subjects

After obtaining written informed consent, subjects (n = 27) underwent a Structured Clinical Interview for DSM-IV (SCID; First et al, 2002) and were carefully screened (Supplementary Text no. 1). Women were excluded because the teratogenicity of iomazenil is not known. A detailed history of cannabis exposure was obtained. Cannabis-naive individuals were excluded to minimize any risk of promoting future cannabis use/abuse. Exclusion criteria included a personal or family history of epilepsy or seizure disorder because of the potential for iomazenil to reduce seizure threshold, a family history of a major Axis 1 disorder, and DSM-IV cannabis or other substance dependence (except nicotine). A general physical and neurological examination, electrocardiogram, and laboratory tests were also conducted. A screening electroencephalogram (EEG) was conducted to exclude subjects with any activity suggestive of seizure disorder. After screening, subjects were instructed to refrain from alcohol, caffeinated beverages, illicit drugs (other than cannabis), or prescription drugs not approved by the research team for 1 week before the study and throughout study participation. Compliance with the alcohol and drug prohibi- tions was formally assessed at every screening visit and on the morning of each test day (×4), by asking subjects and also testing breath for alcohol, and urine for drugs. Compliance with the caffeine restriction was checked by asking subjects.

Drugs

The preparation, formulation, and storage of THC solution and control are re- ported elsewhere (D'Souza et al, 2004) (Supplementary Text no. 2). THC at a dose of 0.015 mg/kg (1.05 mg in a 70 kg individual) was administered intrave- nously over 10 min into a rapidly flowing intravenous infusion of normal saline.

Iomazenil (Ro 16-0154) is an iodine analog of the benzodiazepine receptor (BZR)-competitive antagonist flu- mazenil. Some of its pharmacologic properties are comparable to those of the BZR-competitive antagonist flumazenil (Beer et al, 1990). However, unlike flumazenil, which blocks the effects of BZR agonists but lacks intrinsic pharmacological effects (Hunkeler et al, 1981), inverse agonists interfere with the function of the BZR in coupling the $GABA_A$ receptor and chloride channel, and have intrinsic pharma- cological effects opposite to those of BZR agonists (Tallman and Gallager, 1985). For example, the BZR inverse agonist FG-7142 is anxiogenic in healthy human subjects (Dorow et al, 1983; Horowski and Dorrow, 2002). BZR inverse agonist drugs bind to extrasynaptic $GABA_A$ receptors (Liang et al, 2004). Iomazenil has high affinity and selectivity for BZRs (K_d = 0.5 nM) (Johnson et al, 1990). In in vitro models,

it inhibits the binding of the weak BZR inverse agonist [^{3}H] Ro15-4513 to γ2- and δ-subunit-containing $GABA_A$ receptors with IC_{50} of 20–30 nM (R Olsen, personal communication, 26 January 2007). Iomazenil produces a net deficit in GABA function; in preclinical studies, it has been shown to behave as a BZR-competitive antagonist with inverse agonist effects (Beer et al, 1990; Hoffmann-La Roche; Schubiger and Hasler, 1989), and in clinical studies, it has been shown to have anxiogenic effects, and proconvulsant effects at higher doses (Randall, 1995; personal communication). Consistent with a role of GABA deficits in the pathophysiology of psychosis, iomazenil has been shown to increase the psychotomimetic effects of the 5-HT_2 partial agonist 1-(m-chlorophenyl)piperazine (m-CPP) in healthy subjects (D'Souza et al, 2006) and schizophrenia patients are more vulnerable to the propsychotic effects of iomazenil (Ahn et al, 2011). Iomazenil was administered intravenously at a dose of 3.7 μg/kg over 10 min.

Blood was sampled repeatedly and assayed for THC and 11-nor-Δ-9-THC-9-COOH (THC-COOH) using gas chromatography mass spectrometry (Shaw et al, 1991) to rule out that any hypothesized worsening of effects with the combination of the two drugs was not merely a result of iomazenil increasing THC levels.

Behavioral and Subjective Measures

Psychosis-relevant symptoms were assessed using the PANSS (Kay et al, 1989). Perceptual alterations were measured using the Clinician Administered Dissociative Symptoms Scale (CADSS) (Bremner et al, 1998). 'High' and other subjective effects associated with cannabis intoxication were measured using a self-reported visual analog scale (VAS) (0–100). These assessments were administered at baseline (−60 min), +70 min and +240 min timepoints, where timepoint 0 min denotes the beginning of the THC infusion. The baseline rating was to assess the predrug state. Subsequent ratings covered the entire time period between the current and past timepoint, for example,

+70 covered the time period from baseline to +70 min. The same research coordinators rated all 4 test days for each subject. Inter-rater reliability sessions were conducted every 1–2 months over the time period that this study was conducted (~4 years) and, for example, intraclass correlation coefficients for the PANSS were consistently 40.85 (Table 1).

Table 1 Schedule of Test Day

Time	Procedure
Screening (-4 weeks before test day)	• Medical and psychiatric history, SCID, cannabis/drug/alcohol use, confirmation of history with collateral • Lifetime marijuana use assessment • Chemistry, hematology, urine toxicology, EKG, vital signs, height, and weight • Baseline safety EEG to rule out risk for seizures
– 120	• Confirm adherence to prestudy prohibitions: • Last use of tobacco, cannabis, alcohol, caffeine, other drugs, medications, and supplements • Breathalyzer • Urine toxicology • Confirm that subjects have not had any recent psychosocial stressors? • Confirm that subject has fasted since midnight? • Vital signs: blood pressure and heart rate • Standard light breakfast • Insert two IV lines: identify arms for iomazenil and THC infusions
– 90	• Set up EEG cap • Vital signs: blood pressure and heart rate
– 60	• Ratings: PANSS, CADSS, and VAS • Blood sampling for THC/THC-COOH assay
– 15	• Vital signs: blood pressure and heart rate
– 10	• Intravenous iomazenil 3.7 μg/kg over 10 min
0	• Intravenous THC (0.015 mg/kg) or placebo over 10 min
+5	• Vital signs: blood pressure and heart rate
+10	• Vital signs: blood pressure and heart rate • Blood sampling for THC/THC-COOH assay
+25	• Event-related potentials: P300 • Vital signs: blood pressure and heart rate
+45	• Vital signs: blood pressure and heart rate
+70	• Ratings: PANSS, CADSS, and VAS
+80	• Vital signs: blood pressure and heart rate • Blood sampling for THC/THC-COOH
+240	• Vital signs: blood pressure and heart rate • Ratings: PANSS, CADSS, and VAS • Blood sampling for THC/THC-COOH assay • Safety check list: ○ MMSE ○ Field sobriety test ○ Exit interview ○ Discharge instructions

Abbreviations: SCID, Structured Clinician Interview; EEG, electroencephalography; NPO, nil per oral; PANSS, Positive and Negative Syndrome Scale; CADSS, Clinician Administered Dissociative Symptoms Scale; VAS, Visual Analog Scale; MMSE, Mini Mental State Examination. Safety follow-up: 1 and 3 months after last test day for cannabis use and psychiatric symptoms.

General Procedure and Test Days

Test days were separated by 3 days to minimize carryover effects given the half-life of THC. Subjects fasted overnight, reported to the test facility around 0800 hours, and were provided a standard breakfast. Urine toxicology was conducted on the morning of each test day to rule out recent illicit drug use. In-study safety procedures are described elsewhere (D'Souza et al, 2004). Behavioral and subjective ratings, vital signs, and blood sampling were repeated several times before and after drug administration, while psychophysiological data were collected only once per test day. A field sobriety test, mental state examination, and exit interview were conducted at the end of each test day. Prospective safety assessments were performed at 1 and 3 months after the last test session and after they had received payment for participation to query their use of cannabis.

EEG Recording

EEG data were collected in an acoustically shielded booth, and recording was carried out with the commercially available Active Two Acquisition System (Biosemi, The Netherlands). A sampling rate of 1024 Hz was used, with online low-pass filter of 256 Hz to prevent aliasing of high frequencies. A 64-channel electrode cap according to the extended 10-20 system was used, along with additional electrodes to record the vertical and horizontal electro-oculogram. All electrodes were referenced during recording to a common-mode signal electrode between POz and PO3 and then subsequently re-referenced to the nose offline.

EEG Task

A three-stimulus auditory oddball task adapted from our previous study was used (D'Souza et al, 2012). Briefly, a random series of infrequent (8.33%) 'target' tones (1000 Hz sine wave), frequent (83.33%) 'standard' tones (20, 30, or 40 Hz click trains), and infrequent distractor sounds (8.33%) were presented with a 1250 interstimulus interval in three separate blocks. Distractors included a set of novel everyday natural and manmade sounds (Friedman et al, 1993). Target tones and standard click trains were 500 ms in duration, whereas distractor stimuli ranged in duration from 175 to 250 ms. All stimuli were presented binaurally using at an intensity of 80 dB SPL.

In each block, participants were asked to press a response key to the target stimuli with the index finger of their preferred hand. Each block was comprised of 15 targets, 15 distractor stimuli, and 150 standards (20 Hz standard click trains for Block 1, 30 Hz standard click trains for Block 2, and 40 Hz standard click trains for Block 3). The order of blocks was randomized for each test day. To maximize event-related potential (ERP) signal-to-noise-ratio, target and distractor stimuli were averaged from all three blocks. Thus, in total, there were 45 targets, 45 distractors, and 450 standards.

EEG Signal Processing

EEG data were first bandpass filtered from 0.1 to 100 Hz (24 dB/oct) and notch filtered at 60 Hz. The recorded EEG was then segmented into epochs consisting of a 100 ms baseline and ending 1000 ms after stimulus onset. Ocular movement correction was applied using Gratton's algorithm (Gratton et al, 1983). After baseline correction, any trial with a voltage 4 ± 100 μV was excluded from analysis. Finally, the data were low-pass filtered (12 Hz cutoff, 24 dB/oct) and single trials were averaged before peak detection of ERPs.

For target and novel stimuli, the P300b and P300a, respectively, were identified as the largest positive voltage peak between 250 and 400 ms after stimulus onset using an automated algorithm. To assess primary sensory processing and registration, the N100 component to both target and novel stimuli was examined. The N100 was defined as the largest negative voltage peak between 50 and 150 ms after stimulus onset. For statistical analysis, data were used for each component where amplitude was largest as described previously (Pz for P300b; Cz for P300a; Cz for N100) (D'Souza et al, 2012). Processing and analysis of distractor stimuli will be reported elsewhere. All EEG processing was performed using commercially available software (Analyzer 2.0; Brain Products GmbH, Gilching, Germany).

Statistical Analysis

Initially, data were examined descriptively using means, SDs, and graphs. Each outcome was assessed visually for normal- ity using histograms and normal probability plots. As THC peak effects were captured 70 min after drug administration, and because at other timepoints there was little difference from the minimum score, each behavioral and subjective outcome was expressed as peak change from baseline using methods described elsewhere (D'Souza et al, 2012). The data were analyzed using linear mixed models with drug condition: (1) active iomazenil, placebo THC, (2) active iomazenil, active THC, (3) placebo iomazenil, active THC, and (4) placebo iomazenil, placebo THC as a within-subjects factor. Tukey's post-hoc procedure was used to determine significant pair-wise group differences between each of the four conditions. The correlation between repeated measures on an individual was modeled using random-effects and/or structured variance–covariance matrices. The best fitting variance–covariance structure was determined using information criteria. In the above models, potential covariates (eg, frequency of and days since last use of cannabis) were entered into the model in turn but were not significant and dropped for parsimony. The mixed-effects approach is ad- vantageous as it is unaffected by randomly missing data and allows greater flexibility in modeling the correlation structure of repeated-measures data (Gueorguieva and Krystal, 2004). Models similar to above were used to analyze P300a and P300b data. All analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC) (Table 2).

Table 2 Sample Demographics

	Mean	**SD**
Age (years)	25.44	7.41
Weight (kg)	80.98	12.19
Height (cm)	178.87	6.96
IQ (NART)	116.85	5.27
Psychosis proneness		
- Wisconsin Psychosis Proneness	27.3	10.75
- SPQ (Schizotypal Personality Questionnaire)	5.87	5.12
Average number of alcoholic drinks per week	5.8	5.9
Cannabis use		
- Age at first use	17.27	2.35
- Frequency of use in past 30 days (no. of days)	6.22	7.73
- Lifetime frequency of use (no. of days)	296.75	266.26
	Total n	
Handedness (no.)	22	
- Right		
- Left	4	
- Ambidextrous	1	
Cigarette smoker	4	
- Yes		
- No	23	
No. of subjects who ever tried drugs other than cannabis		
- None	7	
- Hallucinogens	15	
- Stimulants	8	
- Opiates	2	
- Inhalants	0	

*None of the subjects met the criteria for abuse or dependence of alcohol and illicit drugs of abuse.

Table 3 Behavioral and Subjective Effects of THC and Iomazenil Mean (SD) Scores on the PANSS, CADSS, and VAS Across Drug Condition and Time, Along With Post-Hoc Contrasts

Condition	Timepoint	N	Mean	SD	Significant post-hoc contrasts
PANSS total score					
IOM−/THC−	−60	23	28.83	0.78	IOM+/THC+ *vs* IOM−/THC+ ($p=0.038$)
	70	23	33.26	6.33	
	240	23	28.17	6.64	IOM+/THC+ *vs* IOM+/THC− ($p=0.003$)
IOM+/THC−	−60	20	28.65	0.59	
	70	20	38.1	8.84	IOM+/THC+ *vs* IOM−/THC− ($p<0.0001$)
	240	20	28.5	0.69	
IOM−/THC+	−60	23	28.83	0.78	IOM−/THC+ *vs* IOM−/THC− ($p=0.005$)
	70	23	40.83	7.69	
	240	23	28.96	1.3	
IOM+/THC+	−60	23	28.7	0.63	
	70	21	46.19	9.79	
	240	21	29.19	1.89	
CADSS patient-rated					
IOM−/THC−	−60	23	0.17	0.49	
	70	23	1.26	3.09	IOM+/THC+ *vs* IOM+/THC− ($p=0.029$)
	240	23	0.04	0.21	
IOM+/THC−	−60	20	0	0	IOM+/THC+ *vs* IOM−/THC− ($p=0.001$)
	70	20	4.65	7.11	
	240	20	0.15	0.49	IOM−/THC+ *vs* IOM−/THC− ($p=0.022$)
IOM−/THC+	−60	23	0.04	0.21	
	70	23	6.22	6.56	
	240	23	0.04	0.21	
IOM+/THC+	−60	23	0.04	0.21	
	70	21	9.14	8.05	
	240	21	0.14	0.48	
CADSS clinician-rated					
IOM−/THC−	−60	23	0	0	IOM+/THC+ *vs* IOM−/THC− ($p=0.003$)
	70	23	0.91	1.65	
	240	23	0	0	IOM−/THC+ *vs* IOM−/THC− ($p=0.06$)
IOM+/THC−	−60	20	0.05	0.22	
	70	20	1.65	2.16	
	240	20	0	0	
IOM−/THC+	−60	23	0	0	
	70	23	2.96	3.61	
	240	23	0	0	
IOM+/THC+	−60	23	0	0	
	70	21	2.95	2.22	
	240	21	0	0	
VAS anxious					
IOM−/THC−	−60	23	3.62	7.57	IOM+/THC+*vs* THC alone ($p=0.039$)
	70	23	2.78	8.4	
	240	23	0.32	0.79	IOM+/THC+ *vs* IOM−/THC− ($p=0.014$)
IOM+/THC−	−60	20	4.26	9.21	
	70	20	16.37	25.89	IOM+/THC− *vs* IOM−/THC− ($p=0.053$)
	240	20	1.63	5.4	
IOM−/THC+	−60	23	3.68	8.94	
	70	23	4.61	8.36	
	240	23	0.3	0.85	
IOM+/THC+	−60	23	1.72	3.92	
	70	21	16.78	24.82	
	240	21	0.25	0.64	
VAS high					
IOM−/THC−	−60	23	0.5	2.08	IOM+/THC+ *vs* IOM+/THC− ($p=0.0001$)
	70	23	7.38	19.12	
	240	23	0.93	2.46	IOM+/THC+ *vs* IOM−/THC− ($p<0.0001$)
IOM+/THC−	−60	19	0.42	1.17	
	70	20	11.68	23.08	IOM−/THC+ *vs* IOM+/THC− ($p=0.004$)
	240	20	0.08	0.18	
IOM−/THC+	−60	23	0.32	1.06	IOM−/THC+ *vs* IOM−/THC− ($p=0.0001$)
	70	23	36.32	29.86	
	240	23	0.52	1.2	
IOM+/THC+	−60	23	0.53	1.69	
	70	21	44.33	33.03	
	240	21	0.38	0.8	

Results

Of the 27 male subjects who were enrolled in the study, 21 completed all 4 test days. EEG data was collected in 23 subjects. The demographic characteristics of the sample are listed in Tables 2 and 3.

Plasma Level of THC and THC-COOH

There were no significant effects of iomazenil on plasma levels of THC (drug condition × time, p = 0.46) and THC- COOH (drug condition × time, p = 0.18) (Supplementary Figure 1).

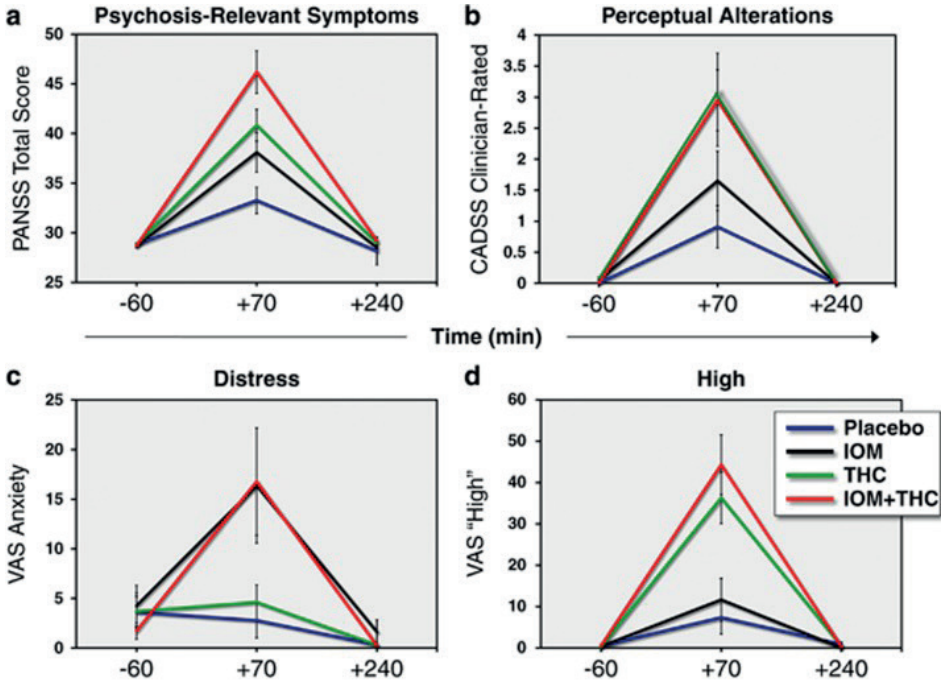

Figure 1

Mean (± SEM) data for each drug condition at baseline (–60 min), +70 min, and +240 min timepoints for the psychosis-relevant symptoms ((a) Total Positive and Negative Syndrome Scale (PANSS)), perceptual alterations ((b) patient-rated Clinician Administered Dissociative Symptoms Scale (CADSS)), distress ((c) VAS Anxiety), and high ((d) VAS High). Placebo, blue line; iomazenil, black line; THC, green line and the combination of iomazenil; and THC: red line. Note that the figure shows mean (SD) data from all time- points, whereas the analyses were conducted on the peak change from baseline.

Behavioral and Subjective Effects

For all behavioral and subjective measures, the main analysis of interest was the contrast between the conditions of combined iomazenil and THC (+IOM+THC), and THC alone (– IOM+THC). Mean (SD) scores on the PANSS (Figure 1), CADSS, and VAS for each drug condition and timepoint are presented in Table 3. Note that even though the statistical analyses were for peak change from baseline, in the figures, all timepoints are shown.

Positive and Negative Syndrome Scale. There was a significant drug condition effect ($F_{3,58}$=12.93, p<0.0001) on the peak change from baseline in Total PANSS scores. Relative to placebo, the combination of iomazenil and THC (IOM +/THC+) produced significant increases (p_{adj} < 0.0001) in Total PANSS scores. Relative to placebo, THC alone (IOM– / THC+) produced significant increases (p_{adj}=0.005) in Total PANSS scores, while iomazenil did not. Furthermore, there were significantly (p_{adj}=0.04) greater increases in Total PANSS scores for the IOM+/THC+ condition compared with IOM– /THC+ (Figure 1a).

Clinician Administered Dissociative Symptoms Scale. There was a significant drug condition effect ($F_{3,59}$= 5.3, p<0.003) on the peak change from baseline in clinician-rated CADSS subscale scores. However, relative to placebo, only IOM+/THC+ produced a significant increase in the clinician-rated CADSS subscale scores (p_{adj} = 0.003), while the increase for IOM – /THC+ trended towards significance (p_{adj} =0.06). The difference between IOM+/THC+ and IOM – /THC+ was not significant.

There was a significant drug condition effect ($F_{3,58}$ =5.82, p<0.0015) on the peak change from baseline in patient-rated CADSS subscale scores. Relative to placebo, IOM+/THC+ (p_{adj} < 0.0006) and IOM – /THC (p_{adj} = 0.02) produced significant increases in patient-rated CADSS scores (Figure 1b). Although the change in patient-rated CADSS scores was greater on the IOM+/THC+ relative to IOM – / THC (unadjusted p= 0.054), the difference was not statistically significant after Tukey's adjustment (p_{adj} = 0.2).

Feeling States (VAS) 'Anxious'. There was a significant drug condition effect ($F_{3,58}$= 4.82, p<0.0046) on the peak change from baseline in self-reported 'anxious' scores. Relative to placebo, only IOM +/THC+ produced greater increases in VAS 'anxious' scores (p_{adj} =0.01) (Figure 1c). Furthermore, IOM+/THC+ produced greater increases in VAS 'anxious' scores relative to IOM – /THC+ (p_{adj} = 0.04).

'High'. There was a significant drug condition effect ($F_{3,57}$= 14.27, p<0.0001) on the peak change from baseline in self-reported 'high' or 'stoned' scores. Relative to placebo, both IOM – /THC+ (p_{adj} < 0.0001) and IOM+/THC+ (p_{adj} < 0.0001), but

not iomazenil alone, produced significant increases in VAS 'high' scores (Figure 1d). Furthermore, there were no significant differences between the IOM – / THC+ and IOM+/THC+ conditions.

EEG Measures

P300b. Relative to placebo, only IOM+/THC+ produced a significant (p = 0.023) decrease in target P300b amplitude at Pz (Figure 2; Table 4). Furthermore, there was a significant linear trend ($F_{1,44}$= 6.02, p = 0.018) for drug condition (IOM – /THC –>IOM+/THC –>IOM – /THC+> IOM+/THC+). There were no significant effects of any of the drug conditions on target P300b latency measured at Pz (Table 4).

P300a. Relative to IOM – /THC –, both IOM+/THC+ and IOM – /THC+ produced trend level (p =0.08) reductions in P3a amplitude measured at Cz. Furthermore, there was a trend towards a linear trend (F1,44 =3.11, p =0.085) for drug condition (IOM – / THC –>IOM+/THC –>IOM – /THC +>IOM+/THC+). Relative to IOM – /THC–, only IOM+/THC+ produced a trend levels reduction in (p =0.08) P300a latency measured at Cz (Table 4).

There were no correlations between the effects of IOM +/THC+ or IOM – /THC+ on PANSS outcomes and both P3b and P3a amplitudes (Supplementary Table 1).

N100. There were no significant effects of THC, IOM, or their combination on N100 amplitude or latency for both target and novel stimuli measured at Cz.

Safety

There were no serious adverse events that occurred on test days. Prospective poststudy safety assessments (described in Supplementary Text no. 3) did not reveal any evidence, suggestive of an increase in cannabis use or psychosis outcomes.

Table 4 Electrophysiological Effects of THC and Iomazenil

Condition	N	Mean	SD	Only significant contrasts
P3b amplitude (Pz)				
IOM−/THC−	17	10.23	5.81	+IOM+THC *vs* PLA+PLA: DF=44, *t*-value=2.34, *p*=0.024
IOM+/THC−	16	9.24	6.32	
IOM−/THC+	19	8.33	6.36	
IOM+/THC+	16	7.73	5.48	
P3b latency (Pz)				
IOM−/THC−	17	328.97	35.8	
IOM+/THC−	16	325.46	26.95	
IOM−/THC+	19	342.17	21.38	
IOM+/THC+	16	327.55	24.69	
P3a amplitude (Cz)				
PLA+PLA	17	11.18	7.16	IOM+THC *vs* PLA+PLA: DF=44, *t*-value=0.18, *p*=0.08
IOM+PLA	16	8.55	4.56	PLA+THC *vs* PLA+PLA: DF=44, *t*-value=1.79, *p*=0.08
PLA+THC	19	7.79	6.4	
IOM+THC	16	8.29	5.36	
P3a latency (Cz)				
IOM−/THC−	17	325.98	23.2	
IOM+/THC−	16	320.92	29.03	
IOM−/THC+	19	322.18	28.5	
IOM+/THC+	16	312.96	18.46	
Target N100 amplitude (Cz)				
IOM−/THC−	17	−5.97	3.53	
IOM+/THC−	16	−5.56	3.32	
IOM−/THC+	19	−5.37	2.38	
IOM+/THC+	16	−6.99	3.86	
Target N100 latency (Cz)				
IOM−/THC−	17	103.36	11.06	
IOM+/THC−	16	107.38	16.77	
IOM−/THC+	19	108.95	14.01	
IOM+/THC+	16	109.3	13.59	
Novel N100 amplitude (Cz)				
IOM−/THC−	17	−6.13	4	
IOM+/THC−	16	−5.89	3.93	
IOM−/THC+	19	−5.36	2.55	
IOM+/THC+	16	−5.91	2.9	
Novel N100 latency (Cz)				
IOM−/THC−	17	107.12	14.24	
IOM+/THC−	16	111.94	10.34	
IOM−/THC+	19	106.64	18.48	
IOM+/THC+	16	106.36	12.56	

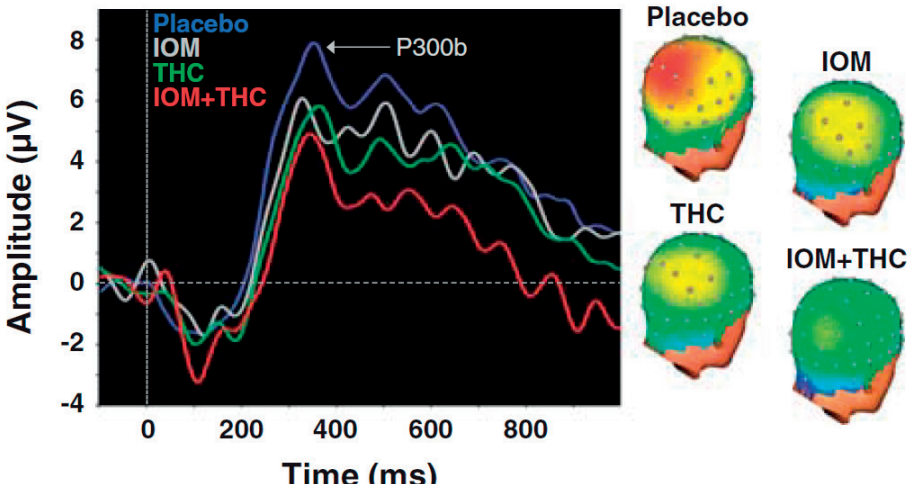

Figure 2

(Left) Grand-averaged target P300b waveforms at electrode Pz for EEG runs across drug conditions. (Right) Topographic voltage maps from the peak grand-averaged P300b for EEG runs across drug condi- tions. Placebo, blue line; iomazenil, gray line; THC, green line and the combination of Iomazenil; and THC, red line.

Discussion

This is the first study to our knowledge that examines the effects of THC in the backdrop of a pharmacologically induced GABA deficit in humans. The results showed that pretreatment with IOM exacerbated several of the behavioral, subjective, and psychophysiological effects of THC in healthy humans. When pretreated with IOM, THC induced significantly greater psychosis-relevant symptoms, as captured by the Total PANSS, compared with the THC-alone condition. Similarly, only with IOM pretreatment did THC significantly increase perceptual alterations as captured by the patient-rated CADSS. Furthermore, only when pretreated with IOM did THC induce distress as captured by VAS 'anxiety' scores. Finally, only the combination of IOM+THC reduced THC-induced P300b amplitude.

The combination of IOM and THC did not increase measures of euphoria (VAS 'high') compared with THC alone. Furthermore, there were no differences in THC blood levels across conditions. Taken together, these findings suggest a pharmacodynamic rather than a pharmacokinetic interaction.

The close interplay between the CB and GABAergic systems in several brain regions provides a mechanistic framework to understand the study findings. In the cerebral cortex and hippocampus, presynaptic CB1-Rs primarily inhibit the release of GABA from CCK – interneurons (Eggan et al, 2010). IOM likely causes a net reduction in $GABA_A$

function across various interneuron types (eg, PV, SST, CCK, and so on). However, as PV-positive interneurons are thought to be the primary GABAergic subtype involved in psychosis (Glausier et al, 2014; Lewis et al, 2012), it is tempting to speculate if IOM exacerbates the psychotomimetic effects of THC by disrupting inputs from PV cells onto PCs. Indeed, several lines of evidence suggest that CB1-Rpositive CCK interneurons and PV cells work in concert to modulate the synchronized output of PCs (Bartos and Elgueta, 2012; Klausberger et al, 2005). Put another way, THC-induced activation of CB-1Rs on the axon terminals of CCK containing GABA neurons reduces GABA release, resulting in disinhibition of PC activity. If this were to occur in the presence of a GABA deficit, as might be produced by IOM (and as might occur in schizophrenia), it would lead to further disinhibition and desynchronization of PC activity, which would lead to the perturbation of gating and associative functions and culminate in psychotic symptoms.

Pretreatment with IOM has been shown to enhance the psychotomimetic effects of serotonergic (m-CPP) (D'Souza et al, 2006), dopaminergic (amphetamine) (K Ahn, personal communication), glutamatergic (ketamine) (H Gunduz- Bruce, personal communication), and now cannabinoidergic (THC) agents. Furthermore, when ad- ministered alone, IOM has been shown to produce small increases in psychosis in schizophrenia patients but not in healthy controls (Ahn et al, 2011). Collectively, these findings highlight the contributions of GABAergic deficits to the pathophysiology of schizophrenia.

The combination of IOM and THC also caused the largest reductions in the P300b. Exogenous cannabinoids have been shown to reduce the amplitude of the P300b (D'Souza et al, 2012; Roser et al, 2008). Reductions in P300 amplitude and increased latencies have been observed in a number of other neuropsychiatric disorders including schizophrenia (reviewed in Bramon et al, 2004; Jeon and Polich, 2003). Both GABAergic and glutamatergic systems have been strongly implicated in contributing to the P300 (Watson et al, 2009). In fact, it is now recognized that ERPs such as the P300 are generated by inhibitory and excitatory postsynaptic potentials, which are primarily driven by the release of glutamate and GABA (Luck et al, 2011). It is likely that normal P300 generation requires an optimal level of inhibitory–excitatory balance, and any perturbation above or below the optimum range can disrupt the neural networks involved in context updating, working memory, and the allocation of attentional resources. Future research is needed to determine how GABA-CB effects on the P300 are related to the psychosis-enhancing effects of IOM and THC.

These results lend support to the hypothesis that a pharmacologically induced GABA deficit would enhance the psychosis-relevant effects of THC in healthy adults. These data suggest that the enhanced vulnerability to cannabis and THC in schizophrenia

patients (D'Souza et al, 2005) may be explained by underlying GABA deficits. Although admittedly speculative, it may be inferred that psychosis-prone individuals who appear sensitive to the psychosis-inducing effects of cannabis may also have GABAergic deficits. However, to our knowledge, the functional state of the GABA system has not been examined in psychosis prone individuals.

These data add to a growing body of evidence from epidemiologic and experimental studies that have identified other factors that modulate the acute response to cannabis and THC, respectively. Caspi et al, (2005) demonstrated that polymorphisms of the gene encoding catechol-O-methyltransferase (COMT), which is critical for the removal of dopamine (DA) in the prefrontal cortex, influenced the risk for psychosis outcomes in later life, following cannabis exposure in adolescence. Henquet et al, (2006) then showed in an experimental study that polymorphisms of the gene encoding COMT-mediated differential sensitivity to the acute psychotomimetic effects of THC (Henquet et al, 2006). Similarly, epidemiologic studies have shown that variation in the gene for protein kinase C (AKT1), an integral component of the DA signaling cascade, influences the risk of associated psychosis outcomes with cannabis use (Di Forti et al, 2012; van Winkel et al, 2011a; van Winkel et al, 2011b). In an experimental study, Bhattacharyya et al, (2012) showed that polymorphisms of the genes for AKT1 and the dopamine transporter (DAT1) influence the psychotomimetic and neurophysiological response to THC. Finally, in a recent study, Bhattacharyya et al, (2014) also showed that the AKT1 genotype mediates the sensitivity to THC-induced impairments in psychomotor control.

Strengths, Limitations, and Conclusions

The intravenous route of drug administration, and weight-adjusted doses address the inter- and intraindividual variability associated with oral or smoked THC. The random- ized, placebo-controlled, double-blind, repeated-measure, 2 (active or placebo THC) ×2 (active or placebo iomazenil), within-subjects design is both powerful and efficient. The behavioral and cognitive outcome measures were complemented by a psychophysi- ological measure that allows a more proximal index of neuronal activity and interaction of CB and GABA systems. Further, ERPs afford near-perfect temporal resolution that is not afforded by other approaches. However, while the proposed study may have limited social relevance, as cannabis is not typically used intravenously, the strengths of the intravenous paradigm outweigh its limited social relevance. Finally, the study was not powered to evaluate interactive effects on all the subscales of the outcome measures or the influence of cannabis exposure on the interactions between IOM and THC.

Future Directions

Whether these findings can be generalized to women needs further study. These findings need replication in a larger sample that is adequately powered to examine the outcome measures in further detail, for example, items of the PANSS. The animal literature shows that GABA and CB systems modulate gamma range (β)-band neural oscillations, which are thought to have a key role in a number of processes that are altered in schizophrenia (Uhlhaas et al, 2009), including sensory registration, the integration and binding of perceptual features, associative learning, and conscious awareness; it will be important to study the interplay between CB and GABA systems on (β)-band neural oscillations in humans. The development and availability of reliable and valid in vivo methods to determine GABAergic deficits in schizophrenia will permit a more direct approach to determining the contribution of GABAergic deficits to vulnerability to the psychosis-inducing effects of THC in schizophrenia. Finally, the availability of drugs that target specific GABA interneurons will permit a more refined testing of the hypothesis.

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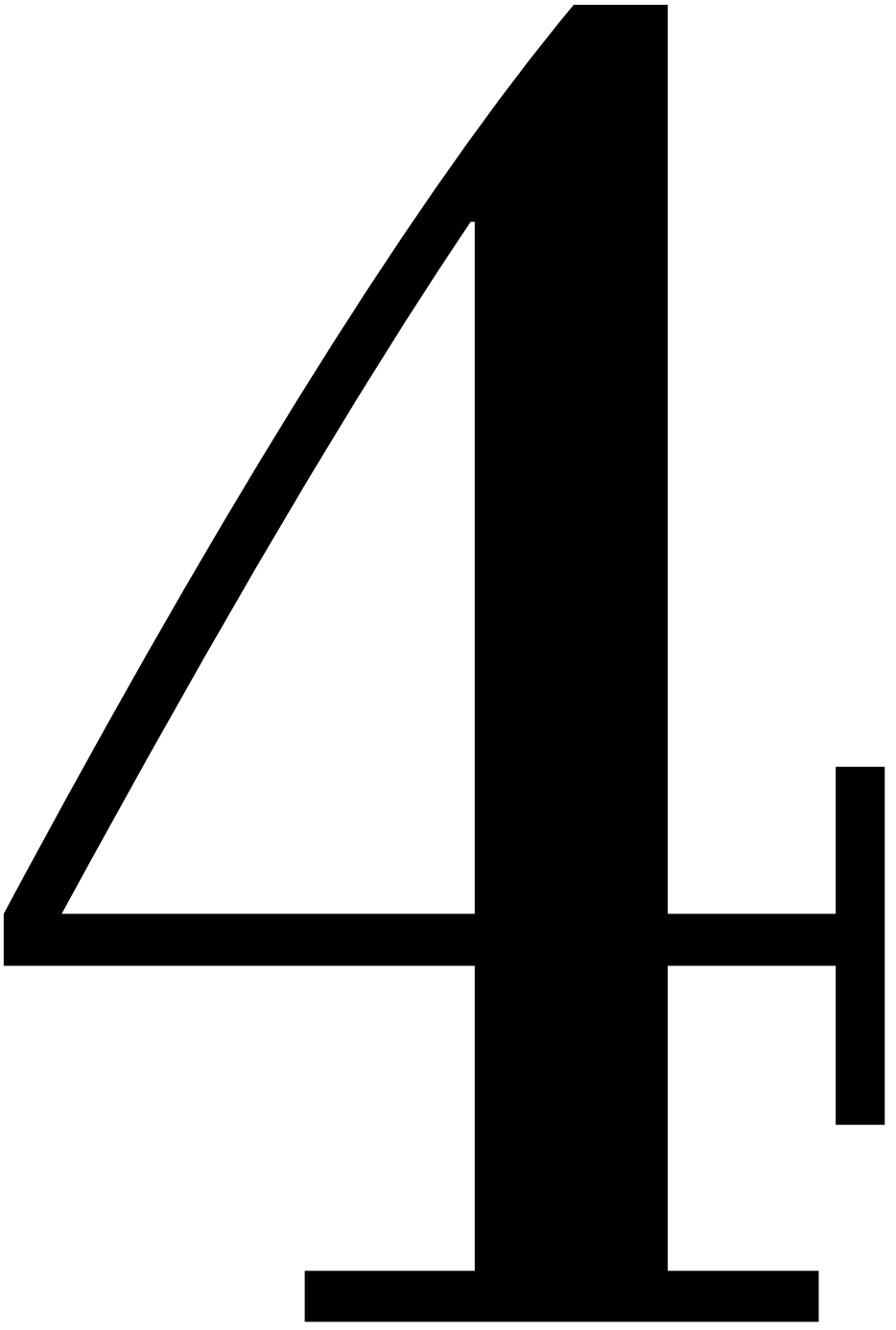

Chapter 4

Tetrahydrocannabinol (THC) impairs encoding but not retrieval of verbal information

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Abstract

Cannabis and agonists of the brain cannabinoid receptor (CB1R) produce acute memory impairments in humans. However, the extent to which cannabinoids impair the component processes of encoding and retrieval has not been established in humans. The objective of this analysis was to determine whether the administration of Δ^9-Tetrahydrocannabinol (THC), the principal psychoactive constituent of cannabis, impairs encoding and/or retrieval of verbal information.

Materials and methods: Healthy subjects were recruited from the community. Subjects were administered the Rey-Auditory Verbal Learning Test (RAVLT) either before administration of THC (experiment #1) (n = 38) or while under the influence of THC (experiment #2) (n = 57). Immediate and delayed recall on the RAVLT was compared. Subjects received intravenous THC, in a placebo-controlled, double-blind, randomized manner at doses known to produce behavioral and subjective effects consistent with cannabis intoxication.

Results: Total immediate recall, short delayed recall, and long delayed recall were reduced in a statistically significant manner only when the RAVLT was administered to subjects while they were under the influence of THC (experiment #2) and not when the RAVLT was administered prior.

Conclusions: THC acutely interferes with encoding of verbal memory without interfer- ing with retrieval. These data suggest that learning information prior to the use of cannabis or cannabinoids is not likely to disrupt recall of that information. Future studies will be necessary to determine whether THC impairs encoding of non-verbal information, to what extent THC impairs memory consolidation, and the role of other cannabinoids in the memory-impairing effects of cannabis.

Clinical Trial Information:: Cannabinoids, Neural Synchrony, and Information Processing (THC-Gamma)

http://clinicaltrials.gov/ct2/show/study/NCT00708994 NCT00708994

Pharmacogenetics of Cannabinoid Response http://clinicaltrials.gov/ct2/show/NCT00678730

NCT00678730

Introduction

Cannabis is the most commonly used drug used worldwide, with an estimated 183 mil- lion people having used the drug in 2014 (UNODC,2016). In 2010, an estimated 13 million people were cannabis dependent accounting for global burden of disease of 2 million disease-adjusted life years (Degenhardt et al., 2013).The age at initiation of can- nabis use has been progressively getting younger. In 2014, a total of 2.5 million persons 12 years or older in the US had used cannabis for the first time during the preceding 12 months, an average of approxi- mately 7000 new users each day (CBHSQ, 2015). The emerging trend of legalization of "medical" cannabis in the US, Canada, Europe and other parts of the world, for both medical and psychiatric indications (Wilkinson et al., 2016a, 2016b), the legalization of recreational can- nabis use (Bly, 2012), the earlier onset of cannabis use (Ernst et al., 2012), the increasing potency of cannabis (Meh- medic et al., 2010) and the recreational use of highly potent synthetic cannabinoid receptor agonists such as Spice and K-2 (Johnson et al., 2011; Vardakou et al., 2010), underscores the need to further understand the acute effects of these compounds.

In particular, we need to understand the acute effects of these drugs in young people who represent the overwhelming majority of cannabis users. Cannabis use is most prevalent among people aged 18 to 25 (with 19.8% using it in the past month). 7.0% of people aged 12 to 17 re- ported cannabis use in the past month (Johnston et al., 2016). Among people aged 12 or older, past month cannabis use increased from 6.2% in 2002 to 8.3% (~ 22.2 million people) in 2015 (CBHSQ, 2015). Importantly, a significant number of people in this demographic are likely to be in high school and college during which they have to learn new information related to their academic demands. Cannabis and cannabinoid receptor (CB1R) agonists such as tetrahydrocannabinol (THC) have been shown to acutely impair learning and memory in humans (reviewed in Ranganathan and D'Souza (2006)). Furthermore, cannabis use during adolescence has been reported to be associated with a higher probability of dropping out from high school, university non-enrolment, and failure to obtain (Silins et al., 2015). Thus, understanding to what extent and precisely how recreational use of cannabis and cannabinoids interfere with learning and memory in this population is of significant public health importance.

The most well-studied acute cognitive effects of CB1R agonists are on declarative verbal memory (Ranganathan and D'Souza, 2006).

Verbal memory is typically measured with verbal learning tasks that require subjects to learn a supraspan list of words and recall it several times immediately and after a delay. Declarative learning and memory involves the processes of encoding, consolidation,

and retrieval. These processes may not be entirely dissociable, but nevertheless remain important constructs in understanding learning and memory (Bernard et al., 2001; Nyberg et al., 2002). Encoding refers to the initial stage of processing during which the acquisition of information occurs. En- coding is followed by a series of changes that consolidate the new in- formation in order to protect against disruption and decay. Retrieval refers to the access or recall of previously encoded memories. The effects of a drug on verbal learning and recall may be a consequence of the drug affecting encoding, consolidation, retrieval, or all three.

A number of laboratory studies in humans have demonstrated that THC results in an impairment in immediate- and delayed-recall among infrequent users (Curran et al., 2002; D'Souza et al., 2004; Hart et al., 2010, 2001; Solowij and Battisti, 2008). The results from animal studies suggest that THC and other cannabinoid 1 (CB1R) receptor agonists disrupt memory (Goonawardena et al., 2011; Hampson and Deadwyler, 1998; Kruk-Slomka et al., 2016; Shiri et al., 2016; Kruk-Slomka and Biala, 2016; Wise et al., 2012). However, to what extent cannabinoids affect different memory subprocesses is still under study. Cannabinoid agonists have been shown to disrupt encoding (Hampson and Deadwyler, 2000; Hampson et al., 2011) and when administered post training (i.e. after encoding) caused an impairment in retrieval (Mackowiak et al., 2009; Yim et al., 2008).

Whether THC differentially effects encoding, consolidation, or retrieval of verbal memory has however, not been studied in humans. Although not specifically related to verbal memory, a few studies have attempted to examine the effects of THC on encoding. Ballard et al. (2013) found that administration of THC prior to encoding on an emotional memory task resulted in impairment in encoding. In another study, Bossong et al. (2012) showed that THC disrupted encoding and recall on a pictorial memory task. However, the design of the studies did not make it possible to disentangle the effects on the different phases i.e. encoding, consolidation and retrieval.

The effect of a drug on encoding, consolidation, retrieval during a verbal memory task can be studied by adjusting the timing of drug administration relative to the different phases of the task: learning list, immediate recall, delayed-recall and delayed recognition recall. The accuracy of immediate-recall following, or the rate of learning during, the learning trials of the word-list is considered to be a measure of "encoding" of verbal memory. Intact immediate recall with impairments in delayed recall suggests deficits in consolidation and/or retrieval. Impairments in delayed recall with intact delayed recognition recall suggest deficits in retrieval but not consolidation.

Administering a drug before encoding but terminating its effects before retrieval would help isolate drug-induced encoding deficits. However, this is not feasible in the case of

cannabinoids, given the long half-life of THC. An alternative strategy is to administer THC before and after encoding in separate experiments using the verbal learning task and then compare the effects on immediate recall, delayed recall and delayed recognition recall. There are no human studies to date, that have employed a standardized and controlled dose and method of THC administration to determine the extent to which cannabis, THC, and other CB1R agonists interfere with encoding, consolidation, or retrieval of verbal information.

The current study examined the effects of intravenous (i.v.) THC on verbal memory in human volunteers using a standardized drug ad- ministration method to address this gap in the literature. Because CB1R agonists are known to impair long-term potentiation (Ievglevskyi et al., 2012), a putative neural substrate of memory encoding and con- solidation, we hypothesized that the administration of THC would im- pair the encoding of verbal information while sparing retrieval.

Materials and Methods

The study were approved by the institutional review boards at Yale University and Veterans Connecticut Healthcare System (VACHS), West Haven, CT. THC was administered with approval from the US Food and Drug Administration through an Investigational New Drug application (IND # 51,671 [D'Souza]). Informed consent was obtained from subjects prior to study participation.

Study Design

The experiments were double-blind, randomized placebo-controlled by design (Fig. 1). In experiment #1 subjects received THC or placebo after the learning trials of a word list (post-encoding THC condition). In experiment #2 subjects received THC or placebo before the learning trials of the word list (pre-encoding THC condition). The effects of THC induced "altered state" was measured on the item "high" on a 0–100 visual analog scale (VAS). Verbal memory was measured using the Rey Auditory Verbal Learning Test (RAVLT), a task sensitive to hippocampal function that has five alternate versions (Rosenberg et al., 1984; Ryan et al., 1986), none of which are semantically organized.

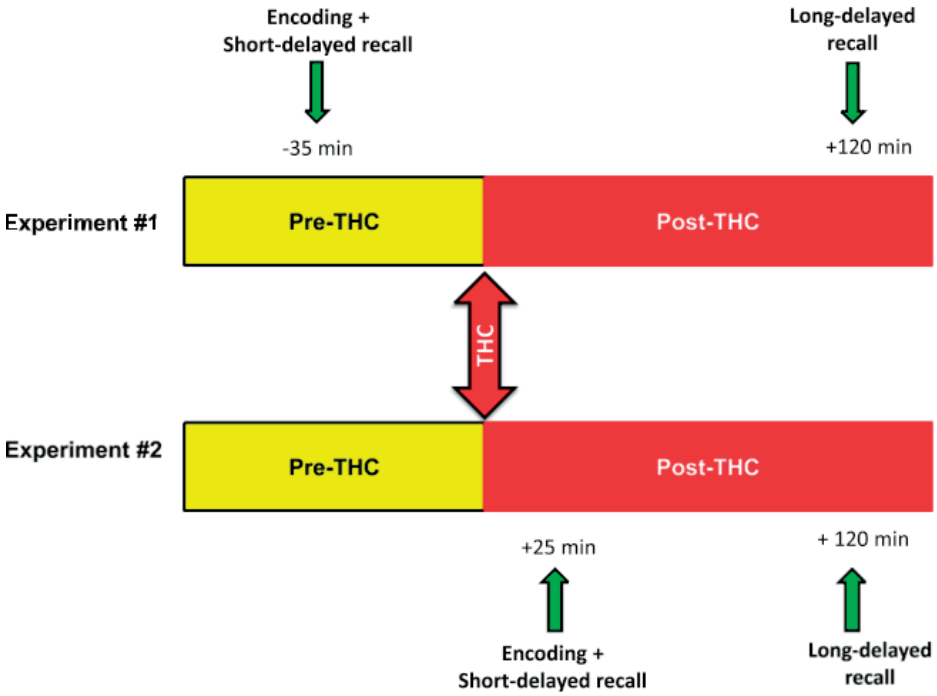

Figure 1

Experimental design. Figure represents the designs of experiment #1 (top panel) and experiment #2 (bottom panel). In experiment #1 immediate recall was assessed prior to THC/placebo administration and long delayed free recall was assessed after THC/ placebo administration. In experiment #2 immediate as well as delayed recall were as- sessed only after THC/placebo administration.

Screening

Psychiatrically and physically healthy subjects were recruited from the local community using advertisements and word of mouth. After completing a brief phone screening, eligible healthy subjects underwent an in-person screening that included a medical history, physical exam, laboratory tests including EKG, psychiatric interview using the Structured Clinical Interview for DSM-IV Diagnoses (SCID), intelligence tests, and cannabis use history.

Inclusion and exclusion criteria

Inclusion criteria were: (1) 18–55 years old; (2) able to provide informed consent; (3) good physical and mental health as determined by history, the Structured Clinical Interview for DSM-IV TR (SCID-NP) and collateral information, physical and laboratory examinations, and ECG and vital signs recordings; and (4) cannabis use at least once in their lifetime.

Exclusion criteria were: (1) diagnosis of DSM-IV TR alcohol or substance dependence (including cannabis) except nicotine dependence in the past year; (2) current or lifetime major Axis I or Axis II diagnoses; (3) positive pregnancy test; (4) recent (< 6 weeks) major stressors (including problems with the primary support group, problems related to the social environment, educational problems, occupational problems, housing problems, economic problems, problems with access to health care services, and problems related to interaction with the legal system or criminal behavior); (5) history of counseling, except for a life circumstance disorder (e.g., bereavement, divorce); (6) history of any psychotropic medication use except sleep medication for > 1 month; (7) major Axis I diagnosis in first degree relative (assessed through subject's self-report); (8) IQ < 80; (9) less than high school education; and (10) positive urine toxicology (other than cannabis) on test day mornings.

A collateral informant identified by the subject was contacted to corroborate the subject's psychiatric and medical history. Subjects were instructed to refrain from using illicit drugs (except cannabis) for two weeks, consuming caffeinated beverages and alcohol for 1 week, and to refrain from cannabis use for a minimum of 24 h prior to each test day until study completion. The subjects recruited were not regular can- nabis users (i.e. were not daily users) to avoid the possibility that a subject would be experiencing symptoms of withdrawal prior to a THC challenge. Frequency of cannabis use was measured at screening by self-report of number of days used in the past 30 days.

Study medication

Subjects received THC or placebo (in ethanol vehicle) infused over 15 min into a rapidly flowing normal saline infusion.

Justification of THC dose and route of administration

The i.v. route of administration was chosen to reduce inter- and intra-individual variability in plasma THC levels observed with the inhaled route and to mimic the time course of plasma THC levels as- sociated with the "high" as discussed in (D'Souza et al., 2004). Even with standardized paced smoking procedures, subjects are able to ti- trate the dose of cannabinoids they receive and in doing so, negate attempts to deliver a uniform dose (Ilan et al., 2005). The choice of THC rather than cannabis is to address the confounding effects of other cannabinoids, and flavonoids present in cannabis.

Subjects received THC (0.03 mg/kg in experiment #1–0.05 mg/kg in experiment #2) or placebo, given intravenously into a rapidly flowing i.v. infusion of normal saline. The formulation of THC and placebo were as described previously in (D'Souza et al., 2004). This dose range been shown in previous studies at our center to produce behavioral, subjective, cognitive and physiological effects consistent with the effects of cannabis (D'Souza et al., 2004, 2005, 2012). THC induced "altered state" was

measured as "high" on a VAS for subjective effects in both experiments and are reported below (Fig. 3). Expectancy effects were minimized as described elsewhere, by informing subjects that they may receive one of many possible doses on each test day (D'Souza et al., 2012).

Test days

Subjects reported to the test facility at 8 am after an overnight fast. Negative urine toxicology and pregnancy tests were confirmed on each test morning. They were provided a standardized breakfast, after which i.v. lines were inserted. Baseline ratings were completed followed by the THC or placebo infusion. Subjects completed two test days over a two- to three-week period, separated by at least 72 h in order to limit residual effects of THC.

Details of RAVLT administration and measures

The RAVLT was administered following a standardized method. First, a list of 15 words (list A) was read aloud to subjects five times. At the end of each reading (trial), subjects were asked to recall as many words as they could (trials 1–5). After five trials of list A, a second list of 15 words (list B: interference list) was read to the subjects and they were then asked to recall words from this list, (interference; trial 6). Following this, subjects were asked to recall words they had learned from List A without cues (short delayed recall; trial 7). Finally, after 30 min, subjects were asked to again recall words from list A without cues (long delayed recall; trial 8). The following outcomes were as- sessed:

1. Total Immediate Recall (the sum of total words correctly recalled in trials 1 to 5).

2. Rate of Learning (increase in recall across learning trials: trial 5 minus trial 1).

3. Short Delayed Recall (trial 7) 5 min after trial 5.

4. Long Delayed Recall (trial 8) 30 min after trial 5

Alternate versions of the RAVLT were administered on each test day and counterbalanced across subjects in order to avoid practice effects. The test-day administration of the RAVLT relative to THC infusion is depicted in Fig. 1.

Experiment #1

All five trials of list A, list B recall, and short delayed recall (trials 1 to 7) trials of the RAVLT were administered 35 min before infusion of THC. The long delayed recall (trial 8) was administered two hours after THC infusion.

Experiment #2

All five trials of list A, list B recall, and short delayed recall (trials 1 to 7) trials of the RAVLT were administered 25 min after infusion of THC. The long delayed recall (trial 8) was administered 95 min after short delayed recall trials, in order to standardize the experiments such that, in both experiments, delayed recall was tested two hours (120 min) after the start of THC infusion.

Measurement of "high" on the visual analog scale (VAS)

The VAS was administered at – 120, – 30, + 15, + 60, + 300 min. The peak change was taken as the highest value at any time point.

Data analysis

Initially, data were examined descriptively using means, standard deviations, and graphs. Based on examination of normal probability and residual plots and Kolmogorov-Smirnov test statistics, each out- come appeared sufficiently normal for parametric analyses. Outcomes were assessed using linear mixed models with drug (active vs. placebo) included as a within-subject factor and experiment (#1 vs. #2) as a between-subjects factor. The best-fitting variance-covariance structure was selected based on information criteria. Post-hoc tests were per- formed to determine the nature of any significant study by THC dose interaction effect by constructing linear contrasts where THC effects were tested within each study. Contrasts were constructed using the CONTRAST statement in SAS PROC MIXED and tested with F-tests. In the above models, age, gender, education, IQ, and cannabis use in the past 30 days were considered as covariates but did not change results and dropped for parsimony. Order effects were tested; none were pre- sent. All tests were two-sided and considered significant at the alpha = 0.05 threshold. All analyses were conducted using SAS, version 9.3 (Cary,NC).

Results

Subject characteristics

A total of 38 subjects participated in experiment #1 (i.e. THC ad- ministered after encoding) and 57 in experiment #2 (i.e THC ad- ministered before encoding). Additionally, 16 subjects who participated in experiment #1 also participated in experiment #2. There were no significant differences between experiment #1 and #2 on demographic variables (Table 1).

Table 1 Subject Characteristics

	Experiment #1 Mean (SD)	**Experiment #2 Mean (SD)**	**p-value**
Age	25.7 (7.6)	24.8 (7.9)	0.61
IQ	115.5 (4.72)	115.9 (5.6)	0.67
Education (yrs)	15.2 (2.2)	14.7 (1.9)	0.29
Gender %			
Female	34.2	24.6	0.28
Male	65.8	75.4	
Cannabis use in past 30 days:			
# of days	N (%)	N (%)	
0	7 (18.4)	24 (42.1)	0.33
1-3	5 (13.2)	12 (21.1)	
4-8	5 (13.2)	6 (10.5)	
9-15	6 (15.8)	8 (14)	
15-29	2 (5.3)	1 (1.8)	
Missing	13 (34.2)	6 (10.5)	

Performance on RAVLT

Immediate recall [sum of trials 1 to 5] (Fig. 2A)

In experiment #1, subjects recalled 59.17 ± 10.08 words on the THC condition and 59.40 ± 9.41 words on the placebo condition ($p = 0.99$). In experiment #2, subjects recalled 44.86 ± 13.09 words on the THC condition and 55.50 ± 9.64 words on the placebo condition ($p < 0.0001$).

There were significant main effects of drug [$F(1,93) = 26.5$, $p < 0.0001$] and experiment [$F(1,93) = 17.8$, $p < 0.0001$], and a significant drug x experiment interactive effect [$F(1113) = 26.7$, $p < 0.0001$]. Post hoc analyses revealed that im- mediate recall was impaired by THC condition only in experiment #2 ($p < 0.0001$), but not in experiment #1 ($p = 0.99$) (Table 2).

Rate of learning [trial 5 minus 1] (Fig. 2B)

In experiment #1, the learning rate was 6.03 ± 2.08 word/trial on the THC condition and 5.37 ± 1.77 word/trial on the placebo condition ($p = 0.18$). In experiment #2 the learning rate was 5.91 ± 2.88 word/trial on the THC condition and 5.52 ± 1.85 word/trial on the placebo condition ($p = 0.31$). There were no significant main effects of drug [$F(1,93) = 2.8$, $p = 0.09$] or experiment [$F(1,93) = 0$, $p = 0.9$], or significant drug x experiment interactive effects [$F(1,93) = 0.1$, $p = 0.68$], indicating that THC did not affect the rate of learning across the five trials (Tables 2).

Table 2 AVLT scores

	THC Mean (SD)	Placebo Mean (SD)	p-value
Experiment #1 (post-encoding THC condition)			
Total Immediate Recall	59.17 (10.08)	59.40 (9.41)	0.99
Short Delayed Recall(trial 7)	13.14 (2.24)	12.80 (2.62)	0.54
Long Delayed Recall(trial 8)	10.79 (3.61)	11.00 (3.79)	0.67
Rate of Learning (trial 5-1)	6.03 (2.08)	5.37 (1.77)	0.18
Experiment #2 (pre-encoding THC condition)			
Total Immediate Recall	44.86 (13.09)	55.50 (9.64)	<0.0001
Short Delayed Recall(trial 7)	9.33 (4.22)	11.69 (2.72)	<0.0001
Long Delayed Recall(trial 8)	9.04 (4.29)	11.45 (2.90)	<0.0001
Rate of Learning (trial 5-1)	5.91 (2.88)	5.52 (1.85)	0.31

AVLT: Rev Auditory Verbal Learning Test; THC: Delta-9- tetrahydrocannabinol

Short delayed recall [trial 7] (Fig. 2C)

In experiment #1, on the short delayed recall, subjects recalled 13.14 ± 2.24 words on the THC condition and 12.80 ± 2.62 words on the placebo condition ($p = 0.54$). In experiment #2, subjects re- called 9.33 ± 4.22 words on the THC condition and 11.69 ± 2.72 words on the placebo condition ($p < 0.0001$). There were significant main effects of drug [$F(1,93) = 10.1$, $p = 0.001$] and experiment [$F(1,93) = 16.53$, $p < 0.0001$], and a significant drug × experiment interaction effect [$F(1,93) = 17.19$, $p < 0.0001$]. Post hoc analyses revealed that short delayed recall was impaired by THC only in experiment #2 (< 0.0001).

Long delayed recall [trial 8] (Fig. 2D)

In experiment #1, on the long delayed recall, subjects recalled 10.79 ± 3.61 words on the THC condition and 11.00 ± 3.79 words on the placebo condition ($p = 0.67$). In experiment #2, subjects re- called 9.04 ± 4.29 words on the THC condition and 11.45 ± 2.90 words on the placebo condition ($p < 0.0001$). There was no significant main effect of experiment [$F(1,92) = 1$, $p = 0.32$] observed. However, there was a significant main effect of drug [$F(1,92) = 13.9$, $p = 0.0003$] and a significant drug x experiment interactive effect [$F(1,92) = 9.32$, $p = 0.003$. Post hoc analyses revealed that long delayed recall was impaired by THC only in experiment #2 (< 0.0001).

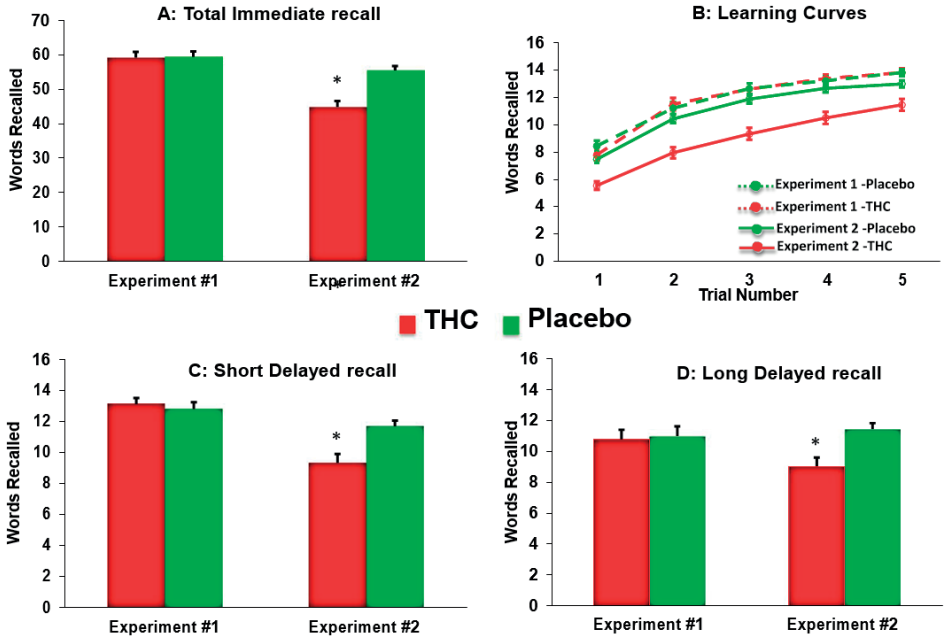

Figure 2 Effects of THC and placebo on Rey-Auditory Verbal Learning Test outcomes:

A) Total immediate Recall, B) Learning Curves, C) Short Delayed Recall and D) Long Delayed Recall. Error bars represent standard error.

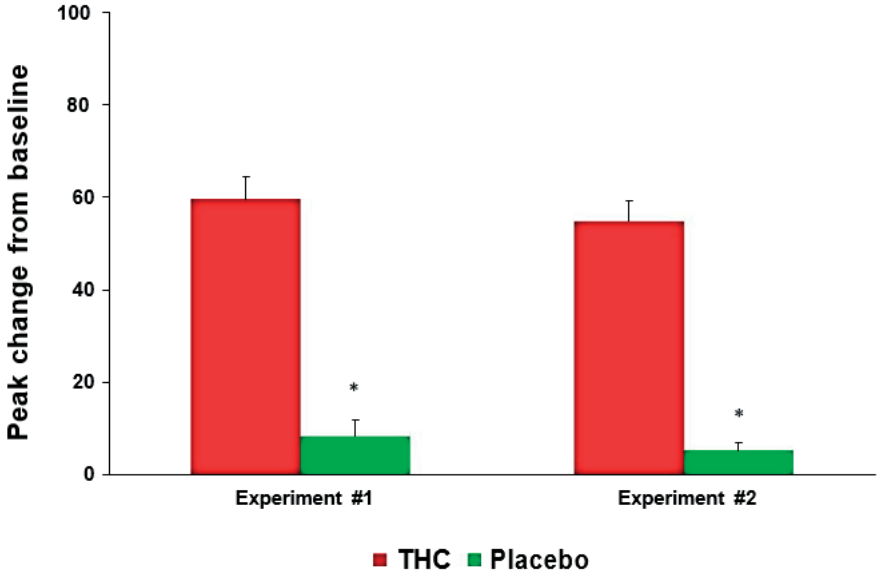

Figure 3

Effects of THC and placebo on self-reported "high" measured by a Visual Analog Scale (0 – 100).

3.3. Subjective effects (Fig. 3)

Both experiments produced similar THC induced subjective effects as measured by "high" on the VAS. The VAS "high" mean ± SD were 61.21 ± 28.64 with THC vs 9.97 ± 21.05 with placebo in experiment #1; and 58.44 ± 28.37 with THC vs 8.58 ± 15.34 with placebo in experiment #2. As expected there was a significant effect of dose (active THC versus placebo) [$F(1,92) = 204.9$, $p < 0.0001$] indicating that THC produces an altered state compared to placebo. There were no significant main effects of experiment [$F(1,92) = 0.86$, $p = 0.35$] on peak change from baseline on VAS "high" and no experiment X drug interaction [$F(1,92) = 0.04$, $p = 0.83$].

Subgroup analyses

To eliminate the possibility that the differences in learning and memory could have been related to differences in characteristics of the samples, we reanalyzed the data collected in 16 subjects who participated in both experiments. The findings persist that recall was impaired only in experiment #2 when the RAVLT was administered to subjects under the influence of THC.

Table 2 AVLT scores

	THC Mean (SD)	Placebo Mean (SD)	p-value
Experiment #1 (post-encoding THC condition)			
Total Immediate Recall	59.17 (10.08)	59.40 (9.41)	0.99
Short Delayed Recall(trial 7)	13.14 (2.24)	12.80 (2.62)	0.54
Long Delayed Recall(trial 8)	10.79 (3.61)	11.00 (3.79)	0.67
Rate of Learning (trial 5-1)	6.03 (2.08)	5.37 (1.77)	0.18
Experiment #2 (pre-encoding THC condition)			
Total Immediate Recall	44.86 (13.09)	55.50 (9.64)	<0.0001
Short Delayed Recall(trial 7)	9.33 (4.22)	11.69 (2.72)	<0.0001
Long Delayed Recall(trial 8)	9.04 (4.29)	11.45 (2.90)	<0.0001
Rate of Learning (trial 5-1)	5.91 (2.88)	5.52 (1.85)	0.31

AVLT: Rev Auditory Verbal Learning Test; THC: Delta-9- tetrahydrocannabinol

Discussion

These experiments are among the first attempts in humans to investigate the effects of i.v. THC on encoding, consolidation and retrieval of verbal memory. Both the immediate and delayed recall of verbal information that was presented prior to the administration of THC (i.e., encoding prior to THC administration) were unimpaired, even when delayed recall occurred under the influence of THC (long delayed recall in experiment #1). However, when verbal information was presented under the influence of THC (i.e., encoding after THC administration), immediate and delayed recall were impaired. These results support the hypothesis that THC specifically impairs encoding but not retrieval of verbal information.

The dose range of THC used in these experiments have been shown in previous studies at our center to impair performance on verbal memory tasks when administered prior to the encoding of information (D'Souza et al., 2004, 2005, D'Souza et al., 2012). Furthermore, the dose range also produces subjective and other effects consistent with the known effects of cannabis. Thus the relative lack of effects of THC on recall cannot be explained by dose, but rather by the timing of ad- ministration in relation to encoding.

Of note, the impairments in immediate as well as delayed recall in experiment #2 were statistically as well as clinically significant. Using a criterion of > 1 SD below the mean (Solowij, 1998), 47.8% of subjects in experiment #2 showed clinically significant THC-induced impair- ments on total immediate recall and 39.1% showed impairments on long delayed recall. This compares to 14.3% of subjects in experiment #1 who

scored > 1 SD below the mean on immediate and 14.7% on delayed recall. Using this approach, a greater proportion of subjects in experiment #2 showed clinically significant impairments.

Previous studies on the effects of CB1R agonists on memory in hu- mans have used THC or cannabis. These studies had designs that did not permit differentiating effects of cannabinoids on encoding and retrieval. The results of the current study provide evidence that THC impairs encoding but not retrieval of verbal information. These results are consistent with preclinical studies demonstrating that hippocampal firing during encoding is impaired both by THC and by the synthetic CB1R agonist WIN 55,212–2 during a delayed non-match-to-sample task (Hampson and Deadwyler, 1999), such that rats fail to learn under the influence of CB1R agonists. Two human neuroimaging studies have examined the effects of THC on brain activation during an associative memory task (Bhattacharyya et al., 2009a; Bossong et al., 2012). In both studies, THC selectively reduced brain activation during encoding of pictures, supporting our finding of impaired brain activity during en- coding associated with THC despite the fact that neither of the studies detected any impairment in performance when THC was present.

Our findings should be interpreted in the context of neurobiological processes involved in encoding, consolidation and retrieval of verbal memory. Brain regions involved in verbal memory encoding include medial temporal lobe (MTL) structures such as hippocampus (specifically, the posterior hippocampus (Fernandez et al., 1998)); and pre- frontal cortex (PFC) (specifically, the ventrolateral PFC (involved in selecting and maintaining incoming information) and dorsolateral PFC (involved in organizing and forming associations between items during encoding)) (Blumenfeld and Ranganath, 2007; Kim, 2011; Spaniol et al., 2009; Wagner et al., 2016). Brain regions involved in retrieval include left superior parietal and dorsolateral and anterior PFC regions (Kim, 2011; Spaniol et al., 2009). Of note, the hippocampus is less in- volved in retrieval, than in encoding (Spaniol et al., 2009). Phase synchronization of neural oscillations in the theta and gamma frequency is known to play an important role in encoding and retrieval (Babiloni et al., 2009; Shaikhouni et al., 2016; Watrous et al., 2013).

An integrative model of memory encoding, storage and retrieval of declarative information posits that encoding is primarily dependent on the hippocampus, related MTL structures, and PFC. Information is subsequently sparsely distributed to non-hippocampal sites such as the PFC and parietal cortex where different aspects of the memory is stored. This is accomplished through phase synchronization in reverberating neural circuits involving hippocampus and PFC, parietal regions (Daumas et al., 2005; Watrous et al., 2013). As consolidation of memory in extra-hippocampal sites takes place, the hippocampal memory decays, but retains a “memory-trace” that can be activated during retrieval (Kandel et al., 2014; Nadel and Bohbot, 2001; Ryan et

al., 2010). Consistent with this model, a functional imaging study that examined the effects of oral THC on memory on a paired association learning task, found that THC (Bhattacharyya et al., 2009b) impaired MTL function during encoding, and disrupted left dorsoanterior cingulate and medial PFC function during recall (Bhattacharyya et al., 2009b). Another functional imaging study that used a pictoral memory task, noted similar disrupted right MTL and inferior frontal activity during encoding, and increase in activation in bilateral pre- cuneus and cuneus during recall (Bossong et al., 2012). These two studies however did not show impairment on test-performance on memory tasks. So it in unclear if similar findings are seen when there is significant impairment in encoding or retrieval of verbal memory.

Animal studies suggest that the impairment in encoding may be related to THC interference with the endocannabinoid-mediated mod- ulation of synaptic neurotransmission and long term depression, which are critical for learning and memory (Heifets and Castillo, 2009). An additional mechanism is suggested by the critical role of, the en- docannabinoid system to the formation of long-term potentiation in the lateral perforant path (LPP), one of two cortical inputs to the hippo- campus, that is also implicated in the formation of episodic memory in humans (Reagh and Yassa, 2014; Wang et al., 2016)

CB1 receptors are expressed in high density in the hippocampus, PFC and parietal regions (Eggan and Lewis, 2007). In the hippocampus, CB1 receptors are predominantly located on GABAergic interneurons and on glutamatergic axon terminals (Kruk-Slomka et al., 2016) THC has also been shown to disrupt phase synchronization of theta (Stone et al., 2012) and gamma oscillations (Cortes-Briones et al., 2015), Given this, it is likely that THC impairs encoding through actions in the left PFC and hippocampus, areas that are known to be rich in CB1 receptors (Mackie, 2005) and caused by abnormal neural synchrony. An additional consideration is the difference between effects of THC from that of endocannabinoids (eCBs). eCBs are produced on demand, actions typically in response to neurotransmitter release, and after producing effects, are quickly inactivated via a retrograde mechanism. In contrast, THC an exocannabinoid, unlike eCBs, is not inactivated immediately and its effects are non-physiological.

Strengths and limitations

Strengths of this study include the design (randomized, double- blind, and placebo-controlled), the standardized delivery of THC, the use of alternative and equivalent versions of the RAVLT in order to avoid practice effects, the inclusion of similar groups, characterization and inclusion of potential confounding variables in the analyses, and the large sample size. Measurement of recent cannabis exposure (over the last 30 days) permitted controlling for tolerance to the effects of THC.

Several limitations need to be considered. The between-subject de- sign is not a powerful as a within- subject design. However, in the 16 subjects who participated in both experiments i.e., within-subjects, the findings prevailed. There were differences in the timing of some of the tasks. The differences in testing delayed recall across experiments were necessitated by a need to minimize confounding due to THC's effects on attention and other psychotomimetic effects. If the same time period was kept between learning and delayed recall across the two studies, delayed recall in experiment #1 would have been measured at the peak of THC's effects on attention and psychotomimesis, while in experiment #2 delayed recall would be tested well after the acute effects of THC had worn off. That is, subjects had to retain information and recall for a longer period of time (155 min) in experiment #1 in contrast to 95 min in experiment #2. This would predict worse performance in experiment #1 unlike what we observed. While the dose of THC used across experiments were similar, they were not identical. It should be noted that at a dose of 0.028 mg/kg (D'Souza et al., 2008), a dose lower than the one used in experiment #1 (0.03 mg/kg), THC produced robust effects on immediate and delayed recall. Thus, in that study (D'Souza et al., 2008), learning occurred under the influence of THC in contrast to experiment #1. That a similar dose of THC produced robust impairments suggests that the lack of impairments in experiment #1 is likely a result of the timing of learning in relation to THC. Plasma THC levels were not measured in this study. However, that in both experiments VAS "high" scores were similar suggest that the level of exposure (dose) was similar across studies. Some subjects studied had been exposed to cannabis in the past 30 days. Thus, one cannot completely rule out the possibility that residual effects of cannabis use influenced memory performance. However, this is unlikely to explain the effects of THC on memory encoding since there were no group differences in cannabis exposure between the two experiments. Measurement of other types of memory (e.g. visual, spatial, emotional) would have further strengthened our results.

Conclusions, implications and future directions

THC impairs recall of information encoded during drug effects. This result suggests that people should avoid learning new information under the influence of CB1R agonists. This is especially relevant to high school and college-age students who have to learn new information related to their academic demands. Young adults consistently have a higher prevalence of cannabis use than other age groups and represent the overwhelming majority of the cannabis-using population (CBHSQ, 2015; Ernst et al., 2012). For example, in the Monitoring the Future survey, 19.8% of young adults aged 18–25 years and 7.0% of adoles- cents aged 12–17 years reported cannabis use in the past month (Johnston et al., 2016). Deficits in learning related to cannabinoids may contribute to the reported reductions in academic performance (Silins et al., 2015).

Future studies should include ecologically valid measures of learning new information. Some evidence suggests a correlation be- tween chronic use of cannabis and poor academic performance (Fergusson et al., 2003; Macleod et al., 2004; Windle and Wiesner, 2004) and also reductions in intelligence quotient (Meier et al., 2012). However, whether the effects of cannabis on academic performance are related to THC-induced deficits in the encoding of verbal information needs to be further studied. Future studies should also be designed to examine the dose related effects of THC on the encoding of non-verbal information and to determine the effects of THC on consolidation of memory.

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Ethics statement

The authors declare that the manuscript is based on original re- search that has not been published. The research was approved by in- stitutional ethics review board and all participants provided informed consent.

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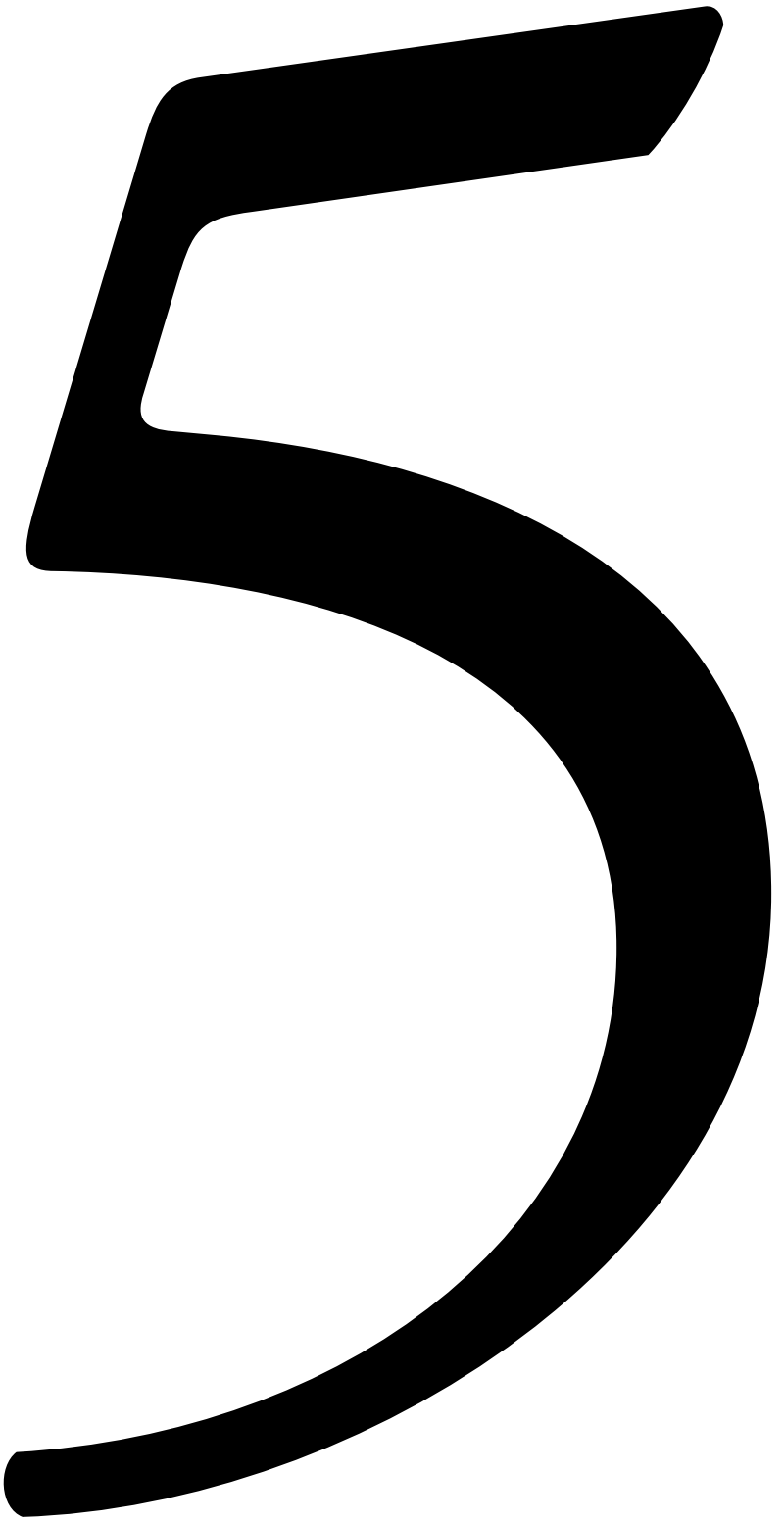

Chapter 5

Age, gender and body-mass-index relationships with in vivo CB1 receptor availability in healthy humans measured with [^{11}C]OMAR PET

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Abstract

Brain cannabinoid 1 receptors (CB1Rs) contribute importantly to the regulation of autonomic tone, appetite, mood and cognition. Inconsistent results have been reported from positron emission tomography (PET) studies using different radioligands to examine relationships between age, gender and body mass index (BMI) and CB1R availability in healthy individuals. In this study, we examined these variables in 58 healthy individuals (age range: 18–55 years; 44 male; BMI=27.01±5.56), the largest cohort of subjects studied to date using the CB1R PET ligand [^{11}C]OMAR. There was a significant decline in CB1R availability (V_T) with age in the pallidum, cerebellum and posterior cingulate. Adjusting for BMI, age-related decline in V_T remained significant in the posterior cingulate among males, and in the cerebellum among women. CB1R availability was higher in women compared to men in the thalamus, pallidum and posterior cingulate. Adjusting for age, CB1R availability negatively correlated with BMI in women but not men. These findings differ from those reported using [^{11}C]OMAR and other radioligands such as [^{18}F]FMPEP-d2 and [^{18}F]MK-9470. Although reasons for these seemingly divergent findings are unclear, the choice of PET radioligand and range of BMI in the current dataset may contribute to the observed differences. This study highlights the need for cross-validation studies using both [^{11}C]OMAR and [^{18}F]FMPEP-d_2 within the same cohort of subjects.

Keywords: Cannabinoid 1 receptor, [^{11}C]OMAR, Age effect, Gender effect, Body-mass index, Healthy humans, Positron Emission Tomography

Introduction

The cannabinoid 1 receptor (CB1R) is a key component of the endocannabinoid system and contributes importantly to the regulation of physiological processes such as autonomic tone, pain, appetite, mood, cognition, and anxiety (Radhakrishnan and Ross, 2018). The receptor is a potential therapeutic target for multiple conditions including obesity, pain disorders, epilepsy, Alzheimer's disease and substance-use disorders (Paez and Campillo, 2018). However, current evidence is mixed regarding relationships between gender, age, body-mass index (BMI) and CB1R availability.

Animal studies have noted age- and gender-specific differences in response to CB1R agonists (Feliszek et al., 2016; Garcia-Cabrerizo and Garcia-Fuster, 2016; Rubino et al., 2015; Silva et al., 2015). For example, in adolescent female (but not male) rats, repeated exposure to the CB1R agonist AM251 prevented developmental decreases in glutamatergic markers within the prefrontal cortex into adulthood (Rubino et al., 2015). Studies of CB1R mRNA and protein expression in rats have shown region-specific changes with age, with age-related increases in some regions and decreases in others (Berrendero et al., 1998a; Liu et al., 2003a). A positron-emission tomography (PET) imaging study in rats, which also included concomitant ex-vivo autoradiography examination of brain tissues, showed a global increase in CB1R availability from adolescence to adulthood (Verdurand et al., 2011). However, it is not clear whether these changes will be the same in humans, as there are notable interspecies differences in CB1R distribution (McPartland et al., 2007).

Data on gender differences in CB1 protein, receptor density, and mRNA expression in animal studies is mixed. While higher CB1 protein has been noted in males compared to females in unstressed conditions (Reich et al., 2009), under conditions of stress males had lower and females had higher CB1 protein (Reich et al., 2009). CB1 receptor density has also been reported to be comparable among males and females (Farquhar et al., 2019). In another study, CB1R mRNA expression was found to be lower in females compared to males in the prefrontal cortex of rats, but there were no differences in the caudate, putamen, nucleus accumbens, ventral tegmental area and hippocampus (Castelli et al., 2014). In contrast, a study in adolescent rats, found that CB1 mRNA level was significantly higher in all brain regions in females compared to males (Xing et al., 2014).

In humans, data regarding potential age and gender influences in CB1R levels are also equivocal. For example, one postmortem study found that CB1R mRNA expression decreases from early life to adult- hood (Long et al., 2012), while another post-mortem study found in- creased CB1R density from childhood to adulthood (Mato et al., 2003). A similar pattern of seemingly discrepant results has also been seen from human

PET imaging studies. A study using the agonist radioligand [^{18}F]MK-9470 found an age-related increase in CB1R binding in the basal ganglia, lateral temporal cortex, and limbic system in females but not males (Van Laere et al., 2008). In contrast, a study using [^{11}C]OMAR reported an age-related decrease in CB1R binding in the globus pallidus in males (Wong et al., 2010). Gender effect was also noted in another study using [^{11}C]OMAR where females displayed greater CB1R binding than males (Normandin et al., 2015). In contrast, a study using [^{18}F]FMPEP-d_2 found 41% higher CB1 availability in males (Laurikainen et al., 2019).

In regard to food intake and body-mass index (Cota et al., 2003; DiPatrizio and Piomelli, 2012), animal studies show that CB1Rs con- tribute importantly to adipogenesis and lipogenesis in diet-induced obesity (Bensaid et al., 2003; Tedesco et al., 2008). The one PET imaging study to date using the CB1R agonist tracer [^{18}F]MK-9470 showed an inverse association between BMI and CB1R availability in the hypothalamus and brainstem (Ceccarini et al., 2016)

The current study aims to examine the relationships between age, gender, BMI and CB1R availability in humans. As prior studies have often been limited by small sample sizes, the potential individual contributions of age, gender and BMI on CB1 receptor availability could not be easily discerned. The current study provides an opportunity to extend prior research by examining CB1R availability, using [^{11}C]OMAR, a reversible, antagonist tracer with a validated kinetic analysis method (Normandin et al., 2015), in the largest sample of healthy human subjects to date.

Methods

Participants

Data were collected from multiple studies using [^{11}C]OMAR PET in healthy adult volunteers (N = 58; mean age =29.12 ± 8.23 years; range: 18–55 years) and combined for analysis in this study. Subjects were recruited from the greater New Haven area by paper and internet advertisements, as well as through personal referrals. All subjects completed a comprehensive screening assessment that included a clinical interview (Structured Clinical Interview for DSM IV Disorders (SCID)), physical ex- amination with medical history, routine blood tests, electrocardiogram and urine toxicology. Women of child bearing potential were required to use effective contraception during participation in the PET scan. Individuals were excluded if evaluation revealed a diagnosis of a current and/or lifetime psychiatric disorder; current or past serious medical or neurological illness (including a history of head injury with loss of consciousness); metal in body which would result in MRI contraindication; a history of substance abuse or dependence or any illicit drug use in the past 3 months.

The study was performed under a repository protocol that allowed a re-analysis of published data from multiple studies that were approved by the Yale Human Investigation Committee, the Yale University Radiation Safety Committee, the Yale-New Haven Hospital Radioactive Drug Research Committee, and the Yale MRI Safety Committee. Although sub- jects were recruited from multiple protocols, all radiochemistry, scanning and blood sampling protocols and data analysis methodology were standardized. The study was performed in accordance with Good Clinical Practice guidelines, regulatory requirements and the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written in- formed consent was initially obtained from all participants at the be- ginning of screening after a full explanation of anticipated study procedures.

Radiochemistry and image acquisition

[^{11}C]OMAR was prepared using previously described procedures (Horti et al., 2006) adapted for automated production on the GE TRACERlab FXC-Pro synthesis module (GE Healthcare, Milwaukee, WI, USA). Radiochemical purity was >90%. The injected activity dose was 552.44 ± 137.80 MBq (14.93 ± 3.72mCi) with mass dose of 0.04 ± 0.04 μg/kg.

Structural MRI (magnetic resonance imaging) was performed using a Siemens 3T system (Siemens Medical Solutions, Malvern, Pennsylvania) and a standard T1-weighted anatomical sequence for purposes of excluding individuals with anatomical abnormalities and anatomically coregistering with PET scans. The dimension and voxel size of MR im- ages were 256 × 256 × 176 voxels and 0.98 × 0.98 × 1.0 mm^3 , respectively.

Subjects underwent dynamic PET scans in a HRRT scanner (Siemens Medical Systems, Knoxville, TN) in 3D mode. Before each PET scan, a transmission scan was acquired for attenuation correction. For each PET scan, [^{11}C]OMAR was administered as a slow bolus intravenous infusion over 1 min, coinciding with the beginning of acquisition. Emission data were obtained in list mode for 120 min. Subject motion was monitored using an optical system (Polaris Vicra, Northern Digital Incorporated, Waterloo, Ontario, Canada) positioned behind the PET scanner, which at 50 ms intervals recorded the three-dimensional position and orientation of the infrared reflective tool mounted rigidly to the subject's head using a Lycra cap and Coban self-adherent wrap. The optical tracking data were synchronized to the listmode emission measurements.

Radioactivity concentration in arterial whole blood was measured continuously during the first 7 min after [^{11}C]OMAR administration using an integrated peristaltic pump and radioactivity detection system (PBS101, Veenstra Instruments, Joure, The Netherlands). Afterward, discrete samples were manually drawn at 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 min after injection. The whole blood and plasma radioactivity in each manual sample was measured using a gamma counter (Wizard

1480, PerkinElmer, Waltham, MA, USA) and converted to con- centration according to the weight and density of the measured aliquot. The ratio of radioactivity in plasma and whole blood was determined from the discrete samples and used to scale the whole blood concentration measured by the continuous counter in the first few minutes after tracer administration. Samples acquired at 5, 15, 30, 60, 90 and 120 min were analyzed by the column-switching high-performance liquid chromatography (HPLC) method (Hilton et al., 2000) to deter- mine the fraction of unmetabolized radiotracer as described previously (Normandin et al., 2015). The unmetabolized parent fraction was calculated as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected and fitted with an inverted gamma function. The parent fraction curve was also normalized by the time-varying extraction efficiency of radioactivity for the corresponding filtered plasma sample. The final metabolite- corrected arterial plasma input function was calculated as the point-by- point product of the total plasma radioactivity concentration curve and the parent fraction curve.

Image processing

Dynamic scan data were reconstructed with corrections for attenuation, normalization, scatter, randoms, and dead time using the ordered subset-expectation maximization (OSEM) algorithm (4 iterations, 16 subsets). PET images were reconstructed into 33 frames, containing 63 axial slices of 128 × 128 voxels each (2.1 × 2.1 × 2.4 mm^3). Motion correction was applied to the dynamic images, using a mutual- information algorithm (FSL-FLIRT version 3.2, Analysis Group, FMRIB, Oxford, UK) by frame-by-frame registration to a summed image (0-10 min post-injection). A summed image was created from the motion- corrected data and registered to the subject's MR anatomical image (6- parameter affine registration) which was then registered to an MR template using a non-linear transform with BioImage Suite.

Regions of interest (ROIs) were defined using the Anatomical Automatic Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Selected regions included those where CB1R binding has been previously associated with age, gender, and BMI (i.e., amygdala, anterior and posterior cingulate cortex, anterior and posterior insula, caudate, cerebellum, frontal cortex, hippocampus, hypothalamus, occipital cortex, parietal cortex, posterior cingulate cortex, putamen, temporal cortex, and thalamus).

To account for possible partial-volume effects, a binary gray-matter mask (GMM) was used with individual MR images segmented with FAST (FMRIB's Automated Segmentation Tool, version 3.1) to obtain masking of gray matter, white matter, and cerebrospinal fluid. The individual GMMs were then applied to the AAL regions to obtain the mean cortical regional values (subcortical areas were not corrected because of small size). Partial volume correction (PVC) was also applied using the Giovacchini

algorithm (Giovacchini et al., 2009). However, since the tissue differentiation for the pallidum was inadequate to implement the Giovacchini algorithm, this ROI was not included in PVC analyses.

Time-activity curves (TACs) were fitted with the multilinear analysis-1 method (MA1) (Ichise et al., 2002) ($t^* = 30$) using the metabolite-corrected arterial input function over 120 min post-injection to estimate regional volume of distribution (V_T) for each ROI.

Statistical Analysis

All outcomes were assessed for normality prior to analysis using nor- mal probability plots and Kolmogorov test statistics. Since BMI was not normally distributed, log transformation was performed. Main and interactive effects of age, gender and BMI across ROIs were examined using linear mixed models with region modeled as a within-subjects factor, gender as a between-subjects factor, and age and BMI as continuous covariates. Post-hoc analyses were performed to examine whether significant interactive effects persisted after controlling for other variables. Similarly, partial correlations of age, gender and BMI with V_T were examined while adjusting for other variables. The interaction between age and region was modeled, and slopes for regions were estimated post-hoc in order to calculate relative change per decade (RCD). Correlations between age and [11C]OMAR V_T were calculated separately for each region, and p values unadjusted for multiple comparisons are reported. All statistical tests were two-tailed and considered statistically significant at $\alpha = 0.05$. All analyses were performed using SAS, version 9.3 (SAS Institute Inc., Cary, NC).

Results

The study sample included 58 healthy volunteers (44 male + 14 female; mean age =29.12 ± 8.23 years; range: 18–55 years; mean BMI = 27.01 ± 5.56, range 18.21–40.20). Males and females did not differ in age, BMI, or [11C]OMAR administration (Table 1).

Table 1 Demographics of the sample

	Male (n = 44)	Female (n = 14)	P value
Age (years)	29.61 ± 8.54	27.57 ± 7.23	0.42
BMI (kg/m^2)	27.53 ± 5.75	25.37 ± 4.71	0.21
Injected activity dose (mCi)	14.79 ± 3.52	15.27 ± 3.86	0.66
Mass dose (μg/kg)	0.04 ± 0.03	0.04 ± 0.03	0.85

Effect of Age on CB1 availability: Examining the multivariate effects of age and gender on CB1 availability adjusting for BMI, there was a significant age x ROI interaction ($F_{(15,53)} = 3.66$, $p < 0.001$). In individual ROIs, a negative effect of age on V_T was seen in the posterior cingulate cortex (unadjusted r = -0.30, p = 0.021; adjusted for gender and BMI r = - 0.29, p = 0.031; RCD = -9%), pallidum (unadjusted r = -0.26, p = 0.050; adjusted for gender and BMI r = -0.25, p = 0.068; RCD = -11%), and cere- bellum (unadjusted r = -0.25, p = 0.060; adjusted for gender and BMI r = -0.24, p = 0.070; RCD = 7%) (Fig. 1 a–c). Post-hoc analysis of gender- specific age effects, adjusted for BMI, revealed that age was negatively correlated with V_T in the posterior cingulate cortex (r = -0.31, p = 0.045) in men, but not in women; and age was negatively correlated with V_T in the cerebellum (r = -0.59, p = 0.03) in women, but not in men. With respect to age effects on [^{11}C]OMAR V_T, analyses of PVC data were consistent in showing a significant effect of age x ROI ($F_{(14,53)} = 2.66$, $p < 0.01$) with and an additional age x gender x ROI interaction ($F_{(14,53)} = 2.03$, p = 0.03). There were negative associations between age and PVC V_T in the posterior cingulate cortex (unadjusted r = -0.32, p = 0.01; adjusted for gender and BMI r = -0.30, p = 0.02; RCD = -11%) and cerebellum (unadjusted r = -0.28, p = 0.03; adjusted for gen- der and BMI r = -0.28, p = 0.04; RCD = -10%) (Supplemental Fig. 2a, b). Gender-specific age effects in the PVC data were also consistent in showing that, adjusting for BMI, age was negatively correlated with V_T in the posterior cingulate cortex (r = -0.29, p = 0.063) in men, but not in women; and age was negatively correlated with V_T in the cerebellum (r = -0.67, p = 0.01) in women, but not in men.

Multivariate effects of BMI and gender adjusting for age: Examining the multivariate effects of BMI and gender on CB1 availability adjusting for age, identified a trend towards significant effect of gender (F (1,53) = 3.60, p = 0.063) and a gender x BMI interaction (F (1,53) = 3.29, p = 0.075) on [^{11}C]OMAR V_T (Tables 2A and 2B). Post-hoc analysis of gender-specific BMI effects, adjusted for age, revealed that BMI was negatively correlated with V_T in women but not men, across the following ROIs: frontal (r = -0.56, p = 0.04), temporal (r = -0.56, p = 0.04), anterior cingulate (r = -0.62, p = 0.03) and thalamus (r = -0.56, p = 0.04).

Table 2A Multivariable results of log(BMI) and Gender effects controlling for Age.

	GM		PVC	
	F	**P**	**F**	**P**
Gender	3.60[a]	0.063	3.83[a]	0.056
Region	1.26[b]	0.262	1.96[c]	0.041
Gender*Region	1.44[b]	0.162	1.82[c]	0.060
log(BMI)	0.71[a]	0.404	0.72[a]	0.398
log(BMI)*Gender	3.29[a]	0.075	3.56[a]	0.065
log(BMI)*Region	1.35[b]	0.208	2.04[c]	0.032
log(BMI)*Gender*Region	1.51[b]	0.137	1.79[c]	0.065
Age	4.18[a]	0.046	0.53[a]	0.470

GM = grey-matter masked V_T ; PVC = partial-volume corrected V_T

[a] df=1,53.

[b] df=15,53.

[c] df=14,53.

Table 2B Multivariable results of Age and Gender effects controlling for log(BMI).

	GM		PVC	
	F	**P**	**F**	**P**
Gender	0.41[a]	0.525	0.26[a]	0.610
Region	12.78[b]	<0.001	10.83[c]	<0.001
Gender*Region	1.18[b]	0.313	1.55[c]	0.124
Age	1.15[a]	0.288	1.18[a]	0.282
Age*Gender	0.12[a]	0.725	0.06[a]	0.801
Age*Region	3.66[b]	0.000	2.66[c]	0.005
Age*Gender*Region	1.47[b]	0.151	2.03[c]	0.033
log(BMI)	1.79[a]	0.187	0.81[a]	0.372

GM = grey-matter masked V_T ; PVC = partial-volume corrected V_T

[a] df=1,53.

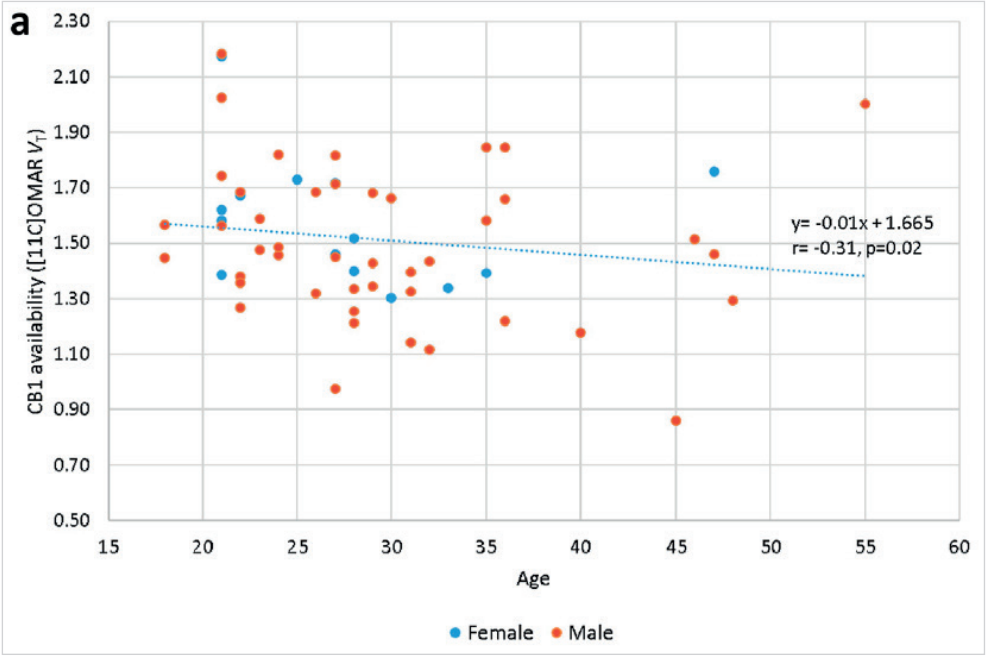
[b] df=15,53.

[c] df=14,53.

Effect of Gender on CB1 availability: With respect to gender effects on [11C]OMAR V_T, analyses of PVC data were largely consistent in showing trends toward an effect of gender (F (1,53) = 3.83, p = 0.056) and a gender x BMI interaction (F (1,53) = 3.56, p = 0.064). [11C]OMAR V_T in regions of interest were numerically higher in females compared to males across all ROIs (mean difference = 7%, range = 3.3-12.5%) (Fig. 2). Females displayed higher V_T in the thalamus (% difference = 11%, unadjusted p = 0.03; adjusted for age and BMI p = 0.051), and pallidum (% difference = 12.5%,

unadjusted p = 0.052; adjusted for age and BMI p = 0.066). PVC data were largely consistent in showing numerically higher V_T in females compared to males across all ROIs (mean absolute difference = 7%, range = 2.5-15.3%) in the posterior cingulate (12%, unad- justed p = 0.051; adjusted for age and BMI p = 0.084) and thalamus (10%, unadjusted p = 0.069, adjusted for age and BMI p = 0.095) (Supplemental Fig. 1).

Effect of BMI on CB1 availability: The PVC analyses showed an additional significant interaction of BMI x ROI (F (14,53) = 2.04, p = 0.03) and a trend toward a gender x BMI x ROI interaction (F (14,53) = 1.79, p = 0.064). Adjusting for age, BMI was negatively correlated with PVC V_T in women but not men, across the following ROIs consistent with the above analysis: frontal (r = -0.55, p = 0.049), temporal (r = -0.60, p = 0.03), thalamus (r = -0.56, p = 0.048) and anterior cingulate (r = -0.48, p = 0.096). Additionally, BMI was negatively correlated with PVC V_T among women in caudate (r= -0.64, p = 0.02) and hippocampus (r = -0.56, p = 0.045).



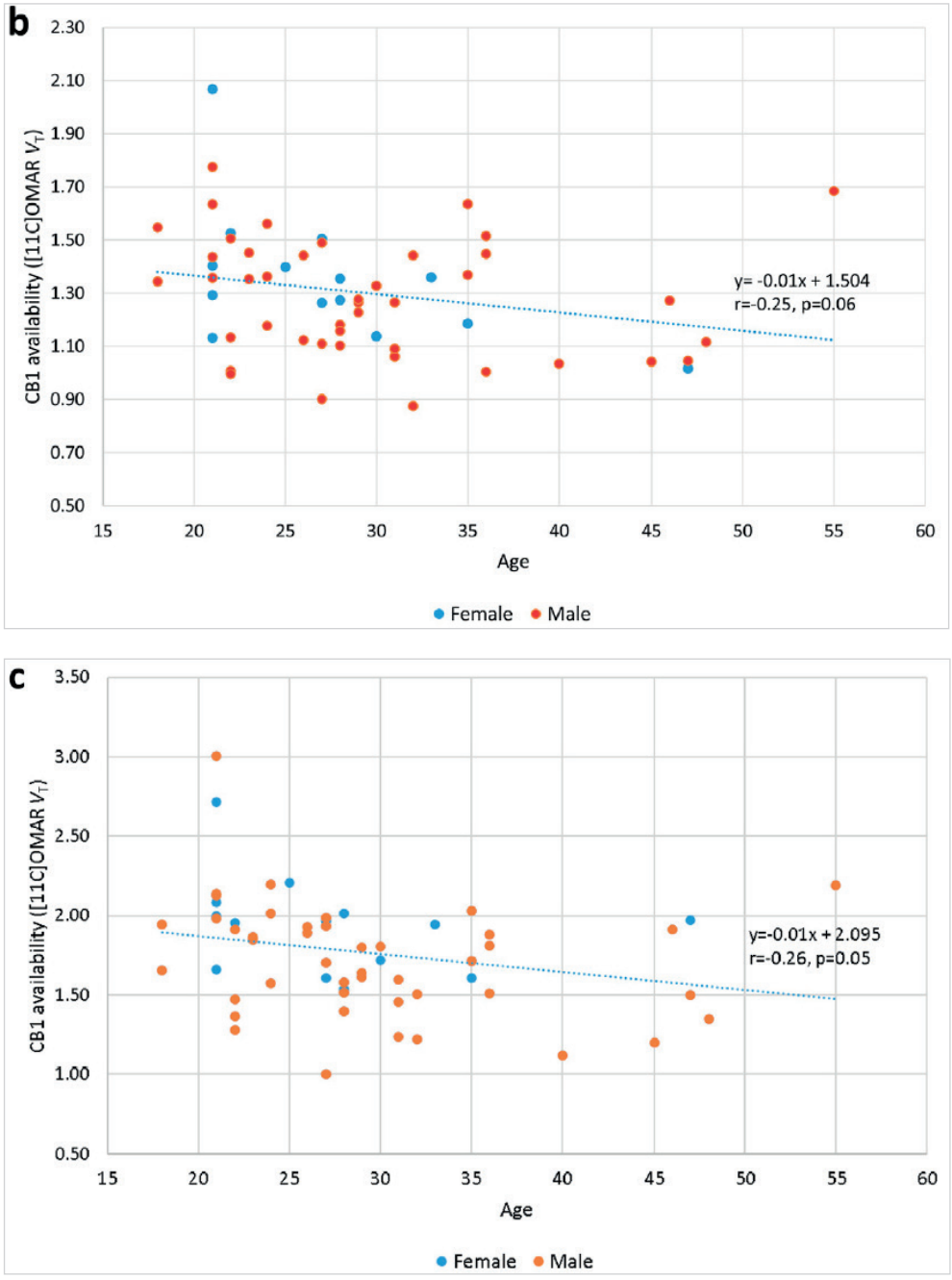


Figure 1.

(a): Correlation between CB1R availability in the posterior cingulate and age. The scatter-plot shows [^{11}C]OMAR V_T values in males (red) and females (blue) across age (in years) on the X- axis. (b): Correlation between CB1R availability in the cerebellum and age. The scatter-plot shows [^{11}C]OMAR V_T values in males (red) and females (blue) across age (in years) on the X-axis. (c): Correlation between CB1R availability in the pallidum and age. The scatter-plot shows [^{11}C]OMAR V_T values in males (red) and females (blue) across age (in years) on the X-axis.

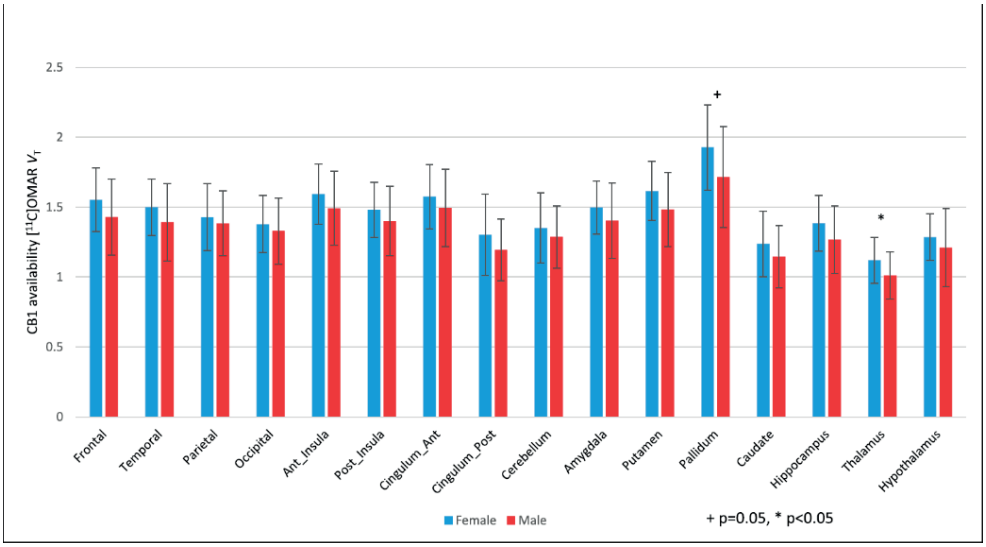


Figure 2.

CB1R availability in healthy males and females. This bar-graph shows average [^{11}C]OMAR V_T values (Y-axis) in males (red bars) and females (blue bars) across regions of interest (ROIs) on the X-axis. The error bars represent standard deviation (SD).

Discussion

This is the first in vivo study in humans to systematically examine age, gender and BMI-related effects on CB1R availability in a large sample (n = 58) using the PET ligand [^{11}C]OMAR. We found significant age effects on regional CB1R availability in the posterior cingulate cortex, cerebellum and pallidum characterized by decreased CB1R availability with increasing age. This was consistent with results following PVC. This is in contrast to a previous study using [^{18}F]MK-9470 (n = 50) (Van Laere et al., 2008) that found higher CB1R with increasing age only in women and in the basal ganglia, lateral temporal cortex, and hippocampus using the semi-quantitative standard uptake value (SUV) of [^{18}F]MK-9470 as outcome measure.

An in vitro autoradiography study of CB1R binding using [^{3}H]CP-55,940 in postmortem human brain samples (n = 3) found decreased CB1R availability with increasing age (Westlake et al., 1994). In an- other study in rats, CB1R mRNA levels were found to decrease in the hippocampus, deep layer VI of the cortex, and hypothalamus, but increase in the dentate gyrus and brainstem with increasing age (Berrendero et al., 1998b). On the other hand, a PET imaging study in mice aged 6, 9, 12 and 15 months found a significant increase in [^{18}F]FMPEP-d_2 binding with increasing age (Takkinen

et al., 2018). Similarly, a study of older-aged rats found that CB1R protein expression was significantly increased in the entorhinal and temporal cortices, but significantly decreased in the post-rhinal cortex, compared to young adult rats (Liu et al., 2003b). Other studies have found no change in CB1R availability with aging (Belue et al., 1995; Wang et al., 2003).

The precise reasons for the effects of age on CB1R in posterior cingulate, pallidum and cerebellum are not known. The posterior cingulate cortex is highly anatomically connected, has a high basal metabolic rate, is a central part of the default mode network and plays an important role in cognition (Leech and Sharp, 2014). The posterior cingulate cortex also shows differential gene expression in cognitively intact elderly individuals underscoring its importance is age-related cognitive decline (Kelley et al., 2022). It is also one of the first regions that show high amyloid deposition in Alzheimer's disease (Insel et al., 2020), and the functional connectivity between posterior cingulate cortex and the cere- bellum is implicated in cognitive impairment (Kang et al., 2021). Given the role of CB1R in cognition, it is possible that these current age-related findings are related. The pallidum is among the ROIs with the highest density of CB1R and also shows a linear volumetric decline with age (Tullo et al., 2019).

While we found evidence for gender-specific age effect with a decrease in CB1R availability in the posterior cingulate cortex in men and cerebellum in women, these findings are limited by the small sam- ple size in women. Regarding overall gender difference, we found that CB1R availability was higher in women compared to men across all ROIs, reaching statistical significance in the thalamus and pallidum. These differences were also present after PVC. The gender difference was smaller in this larger sample (i.e., 10-12% thalamus and pallidum, 7% mean across all ROIs) compared to those from earlier studies using [^{11}C]OMAR (Neumeister et al., 2013 ; Normandin et al., 2015). In the first study (n = 10), [^{11}C]OMAR V_T was found to be significantly greater in women by 23% (Normandin et al., 2015). In contrast, another PET study using [^{18}F]FMPEP- d_2 (n = 22) found 41% higher CB1 availabil- ity in males (Laurikainen et al., 2019). In the PET imaging study using [^{18}F]MK-9470, men were shown to have higher SUV in early adulthood (Van Laere et al., 2008). Findings from animal studies have also appeared mixed in this regard, showing both higher (Burston et al., 2010 ; Mateos et al., 2010) and lower (González et al., 2005) CB1R availability in females compared to males, with evidence of more efficient receptor signaling in females (Ru- bino and Parolaro, 2011). A study using [^{3}H]CP-55,940 binding found that striatal CB1R availability was higher in female than male rats in the striatum at postnatal day (PND) 20, and higher in males than females in the ventral mesencephalon across the lifespan from PND 10-70 (i.e., substantia nigra/ventral tegmental area) (Rodriguez de Fonseca et al., 1993). Another study using [^{3}H]CP-55,940 binding found that CB1R availability was higher in male rats in the hippocampus compared to female rats (Mateos et al., 2010). While

a study showed higher CB1R availability in male than female rats, the difference was no longer significant when female rats were ovariectomized and administered estrodiol exogenously (Castelli et al., 2014). It is possible that the lack of significant gender-related CB1R difference in our study may be related to other variables (e.g., use of oral contraceptives over time) for which information was not available. Another factor that has been shown to influence CB1R is exposure to stress. In a study in rats exposed to chronic unpredictable stress, females showed higher CB1 protein level compared to males, although the level was higher in males compared to females in unstressed conditions (Reich et al., 2009). Exposure to prenatal vs. postnatal stress is another factor that could potentially confound gender-related difference in CB1R availability (Dow- Edwards et al., 2016). Male rats exposed to prenatal stress had lower CB1R levels in the prefrontal cortex, striatum, amygdala and hippocampus, while female rats exposed to prenatal stress showed higher hip- pocampal CB1R levels. Male rats exposed to postnatal stress showed lower prefrontal CB1R levels, while female rats exposed to postnatal stress showed increased CB1R levels in the prefrontal cortex, striatum, amygdala and hippocampus.

Another interesting finding in our analysis is that BMI was found to be negatively correlated with CB1R availability in women but not in men. This was seen across different ROIs (frontal, temporal, thala- mus, anterior cingulate) and was consistent in the analyses with/without PVC. Additionally in the PVC analysis, BMI was also seen to be nega- tively correlated with CB1R availability in the caudate and hippo-cam- pus. In a post-hoc analysis we did not find the inverse association be- tween BMI and CB1R availability in the hypothalamus as reported by another study using [^{18}F] MK-9470 (Ceccarini et al., 2016) (n = 26). Of note, the study only included women and the range of BMI in that study was 18.5-26.6 kg/m 2 compared to 20.1-40.2 kg/m 2 in our study which included both men and women (Supplemental Fig. 3). However, on constraining the analysis to BMI less than 27, a significant positive cor- relation was noted between BMI and CB1R availability in the hypothalamus ($r = 0.45$, $p = 0.02$) (Supplemental Fig. 4). A similar positive correlation between BMI and CB1R availability was seen in anterior insula, posterior insula, putamen, and hippocampus, even after adjusting for age and gender (Supplemental Table 1). This suggests that the normal homeostatic relationship between CB1R availability and BMI is maintained at BMI < 27, consistent with animal studies showing that CB1R availability is strongly associated with BMI. While peripheral CB1R activation has been shown to stimulate adipogenesis and lipogenesis in diet-induced obesity (Bensaid et al., 2003; Tedesco et al., 2008), administration of rimonabant, a central CB1R antagonist, has been associated with reduced food intake in diet-induced obesity (Nogueiras et al., 2008). In male, leptin-deficient, Zucker obese rats, CB1R levels were increased in the cingulate and frontal cortices and decreased in the hypothalamus, com- pared to lean rats (Thanos et al., 2008). Although the reasons for these divergent findings are unclear, the choice of

tracer (Normandin et al., 2015) and range of BMI in the current dataset may contribute to the observed differences.

Other important differences between the published studies may be tracer-related. [^{11}C] OMAR is a "second-generation ", rimonabant- like antagonist radioligand (Horti et al., 2006). [^{18}F]MK-9470 and [^{18}F]FMPEP-d_2, on the other hand, are second-generation radioligands that are potent CB 1 R inverse agonists (Burns et al., 2007 ; Terry et al., 2010). Compared to [^{11}C]OMAR, [^{18}F]FMPEP-d_2 and [^{18}F]MK-9470 have greater affinity, higher lipophilicity, and markedly slower ki- netics (Riano Barros et al., 2014; Sanabria-Bohórquez et al., 2010; Tsujikawa et al., 2014). While both [^{18}F]FMPEP-d_2 and [^{11}C]OMAR exhibit specific binding to the CB1R that can be displaced with rimonabant, it is still possible that the tracers label different receptor conformation states. A substantial portion of CB1Rs undergoes receptor internalization (Hsieh et al., 1999) and exists in at least 2 different conformation states: an active conformation associated with agonist binding that results in activation of G-proteins and an inactive conformation as- sociated with binding to an antagonist (Hua et al., 2017; Shao et al., 2016). While antagonists have equal affinities for the active and inactive conformation states of the CB1R, agonists and inverse agonists preferentially bind to the active conformation state. Thus, as inverse ag- onists, [^{18}F]MK-9470 and [^{18}F]FMPEP-d_2 favor binding to the receptors at their high-affinity conformation whereas [^{11}C]OMAR, an antagonist, binds to both the high- and low-affinity states.

Additionally, a substantial majority of CB1Rs on the neuronal membrane reside in the inactive state, and agonists may shift this proportion of active/inactive conformation towards the active state (Gullapalli et al., 2010). While currently there are no data available on whether [^{18}F]MK-9470 or [^{18}F]FMPEP-d_2 crosses the cell membrane and binds to intracellular CB1Rs, this characteristic of the ligands may underlie the differences in results from PET imaging studies. Thirdly, the effect of endogenous agonists such as anandamide and 2-AG on receptor availability and the proportion of active receptor conformation state cannot be ignored. It is not known whether [^{11}C]OMAR, [^{18}F]MK-9470 and [^{18}F]FMPEP-d_2 can be displaced by endogenous agonists, although [^{11}C]MePPEP, a structural analogue of [^{18}F]FMPEP- d_2, was found not to be displaced by endogenous agonists or synthetic agonists (Terry et al., 2008).

It is thus possible that the differences in radiotracer affinity, lipophilicity, receptor conformation states and receptor-ligand interac- tions are responsible for the different results seen in PET imaging stud- ies with these ligands. In studies of subjects with alcohol-use disorder, CB1R availability following abstinence was found to be higher with [^{11}C]OMAR (Neumeister et al., 2012) and lower with [^{18}F]FMPEP-d_2 (Hirvonen et al., 2012), compared to controls. However, studies in sub- jects with schizophrenia

using [^{11}C]OMAR (Ranganathan et al., 2016), [^{18}F]FMPEP-d_2 and [^{11}C]MePPEP (Borgan et al., 2019) all show lower CB1R availability compared to controls.

The findings from the present study should be viewed within the context of its limitations. The data was pooled from multiple individual studies and had a smaller proportion of women compared to men. We did not document whether women were on oral contraceptive pills or hormonal intrauterine devices and did not measure hormonal levels or level of stress in subjects. These factors can potentially alter CB1 receptor density as hormone levels have been shown to alter CB1 availability (Laurikainen et al., 2019; Riebe et al., 2010). Additionally, the analysis included comparison across 16 ROIs and the limited sample-size precluded adjustment for multiple comparisons. Hence, the possibility of Type-1 error cannot be ruled out. Another caveat is that, while the use of DSM-IV SCID allowed us exclude psychiatric disorders, it did not allow us to exclude personality disorders. Finally, differences in findings with [^{18}F]FMPEP-d_2 and [^{11}C]OMAR would best be addressed by a within-subject, cross-validation study given the multiple potential confounders as discussed above.

Data and code availability statement

Data and code will be made available on reasonable request when in keeping with Yale University's data-sharing policy.

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Credit authorship contribution statement

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.119674.

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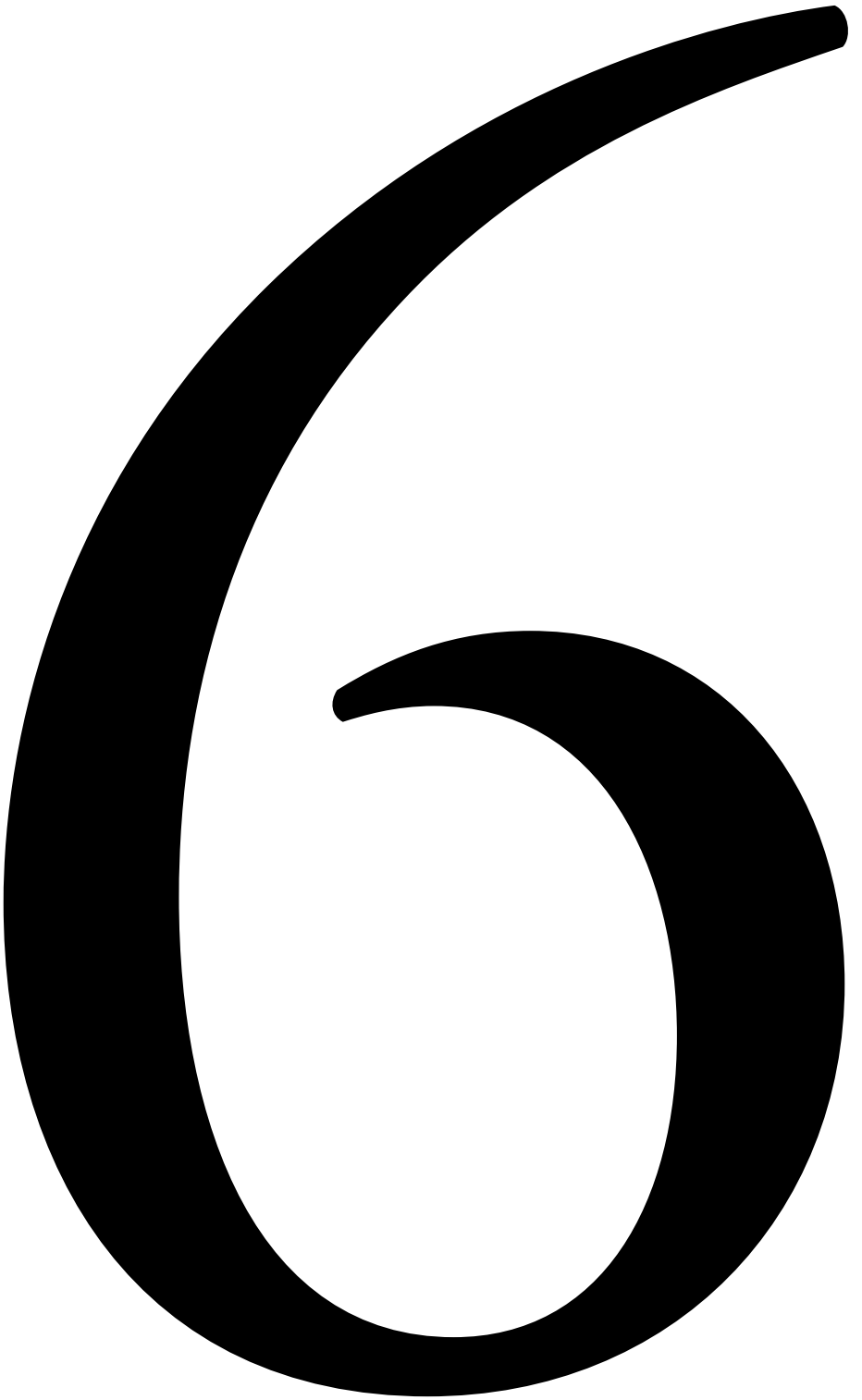

Chapter 6

Preliminary in vivo evidence of lower hippocampal synaptic density in cannabis use disorder

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Abstract

Cannabis is one of the most commonly and widely used psychoactive drugs. The rates of cannabis misuse have been increasing. Therefore, understanding the effects of cannabis use on the brain is important. Adolescent and adult rodents exposed to repeated administration of cannabinoids show persistent microstructural changes in the hippocampus both pre- and post-synaptically. Whether similar alterations exist in human cannabis users, has not yet been demonstrated in vivo. Positron emission tomography (PET) and [^{11}C]UCB-J, a radioligand for the synaptic vesicle glycoprotein 2A (SV2A), were used to study hippocampal synaptic integrity in vivo in an equal number (n = 12) of subjects with DSM-5 cannabis use disorder (CUD) and matched healthy controls (HC). Arterial sampling was used to measure plasma input function. [^{11}C] UCB-J binding potential (BP_{ND}) was estimated using a one-tissue (1T) compartment model with centrum semiovale as the reference region. Hippocampal function was assessed using a verbal memory task. Relative to HCs, CUDs showed significantly lower [^{11}C]UCB-J BP_{ND} in the hippocampus (~10%, p = 0.008, effect size 1.2) and also performed worse on the verbal memory task. These group differences in hippocampal BP_{ND} persisted after correction for volume differences (p = 0.013), and correction for both age and volume (p = 0.03). We demonstrate, for the first time, in vivo evidence of lower hippocampal synaptic density in cannabis use disorder. These results are consistent with the microstructural findings from experimental studies with cannabinoids in animals, and studies of hippocampal macrostructure in human with CUD. Whether the lower hippocampal synaptic density resolves with abstinence warrants further study.

Introduction

Cannabis is one the most commonly and widely used psychoactive drugs [1]. The legalization of "medical" and recreational cannabis use continues to spread globally [2]. The potency of cannabis has been steadily increasing [3]. Furthermore, the rates of cannabis use and cannabis use disorder (CUD) are increasing [4]. Given the above, it is important to understand the impact of cannabis exposure on brain structure and function, and especially on the maturating brain.

Converging lines of evidence suggest that the structure and function of the hippocampus, seems to be more sensitive to the effects of acute and chronic cannabis exposure. In both animals and humans, learning and memory, which are dependent on hippocampal function [5], are disrupted by the acute administration of cannabis and cannabinoid receptor (CB1R) agonists [6] and likewise, verbal memory deficits are one of the most consistently reported deficits in chronic cannabis users [7, 8].

In addition to functional deficits, a range of macrostructural white and gray matter abnormalities have been reported in chronic, heavy cannabis users using magnetic resonance imaging (MRI) [9, 10]. Amongst these, reductions in hippocampal volume appear to be the most consistent findings [11, 12]. Furthermore, hippocampal volume in heavy canna- bis users has been reported to be negatively correlated with the duration and amount of lifetime [11, 13] and recent [14] cannabis use. While human brain imaging studies utilizing MRI have been informative about the effects of cannabis at the macrostructural level, we are unaware of studies examining alterations at the microstructural level.

In preclinical studies, the repeated administration of both phyto and synthetic CB1R agonists to both adolescent and adult rodents results in persistent changes in dendritic morphology and spine density [15–21]. Rubino et al. also demonstrated alterations in presynaptic microstructure, as evidenced by decreased vesicle-associated membrane protein 2 (VAMP2) after chronic cannabinoid administration [16]. These changes seem to be more evident in the hippocampus. Furthermore, in animal studies, repeated exposure to Δ^9-tet- rahydrocannabinol (Δ^9-THC), the principal active constituent of cannabis, leads to both structural (synaptic) and functional (memory) impairments via a CB1R-dependent mechanism [22]. However, to our knowledge, whether exposure to cannabis alters hippocampal synaptic integrity in humans has not been studied in vivo or post-mortem.

It is now possible to investigate the effects of cannabis exposure on synaptic density in vivo with [^{11}C]UCB-J, a positron emission tomography (PET) radioligand which has high specificity for the synaptic vesicle glycoprotein 2A [23, 24]. Synaptic vesicle

glycoproteins 2 (SV2) are integral proteins localized on the surface of synaptic vesicles (Supplemental Fig. S1). SV2A is one of three SV2 isoforms that is ubiquitously and homogenously present in virtually all active synapses across the brain [25]. The number of vesicles per nerve terminal is a stable feature of neurons— the synaptic vesicle pool size is rapidly replenished during neural activity to sustain the capacity for ongoing neuro- transmitter release [26]. SV2 is one of the most mono- dispersed of the main vesicle proteins [27]. UCB-J is derived from levetiracetam that binds to SV2A and [^{11}C] UCB-J has been developed as a radioligand of PET imaging of SV2A in vivo [28]. The SV2A-specific signal measured with [^{11}C]UCB-J PET has been shown to be a proxy quantitative measure of synaptic density [24]. Validation studies have confirmed that postmortem quantification of SV2A is highly correlated with synaptophysin a commonly used synaptic density marker, and furthermore, in vivo binding of [^{11}C]UCB-J PET is highly correlated with postmortem SV2A density [24]. [^{11}C]UCB-J has high specificity, rapid kinetics, high test-retest reproducibility and the ability to produce high-quality parametric maps [23, 28]. It has been used to study synaptic density in depression [29], schizophrenia [30], temporal lobe epilepsy (TLE) [31], Parkinson's Disease (PD) [32], and Alzheimer's disease (AD) [33]. In brief, in schizophrenia [^{11}C] UCB-J binding was significantly lower (~15%) in the frontal and anterior cingulate cortices (Cohen's d = 0.8–0.9), but not the hippocampus [30]. In depression, Holmes et al., found an negative correlation between the severity of depressive symptoms and [^{11}C]UCB-J binding, with more depressed individuals showing lower [^{11}C]UCB-J binding compared to healthy controls [29]. In TLE, Finemma et al. reported a large significant reduction in [^{11}C]UCB-J binding in the epileptogenic temporal lobe relative to the contralateral side. In AD, Chen reported significant reduction in hippocampal [11C]UCB-J binding (–41%) relative to controls [33] and in PD, Matuskey et al. reported lower [^{11}C] UCB-J binding in the substantia nigra (–45%), red nucleus (–31%), and locus coeruleus (–17%) [32].

The purpose of the current study was to compare hippocampal synaptic density in individuals with CUD to matched healthy controls (HCs) using [^{11}C]UCB-J and high resolution research tomography (HRRT). A secondary goal was to compare the groups for performance on a verbal memory task sensitive to hippocampal function, and to explore the relationship between [^{11}C]-UCB-J binding and verbal memory.

Methods

Regulatory

The study was approved by the Human Investigation Committee (Yale HIC# 2000021592), Radiation Safety Committee, Radioactive Drug Research Committee, and MRI Safety Committee of Yale University.

Participants

The study included male and females ≥18 years diagnosed with current DSM-5 CUD and age-, gender-matched HCs. Participants were recruited by word of mouth, advertisements, and flyers. Written informed consent was first obtained before participants were screened with a Structured Clinical Inter- view for DSM-5, Clinician Version, psychiatric and physical examination, blood tests, electrocardiogram, urine toxicology, and pregnancy testing.

Inclusion criteria for CUD participants were: (1) current cannabis consumption ≥30 joints/month or equivalent over the past 6 months, (2) daily cannabis use (>30/month) in the last 30 days, (3) ≥2-year history of regular cannabis use, (4) ≥1000 lifetime exposures to cannabis (estimated # of joints), (5) positive urine screen for cannabinoids (and negative for all other drugs), (6) DSM-5 CUD (≥moder- ate), (7) no other illicit substance use during the past 3 months. Detailed information about last 30-day cannabis use was captured using the Time Line Follow Back for Cannabis (TLFB-C_{30}) [34–36]. Inclusion criteria for HC participants were: (1) a lifetime cannabis exposure less than 20 times, (2) no cannabis use in the past 2 years (self- reported), (3) no history of illicit substance use, and (4) a negative urine drug screen.

Common exclusion criteria include:

(1) Other current DSM-5 substance (including nicotine) or alcohol use disorder;

(2) Lifetime diagnosis of major psychiatric disorder (e.g., DSM-5 neurodevelopmental disorders, schizophrenia spectrum disorders, bipolar, and related disorders etc);

(3) Clinically significant medical conditions or laboratory abnormalities;

(4) Positive drug screen (except for THC-COOH in CUDs);

(5) Weekly alcohol consumption > the National Institute of Alcohol Abuse and Alco- holism (NIAAA) guide- lines;

(6) Consumption of > five cigarettes per day and score of > three on the Fagerstrom Test for Nicotine Dependence;

(7) Pregnancy or lactation;

(8) Unwillingness to practice birth control;

(9) Contraindications for arterial line;

(10) Contraindications for MRI;

(11) Radiation exposure > the allowable annual limit;

(12) BMI > 35 kg/m^2;

(13) Unable to provide informed consent;

(14) Intelligence Quotient <80;

(15) Not fluent in English.

Magnetic resonance imaging

Structural MR imaging was performed on a Siemens Trio 3-T scanner for purposes of excluding individuals with anatomical abnormalities, anatomically co-registering with PET scans and to define regions of interest (ROIs). The dimension and voxel size of MR images were 256 × 256 × 176 voxels and 0.98 × 0.98 × 1.0 mm^3, respectively. MRI scans were acquired using a T1-weighted magnetization-prepared rapid gradient echo sequence with 1 mm^3 isotropic resolution (flip angle = 7°, echo time = 3.34 ms, inver- sion time = 1100 ms, repetition time = 2500 ms). Images were stripped of the skull and meninges (Functional Magnetic Resonance Imaging of the Brain [FMRIB]'s Brain Extraction Tool, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET) before processing.

Radiochemistry and PET acquisition

At the PET Center participants were tested to rule out recent street drug use (other than cannabis in those with CUD); the latter were prohibited from using cannabis beyond 12 am on the day of the PET scan. [^{11}C]UCB-J was synthesized at the Yale PET Center as previously reported [28]. All [^{11}C] UCB-J PET measurements were conducted on the HRRT (Siemens Medical Solutions), which acquires 207 slices (1.2-mm slice separation) with a reconstructed image resolution (full width at half maximum) of ~3 mm. Before each [^{11}C]UCB-J injection, a 6-min transmission scan was performed for attenuation correction. The PET scan (90 min) was acquired following administration

of up to 740 MBq (20 mCi) of [^{11}C]UCB-J. The arterial input function was measured with samples drawn every 10 s for the first 90 s and at 1.75, 2, 2.25, 2.5, 2.75, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 45, 60, 75, 90 min after [^{11}C]UCB-J injection. For each blood sample, plasma was obtained by centrifugation at 4 °C (2930 g for 5 min). Whole blood and plasma were counted in cross-calibrated β-counters (Wizard2; PerkinElmer). Plasma samples at 3, 8, 15, 30, 60, and 90 min after [^{11}C]UCB-J injec- tion were analyzed by column switching HPLC [37] for unmetabolized [^{11}C]UCB-J fraction [28]. Plasma free fraction was measured by ultrafiltration [38].

PET images were reconstructed using MOLAR, a list- mode algorithm [39] with all corrections (e.g., attenuation, normalization, scatter, randoms). Motion correction was based on an optical detector (Vicra, NDI Systems) and was performed on an event-by-event basis directly from the list-mode data [40]. Motion-corrected PET data were co- registered to the participant's T1-weighted 3T MR image using a 6-parameter mutual registration algorithm, which is in turn nonlinearly (BioImage Suite) aligned to a standard MR template in MNI space. The hippocampus and exploratory ROIs were delineated on the Anatomical Automatic Labeling template [41]. Parametric images of distribution volume (V_T) of total ligand uptake in tissue relative to total concentration of ligand in plasma [42] were generated using the one-tissue compartment model (1T) [23]. BP_{ND} (ratio of specifically bound radioligand to that of non-displaceable radioligand in tissue) [42], was calculated using centrum semiovale as the reference region (V_T (ROI)/ V_T (centrum semiovale) – 1). The centrum semiovale has been optimized as a reference region to minimize partial- volume effects [43].

Partial-volume correction (PVC) was applied frame-by- frame based on the Muller-Gartner algorithm [44] following segmentation of MR images using SPM12. All kinetic modeling was performed with in-house developed programs written with IDL 8.0 (ITT Visual Information Solutions). Hippocampal volume was determined from T1-weighted images analyzed using FreeSurfer 6.0.

Verbal memory

The Rey Auditory Verbal Learning Task (RAVLT) is a hippocampal-sensitive verbal memory task [45, 46] that is sensitive to the acute [47], chronic [48], and persistent [8] effects of cannabinoids. Testing was conducted after the PET scan and at least 12 h from the time of last cannabis use.

Statistical analyses

Demographic variables and measures of substance use were summarized descriptively and distribution of the data for each variable was examined with box and whisker

plots and tests of normality. The differences between the CUD and the matched HC groups were examined with t-test for continuous measures and chi-square (X^2) test for discrete variables. The distribution of hippocampal [^{11}C]UCB-J BP_{ND} was examined for normality in both the groups using quantile–quantile plots and Shapiro–Wilk test. As these data were observed to be normally distributed, a parametric t-test was applied to examine the group differences in hippocampal ligand binding. Further, the observed effects in the group difference were corrected for differences in age by including this term in a general linear model. The relationship between hippocampal [^{11}C]UCB-J BP_{ND} and verbal memory measured with total recall score on RAVLT was explored with a scatter plot. No clear relationship was observed between these variables. To examine the hypothesis if the direction of deviation in [^{11}C]UCB-J BP_{ND} in CUD subjects in comparison to HCs would be correlated with the direction of deviation in the RAVLT total recall scores, median values of the control sample were used to binarize the two variables. The relationship between the binarized variables was measured using the phi coefficient [49, 50]. Exploratory analyses for group differences in [^{11}C] UCB-J BP_{ND} in other brain regions were conducted. However, given the exploratory nature of these analyses and the limited sample, no adjustments for multiple comparisons were made. Statistical analysis and plotting were was performed in R version 3.6.1 and R studio 1.2.1335 using package tidyverse [51] and ggpubr [52].

Results

The sample included 12 participants with DSM-5 CUD and an equal number of age- and gender-matched controls (Table 1). The CUD group were using cannabis at least once daily for at least 3 months. Exposure to cannabis was also confirmed by urine toxicology on several visits including, on the morning of the scan. Likewise, urine toxicology and breathalyzer tests were conducted to confirm that subjects had not been exposed to other drugs and alcohol, respectively, on several visits including on the morning of the PET scan.

While there were significant group differences in measures of cannabis exposure, there were no significant group differences in age, injected mass dose, and plasma free fraction of [^{11}C]UCB-J (Table 1). There were no significant group differences in the consumption of alcohol (median interquartile range [IQR]) for number of drinks per week CUD: 2 (3); HCs 0.5 (1.9), p = 0.123) or tobacco (median (IQR) number of cigarettes

in past 30 days CUD: 0 (6); HCs 0 (0), p = 0.346). The injected activity dose of [^{11}C] UCB-J was higher in CUDs than HCs. There were no significant differences in [^{11}C] UCB-J volume of distribution (V_T) in the centrum semiovale between CUD (mean ±

SD) = 4.71 ± 0.54 ml/cm^3, and HC = 4.44 ± 0.60 ml/cm^3, p = 0.26, thus supporting its use as a reference region in the calculation of [^{11}C]UCB-J binding potential (BP_{ND}).

Table 1 Demographics.

	CUD (mean±s.d)	HC (mean±s.d)	Statistic
Age (in years)	24.24 (3.12)	27.32 (4.41)	t=1.97,p=0.06
Male: Female	8:4	7:5	X^2=0, p>0.99
Body mass index (BMI)	27.34 (5.13)	25.54 (2.86)	t=1.06,p=0.3
Education (years)	13.83 (2.16)	15.4 (1.5)	t=2.01,p=0.051
Last 30 days frequency of cannabis use	60.5 (49.6)	0 (0)	n.a.
Last 90 days frequency of cannabis use	121.66 (89.85)	0 (0)	n.a.
Age of initiation of cannabis use (in years)	15.91 (2.26)	n.a.	n.a.
Estimated lifetime frequency of cannabis exposure	1020.83 (984.29)	0.1 (0.31)	n.a.
Injected dose (in MBq)	644.94 (118.16)	505.51 (179.12)	t=−2.25,p=0.04
Specific activity at time of injection (in mCi/mcg)	3.86 (2.3)	4.43 (1.92)	t=0.66,p=0.52
Mass dose (in mcg)	2.01 (1.17)	1.30 (0.97)	t=−1.63,p=0.12
Mass dose (in ng/kg)	26.40 (17.20)	16.70 (11.85)	t=−1.63,p=0.12
Plasma free fraction	0.28 (0.02)	0.29 (0.02)	t=0.26,p=0.80
Hippocampal volume (in ml)	8.26 (0.75)	8.55 (0.96)	t=0.83,p=0.41
Estimated total intracranial volume [eTIV] (in ml)	1518.10 (166.73)	1521.13 (175.78)	t=0.04,p=0.97
Hippocampal volume corrected for eTIV	0.0055 (0.0003)	0.0056 (0.0003)	t=1.19,p=0.25

MBq megabecquerel, mcg micrograms, ng nanograms, ml milliliters, n.a. not applicable.

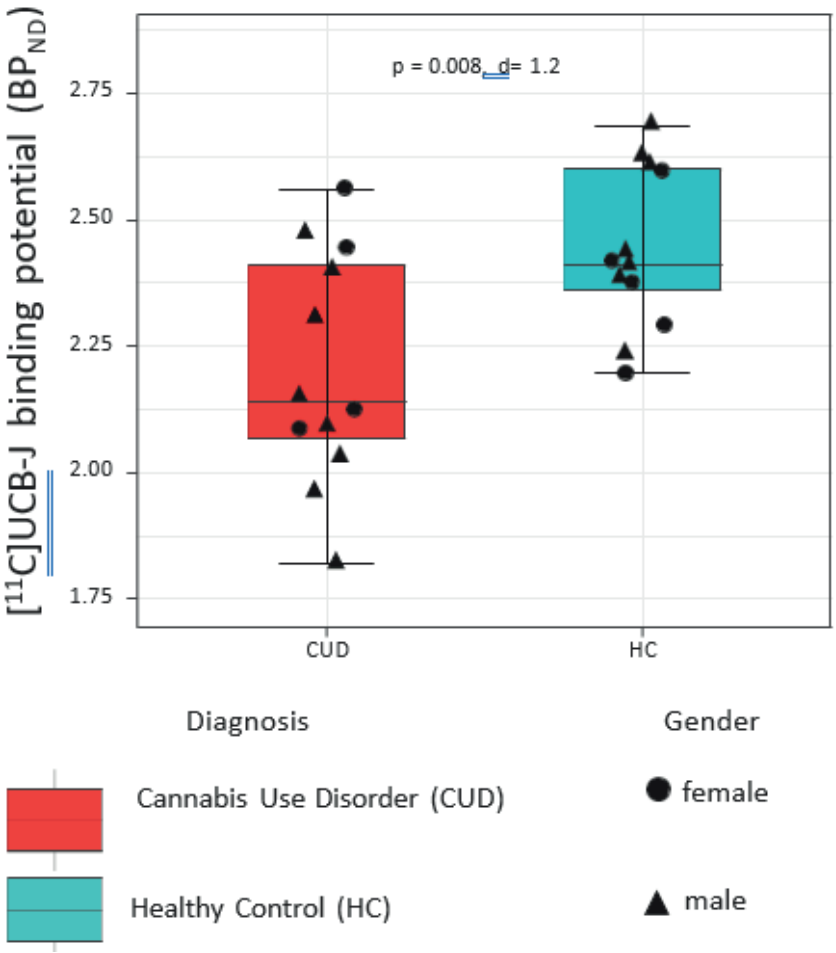

Figure 1

Comparison of Hippocampal synaptic density ([^{11}C]UCB-J BPND) between Cannabis Use Disorder (CUD) and healthy controls (HC). Figure shows a boxplot of hippocampal synaptic density ([^{11}C]UCB-J BP_{ND}) in cannabis use disorder (CUD) vs healthy controls (HC). Within each box, horizontal line denotes median value; boxes extend from the 25th to the 75th percentile of each group's distribution of values. Individual data-points are shown (circle = female, triangle = males). The difference between groups was large (effect size Cohen's d = 1.2) and statistically significant (p = 0.008) on a t-test.

Group differences in hippocampal volume

There were no significant group differences in hippocampal volume, estimated total intracranial volume (eTIV), and hippocampal volume corrected for eTIV (Table 1) measured using MRI.

Group differences in hippocampal [^{11}C]UCB-J binding

Relative to matched HCs, CUD subjects showed significantly lower [^{11}C]UCB-J BP_{ND} in the hippocampus (mean [±SD]—HC = 2.44 [±0.16], CUD = 2.2 [±0.23], t = 2.92,

p = 0.008) (Fig. 1). The difference in BPND was ~10% and the magnitude of the difference was large (effect size d = 1.2). After adjusting for group differences in age (beta = −0.006, SE = 0.01, p = 0.623) in the linear model, the effect of diagnosis on BP_{ND} was significant (beta = 0.21, SE = 0.1, p = 0.02). After applying PVC, the group differences in hippocampal [^{11}C]UCB-J BPND remained significant (mean [±SD]—HC = 3.37 [±0.21], CUD = 3.06 [±0.35], t = 2.71, p = 0.013; effect size d = 1.1) (Supplemental Fig. S2) and this remained significant (beta = 0.29, SE = 0.13, p = 0.03) after correcting for age as a covariate.

Verbal memory and the relationship with [^{11}C]UCB-J binding

Individuals with CUD had significantly lower (t = 2.39, p = 0.03) RAVLT total immediate recall than HCs (Table 2). The group differences were large (effect size d = 1.02). Amongst individuals with CUD, there was a positive correlation (phi coefficient = 0.67) between hippocampal [^{11}C]UCB-J binding and RAVLT total immediate recall (p = 0.02) (Supplemental Fig. S3).

[^{11}C]UCB-J binding and measures of cannabis exposure

Amongst CUDs, there were no significant correlations between hippocampal [^{11}C] UCB-J BP_{ND} and cannabis exposure (measured by the TLFB) in the last 1) 30 days (rho = 0.43, p = 0.16), 2) 90 days (rho = 0.39, p = 0.21), or 3) 180 days (rho = −0.071, p = 0.83).

Table 2 Verbal Learning and Memory (Rey Auditory Verbal Learning Test [RAVLT]).

	CUD mean (SD)	HC mean (SD)	Statistic
Total immediate	44.25 (12.84)	56.6 (11.36)	t = 2.39, p = 0.03
Short delayed recall	10.5 (3.53)	11.9 (3.38)	t = 0.95, p = 0.36
Long delayed recall	9.08 (4.42)	11.8 (3.11)	t = 1.63, p = 0.12
Total intrusions	2.33 (2.53)	1.9 (2.23)	t = −0.42, p = 0.68

Discussion

We show for the first time that [^{11}C]UCB-J binding potential BPND, an in vivo marker of synaptic vesicle glycoprotein 2A levels and, a proxy marker of synaptic nerve terminal density, is significantly lower by 10% in the hippocampus of CUD relative to matched controls. This group difference persists despite adjusting for partial-volume effects.

The group differences in synaptic density were large in magnitude (effects size d = 1.2) and within the range of group differences observed in schizophrenia patients [30] but smaller than those seen in other neuropsychiatric disorders [29, 31, 33]. As hypothesized, synaptic density was lower in the hippocampus.

While not adequately powered, exploratory analyses revealed lower [^{11}C]UCB-J BP_{ND} in other ROIs known to have a high density of CB1Rs (e.g., cerebellum, striatum, anterior cingulate), but these differences were not statistically significant and were of lower magnitude (Supplementary Table 1).

CUD had lower hippocampal VT compared to HCs (Supplementary Table 2), although this difference was small and not statistically significant. However, for all other regions, VT values were higher in CUD than HC, although these differences were also not significant. The centrum semiovale VT values were also numerically higher in CUD than HC. This pattern is supportive of our contention that a region-specific group difference occurs in the hippocampus.

Furthermore, this lower synaptic density in the hippo- campus appears to be specific to CUD given that in a parallel study using identical methods in cocaine use disorder (Angarita et al., personal communication) lower synaptic density was observed in different brain regions (anterior cingulate cortex, ventromedial prefrontal cortex, and medial orbitofrontal cortex) [53]. Lower hippocampal synaptic density in CUD observed in this in vivo study is consistent with microstructural alterations observed in animal studies, the findings of functional deficits and macrostructural alterations in humans, and supports the hypothesis that the hippocampus is especially sensitive to the effects of cannabis. Furthermore, that group differences in hippocampal volume (3.4%) obtained from MRI (using FreeSurfer 6.0 (Table 1)) were not statistically significant, while group differences in [^{11}C]UCB-J BP_{ND} (10%) were, suggests that [^{11}C]UCB-J may be a more sensitive measure of alterations in hippocampal structure than MRI.

While there were significant differences in cannabis exposure there were no significant group differences across a number of variables including alcohol and tobacco exposure, suggesting that lower synaptic density in CUD is related to cannabis. We did

not observe any relationship between cannabis consumption and synaptic density. The primary goal was to study hippocampal synaptic density in CUD. As discussed elsewhere, it is challenging to accurately estimate cannabis consumption [54, 55] given the variability in how cannabis is consumed (smoked, vaped, per oral), the content of THC, the content of CBD, etc. The lack of a correlation between cannabis consumption and [^{11}C]UCB-J BP_{ND} may be explained by the limited range in cannabis exposure in the small sample studied, and the challenges in estimating cannabis consumption. Whether these differences are the consequence of cannabis or pre- existed cannabis exposure cannot be conclusively deter- mined in this study design even though animal experiments conclusively demonstrate that cannabinoids alter hippo- campal synaptic integrity. While there are no postmortem data in humans on the effects of cannabis exposure on synaptic density to correlate with the in vivo findings reported here, our findings are consistent with experimental studies in rodents showing changes in markers of pre- synaptic density (VAMP2) and dendritic morphology and spine density with the administration of both phyto- and synthetic-cannabinoids [15–20, 56, 57].

Consistent with the role of the hippocampus in learning and memory, a modest significant correlation was observed between hippocampal [^{11}C]UCB-J BP_{ND} and verbal memory raising the possibility that lower synaptic density in CUD may have functional correlates. While admittedly speculative, we offer some explanations for the findings of lower hippocampal synaptic density observed in CUD. The principal psychoactive constituent of cannabis, Δ9-THC, produces most of its effects via cannabinoid receptors (CB1R and CB2R). THC induces apoptosis in cultured cortical neurons by activating of the caspase-3 cell death pathway, resulting in DNA fragmentation and programmed cell death [58, 59]; that CB1R antagonists block THC- induced apoptotic effects suggests that a CB1R specific mechanism is involved in the apoptotic process [59]. Similarly, Chen et al., showed in a series of animal studies that the effects of repeated administration on changes in both synaptic structure and memory were via a CB1R- dependent mechanism that involved the inhibition of cyclooxygenase-2, an inflammatory molecule [22].

While the historical view was that CB1Rs and CB2Rs were distributed mainly centrally and peripherally, respectively, it is now established that CB2Rs are also present in the brain [60]. Neurons express primarily CB1Rs, microglia express mostly CB2Rs, and astrocytes express both [61].

THC administration to adolescent mice has been shown to induce microglia activation in the prefrontal cortex at adult age [62]. Furthermore, co-treatment during adolescence with an inhibitor of microglial activation reduced glial activation, the levels of the inflammatory mediators and the memory deficit present in adult animals. A recent PET study with [^{18}F]FEPPA showed that relative to controls, CUDs showed higher levels of

the mitochondrial translocator protein (TSPO), a marker of microglial activation [63]. Furthermore, higher TSPO levels were associated with higher circulating C-reactive protein levels, an inflammatory marker. Thus, lower synaptic density observed in CUD may be mediated, at least in part, by increased neuroimmune or microglial activation via a CB2R mechanism [62, 64–66].

Strengths, limitations, and future directions

We used [^{11}C]UCB-J, a well-validated ligand, and the highest resolution PET scanner available. We also used binding potential (BP_{ND}), the preferred outcome measure in PET studies. While animal experiments conclusively demonstrate that cannabinoids alter synaptic integrity, our study design does not allow us to determine whether lower synaptic density in CUD is a consequence of cannabis use or were present before the initiation of cannabis. Additional studies measuring synaptic density after some period of abstinence will help determine whether it recovers with abstinence in CUD and if so, how long it takes. The limited sample size may have made it challenging to detect regional differences and the effects of gender, dose of exposure, and the timing of initiation of cannabis use. Thus, a larger sample size with more females and males, a wider range of cannabis exposure, and a wider range in the age of initiation of cannabis use (early, mid, or late adolescence) will help more conclusively determine these effects. Lastly, understanding the mechanisms by which exposure to cannabis produces synaptic deficits may open up possibilities for developing treatments.

Implications

The findings of this study have implications for cannabis users especially at a time when liberalization of cannabis laws are sweeping across the globe [2, 67] and the use of cannabis has increased [1, 68]. It will be important for the public to be aware of the findings of this study that were previously not known. Given that cannabis use is typically initiated during adolescence and young adulthood [69] when the brain is still maturing [70], the findings are particularly relevant to youth. Both observational studies in humans and experimental studies in animals suggest a specific vulnerability of the adolescent brain to cannabis effects [71]. The endocannabinoid (eCB) system, which influences several brain developmental processes, undergoes significant changes during different periods of brain development [72–74]. Stimulation of the eCB system in a nonphysiological manner, as would be the case with cannabis exposure, during adolescence could have long lasting consequences on brain development depending on the neurodevelopmental window during which the exposure occurs [72, 75]. If cannabis exposure leads to lower hippocampal synaptic density, it may have consequences for net- work organization and function in cannabis users. The lower synaptic contacts and/or less efficient synaptic connections throughout the hippocampus in THC treated animals may represent the molecular underpinning of the cognitive deficits induced by adolescent THC treatment [16].

Conclusions

We demonstrate for the first-time in vivo evidence of altera- tions in hippocampal synaptic density in individuals with current CUD. These results are consistent with the micro- structural findings from experimental studies with cannabinoids in animals, and studies in humans of hippocampal macrostructure. Whether the lower hippocampal synaptic density resolves with abstinence warrants further study.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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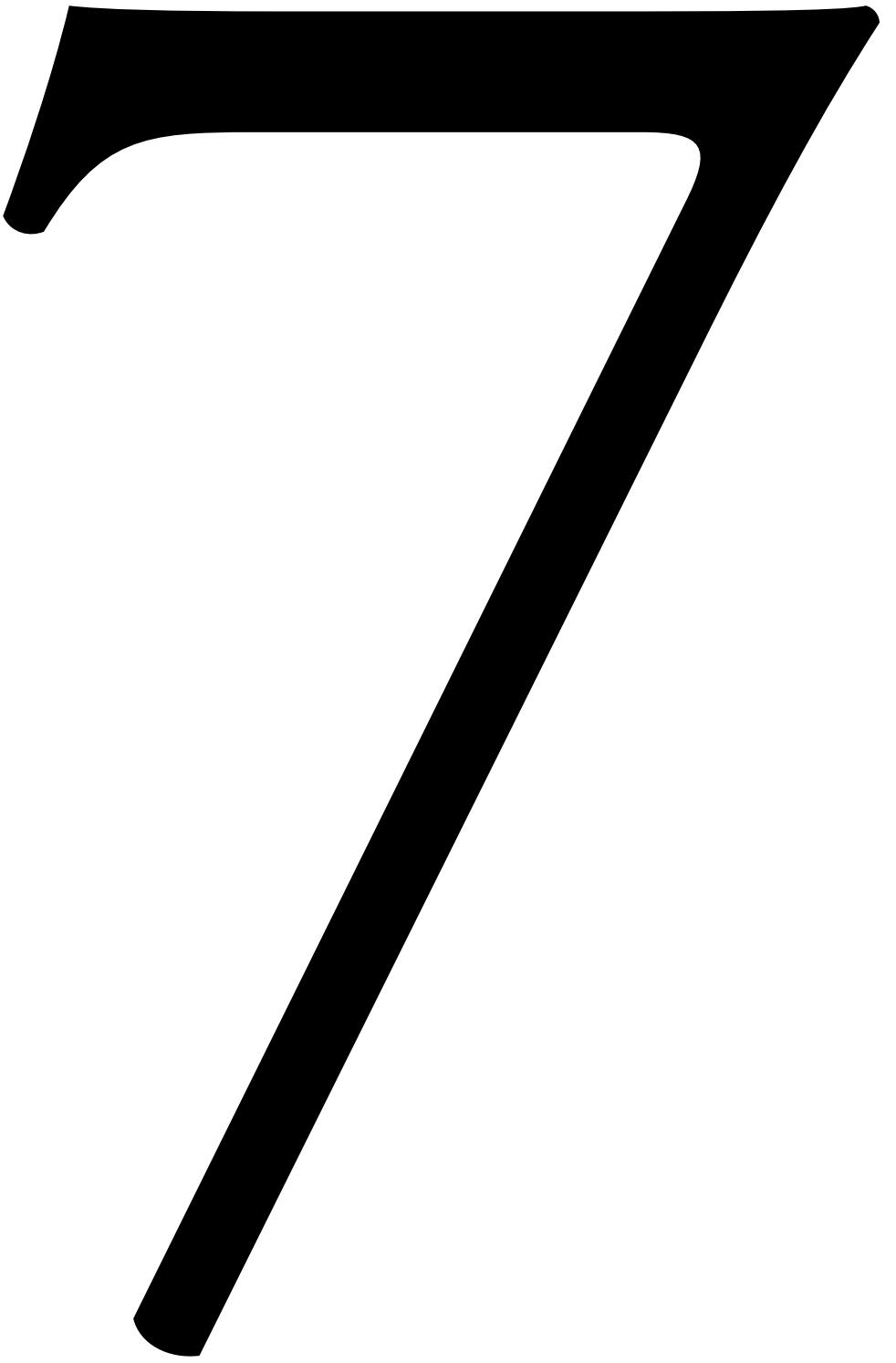

Chapter 7

Interaction between environmental and familial affective risk impacts psychosis admixture in states of affective dysregulation

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Abstract

Background

Evidence suggests that cannabis use, childhood adversity, and urbanicity, in interaction with proxy measures of genetic risk, may facilitate onset of psychosis in the sense of early affective dysregulation becoming 'complicated' by, first, attenuated psychosis and, eventually, full-blown psychotic symptoms.

Methods

Data were derived from three waves of the second Netherlands Mental Health Survey and Incidence Study (NEMESIS-2). The impact of environmental risk factors (cannabis use, childhood adversity, and urbanicity) was analyzed across severity levels of psychopath- ology defined by the degree to which affective dysregulation was 'complicated' by low-grade psychotic experiences ('attenuated psychosis' – moderately severe) and, overt psychotic symptoms leading to help-seeking ('clinical psychosis' – most severe). Familial and non-familial strata were defined based on family history of (mostly) affective disorder and used as a proxy for genetic risk in models of family history × environmental risk interaction.

Results

In proxy gene–environment interaction analysis, childhood adversity and cannabis use, and to a lesser extent urbanicity, displayed greater-than-additive risk if there was also evidence of familial affective liability. In addition, the interaction contrast ratio grew progressively greater across severity levels of psychosis admixture (none, attenuated psychosis, clinical psychosis) complicating affective dysregulation.

Conclusion

Known environmental risks interact with familial evidence of affective liability in driving the level of psychosis admixture in states of early affective dysregulation in the general population, constituting an affective pathway to psychosis. There is interest in decomposing family history of affective liability into the environmental and genetic components that under- lie the interactions as shown here.

Introduction

The conceptualization of psychosis as an extended phenotype encompasses both psychosis spectrum disorder (any psychotic disorder) and subthreshold psychotic symptoms that are experienced by individuals in the general population (van Os, 2016). About 7% of the general population may experience subthreshold psychotic experiences (Linscott and van Os, 2013). Subthreshold psychotic experiences are transitory in around 80%, persistent in around 20% and evolve into a psychotic disorder in 7%, with an annual transition rate of 0.5–1% (Linscott and van Os, 2013).

The pathway leading to psychotic disorder remains unknown. It is likely to involve a complex dynamic interaction between familial genetic risk, non-genetic factors such as exposure to environmental risks and interactions within and between symptoms themselves (Smeets et al., 2012; van Os, 2013). There is accumulating evidence that the earliest pathway to psychosis involves an interaction between 'affective dysregulation' (Myin-Germeys and van Os, 2007; Kramer et al., 2014) and 'aberrant salience' (Reininghaus et al., 2016). Studies suggest that psychosis in a group of patients begins when, first, affective dysregulation, under the influence of genetic and environmental risk factors, becomes 'complicated' by aberrant salience, which in turn, under the influence of the same genetic and environmental risk factors, may progress to full-blown psychotic symptoms (Hafner et al., 1999; Guloksuz et al., 2015, 2016; van Os and Reininghaus, 2016). Genetic and environmental risk factors thus may operate by impacting the degree of psychosis admixture (none, attenuated psychosis, overt psychotic symptoms) in initially simple states of affective dysregulation.

The role of affective dysregulation in the onset of psychosis derives from experimental and observational studies (Garety et al., 2001; Hanssen et al., 2005; Freeman et al., 2013a, 2013b; Bird et al., 2017), as well as experience sampling studies that found that a primary increase in stress-reactivity was associated with subsequent increase in psychotic experiences (Myin- Germeys and van Os, 2007; Kramer et al., 2014; Klippel et al., 2017). In a recent experience sampling study, increased negative affect was found to result in later paranoia over the subsequent 180 min (Kramer et al., 2014). A network analysis of psychopath- ology similarly found that affective symptoms were likely to be on the pathway between environmental exposure (childhood adversity and cannabis use) and clinical psychosis (Isvoranu et al., 2016a, 2016b). Affective dysregulation has also been found to mediate the association between childhood adversity and psychosis (van Nierop et al., 2014) and, conversely, childhood adversity is associated with greater stress-reactivity in individuals at risk of psychosis (Veling et al., 2016; Klippel et al., 2017).

Aberrant salience, or the attribution of salience to typically non-salient stimuli, is a concept rooted in the dopaminergic hypothesis of psychosis (Kapur et al., 2005; Winton-Brown et al., 2014). The concept suggests that a primary dopaminergic dysfunction results in attention and action-selection being redirected to irrelevant internal or external stimuli or being directed diffusely, leading to sensory overload. This state of aberrant salience leads to delusion formation, in an attempt by the individual to make sense of the experience (Winton-Brown et al., 2014). Experience of aberrant salience was found to be associated with greater risk of psychotic experiences in at-risk individuals (Reininghaus et al., 2016). Psychotic experiences or attenuated psychosis has been shown to predict conversion to psychotic dis- order (Brucato et al., 2017; Crump et al., 2017), suggesting that they lie on a spectrum of increasing psychopathology.

Among the environmental risk factors, childhood adversity (Varese et al., 2012), urbanicity (Vassos et al., 2012; Heinz et al., 2013), and regular cannabis use (D'Souza et al., 2016; Marconi et al., 2016) have been shown to increase the risk of developing psychotic experiences, persistent psychotic symptoms, and psychotic disorders in epidemiological studies. There is also emerging evidence that some environmental risk factors have stronger effects if there is also evidence of (proxy) genetic risk (van Os et al., 2010). In the current study, we wished to examine the role of environmental risk factors in relation to the earliest ontogenesis of psychosis, defined as the degree of psychosis admixture 'complicating' an early state of affective dysregulation.

We wished to test the hypothesis that the association with known environmental risk factors (cannabis use, childhood adversity, and urbanicity) would grow progressively stronger across higher levels of psychosis admixture occurring across more severe affective states, in interaction with evidence of proxy genetic risk. In this analysis, we used evidence of familial affective dysregulation as a proxy for genetic liability, given earlier evidence that (i) molecular genetic risk for schizophrenia can be modeled through affective dysregulation in the relatives (van Os et al., 2017); (ii) evidence indicating overlap in genetic risk for affective and psychotic disorder (Cardno et al., 1999; Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013); and (iii) evidence that family history of affective dysregulation is an indicator of more severe illness (Milne et al., 2009). Thus, in this analysis, we sought to examine whether child- hood adversity, urbanicity, and cannabis use (i) impacted, in a dose–response fashion, the level of psychosis admixture (from none to attenuated psychosis to overt psychotic symptoms) across different severity states of affective dysregulation, (ii) interacted with proxy genetic risk, showing, for a given level of psychosis admixture, greater effect size in the familial stratum compared with the non-familial stratum.

Method

Sample

Data were derived from three waves of the second Netherlands Mental Health Survey and Incidence Study (NEMESIS-2), a longitudinal study of the prevalence, incidence, course, and con- sequences of psychiatric disorders in the Dutch general population. The study was approved by the standing medical ethics committee. Participants were selected based on a multistage random sampling procedure, resulting in a sample that was representative of the Dutch adult population in terms of age, region, and population density. Participants were included between the ages of 18 and 65; insufficient fluency in Dutch was an exclusion criterion. The participants were interviewed at home by trained interviewers, who were not clinicians, with the Composite International Diagnostic Interview (CIDI) version 3.0 (Alonso et al., 2004; de Graaf et al., 2008) and additional questionnaires. A more detailed description of NEMESIS-2 methodology is presented elsewhere (de Graaf et al., 2010, 2012).

In the first wave (T0), a total of 6646 persons aged 18–64 years were included. Participants were approached for two follow-up surveys, respectively, 3 years (T1) and 6 years (T2) after baseline. At T1, 5303 persons were interviewed again (response rate 80.4%; excluding those who deceased). At T2, 4618 persons were inter- viewed (response rate 87.8%). Attrition (T0–T1 and T1–T2) was not associated with any of the 12-month mental disorders at T0 (controlled for sociodemographic factors), except for alcohol and drug dependence at T1, which was significantly associated with attrition at T2 (de Graaf et al., 2013, 2015). The mean period between the baseline interview and second follow-up interview was 6 years and 6 days. A more comprehensive description of the design can be found elsewhere (van Nierop et al., 2015).

Assessment of psychopathology

Affective dysregulation

Depressive, manic, and anxiety symptoms were assessed with CIDI 3.0 (de Graaf et al., 2008). As described elsewhere (van Nierop et al., 2015), affective dysregulation was coded as a binary variable [i.e. considered present if participants experienced at least one of the CIDI 3.0 core symptoms of depressive episode, panic disorder, social phobia, generalized anxiety disorder, and manic episode, assessed at baseline (assessing lifetime occurrence) and each follow-up visit (assessing interval occurrence)].

Psychosis

Presence of psychotic experiences was assessed using a questionnaire based on CIDI 1.1 specifically developed for the purpose of assessing psychotic symptoms, since studies

on earlier CIDI ver- sions concluded that the instrument did not adequately measure psychotic symptoms (Andrews and Peters, 1998). Participants were asked at baseline and each follow-up whether they had experienced any of a list of 20 positive psychotic symptoms (van Nierop et al., 2015). For each symptom category, symptoms were considered present when participants endorsed at least one symptom. All symptoms were assessed using 'yes' or 'no' questions, and sum scores were obtained by adding reported psychotic symptoms. If symptoms were endorsed, subjects were asked whether they had sought help for these symptoms.

Family history of affective disorders

Family history was assessed as a person-level characteristic in two stages. First, the following psychiatric diagnoses were assessed by self-report in participants who had screened positive for affective dysregulation: depression, mania, and anxiety disorders (panic disorder, social phobia, agoraphobia, generalized anxiety dis- order). A total of 44% of the sample thus screened positive at any of the three interview waves. Second, at the first follow-up, self-reported parental history of 'severe anxiety or phobias', 'severe depression', and 'delusions or hallucinations' were assessed in the entire sample: an additional 20% thus screened positive, bringing the total screening positive for family history at 64% (hereafter called 'FH'). Using these two sources of information, the proportion of the sample in which family history could be assessed was 94%.

Strata of psychopathology

Level of psychopathology was defined based on the degree of admixture of affective and psychotic symptom dimensions and the severity of psychotic experiences: 0 = no symptoms, 1 = any psychotic experience but no affective dysregulation, 2 = affective dysregulation but no psychotic experience, 3 = affective dysregulation and one or two psychotic experiences that did not require help-seeking for psychotic experiences (hereafter: 'attenuated psychosis'), 4 = affective dysregulation and psychotic experience in more than two domains that did not require help-seeking for psychotic experiences or affective dysregulation and any psychotic experience that required help-seeking for psychotic experiences (hereafter: 'clinical psychosis').

Each of these five levels of psychopathology was combined with FH to construct 10 vulnerability strata.

Exposure to environmental risks

Childhood adversity

Childhood adversity was assessed using a questionnaire based on the NEMESIS-1 trauma questionnaire (de Graaf et al., 2010). Whenever a subject reported having expe-

rienced one of five types of childhood adversity [two times or more emotional neglect (not listened to, ignored, or unsupported), physical abuse (kicked, hit, bitten, or hurt with object or hot water), psychological abuse (yelled at, insulted, unjustly punished/ treated, threatened, belittled, or blackmailed), peer victimization (bullying), and one time or more sexual abuse (any unwanted sexual experience) before the age of 16], they were asked to state how often it had occurred. The item 'sexual abuse' was rated on a scale of 1 (once) to 5 (very often), while all other items (namely, emotional neglect, physical abuse, psychological abuse, and peer victimization or bullying) were rated and on a scale of 1 (sometimes) to 5 (very often). Conforming with previous work in this area, the childhood adversity score was dichotomized at the 80th percentile (van Dam et al., 2015).

Cannabis use

Cannabis use was assessed in the section Illegal Substance Use of the CIDI 3.0. Conforming with previous work, the cut-off of use of once per week or more in the period of most frequent use was used to define a binary variable for regular cannabis use (van Winkel et al., 2011).

Urbanicity

The extent of the exposure to urban environment until age 16 years was constructed at five levels based on the Dutch classification or population density: (1) countryside (distances to amenities is larger), (2) village (<25 000 inhabitants), (3) small city (25 000–50 000 inhabitants), (4) medium city (50 000–100 000 inhabitants), (5) large city (>100 000 inhabitants). Consistent with previous work, the cut-off of >50 000 inhabitants was used to define the binary variable of urban area (Guloksuz et al., 2015).

Statistical Analysis

All analyses were performed using STATA, version 14. The level of significance (α) was set at 0.05. In line with previous analyses in this sample (Guloksuz et al., 2015), cross-sectional data ana- lysis was performed using the 'long format' [each individual con- tributing three observations (T0, T1, and T2)], adding precision to the estimates. Consistent with previous work in this area (Morgan et al., 2014; Guloksuz et al., 2015), the five psychopath- ology outcomes were modeled as a function of the joint effects of FH and each environmental risk factor (regular cannabis use, childhood adversity, urbanicity) under a model of additive inter- action, expressed as the interaction contrast ratio (ICR), calculated with the NLCOM option in Stata. The ICR is the excess risk due to interaction relative to the risk without exposure. The ICR method as suggested by Knol et al. (2007) allows use of odds ratios (ORs) derived from logistic models to estimate the relative excess risk as a result of synergy for combinations of dichotomous,

ordinal, and continuous exposures (i.e. ICR = OR (exposure A and exposure B) – OR (exposure A only) – OR (exposure B only) + 1. An ICR greater than zero is defined as a positive deviation from additivity.

To test our hypotheses on synergism, the combination of each environmental factor (childhood trauma, urbanicity, cannabis use) and family history of affective dysregulation were included as independent variables (three dummy variables with non- exposed state as the reference category), and strata of psychopath- ology was included as the dependent variable in logistic models (Knol et al., 2007). Using the ORs derived from these models, ICRs (e.g. ICR = OR (childhood trauma and psychosis expression) – OR (childhood trauma) – OR (psychosis expression) + 1) for each model were calculated using the Stata NLCOM command.

All analyses were corrected for sex, age, education [(1) primary school, (2) lower secondary education, (3) higher secondary edu- cation, (4) higher professional education), and first-generation minority status (dichotomized as born in the Netherlands v. other).

Results

The sample size for the analysis was 16 140 observations from surveys of 6646 participants at three time points (T0, T1, and T2) [n = 6646 in the first wave (T0), n = 5303 in second wave (T1), and n = 4618 in third wave (T3)]. The demographics of the NEMESIS-2 sample has been published elsewhere (de Graaf et al., 2010, 2012). Pertinent to this paper, the descriptive data in the different strata with respect to age, sex, minority status, and education are detailed in Table 1. Figure 1a–c shows the rates of regular cannabis use, childhood adversity, and urbanicity across strata of five increasing levels of psychopathology complicating affective dysregulation and two levels of family history. The figures show that (i) for each environmental risk and for each of the five outcome levels, the exposure rate is higher in the familial history stratum as compared with the non-familial history stratum; and (ii) for childhood adversity and cannabis use, but not urbanicity, exposure rate is progressively higher with higher levels of psychosis admixture complicating affective dysregulation.

Table 1. Descriptive data per strata

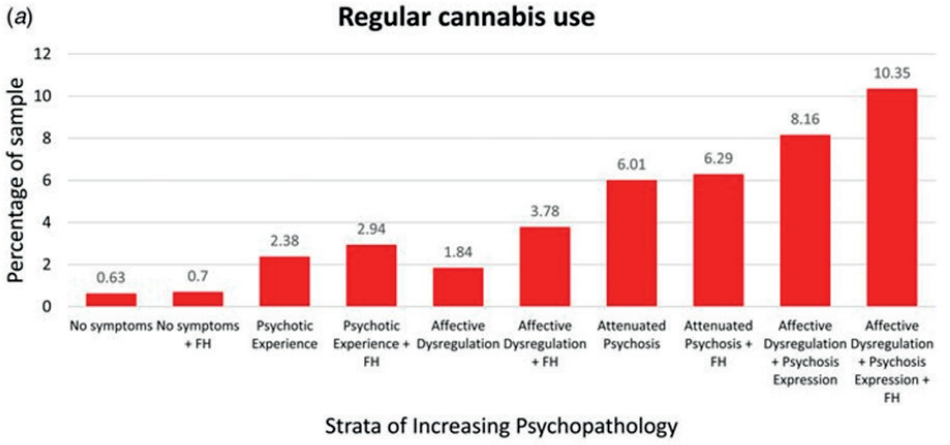
	N (%)	Age (mean±S.D.)	Female n (%)	Minority Status n (%)	Educationa (mean±S.D.)
No symptoms	4363 (27.03%)	49.34 ± 12.90	2044 (46.85%)	294 (6.74%)	3.04 ± 0.90
No symptoms + FH	2624 (16.26%)	49.00 ± 12.49	1457 (55.33)	117 (4.46%)	3.10 ± 0.89
Psychosis expression	128 (0.79%)	47.88 ± 13.53	69 (53.91%)	9 (7.03%)	2.85 ± 0.85
Psychosis expression + FH	137 (0.85%)	46.54 ± 12.31	84 (61.31)	7 (5.11%)	2.95 ± 0.90
Affective dysregulation	2125 (13.17%)	47.13 ± 12.20	1093 (51.44%)	163 (7.67%)	3.03 ± 0.89
Affective dysregulation + FH	5252 (32.54%)	45.61 ± 12.35	3251 (61.90%)	398 (7.58%)	3.02 ± 0.90
Affective dysregulation + attenuated psychosis	191 (1.18%)	45.85 ± 13.35	106 (55.50%)	25 (13.09%)	2.81 ± 0.90
Affective dysregulation + attenuated psychosis + FH	844 (5.23%)	44.52 ± 12.36	349 (65.05%)	76 (9.00%)	2.84 ± 0.90
Affective dysregulation + psychosis expression	51 (0.32%)	42.92 ± 12.78	23 (45.10%)	9 (17.65%)	2.82 ± 0.91
Affective dysregulation + psychosis expression + FH	425 (2.63%)	43.42 ± 12.80	271 (63.76%)	56 (13.18%)	2.76 ± 0.88
Total	16 140	47.27 ± 12.67	8947 (55.43%)	1154 (7.15%)	3.02 ± 0.90

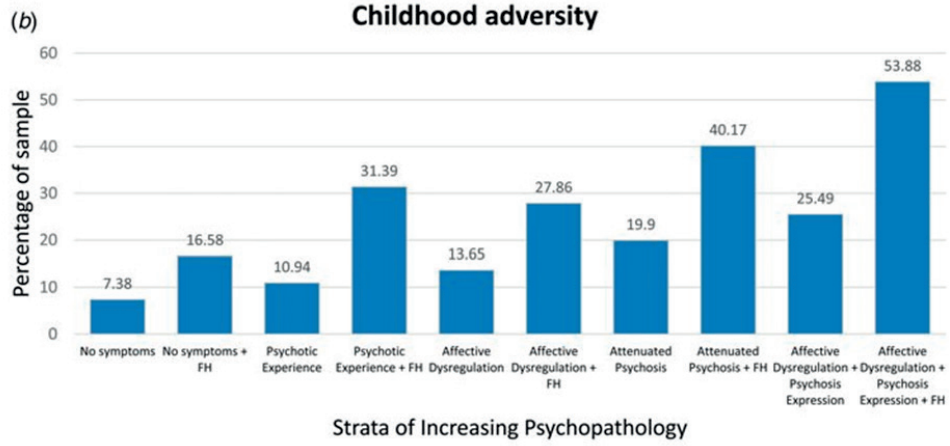
Data from surveys of 6646 participants at three time points (T0, T1, and T2), yielding a total of 16 140 observations for analysis in the 'long' format.

[a]Four-level education: (1) primary school, (2) lower secondary education, (3) higher secondary education, (4) higher professional education, university; FH, family history of affective disorders.

Family history–environment interaction analysis

The FH × environment interaction analyses revealed that, consistent with the results displayed in Fig. 1, the risk associated with environmental exposure was significantly greater if there was also evidence of familial affective liability (Fig. 2). In addition, the greater-than-additive effect of environmental risk in combination with familial affective liability grew progressively greater as psychopathology outcomes were more severe in the sense of more psychosis admixture complicating affective dysregulation (Table 2).


(a)
Regular cannabis use
Percentage of sample
12
10
8
6
4
2
0
0.63
0.7
2.38
2.94
1.84
3.78
6.01
6.29
8.16
10.35
No symptoms
No symptoms + FH
Psychotic Experience
Psychotic Experience + FH
Affective Dysregulation
Affective Dysregulation + FH
Attenuated Psychosis
Attenuated Psychosis + FH
Affective Dysregulation + Psychosis Expression
Affective Dysregulation + Psychosis Expression + FH
Strata of Increasing Psychopathology


(b)
Childhood adversity
Percentage of sample
60
50
40
30
20
10
0
7.38
16.58
10.94
31.39
13.65
27.86
19.9
40.17
25.49
53.88
No symptoms
No symptoms + FH
Psychotic Experience
Psychotic Experience + FH
Affective Dysregulation
Affective Dysregulation + FH
Attenuated Psychosis
Attenuated Psychosis + FH
Affective Dysregulation + Psychosis Expression
Affective Dysregulation + Psychosis Expression + FH
Strata of Increasing Psychopathology

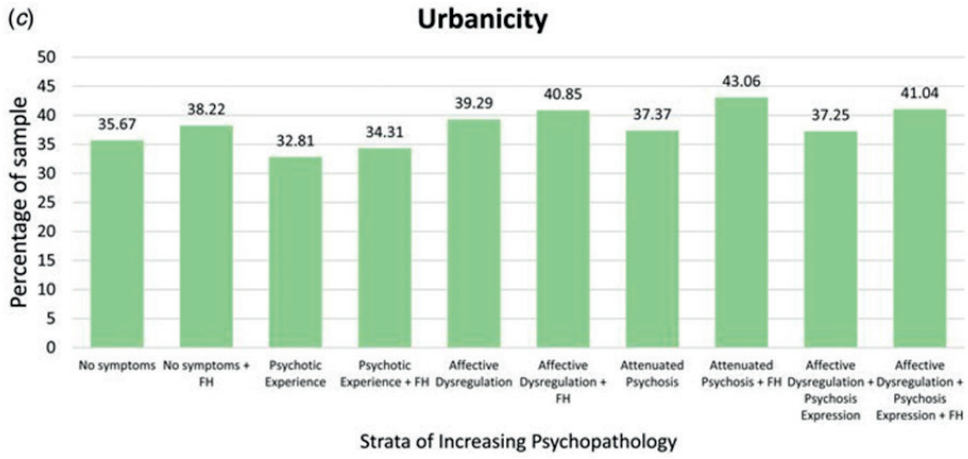


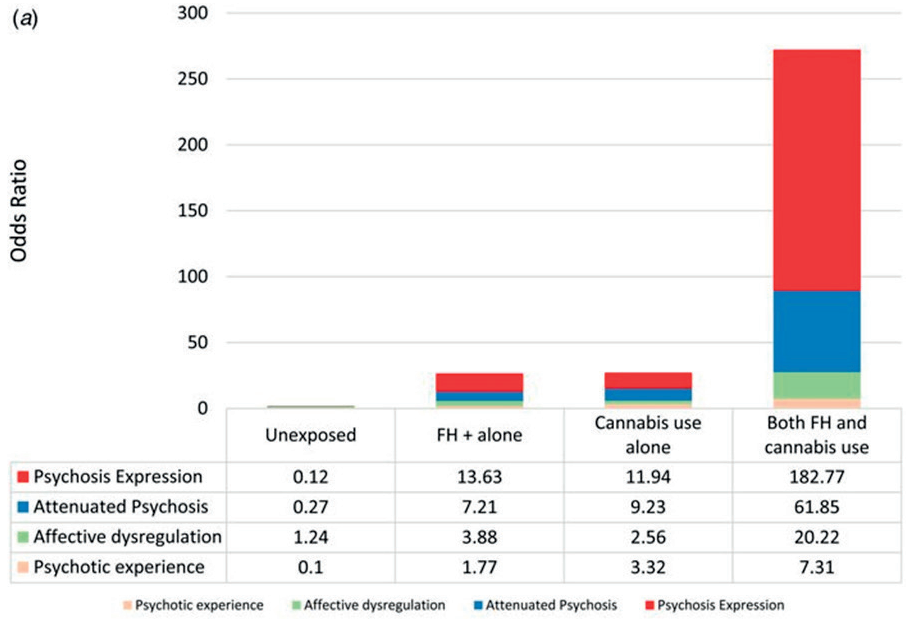
Figure 1.

(a) Depicts rates of regular cannabis use across strata of increasing levels of psycho- pathology complicating affective dysregulation. Rates of regular cannabis use is greater with increasing levels of psychopathology.

(b) Depicts rates of childhood adversity across strata of increasing levels of psycho- pathology complicating affective dysregulation. Rates of childhood adversity is greater with increasing levels of psychopathology.

(c) Depicts rates of urbanicity across strata of increasing levels of psychopathology complicating affective dysregulation. Rates of urbanicity did not increase with increasing levels of psychopathology.

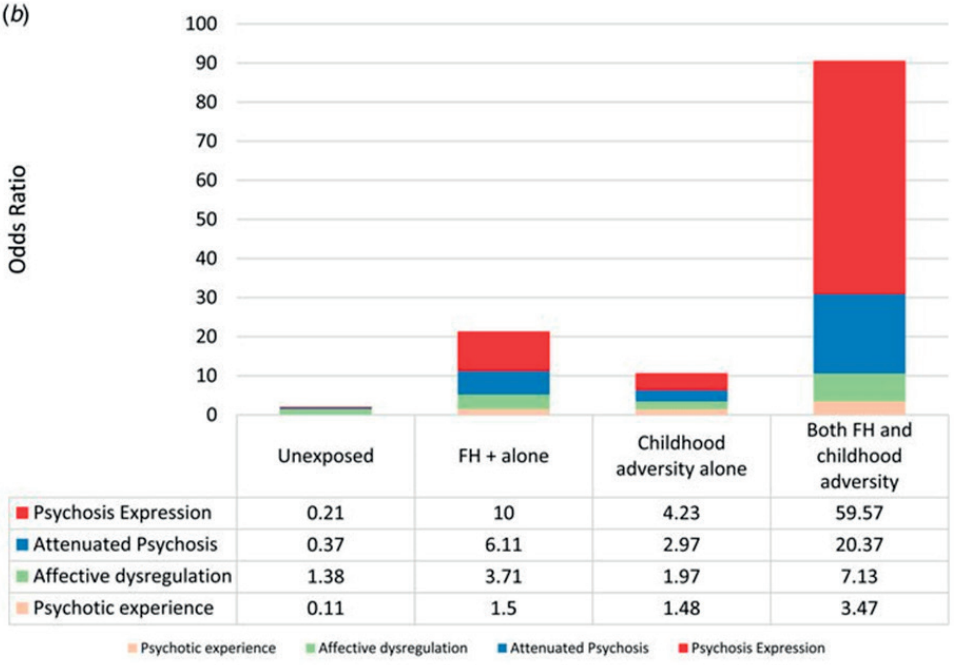
(a)



	Unexposed	FH + alone	Cannabis use alone	Both FH and cannabis use
Psychosis Expression	0.12	13.63	11.94	182.77
Attenuated Psychosis	0.27	7.21	9.23	61.85
Affective dysregulation	1.24	3.88	2.56	20.22
Psychotic experience	0.1	1.77	3.32	7.31

FH= family history of affective disorder

(b)



	Unexposed	FH + alone	Childhood adversity alone	Both FH and childhood adversity
Psychosis Expression	0.21	10	4.23	59.57
Attenuated Psychosis	0.37	6.11	2.97	20.37
Affective dysregulation	1.38	3.71	1.97	7.13
Psychotic experience	0.11	1.5	1.48	3.47

FH= family history of affective disorder

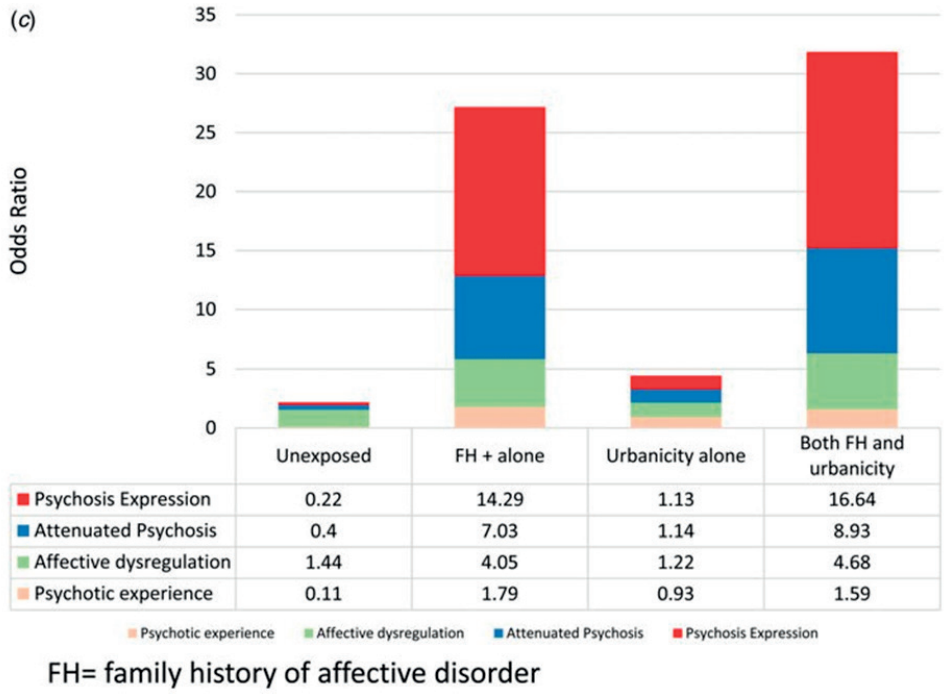


Figure 2.

(a) Depicts individual and joint effects of FH and cannabis use on risk across levels of psychopathology. The risk with both FH + cannabis use is greater than the individual risks with FH alone and cannabis use alone. (b) Depicts individual and joint effects of FH and child- hood adversity use on risk across levels of psychopath- ology. The risk with both FH + childhood adversity is greater than the individual risks with FH alone and childhood adversity alone. (c) Depicts individual and joint effects of FH and urbanicity on risk across levels of psychopathology. The risk with both FH + urbanicity use is greater than the individual risks with FH alone and urbanicity alone.

Table 2. Greater-than-additive risk of family history of affective dysregulation and environmental exposure across strata of increasing psychopathology

Levels of psychopathology → / Exposure to risk ↓	Psychotic experience			Affective dysregulation			Attenuated psychosis			Psychosis expression		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Cannabis use												
Unexposed	0.10	0.05–0.21	<0.001	1.24	0.97–1.59	0.08	0.27	0.16–0.44	<0.001	0.12	0.06–0.24	<0.001
FH + alone	1.77	1.34–2.33	<0.001	3.88	3.58–4.21	<0.001	7.21	5.92–8.79	<0.001	13.63	9.69–19.19	<0.001
Cannabis use alone	3.32	1.04–10.58	0.04	2.56	1.56–4.20	<0.001	9.23	4.35- 19.58	<0.001	11.94	3.79–33.66	<0.001
Both FH and cannabis use	7.31	2.40–22.27	<0.001	20.22	12.40–32.98	<0.001	61.85	34.64–110.44	<0.001	182.77	93.61–356.84	<0.001
Interaction contrast ratio (ICR)	3.22	−5.60 to 12.05	0.48	14.78	4.86 to 24.71	0.003	46.41	10.70 to 82.12	0.01	158.19	39.12–277.27	0.009
Childhood adversity												
Unexposed	0.11	0.05–0.23	<0.001	1.38	1.09–1.75	0.008	0.37	0.23–0.59	<0.001	0.21	0.10–0.43	<0.001
FH + alone	1.50	1.11–2.03	0.008	3.71	3.40–4.05	<0.001	6.11	4.93–7.58	<0.001	10.00	6.83–14.63	<0.001
Childhood adversity alone	1.48	0.84–2.62	0.17	1.97	1.64–2.38	<0.001	2.97	1.95–4.51	<0.001	4.23	2.15–8.34	<0.001
Both FH and childhood adversity	3.47	2.30–5.24	<0.001	7.13	6.23–8.15	<0.001	20.37	15.90–26.10	<0.001	59.57	40.06–88.59	<0.001
Interaction contrast ratio (ICR)	1.49	−0.01 to 2.98	0.05	2.45	1.50 to 3.39	<0.001	12.29	8.12 to 16.46	<0.001	46.34	26.40–66.29	<0.001
Urbanicity												
Unexposed	0.11	0.05–0.23	<0.001	1.44	1.13–1.82	0.003	0.40	0.25–0.66	<0.001	0.22	0.11–0.45	<0.001
FH + alone	1.79	1.28–2.50	0.001	4.05	3.65–4.50	<0.001	7.03	5.54–8.91	<0.001	14.29	9.57–21.33	<0.001
Urbanicity alone	0.93	0.63–1.38	0.72	1.22	1.08–1.38	0.002	1.14	0.80–1.61	0.47	1.13	0.61–2.08	0.70
Both FH and urbanicity	1.59	1.06–2.38	0.03	4.68	4.18–5.24	<0.001	8.93	6.98–11.43	<0.001	16.64	10.96–25.26	<0.001
Interaction contrast ratio (ICR)	−0.13	−0.90 to 0.63	0.73	0.41	−0.09 to 0.90	0.11	1.77	0.23 to 3.31	0.02	2.22	−1.94 to 6.39	0.30

Discussion

Findings

This study investigated the association between the level of psychosis admixture in affective dysregulation and environmental risks, and to what degree these risks were conditional on affective familial liability. The main findings were: (i) for a given stratum of psychosis admixture, the association with environmental risk factors was greater-than-additive if there was also evidence of familial affective liability; (ii) the ICR grew progressively greater over the five psychopathology levels indicative of greater severity due to greater psychosis admixture complicating affective dysregulation.

Affective pathway to psychosis

There is growing evidence that affective dysregulation is an early sign of psychosis, representing the mildest form along the severity dimension of psychosis (Kelleher et al., 2012; Wigman et al., 2012). Among the so-called clinical high-risk populations, affect- ive dysregulation is more prevalent in the group with high risk of 'conversion' to psychotic disorder (Addington et al., 2007), i.e. the most severe psychopathological state. The association between affective dysregulation and psychosis has long been demonstrated in studies across the psychosis spectrum including among patients with psychotic disorders (McMillan et al., 2009), those with sub- threshold psychotic experiences (van Rossum et al., 2011; Wigman et al., 2012; Stochl et al., 2015), clinical high-risk populations (Fusar-Poli et al., 2014), and prodromal samples (Hafner et al., 1999). In agreement with the current analyses, previous work suggests that early states of affective dysregulation may progress to more severe states characterized by psychotic symptoms following exposure to environmental risk factors (van Os and Reininghaus, 2016). Additionally, recent studies suggest that the relationship between affective dysregulation and psychosis is not dependent on any particular mood state, i.e. anxiety, depression, or mania (Krabbendam et al., 2005; van Rossum et al., 2011; Armando et al., 2013). In one study, Armando et al. (2013) exam- ined anxiety and depressive symptoms and found that they both correlated with psychosis risk (anxiety correlated with increased CAPE positive symptom score, and depressive symptoms correlated with CAPE negative symptom score). van Rossum et al. (2011) measured depressive symptoms and hypomanic symptoms and found that they were both associated with greater risk of psychotic experiences, i.e. there was no differential effect of a particular affective state on risk of psychotic experiences. Krabbendam et al. (2005) showed that in people with hallucinatory experience, depressive symptoms increase the risk of development of a psychotic disorder, in agreement with the idea that affective dysregulation increases risk along the dimension of increasing psychopathology.

Environmental risk factors such as cannabis use, childhood adversity, and urbanicity have been shown to increase the risk of psychosis admixture among those with affective

disorders (Guloksuz et al., 2015, 2016); childhood adversity has been associated with an increased risk of admixture of psychotic and non-psychotic psychopathology (van Nierop et al., 2015); and environmental risk factors were shown to act additively in increasing the risk of psychosis (Cougnard et al., 2007).

Although the current analysis does not inform on temporal order, the findings are compatible with the view that progression from an early state of affective dysregulation to more severe states characterized by admixture with, first, attenuated psychosis and, subsequently, clinical psychosis is associated with progressively greater level of exposure to some environmental risks. The current study suggests a complex relationship between affective dysregulation, cannabis use and childhood adversity, and clinical psychosis.

The finding that cannabis use and childhood adversity, and to a lesser degree urbanicity, are associated with increased risk of clinical psychosis in those with affective dysregulation, in interaction with the concomitant presence of familial affective liability, suggests some testable hypotheses. First, it is noteworthy that childhood adversity, cannabis use, and urban environment affect individuals at distinct neurodevelopmental stages, namely during childhood (adversity) and adolescence (cannabis use), or both (urban environment) (Pedersen and Mortensen, 2001; van Os et al., 2010). Yet, they all appear to be associated with increased risk across all strata of psychosis admixture, particularly childhood adversity and cannabis use. This raises the question whether cannabis use and childhood adversity mediate risk via a final common neurobiological pathway, or if they are both environmental risk factors that are mediated by a common latent risk variable. Second, while child- hood adversity has been shown to increase stress-reactivity and cannabis use has been shown to be associated with greater affective dysregulation (Dorard et al., 2008), the direction of causality remains an open question. Novel analytic techniques such as machine learning, deep phenotyping, and ecological momentary assessment strategies may help answer these questions. Third, the dimensional relationship between affective dys- regulation and psychosis risk warrants further study. While psychosis and affective dysregulation are traditionally considered orthogonal, our results show that affective dysregulation is able to increase psychosis risk irrespective of their dimensional relationship, as also noted in other studies (Krabbendam et al., 2005).

Gene–environment interplay

While earlier studies have shown that affective dysregulation is associated with expression of psychosis in a bidirectional, dose– response fashion (van Rossum et al., 2011), the present study extends this association to familial affective liability as well. Research suggests that risk for psychosis is pleiotropically distributed across currently defined diagnostic boundaries (Cardno et al., 1999; Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013). A recent study from the GROUP cohort shows

that the polygenic risk score for psychosis was associated with affective dysregulation in both controls and the relatives of patients with a psychotic disorder, supporting this premise (van Os et al., 2017). Another recent study showed that non-psychotic mental health complaints, particularly depression, in a general population twin sample were associated with polygenic risk for schizophrenia (Nivard et al., 2017).

The results indicate that the effect sizes of cannabis use and childhood adversity, and to a lesser extent urban environment, on the level of psychosis admixture were strongly moderated by the presence of familial affective liability. These findings are in agreement with earlier work showing moderation of cannabis use and urban environment by variables indexing familial loading of psychosis (van Os et al., 2010; Genetic Risk and Outcome in Psychosis Investigators, 2011). As the terms making up the inter- action (FH × cannabis use, FH × childhood adversity, and FH × urbanicity) were all significantly associated with each other, two explanations can be invoked to explain this finding. First, familial affective liability may reflect pleiotropic genetic risk which may render individuals more sensitive to environmental risks under a model of gene–environment interaction. Second, familial affect- ive liability may reflect a high-risk environment associated with greater probability of cannabis use and childhood adversity under a model of gene–environment correlation. It is not possible to distinguish between the two in the current data set but both would be clinically relevant and both may apply to a degree (Van Os and Sham, 2003).

Gene–environment interplay in the affective pathway to psychosis

Another important finding of this study is that among those with affective dysregulation, gene–environment interplay is more prominent at the more severe level of psychosis admixture, i.e. clinical psychosis, but less at lower levels of severity such as attenuated psychosis. In the formal gene × environment ICR analysis, cannabis use and childhood adversity, and to a lesser extent urbanicity, was associated with a greater-than-additive risk at the level of clinical psychosis. This pattern of simple additivity at lower levels of psychopathology, and greater-than-additive risk at higher level of psychopathology (namely, clinical psychosis) is interesting, as it suggests that clinical relevance occurs when there is more-than-additivity. This points to the relevance of the quality and intensity of the factors associated with environmental and genetic risk, and not merely their presence.

Strengths and limitations

These findings should be interpreted in the light of several strengths and limitations. In terms of strengths, the present study has the advantage of a large number of participants, representative of the Dutch population, with direct questioning of reported psychotic experiences by trained interviewers, which is especially important in general population studies (Linscott and van Os, 2010; van Nierop et al., 2012). The study

incorporates symptom assessments across diagnostic boundaries, providing an opportunity to examine multiple strata of increasing psycho- pathology and familial risk as is present in the general population.

The most obvious limitation of the current analysis is its cross- sectional nature, precluding conclusions of causality. Another limitation, although difficult to avoid, is the retrospective nature of the information on childhood adversity, which may have resulted in increased random error. Previous work has found that recall of childhood adversity is reliable, including in individuals with psychotic disorders (Fisher et al., 2011). Similarly, the assessment of cannabis use and urbanicity was based on CIDI questions and might be subject to decreased precision in reporting of use. Additionally, assessment of familial risk was by different short instruments where the respondent reported whether parents had mental health problems, and is hence limited by the respondent's knowledge of the same. The NEMESIS study did not include family history of all possible mental disorders since they are of relatively low prevalence. This however does not limit the interpretation of our analysis since the primary question in this paper was focused mainly on the group with affective psychopath- ology, i.e. whether cannabis use and other risks were associated with increasing psychopathology in the psychosis dimension in this group.

Conclusions

In conclusion, the current study provides evidence that exposure to regular cannabis use and childhood adversity, and to a lesser extent urban environment, results in increasing levels of psychosis admixture in early states of affective dysregulation, supporting the model of an affective pathway to psychosis. In addition, the results suggest that there is interplay between familial affective liability and environmental risks, possibly in the direction of synergism. The findings imply that a multidimensional staging of severity cutting across traditional diagnostic clusters extended by means of genetic and environmental risk tiers may provide a suitable framework to gain insight into early psychopathology that often emerges as a mixed bag of subtle symptoms and may further progress to a more distinct and severe clinical syndrome, such as psychosis spectrum disorder (Guloksuz and van Os, 2017).

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Conflict of interest

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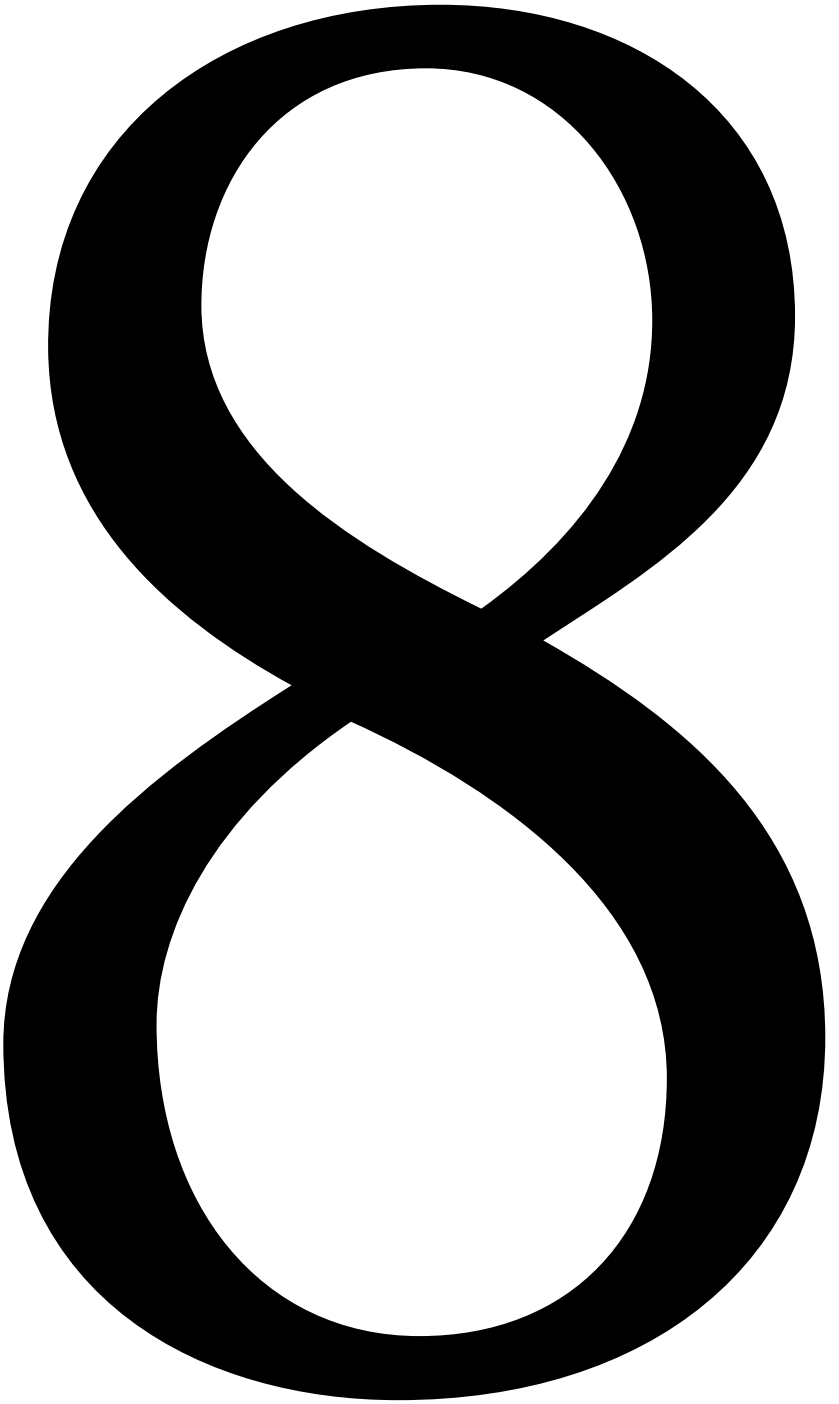

Chapter 8

Bidirectional relationships between cannabis use, anxiety and depressive symptoms in the mediation of the association with psychotic experience: further support for an affective pathway to psychosis

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Abstract

Background

Empirical evidence suggests that people use cannabis to ameliorate anxiety and depressive symptoms, yet cannabis also acutely worsens psychosis and affective symptoms. However, the temporal relationship between cannabis use, anxiety and depressive symptoms and psychotic experiences (PE) in longitudinal studies is unclear. This may be informed by examination of mutually mediating roles of cannabis, anxiety and depressive symptoms in the emergence of PE.

Methods

Data were derived from the second longitudinal Netherlands Mental Health Survey and Incidence Study. Mediation analysis was performed to examine the relationship between cannabis use, anxiety/depressive symptoms and PE, using KHB logit in STATA while adjusting for age, sex and education status.

Results

Cannabis use was found to mediate the relationship between preceding anxiety, depressive symptoms and later PE incidence, but the indirect contribution of cannabis use was small (for anxiety: % of total effect attributable to cannabis use = 1.00%; for depression: % of total effect attributable to cannabis use = 1.4%). Interestingly, anxiety and depressive symptoms were found to mediate the relationship between preceding cannabis use and later PE incidence to a greater degree (% of total effect attributable to anxiety = 17%; % of total effect attributable to depression = 37%).

Conclusion

This first longitudinal cohort study examining the mediational relationship between cannabis use, anxiety/depressive symptoms and PE, shows that there is a bidirectional relationship between cannabis use, anxiety/depressive symptoms and PE. However, the contribution of anxiety/depressive symptoms as a mediator was greater than that of cannabis.

Keywords: Affective dysregulation; bidirectional relationship; cannabis; environment; longitudinal cohort; mediation analysis; population survey; psychosis; risk factors

Introduction

Epidemiological studies have consistently shown an association between cannabis use and the emergence of psychosis (Di Forti et al., 2019a; 2019b; Pries et al., 2018). Mendelian randomization studies examining the association between genetic predisposition, cannabis use and psychosis have found a bidirectional relationship i.e. genetic vulnerability predisposes to earlier cannabis use and cannabis use predisposes to psychosis among those with genetic vulnerability (Gillespie & Kendler, 2021). Patients with psychosis also have high rates of cannabis use (Boydell et al., 2006). The reasons for these seemingly counterintuitive findings of greater risk of psychosis with cannabis use and high rates of cannabis use among patients with psychosis are not fully understood. One possible explanation for these discrepant findings is that cannabis use ameliorates anxiety and depressive symptoms while also increasing the risk for psychotic experiences (PE).

While it is difficult to test this hypothesis in patients who have already developed psychosis, a general population, longitudinal sample such as the Netherlands Mental Health Survey and Incidence Study-2 (NEMESIS-2), provides a unique opportunity to examine this question. Approximately 5–8% of the general population may report PE (Linscott & van Os, 2013). We have previously shown that anxiety and depressive symptoms increase the risk of PE in interaction with environmental risk factors such as cannabis use and childhood adversity, consistent with an 'affective pathway' to psychosis (Guloksuz et al., 2015; Pries et al., 2018; Radhakrishnan et al., 2019; van Os et al., 2020). The important role of an 'affective pathway' to psychosis is also supported by experimental and observational studies (Bird, Waite, Rowsell, Fergusson, & Freeman, 2017; Freeman et al., 2013a, 2013b; Garety, Kuipers, Fowler, Freeman, & Bebbington, 2001; Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005) where anxiety, depressive symptoms and worry is associated with paranoia and anomalous perceptual experiences, as well as experience sampling studies showing that increased stress-reactivity and negative affect are associated with subsequent increase in PE (Kramer et al., 2014; Myin-Germeys & van Os, 2007). A network analysis of psychopathology showed that affective symptoms were on the pathway between environmental risk-factors such as cannabis use and psychosis expression (Isvoranu et al., 2017). More recently, depression was shown to contribute to greater than multiplicative effect to the association between polygenic risk of schizophrenia, psychosis and childhood trauma (van Os et al., 2020). Additionally, it has been shown recently in the NEMESIS-2 data, that a fixed-effects model yields reliable estimates when examining the causal association between cannabis use and psychosis, and that the direction of causality is from cannabis use to psychosis i.e. cannabis use precedes the onset of psychosis (van Os et al., 2021a, 2021b). The question then arises whether the causal association between cannabis use and PE is mediated by anxiety/depressive symptoms or whether the relationship is bidirectional.

The presence of a bidirectional relationship between cannabis use and anxiety/depressive symptoms in the relationship to PE can be examined using mediation analysis. In this regard, we sought to examine the mediational relationship between cannabis use, anxiety/depressive symptoms, and PE. We hypothesized that cannabis use mediates the relationship between anxiety/depressive symptoms and PE. We also examined whether anxiety/depressive symptoms mediated the relationship between cannabis use and PE.

Method

Study Population

The Netherlands Mental Health Survey and Incidence Study-2 (NEMESIS-2) is a longitudinal cohort study comprising 4 waves that examines the prevalence, incidence, course, and consequences of psychiatric disorders in the Dutch general population. The first wave (T0) that was conducted from 2007 to 2009, enrolled 6646 participants (response rate 65.1%; average interview duration: 95 min). They were followed up at year 3 (T1), year 6 (T2), and year 9 (T3) with successive response rates of 80.4% (n = 5303; excluding those who deceased; interview duration: 84 min), 87.8% (n = 4618; interview duration: 83 min), and 87.7% (n = 4007; interview duration: 101 min), respectively. The study was approved by the Medical Ethics Review Committee for Institutions on Mental Health Care and written informed consent was obtained from participants at each wave. A multistage random sampling procedure was applied to ensure representativeness of the sample to the general population in terms of age (between the ages of 18 and 65 at baseline), region, and population density. All participants were required to be literate in Dutch. The Composite International Diagnostic Interview (CIDI) version 3.0 [de Graaf, Ormel, ten Have, Burger, & Buist-Bouwman, 2008; Alonso J, et al., 2004] and additional questionnaires were administered by non-clinician, trained interviewers during home visits. Additional details of NEMESIS-2 are provided elsewhere (de Graaf, Ten Have, & van Dorsselaer, 2010; de Graaf, ten Have, van Gool, & van Dorsselaer, 2012). Data from all four waves were utilized. While there was some attrition between T0 and T3, this was not significantly associated with any of the individual 12-month mental disorders at T0 after controlling for sociodemographic characteristics (Nuyen et al., 2020).

Outcome measure: psychotic experiences (PE)

PE were assessed using a 20-item binary-response questionnaire based on CIDI 1.1 as used elsewhere (Pries et al., 2018; Radhakrishnan et al., 2019; van Nierop et al., 2015), since earlier CIDI versions were found not to be adequate for capturing positive psychotic symptomatology. The primary binary outcome was the presence of any self-report PE conforming to previous studies (Guloksuz et al., 2020; Pries et al., 2021, 2020; Radhakrishnan et al., 2019).

Assessment of anxiety and depressive symptoms

Symptoms of anxiety disorders including social phobia, specific phobia, panic disorder, generalized anxiety disorder, agoraphobia without panic disorder and symptoms of depressive disorders were assessed using CIDI 3.0 at each visit (de Graaf et al., 2008). Conforming to previous publications, we used two binary variables for depressive and anxiety symptoms, respectively (e.g. considered present if participants experienced at least one of the CIDI 3.0 core symptoms of depressive (or anxiety) disorder at each time-point) (Pries et al., 2018; Radhakrishnan et al., 2019).

Assessment of cannabis use

The frequency of cannabis use over the last year was assessed using the section Illegal Substance Use of the CIDI 3.0 at each visit as used previously (Radhakrishnan et al., 2019). If subjects reported cannabis use, they were rated on frequency of use on a scale of 1 (never) to 7 (every day). A binary variable (absent = '0' and present = '1') was constructed by using the cut-off value of once per week or more in the previous year, consistent with other studies (Pries et al., 2021, 2020; Radhakrishnan et al., 2019).

Statistical analysis

All analyses were performed using Stata, version 16 (StataCorp., 2019). The level of significance (α) was set at 0.05. To establish mediation, the following assumptions should be met [detailed in Baron and Kenny (1986), Gelfand, Mensinger, and Tenhave (2009)]: (1) The independent variable should be associated with the outcome; (2) There should be a association between the independent variable and the mediator, and between the mediator and the outcome; (3) The independent variable, mediated through the mediator, should be associated with the outcome. For mediation, the total effect of the independent variable on the outcome should be, at least partly, explained by the mediator (indirect effect). In the mediation model, the direct effect of the independent variable should decrease.

Given that mediation requires temporal precedence from independent variable to me- diator to outcome, thus requiring absence of the outcome at the previous wave T–1, PE incident at time point T was modeled as a function of independent variable and mediator assessed over the interval T–1 to T, conforming to previous analyses conducted in the NEMESIS-2 (van Os et al., 2021a, 2021b).

Logistic regression models were performed to analyze whether there was an association between the presence of affective dysregulation (any anxiety symptom and any depressive symptom, separately) at the preceding time point (T–1) and PE outcomes. Consequently, the KHB procedure, developed for estimating mediation in logistic regression models, was applied to estimate the mediation effect of cannabis use (Fig. 1a). Logistic regression models were performed to analyze whether there was an association between

cannabis use in the previous year and PE outcomes. Subsequently, the KHB procedure was applied to estimate the mediation effect of the presence of affective dysregulation (any anxiety symptom and any depressive symptom, separately) (Fig. 1b). To correct for clustering of multiple observations within subjects, the CLUSTER option was applied in all logistic regression models to estimate cluster-robust standard errors.

All analyses were corrected for sex, age, education (1) primary school, (2) lower secondary education, (3) higher secondary education, (4) higher professional education).

Results

This analysis used data from 6646 participants at four time points (T0, T1, T2, and T3) [n = 6646 at baseline (T0), n = 5303 in the first wave (T1), n = 4618 in the second wave (T2) and n = 4007 in the third wave (T3)]. Table 1 reports the demographics of the NEMESIS-2 at each time point.

Cannabis use as mediator for the association between anxiety symptoms and psychotic experience

Preceding anxiety symptoms were associated with cannabis use (OR 2.55, 95% CI 1.78–3.66, $p < 0.001$) and any self-reported PE (OR 1.99, 95% CI 1.65–2.42, $p < 0.001$); cannabis use was associated with self-reported PE (OR 2.36, 95% CI 1.25–4.46, $p = 0.008$). The mediation model revealed that cannabis use mediated the association between preceding anxiety symptoms and selfreported PE but explained only 1% of the relationship (Total effect of anxiety OR 1.97, 95% CI 1.63–2.39, $p < 0.001$; direct effect of anxiety OR 1.96, 95% CI 1.62–2.37, $p < 0.001$; indirect effect attributable to cannabis use OR 1.01, 95% CI 1.00–1.01, $p = 0.045$; % of total effect attributable to cannabis use = 1%) (Table 2).

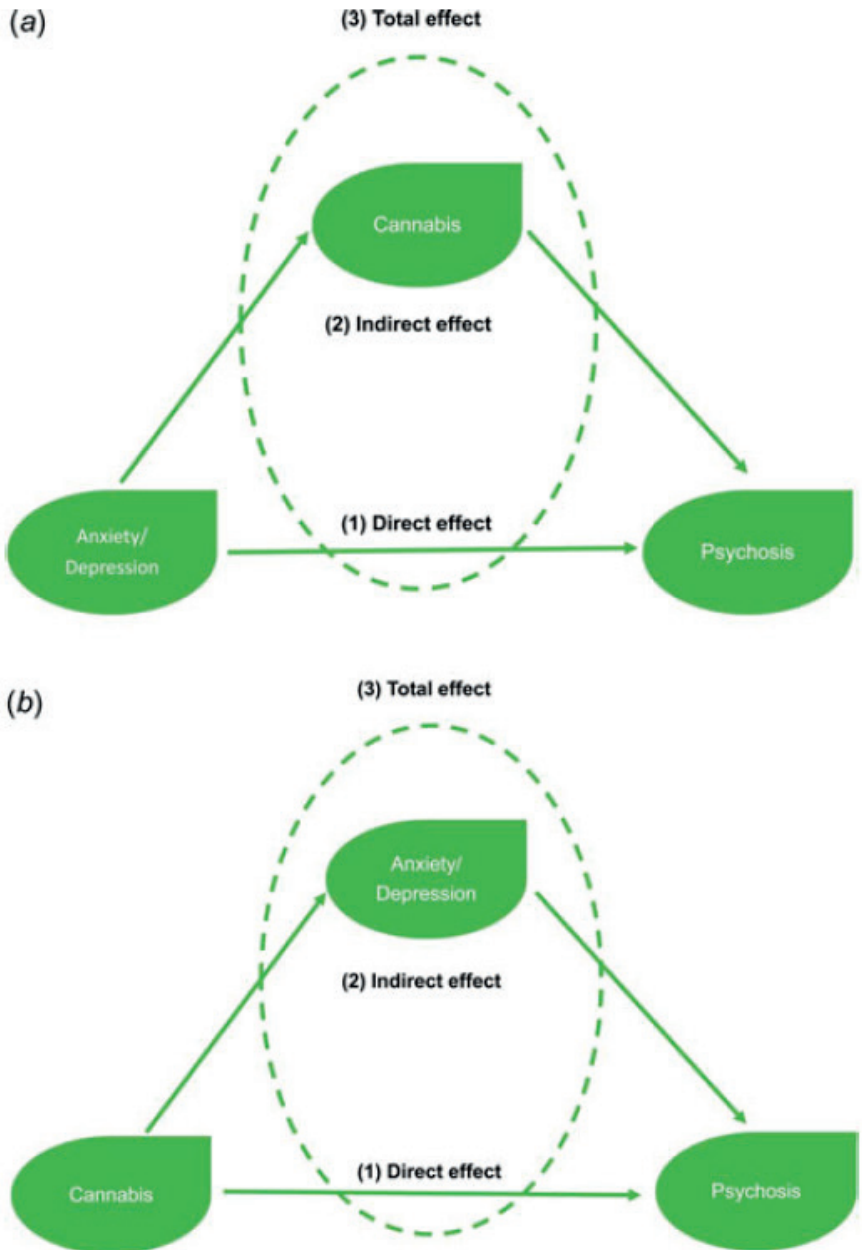

Figure 1.

(a) and (b) illustrate hypothesized mediational model pairs. In (a) the model examines cannabis as a mediator of the effects of Anxiety/Depression on Psychosis outcomes. In (b), the model examines Anxiety/ Depression as a mediator of the effects of Cannabis on Psychosis outcomes.

Anxiety symptoms as mediator for the association between cannabis use and psychotic experience

Preceding cannabis use was associated with anxiety (OR 1.55, 95% CI 1.00–2.39, p = 0.05) and, directionally and with greater effect size, any self-reported PE (OR 1.77, 95% CI 0.76–4.12, p = 0.187); anxiety was associated with self-reported PE (OR 3.05, 95% CI 2.44–3.82, p < 0.001). The mediation model revealed that anxiety explained 17% of the total relationship (Total effect of cannabis OR 1.75, 95% CI 0.75–4.06, p = 0.195; direct effect of cannabis OR 1.56, 95% CI 0.68–3.71, p = 0.281; indirect effect attributable to anxiety OR 1.10, 95% CI 0.99–1.12, p = 0.065; % of total effect attributable to anxiety = 17%) (Table 2).

Cannabis use as the mediator for the association between depressive symptoms and psychotic experience

Preceding depression was associated with cannabis use (OR 3.39, 95% CI 2.37–4.86, p < 0.001) and self-reported PE (OR 1.89, 95% CI 1.56–2.28, p < 0.001); cannabis use was associated with selfreported PE (OR 2.02, 95% CI 1.06–3.85, p = 0.033). The mediation model revealed that cannabis use was found to be a significant mediator of the relationship between depression and selfreported PE, but explained only 1.4% of the relationship (total effect of depression OR 1.86, 95% CI 1.54–2.25, p < 0.001; direct effect of depression OR 1.84, 95% CI 1.52–2.23, p < 0.001; indirect effect attributable to cannabis use OR 1.01, 95% CI 1.00–1.02, p = 0.045; % of total effect attributable to cannabis use = 1.4%) (Table 3).

Depressive symptoms as the mediator for the association between cannabis use and psychotic experiences

Preceding cannabis use was associated with depressive symptoms (OR 2.43, 95% CI 1.58–3.74, p < 0.001) and self-reported PE at a trend level (OR 1.84, 95% CI 0.86–3.98, p = 0.118); depressive symptoms was associated with self-reported PE (OR 2.86, 95% CI 2.27–3.60, p < 0.001). The mediation model revealed that depressive symptoms were found to be a significant mediator of the relationship between cannabis use and self-reported PE, and explained 37% of the relationship (Total effect of cannabis use OR 1.77, 95% CI 0.82–3.85, p = 0.146; direct effect of cannabis use OR 1.44, 95% CI 0.66–3.13, p = 0.359; indirect effect attributable to depressive symptoms OR 1.23, 95% CI 1.11–1.37, p < 0.001; % of total effect attributable to depressive symptoms = 37%) (Table 3).

Table 1. Demographics of the NEMESIS sample

	Baseline (n=6646)	3-year follow up (n=5503)	6-year follow up (n=4618)	9-year follow up (n=4007)
Female sex (%)	3672 (55.3)	2922 (55.1)	2558 (55.4)	2225 (55.5)
Mean age, years (S.D.)	44.3 (12.5)	47.6 (12.4)	50.9 (12.3)	54.1 (12.3)
Education				
- Primary education (%)	332 (5)	226 (4.3)	186 (4)	154 (3.8)
- Lower secondary education (%)	1826 (27.5)	1388 (26.2)	1193 (25.8)	1022 (25.5)
- Higher secondary education (%)	2145 (32.3)	1728 (32.6)	1479 (32)	1274 (31.8)
- Higher professional education, university degree (%)	2343 (35.3)	1961 (37)	1760 (38.1)	1557 (38.9)
Regular cannabis use in past year (%)	118 (2.2)	77 (1.5)	53 (1.2)	60 (1.5)
Presence of any anxiety/depression symptoms at each time point (%)[a]	3565 (53.6%)	1519 (27.6%)	1347 (29.2%)	1027 (25.6%)

Data are given as number (percentage) unless otherwise indicated.

[a]Baseline covers lifetime, and each follow-up covers the preceding period.

Table 2. Results of decomposition of total effect, direct effect and indirect effect of the association between cannabis use, anxiety, and incidence psychosis using the Karlson–Holm–Breen (KHB) method

	Association between preceding anxiety and incidence psychosis with cannabis use as the mediator	p value	Association between preceding cannabis use and incidence psychosis with anxiety as the mediator	p value
	OR (95% CI)		OR (95% CI)	
Total effect (Reduced)	1.97 (1.63–2.39)	<0.001	1.75 (0.75–4.06)	0.195
Direct effect (Full)	1.96 (1.62–2.37)	<0.001	1.59 (0.68–3.71)	0.281
Indirect effect (Diff)	1.01 (1.00–1.01)	0.045	1.10 (0.99–1.21)	0.065
Confounding Ratio (total effect/direct effect)	1.01		1.2	
Confounding % (attributable to the mediator)	1		16.66	
Pseudo R^2	0.02		0.04	

Table 3. Results of decomposition of total effect, direct effect and indirect effect of the association between cannabis use, depression, and incidence psychosis using the Karlson–Holm–Breen (KHB) method

	Association between preceding depression and incidence psychosis with cannabis use as the mediator	p value	Association between preceding cannabis use and incidence psychosis with depression as the mediator	p value
	OR (95% CI)		OR (95% CI)	
Total effect (Reduced)	1.86 (1.54–2.25)	<0.001	1.77 (0.82–3.85)	0.146
Direct effect (Full)	1.84 (1.52–2.23)	<0.001	1.44 (0.66–3.13)	0.359
Indirect effect (Diff)	1.01 (1.00–1.02)	0.045	1.23 (1.11–1.37)	<0.001
Confounding Ratio (total effect/direct effect)	1.01		1.58	
Confounding % (attributable to the mediator)	1.39		36.65	
Pseudo R^2	0.02		0.04	

Discussion

In this analysis of the NEMESIS-2 dataset, a large longitudinal cohort among the general population, we examined the mediational relationship between cannabis use, anxiety/depression symptoms (i.e. affective dysregulation) and PE. Our analysis showed that the mediational relationship between cannabis use, anxiety/depressive symptoms (affective dysregulation) and PE was bidirectional i.e. anxiety/depressive symptoms mediated the relationship between cannabis use and PE; and cannabis use mediated the relationship between anxiety/depressive symptoms and PE. However, although the relationship was bidirectional, the contribution of affective dysregulation in mediating the relationship between cannabis use and PE over time was greater than the mediational contribution of cannabis. These results lend further support for the presence of an 'affective pathway' to psychosis (Guloksuz et al., 2015; Pries et al., 2018; Radhakrishnan et al., 2019; van Os et al., 2020).

This is the first study to our knowledge, to show a bidirectional mediational relationship between cannabis use, affective dysregulation and psychosis in a general population sample. The directionality of increased anxiety mediating the association between cannabis use and psychosis is consistent with human laboratory challenge studies using delta-9 tetrahydrocannabinol (THC), the primary psychoactive ingredient, which show that administration of THC results in a significant increase in anxiety and psychosislike experience (Ganesh et al., 2020; Hindley et al., 2020). Interestingly, preceding anxiety (pharmacologically induced using the GABA inverse agonist, Iomazenil) has also been

shown to increase the psychosis-like effects of THC (Radhakrishnan et al., 2015). In epidemiological studies, cannabis use has been associated with greater depression in the general population (Rabiee et al., 2020), and among first-episode psychosis (Elowe, Golay, Baumann, Solida-Tozzi, & Conus, 2020), consistent with the directionality of cannabis use resulting in greater depression in our analysis. Animal studies show that THC has biphasic effects, i.e. low-dose THC is anxiolytic while high-dose THC is anxiogenic al (Salviato et al., 2021). While this has not been shown in human studies, an anxiolytic and antidepressant response to cannabis at low doses may explain the directionality of preceding anxiety, depressive symptoms resulting in increased cannabis use. These results are also consistent with the seemingly contradictory clinical observation that cannabis use is associated with psychosis, and people with psychosis are also at high risk of cannabis use.

However, as noted earlier, the contribution of cannabis use as a mediator of the relationship between affective dysregulation and PE was small in our analysis. These results hence do not support a self-medication hypothesis, but rather suggest that cannabis use results in increased affective dysregulation, supporting an 'affective pathway' to psychosis (Radhakrishnan et al., 2019; van Os et al., 2020).

Our results are consistent with studies examining the interactive effects of psychosis risk using schizophrenia polygenic risk score (PRS-SZ) for psychosis, cannabis use and affective dysregulation (Di Forti et al., 2019a, 2019b; Guloksuz et al., 2019; van Os et al., 2020). van Os et al., recently found that the relationship between PRS-SZ and psychosis showed significant dependence on the concomitant presence of affective dysregulation in 2 independent cohorts, NEMESIS-2 and EUGEI (van Os et al., 2020). This relationship was particularly evident for delusional ideation but not for hallucinatory experiences (van Os et al., 2020). A similar relationship was also with childhood trauma, such that the relationship between PRS-SZ and childhood trauma showed significant dependence on the concomitant presence of affective dysregulation, and was particularly evident for delusional ideation (van Os et al., 2020). Cannabis-induced dysphoria was also shown to mediate the relationship between childhood trauma and psychotic experiences (Carlyle et al., 2021).

The common biological mechanism that may explain this relationship remains to be elucidated. Interestingly, cannabis use, childhood trauma and affective dysregulation result in impairment in hippocampal structure and function (Barch et al., 2020; D'Souza et al., 2020; Treadway et al., 2015; Yuan et al., 2020; Zeredo et al., 2019). Abnormalities in hippocampal microstructure have also been seen in schizophrenia using PET imaging (Radhakrishnan et al., 2021). One hypothesis is that hippocampal dysfunction, via the hippocampal-striatal-midbrain network, leads to aberrant signaling of salience (Modinos et al., 2020) and hence, may represent a common biological

mechanism leading to delusion formation, especially in those with affective dysregulation (Rauschenberg et al., 2021).

Strengths and limitations

These findings should be interpreted in the light of several strengths and limitations. In terms of strengths, the present study has the advantage of a large number of participants representative of the Dutch population (Linscott & van Os, 2010; van Nierop et al., 2012). The analysis was conducted among 6646 participants at four time points. The study incorporated thorough symptom assessments across diagnostic boundaries providing an opportunity to examine trans-syndromal psychopathology in the general population. One limitation of the study is that the assessment of cannabis use was based on a questionnaire and might be subject to recall-bias and under-reporting. Similarly, the assessment of anxiety and depressive symptoms were based on CIDI 3.0 rather than clinical scales that specifically assess the severity of anxiety and depression, such as. Additionally, the current analysis could not answer the question of whether within-subject effects were different than between-subject effects as noted earlier (van Os et al., 2021a, 2021b). Another limitation is that the average age of participants at baseline in our sample was 44.3 years. This can in part account for the relatively lower contribution of cannabis use on the outcome compared to that of anxiety and depression. The impact of cannabis use on psychosis risk is greater in adolescence and it decreases over time. The information regarding the potency of cannabis was also not available in this sample. Additionally, cannabis use was coded as a dichotomous variable which limits the range of this measure in identifying whether more frequent use has a higher impact on psychosis.

Conclusion

In this large, general population sample, we found that the mediational relationship between cannabis use, anxiety/depressive symptoms (affective dysregulation) and PE is bidirectional. The contribution of affective dysregulation in mediating the relationship between cannabis use and PE was far greater than the mediational contribution of cannabis. This study provides further support for the existence of an 'affective pathway' to psychosis. Whether interventions addressing affective dysregulation among cannabis-using individuals can reduce the risk of the emergence of PE remains to be verified.

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Conflict of interest

Rajiv Radhakrishnan, Sinan Guloksuz, Lotta-Katrin Pries, Saskia van Dorsselaer, Margreet ten Have, Ron de Graaf, Jim van Os, and Maarten Bak do not have any conflicts of interest and have received no funding or compensation from companies. Rajiv Radhakrishnan is supported by Dana Foundation David Mahoney program, NIMH (grants R21 MH115316-02 and R21 MH123870-01A1), CTSA Grant Number UL1 TR001863 from the National Center for Advancing Translational Science, components of the National Institutes of Health (NIH), and NIH roadmap for Medical Research. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official view of NIH.

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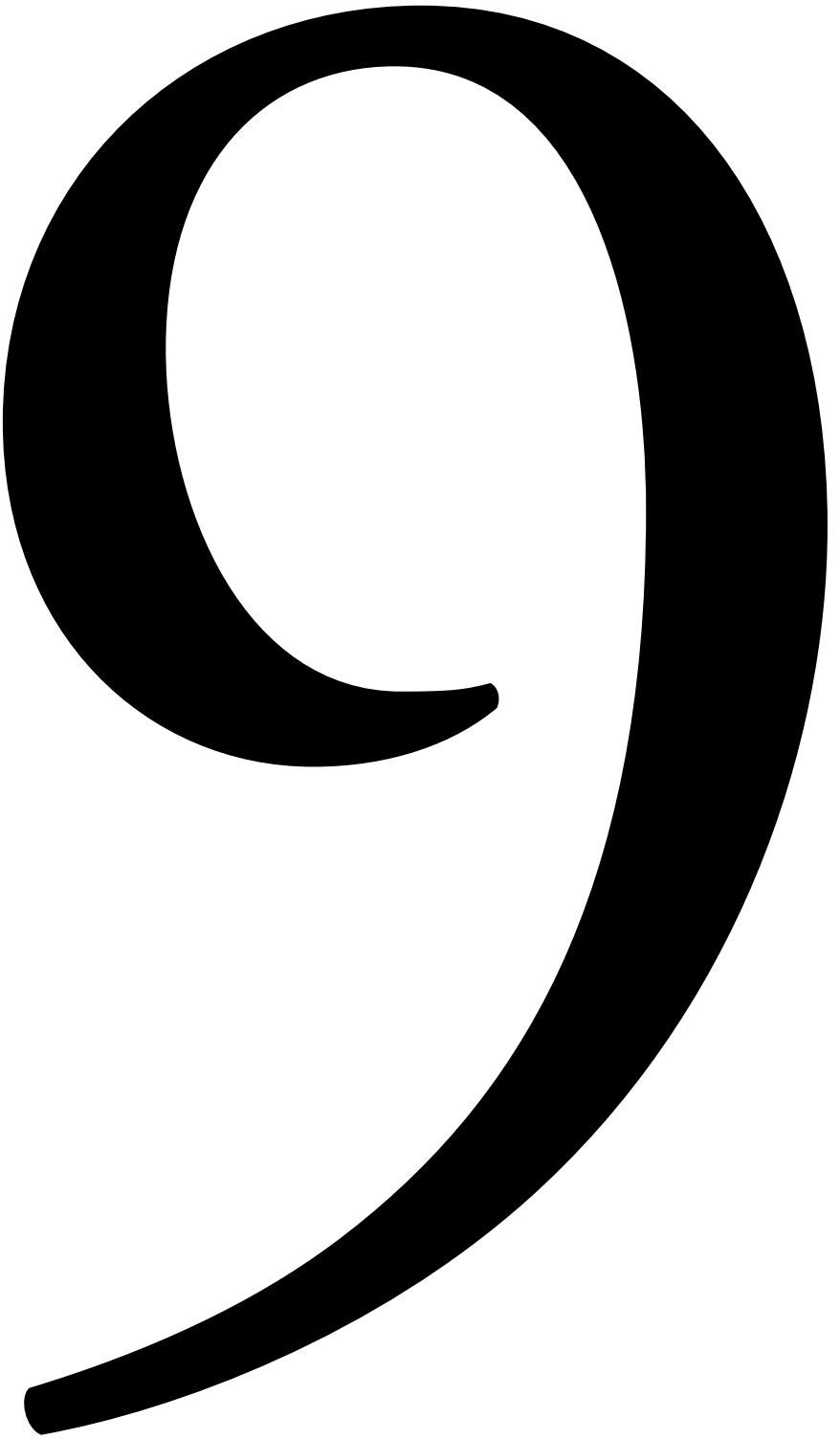

Chapter 9

General Discussion

The overall aim of this thesis was to investigate the association between cannabis use and psychosis. In order to understand this complex association, this broad topic was examined using several strategies, ranging from epidemiological studies to laboratory challenge paradigm and PET imaging.

Defining the cannabis-psychosis risk state:

Given the increasing prevalence of cannabis use across the world, there is a great need to identify individuals who are at risk of developing psychosis with continuing cannabis use.

Chapter 2 reviews the literature on cannabis-psychosis risk syndrome, including concepts of schizotypy and clinical-high risk (CHR) and examines their association with cannabis use. Notably, these conceptualizations of psychosis-proneness have focused largely on the positive and negative symptoms of psychosis. With the advent of large, genome-wide association studies (GWAS) in schizophrenia, psychosis proneness has been defined in terms of genetic risk independent of the presence of less intense symptoms resembling psychosis i.e. highest tertile of polygenic risk score for schizophrenia.

Cannabis has been found to have similar effects across different conceptualizations of psychosis-proneness. In cross-sectional studies, cannabis use was associated with higher schizotypy scores on domains of positive schizotypy, disorganization and to a lesser extent on negative schizotypy (Szoke et al, 2014, O'Tuathaigh et al., 2020). The evidence for the effect of age on negative schizotypy is mixed with both increase and decreased negative schizotypy over time reported (Albertella et al., 2018).

There are few experimental studies on the effects of cannabis on CHR/UHR. Cannabis use was associated with transient increase in paranoia and anxiety and a decrease in cognitive performance.

Rates of cannabis use are high among CHR/UHR with approximately 30-50% noting current and lifetime cannabis use. CHR with a lifetime history of cannabis use have been found to have less social anhedonia and better social functioning compared to CHR who did not use cannabis. It is unclear whether there's a neurobiological basis for this finding or whether it is confounded by the fact that the procurement and use of cannabis requires adequate social functioning.

Cannabis use was also associated with higher rate of conversion from schizotypy to schizophrenia (Hjorthoj et al., 2018) and from CHR/UHR to psychosis. Siblings of people with psychotic disorder also showed greater sensitivity to the effects of cannabis use on positive and negative schizotypy (Genetic and Outcome in Psychosis, 2011).

The important finding that psychosis exists as a continuum in the general population (Johns & van Os, 2001) led to studies to leverage the large sample sizes in general population samples. Cannabis use was associated with higher rates of psychosis expression (van Os et al., 2002). The picture that emerges from general population

samples is that cannabis use interacts with other environmental risk factors (such as childhood trauma and urbanicity); and genetic risk resulting in the expression of psychosis (Pries et al., 2018). Cannabis use and genetic risk (measured by schizophrenia polygenic risk score) has been shown to contribute independently and to also interact additively to increase risk of schizophrenia.

The question of whether the association between cannabis use and schizophrenia is causal remains an open question. Current evidence is mixed with some studies suggesting a causal association while genetic studies utilizing Mendelian randomization find that the relationship is bidirectional (van Os et al., 2021.Gage et al., 2017; Pasman et al., 2018).

The biological basis for this relationship remains speculative and includes increased striatal dopamine release, information processing abnormalities (aberrant salience, jumping-to-conclusions, working memory deficits, altered face-processing), electroencephalographic abnormalities associated with face-processing and alterations in brain synaptic density.

Understanding the link between cannabis and psychosis through experimental research

In an effort to better understand the link between cannabis and psychosis through experimental research, in **Chapter 3,** I examined whether cannabis-psychosis risk can be identified by experimental, laboratory challenge-studies. Using Iomazenil, a GABA inverse-agonist, I showed that pharmacologically-induced GABA deficit increased the psychosis-like effects of delta-9 tetrahyrocannabinol (THC), the main psychoactive ingredient in cannabis. This challenge paradigm offers the opportunity to test cannabis-psychosis risk at the individual participant level. The results showed that pretreatment with pharmacologically induced GABA-deficit (using Iomazenil) exacerbated psychosis-like effects and anxiety with low-dose THC in a general population sample of healthy individuals. This was also associated with changes in electroencephalography (EEG) of reduced P300b amplitude, reflective of altered excitatory-inhibitory balance. This is particularly relevant since cannabinoid CB1 receptors are expressed presynaptically inhibiting the release of GABA from specific interneuron types, and reduction in P300 amplitude has been seen in schizophrenia.

While cannabis-psychosis risk has predominantly been defined in terms of positive psychotic symptoms, cognitive deficits, especially those related to verbal memory have been shown to be significantly impacted in schizophrenia.

In **Chapter 4**, I examined whether THC impairs verbal memory similar to that in schizophrenia and may hence serve as a laboratory challenge paradigm that identifies risk. I found that THC impairs encoding of information.

Verbal memory comprises of three key distinct processes, namely encoding, consolidation, and retrieval. In an experimental, laboratory challenge paradigm, using the Rey-Auditory Verbal Learning Test (RAVLT), the effects of THC on the distinct processes of encoding, consolidation and retrieval were examined. The results showed that when encoding was presented under the effects of THC, both consolidation and retrieval were impaired. However, when THC was presented following encoding, consolidation and retrieval were not impaired.

An integrative model of memory encoding, storage and retrieval of declarative information posits that encoding is primarily dependent on the hippocampus, related medial-temporal lobe structures, and prefrontal cortex (PFC). Information is subsequently sparsely distributed to non-hippocampal sites such as the PFC and parietal cortex where different aspects of the memory is stored. This is accomplished through phase synchronization in reverberating neural circuits involving hippocampus, PFC, and parietal cortical regions (Daumas et al., 2005; Watrous et al., 2013). As consolidation of memory in extra-hippocampal sites takes place, the hippocampal memory decays, but retains a "memory-trace" that can be activated during retrieval (Kandel et al., 2014; Nadel and Bohbot, 2001; Ryan et al., 2010). Phase synchronization of neural oscillations in the theta and gamma frequency is known to play an important role in encoding and retrieval (Babiloni et al., 2009; Shaikhouni et al., 2016; Watrous et al., 2013). The changes in EEG with THC noted in **Chapter 2**, is relevant to the importance of phase synchronization to memory consolidation. Integrating these findings, Chapter 3 suggests that THC impairs encoding through effects on CB1 receptors in the left PFC and hippocampus, areas that are known to be rich in CB1 receptors (Mackie, 2005) and via abnormal neural synchrony.

This raises the question of whether there are abnormalities in CB1 receptors in the hippocampus and PFC in schizophrenia and whether these contribute to the risk of psychosis.

Understanding the link between cannabis and psychosis through Positron Emission Tomography (PET) imaging

Since the effects of THC are mediated via the cannabinoid CB1 receptor, in **Chapter 5**, I examined the effects of age, sex and body-mass index on CB1 receptor availability using the PET ligand [^{11}C]OMAR. CB1 receptors were found to be higher

at younger age, providing a biological mechanism that explains why adolescents may be more vulnerable to the effects of cannabis. I found significant age effects on regional CB1 receptor availability in the posterior cingulate cortex, cerebellum and pallidum characterized by higher receptor availability at younger age and lower CB1R availability with increasing age. CB1 receptor availability was also higher among women compared to men across all brain regions.

These findings are relevant to the finding of increased vulnerability to psychosis risk during young age, especially adolescence and among female sex. Cannabis use has been shown to be associated with earlier ages of psychosis onset in women as compared to men (Allegri et al, 2013). While the typical, the age of psychosis onset is later in women, this gender gap is diminished in cannabis users (Donoghue et al, 2014). Increased frequency of cannabis use has also been shown to be associated with an increase in psychotic disorders, especially in women (Compton et al, 2009).

The posterior cingulate cortex is highly anatomically connected, has a high basal metabolic rate, is a central part of the default mode network and plays an important role in cognition (Leech and Sharp, 2014). It is also one of the first regions that show high amyloid deposition in Alzheimer's disease (Insel et al., 2020), and the functional connectivity between posterior cingulate cortex and the cerebellum is implicated in cognitive impairment (Kang et al., 2021). Given the role of CB1 receptors in cognition, it is possible that these age-related findings are related. The pallidum is among the brain regions with the highest density of CB1R and also shows a linear volumetric decline with age (Tullo et al., 2019).

Given the cognitive effects of cannabis, I examined the effects of cannabis on brain synaptic density using [11C]UCB-J PET imaging in **Chapter 6**. I found that chronic, heavy cannabis use was associated with lower hippocampal synaptic density and this correlated with lower verbal memory performance.

In order to characterize the effects of cannabis on brain synaptic density, I evaluated people with cannabis use disorder using the PET ligand, [^{11}C]UCB-J. [11C]UCB-J has high specificity for synaptic vesicle glycoprotein 2A (SV2A) which is an integral protein expressed on synaptic vesicles. SV2A is the most mono-dispersed subtype of synaptic vesicle glycoproteins with 95% of vesicles expressing ~5 copies per vesicle. It is hence a proxy quantitative marker of synaptic density. Cannabis-use disorder was associated with lower synaptic density in brain regions thought to be high in CB1 receptor availability, and was statistically significant in the hippocampus (10% lower). Lower hippocampal synaptic density was also associated with worse verbal memory, consistent with the findings in Chapter 2. Reductions in synaptic density including in the hippocampus, have also been shown in schizophrenia (Radhakrishnan, et al, 2021)

Given the presence of lower hippocampal synaptic density in schizophrenia as well, and the role of hippocampus in the verbal memory effects of THC, this brain region may hence serve as a point of convergence of cannabis-psychosis risk.

Understanding the link between cannabis and psychosis through epidemiological research

In **Chapter 7**, I examined the interaction between environmental risk factors and familial affective liability in influencing cannabis-psychosis risk in the NEMESIS cohort. I found that childhood adversity and cannabis use, and to a lesser extent urbanicity, displayed greater-than-additive risk if there was also evidence of familial affective liability; and the risk was progressively greater across increasing levels of psychosis severity (none, attenuated psychosis, clinical psychosis) complicating affective dysregulation.

These findings provide evidence for the importance of familial affective liability in increasing psychosis risk and additional support for an affective pathway to psychosis.

Affective dysregulation is known to be present in early stages of psychosis, including in CHR/UHR. Environmental risk factors such as cannabis use, childhood adversity, and urbanicity have been shown to increase the risk of psychosis admixture among those with affective disorders (Guloksuz et al., 2015, 2016). Additionally, environmental risk factors were shown to act additively in increasing the risk of psychosis (Cougnard et al., 2007). The interaction between affective familial liability and environmental risk factors is an important contribution of this work.

While childhood adversity, cannabis use, and urban environment affect individuals at distinct neurodevelopmental stages, i.e. during childhood (adversity), adolescence (cannabis use), or both (urban environment). Yet, they all appear to be associated with increased risk across all strata of psychosis admixture, particularly childhood adversity and cannabis use. This raises the question whether cannabis use and childhood adversity mediate risk via a final common neurobiological pathway, or if they are both environmental risk factors that are mediated by a common latent risk variable. Second, while childhood adversity has been shown to increase stress-reactivity and cannabis use has been shown to be associated with greater affective dysregulation, the direction of causality remains an open question.

Finally, in **Chapter 8** I examined the mediational relationship between cannabis use, anxiety/depressive symptoms and psychotic experience, and showed that there is a bidirectional relationship between cannabis use, anxiety/depressive symptoms and

psychotic experiences. However, the contribution of anxiety/depressive symptoms as a mediator was greater than that of cannabis.

Our analysis showed that the mediational relationship between cannabis use, anxiety/depressive symptoms (affective dysregulation) and psychosis expression was bidirectional i.e. anxiety/depressive symptoms mediated the relationship between cannabis use and psychosis expression; and cannabis use mediated the relationship between anxiety/depressive symptoms and psychosis expression. However, although the relationship was bidirectional, the contribution of affective dysregulation in mediating the relationship between cannabis use and psychosis expression over time was greater than the mediational contribution of cannabis. These results lend further support for the presence of an 'affective pathway' to psychosis (Guloksuz et al., 2015; Pries et al., 2018; Radhakrishnan et al., 2019; van Os et al., 2020).

The directionality of increased anxiety mediating the association between cannabis use and psychosis has been noted in human laboratory challenge studies with THC i.e. the administration of THC results in a significant increase in anxiety and psychosis-like experience (Ganesh et al., 2020; Hindley et al., 2020). This is also consistent with the findings in Chapter 3, where preceding anxiety (pharmacologically induced using the GABA inverse agonist, Iomazenil) was seen to increase the psychosis-like effects of THC.

Of note, cannabis use, childhood trauma and affective dysregulation result in impairment in hippocampal structure and function (Barch et al., 2020; D'Souza et al., 2020; Treadway et al., 2015; Yuan et al., 2020; Zeredo et al., 2019). This is consistent with our findings on hippocampal synaptic density reductions with chronic cannabis use in Chapter 7. One hypothesis is that hippocampal dysfunction, via the hippocampal-striatal-midbrain network, leads to aberrant signaling of salience (Modinos et al., 2020) and hence, may represent a common biological mechanism leading to delusion formation, especially in those with affective dysregulation (Rauschenberg et al., 2021).

Integrative model of cannabis-associated psychosis risk

Synthesizing the multi-modal findings in this thesis yields a coherent, testable model:

- Neurodevelopmental vulnerability: Psychosis proneness, schizotypy and ultra-high risk for psychosis (UHR)(Chapter 2) define a neurodevelopmental vulnerability phenotype. Elevated CB1 receptor density in adolescence and early adulthood (Chapter 5) amplifies the impact of THC on GABAergic interneurons, shifting the excitatory–inhibitory balance (Chapter 3).

- Acute circuit disruption: CB1-mediated suppression of GABA release impairs hippocampal–prefrontal encoding circuits, producing the encoding-specific verbal-memory deficit (Chapter 4) and reduced P300 amplitude. Concurrently, anxiogenic effects of THC heighten affective dysregulation.

- Chronic structural consequence: Repeated exposure leads to measurable reductions in hippocampal synaptic density (Chapter 6), which further compromise memory and the hippocampal–striatal–midbrain network that signals salience.

- Amplification by co-occurring risks: Childhood adversity and urbanicity, acting on overlapping developmental vulnerability windows, interact with familial affective liability (Chapter 7). The resulting affective dysregulation both mediates and is mediated by cannabis use (Chapter 8), creating a positive-feedback loop that progressively elevates psychosis expression along a continuum.

- Final common pathway: Hippocampal dysfunction—whether induced by acute CB1 signaling, chronic synaptic loss, early adversity, or affective stress—disrupts salience attribution and thereby promotes delusion formation, particularly in individuals already carrying genetic or familial liability.

This model accounts for the epidemiological dose–response relationship, the greater vulnerability of adolescents and of those with pre-existing affective or genetic risk, the paradoxical social-functioning findings in CHR cohorts, and the shared hippocampal pathology observed in both chronic cannabis use and schizophrenia.

It also generates clear experimental predictions: interventions that restore hippocampal synaptic integrity, normalize GABA–glutamate balance, or interrupt the affective–cannabis feedback loop should attenuate risk.

Methodological Issues and Future Perspective

The strength of this thesis is the multimodal approach to understand the link between cannabis use and psychosis. Additionally using diverse experimental designs ranging from human laboratory-challenge studies, cross-sectional imaging studies and general population longitudinal studies, this thesis attempts to understand the biological basis of the association between cannabis and psychosis.

The human laboratory challenge studies (**chapter 3, 4**) provided a unique opportunity to examine the effects of THC (the psychoactive ingredient in cannabis) in a controlled setting, namely on specific aspects of verbal memory and to test the hypothesis that

psychosis-like effects are exaggerated in the presence of GABA-deficit. The intravenous route of drug administration, and weight-adjusted dosing address the inter- and intra-individual variability associated with oral or smoked cannabis.

The randomized, placebo-controlled, double-blind, repeated-measure, within-subjects design used to test the hypothesis of GABA deficit worsening the psychosis-like effects of THC **(Chapter 3)** is both powerful and efficient. It was also possible to concurrently examine psychophysiological measure (using EEG) that allows a more proximal index of neuronal activity and interaction of the cannabinoid and GABA systems. The limitations of this approach include the limited real-world social relevance, since cannabis is not typically used intravenously. Additionally, the sample size of the study while well-powered for hypothesis-testing, was not adequate to examine effects of sex.

In examining the effects of THC on verbal memory processes **(Chapter 4)**, used a between-subject design. However, in 16 subjects who participated in both experiments i.e., within-subjects, the findings were consistent with the final results. Of note, some participants noted exposure to cannabis in the past 30 days. Thus, one cannot completely rule out the possibility that residual effects of cannabis use influenced memory performance. However, this is unlikely to explain the effects of THC on memory encoding since there were no group differences in cannabis exposure between the two experiments. While I examined verbal memory, assessment of other types of memory (e.g. visual, spatial, emotional) may have answered the question of specificity of our results to verbal memory.

The PET imaging studies **(Chapter 5, 6)** leveraged the use of state-of-the art techniques to examine the factors that influence cannabinoid CB1 receptor availability and the effects of cannabis use on brain synaptic density by studying individuals with cannabis use disorder. While these studies provide an in-depth view of the sensitivity of brain cannabinoid system and brain changes associated with chronic cannabis use, the data was pooled from multiple individual studies and had a smaller proportion of women compared to men. In **Chapter 5**, there were multiple potential confounders that I was unable to assess, including whether women were on oral contraceptive pills or hormonal intrauterine devices and I did not measure hormonal levels or level of stress in subjects. These factors can potentially alter CB1 receptor density as hormone levels have been shown to alter CB1 availability (Laurikainen et al., 2019; Riebe et al., 2010). Another caveat is that, while the use of DSM-IV SCID allowed us exclude psychiatric disorders, it did not allow us to exclude personality disorders, and specific domains of personality such as impulsivity and aggression have been shown to be correlated with CB1 receptor levels.

In the examination of synaptic changes in cannabis use disorder **(Chapter 6)**, our study design does not allow us to determine whether lower synaptic density in CUD is a consequence of cannabis use or were present before the initiation of cannabis. The limited sample size made it challenging to examine the effects of sex, dose of exposure, and the timing of initiation of cannabis use. Thus, a larger sample size with more females and males, a wider range of cannabis exposure, and a wider range in the age of initiation of cannabis use (early, mid, or late adolescence) will help to more conclusively determine these effects.

The general population sample studies **(Chapter 7, 8)** were undertaken with the goal of examining the interactive effects of risk factors and the causal relationship between cannabis use and psychosis expression. The limitation of the analysis in **Chapter 7** is its cross- sectional nature, precluding conclusions of causality. Another limitation, is the retrospective nature of the information on childhood adversity, although the recall of childhood adversity has been shown to be reliable, including in individuals with psychotic disorders (Fisher et al., 2011). Similarly, the assessment of cannabis use and urbanicity was based on responses to questions on CIDI 3.0 and might be subject to decreased precision in reporting of use. Additionally, assessment of familial risk was by different short instruments where the respondent reported whether parents had mental health problems, and is hence limited by the respondent's knowledge of the same. The NEMESIS study did not include family history of all possible mental disorders since they are of relatively low prevalence.

In **Chapter 8**, I examined whether there was a causal relationship between cannabis use and psychosis. The analysis was conducted among 6646 participants at four time points incorporating thorough symptom assessments across diagnostic boundaries. Limitations of the study include that the assessment of cannabis use was based on a questionnaire and might be subject to recall-bias and under-reporting. Similarly, the assessment of anxiety and depressive symptoms were based on CIDI 3.0 rather than clinical scales that specifically assess the severity of anxiety and depression. Another limitation is that the average age of participants at baseline in our sample was 44.3 years. This can in part account for the relatively lower contribution of cannabis use on the outcome compared to that of anxiety and depression. The impact of cannabis use on psychosis risk is greater in adolescence and it decreases over time. The information regarding the potency of cannabis was also not available in this sample. Additionally, cannabis use was coded as a dichotomous variable which limits the range of this measure in identifying whether more frequent use has a higher impact on psychosis.

It is notable that in the field, in general, human laboratory challenge studies and PET imaging studies do not capture exposure to psychosis risk factors. Incorporating

ex- posure to risk factors (such as using exposome scores or polygenic risk scores) may enhance the test of biological hypotheses in these study designs.

Similarly, general population epidemiological studies do not always include a biological perspective. Such an effort may involve characterizing risk factors based on common biological pathways that may mediate their effects. For example, as I show in this thesis, the hippocampus may be a common biological region of interest. Hence examination of cognitive measures related to hippocampal function may provide additional valuable data in general population studies. As another example, many of the risk factors involved in the association between cannabis and psychosis converge on an affective pathway to psychosis and on a common inflammatory pathway (Radhakrishnan et al, 2017). Incorporating biological measures related to affective function and inflammatory pathways may hence help parse out subgroups within the heterogeneous mix of disorders that are currently grouped as schizophrenia.

Another area that has been less well examined is the biological basis for the association between cannabis use and negative symptoms of schizophrenia. Chronic cannabis use is known result in a protracted amotivation syndrome reminiscent of negative symptoms of schizophrenia, and distinct from depression.

Conclusions

In this thesis, I have explored the relationship between cannabis and psychosis using 3 different paradigms. In the first part of the thesis, I examined the biological mechanisms underlying the effects of cannabis using a human laboratory challenge paradigm. In the second part of the thesis, I examined the specific in vivo brain changes associated with the cannabinoid system and chronic exposure to cannabis. In the third part of the thesis, I examined the interactive effects of cannabis and psychosis risk factors in a general population epidemiological sample and attempted to address the causal relationship between cannabis use and psychosis.

Taken together, the findings indicate that cannabis is neither a necessary nor a sufficient cause of psychosis, but a moderator and mediator that acts on a neurodevelopmentally sensitive, sexually dimorphic CB1 system, preferentially disrupts hippocampal circuitry, and amplifies affective and environmental liabilities. The hippocampus emerges as a critical node of convergence: acute CB1-mediated encoding deficits, chronic synaptic loss, and stress- or trauma-related hippocampal compromise collectively impair salience processing and thereby facilitate the transition from attenuated psychotic experiences to clinical psychosis. Recognition of this multi-level pathway reframes prevention and early-intervention strategies—targeting not only cannabis exposure itself but also the

affective dysregulation and hippocampal vulnerability that amplify its impact. In an era of expanding cannabis legalization, the model supplies a mechanistic rationale for age-restricted policies, for screening high-risk individuals with combined genetic–environmental–biological tools, and for developing interventions aimed at preserving or restoring hippocampal integrity.

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Summary

Summary

In **chapter 1**, a brief overview of research literature on the association between cannabis and psychosis was provided. I cover epidemiological studies examining the association between cannabis use and psychosis, efforts to define the cannabis-psychosis risk syndrome, biological risk factors, interaction between environmental and biological risk factors and examination of whether the relationship between cannabis use and psychosis is causal. Finally, the aims and outline of the thesis were presented.

Chapter 2 reviews the literature on cannabis-psychosis risk syndrome, including concepts of schizotypy and clinical-high risk (CHR) and examines their association with cannabis use. The review identified that while cannabis use increases the risk of conversion to psychosis in schizotypy and CHR, it also resulted in lower negative schizotypy, social anhedonia, and better social functioning. These paradoxical effects may partly explain the counter-intuitive finding of high rates of cannabis use among the CHR population.

In **Chapter 3, I** examined whether cannabis-psychosis risk can be identified by experimental, laboratory challenge-studies. Using Iomazenil, a GABA inverse-agonist, I showed that pharmacologically-induced GABA deficit increased the psychosis-like effects of delta-9 tetrahyrocannabinol (THC), the main psychoactive ingredient in cannabis. This challenge paradigm offers the opportunity to test cannabis-psychosis risk at the individual participant level.

While cannabis-psychosis risk has predominantly been defined in terms of positive psychotic symptoms, cognitive deficits, especially those related to verbal memory have been shown to be significantly impacted in schizophrenia.

In **Chapter 4**, I examined whether THC impairs verbal memory similar to that in schizophrenia and may hence serve as a laboratory challenge paradigm that identifies risk. I found that THC impairs encoding of information but not retrieval, at variance to that seen in schizophrenia.

Since the effects of THC are mediated via the cannabinoid CB1 receptor, in **Chapter 5**, I examined the effects of age, gender and body-mass index on CB1 receptor availability. CB1 receptors were found to be higher at younger age, providing a biological mechanism that explains why adolescents may be more vulnerable to the effects of cannabis.

Given the cognitive effects of cannabis, I examined the effects of cannabis on brain synaptic density using [11C]UCB-J PET imaging in **Chapter 6**. I found that chronic,

heavy cannabis use was associated with lower hippocampal synaptic density and this correlated with lower verbal memory performance. Given the presence of lower hippocampal synaptic density in schizophrenia as well, this brain region may hence serve as a point of convergence of cannabis-psychosis risk.

In **Chapter 7**, I examined the interaction between environmental risk factors and familial affective liability in influencing cannabis-psychosis risk in the NEMESIS cohort. I found that childhood adversity and cannabis use, and to a lesser extent urbanicity, displayed greater-than-additive risk if there was also evidence of familial affective liability; and the risk was progressively greater across increasing levels of psychosis severity (none, attenuated psychosis, clinical psychosis) complicating affective dysregulation.

Finally, in **Chapter 8** I examined the mediational relationship between cannabis use, anxiety/depressive symptoms and psychotic experience, and showed that there is a bidirectional relationship between cannabis use, anxiety/depressive symptoms and psychotic experiences. However, the contribution of anxiety/depressive symptoms as a mediator was greater than that of cannabis.

Samenvatting

In hoofdstuk 1 gaf ik een kort overzicht van de onderzoeksliteratuur over de associatie tussen cannabis en psychose. Ik behandel epidemiologische studies die de relatie tussen cannabisgebruik en psychose onderzoeken, pogingen om het cannabis-psychose risicosyndroom te definiëren, biologische risicofactoren, de interactie tussen omgevings- en biologische risicofactoren en een onderzoek naar of de relatie tussen cannabisgebruik en psychose causaal is. Tot slot werden de doelen en opzet van de thesis gepresenteerd.

Hoofdstuk 2 bespreekt de literatuur over het cannabis-psychose risicosyndroom, inclusief concepten van schizotypie en klinisch hoog risico (CHR), en onderzoekt hun associatie met cannabisgebruik. De review wees uit dat hoewel cannabisgebruik het risico op conversie naar psychose verhoogt bij schizotypie en CHR, het ook leidde tot lagere negatieve schizotypie, sociale anhedonie en betere sociale werking. Deze paradoxale effecten kunnen deels verklaren waarom er verrassend hoge percentages cannabisgebruik zijn onder de CHR-populatie.

In hoofdstuk 3 onderzocht ik of het cannabis-psychose risico kan worden geïdentificeerd door experimentele laboratorium-uitdagingsstudies. Met behulp van Iomazenil, een GABA-inverse-agonist, toonde ik aan dat een farmacologisch geïnduceerd GABA-tekort de psychose-achtige effecten van delta-9-tetrahydrocannabinol (THC),

de belangrijkste psychoactieve stof in cannabis, versterkte. Dit uitdagingsmodel biedt de mogelijkheid om het cannabis-psychose risico op individueel deelnemersniveau te testen.Hoewel het cannabis-psychose risico voornamelijk is gedefinieerd in termen van positieve psychotische symptomen, zijn cognitieve tekorten, vooral die gerelateerd aan verbaal geheugen, significant aangetast bij schizofrenie.

In hoofdstuk 4 onderzocht ik of THC het verbale geheugen aantast op een manier die vergelijkbaar is met schizofrenie en daarmee kan dienen als een laboratorium-uitdagingsmodel om risico's te identificeren. Ik vond dat THC de codering van informatie aantast, maar niet het ophalen ervan, in tegenstelling tot wat wordt gezien bij schizofrenie.

Aangezien de effecten van THC worden gemedieerd via de cannabinoïde CB1-receptor, onderzocht ik in hoofdstuk 5 de effecten van leeftijd, geslacht en body-mass index op de beschikbaarheid van CB1-receptoren. CB1-receptoren bleken hoger te zijn op jongere leeftijd, wat een biologisch mechanisme biedt dat verklaart waarom adolescenten mogelijk kwetsbaarder zijn voor de effecten van cannabis.

Gezien de cognitieve effecten van cannabis onderzocht ik in hoofdstuk 6 de effecten van cannabis op de synapsdichtheid in de hersenen met behulp van [11C]UCB-J PET-beeldvorming. Ik vond dat chronisch, zwaar cannabisgebruik geassocieerd was met een lagere synapsdichtheid in de hippocampus, en dit correleerde met slechtere prestaties op verbaal geheugen. Aangezien een lagere synapsdichtheid in de hippocampus ook aanwezig is bij schizofrenie, kan dit hersengebied dienen als een convergentiepunt van het cannabis-psychose risico.

In hoofdstuk 7 onderzocht ik de interactie tussen omgevingsrisicofactoren en familiale affectieve belasting bij het beïnvloeden van het cannabis-psychose risico in de NEMESIS-cohort. Ik vond dat kindermishandeling en cannabisgebruik, en in mindere mate urbaniteit, een groter-dan-additief risico lieten zien als er ook sprake was van familiale affectieve belasting; het risico was progressief groter naarmate de ernst van de psychose toenam (geen, verzwakte psychose, klinische psychose) en affectieve dysregulatie compliceerde.

Tot slot onderzocht ik in hoofdstuk 8 de mediërende relatie tussen cannabisgebruik, angst-/depressieve symptomen en psychotische ervaringen, en toonde ik aan dat er een bidirectionele relatie bestaat tussen cannabisgebruik, angst-/depressieve symptomen en psychotische ervaringen. De bijdrage van angst-/depressieve symptomen als mediator was echter groter dan die van cannabis.

Impact

Impact

Schizophrenia is a heterogenous group of chronic psychotic disorders of as yet unknown etiology. With a prevalence of approximately 1% worldwide, it is a leading cause of disability and healthcare expenditure. The prevalence of schizophrenia appears to be increasing – it was 20.9 (95% UI: 18.5–23.4) million cases in 2016 compared to 13.1 (95% UI: 11.6–14.8) million cases in 1990 (Charlson et al, 2018). Coincidentally, rates of cannabis use has increased worldwide with many countries legalizing recreational use and the use of cannabis for medical indication.

Converging lines of evidence from epidemiological and experimental studies indicate that cannabis use during adolescence is a risk factor for the development of psychotic illness. This is also evident in recent epidemiological studies examining rates of schizophrenia following legalization of cannabis use (Myran et al, 2025) -- the population-attributable risk fraction (PARF) for cannabis use disorder associated with schizophrenia almost tripled from 3.7 during the pre-legalization period to 10.3% during the legalization period (Myran et al, 2025). Despite the compelling epidemiological and experimental evidence, the specific ways in which cannabis use interacts with other environmental exposures and underlying biological (including genetic and neurobiological) substrates is not known.

This thesis contributes three interlocking advances in examining the question of the association between cannabis use and schizophrenia using 3 different approaches, namely human laboratory challenge studies, neuroimaging studies using PET and general population cohort studies. First, laboratory challenge paradigms demonstrate that a transient GABA deficit markedly amplifies the acute psychosis-like effects of THC and that THC selectively impairs the encoding stage of verbal memory. These findings supply a controlled experimental platform for identifying individual vulnerability and for testing candidate protective agents (e.g., GABA-enhancing compounds or agents that stabilize hippocampal–prefrontal synchrony). Second, PET imaging establishes that CB1 receptor availability is higher in younger brains and in women, and that chronic heavy cannabis use is associated with reduced hippocampal synaptic density that correlates with memory impairment. Because analogous hippocampal synaptic loss occurs in schizophrenia, the work identifies a concrete neurobiological substrate that can be monitored longitudinally and potentially targeted by interventions aimed at synaptic resilience. Third, population-based analyses reveal that cannabis interacts with childhood adversity and familial affective liability in a greater-than-additive fashion, and that affective dysregulation is a bidirectional mediator of the cannabis–psychosis relationship. Collectively these results support an integrated risk model in which developmental CB1 density, hippocampal vulnerability, and affective pathways converge.

Utilizing these diverse approaches, the thesis makes valuable contributions to a better understanding of the neurobiological basis underlying the effects of cannabis, the interactive nature of exposure risk factors and points to convergent brain networks involving the hippocampus that may mediate the association between cannabis use and schizophrenia. This is consistent with a large body of evidence implicating the role of the hippocampus in the development of schizophrenia (Jiang et al, 2024; Mancini et al, 2025, Alkan et al, 2025). The thesis also provides motivation for the use of multimodal methods that combine measurement of exposure risk (such as exposome score) and utilizing a biological framework to inform how risk factors interact to result in psychosis expression, in future studies.

Scientifically, the thesis advances the field by demonstrating the feasibility and value of multi-modal triangulation—experimental pharmacology, molecular imaging, and longitudinal epidemiology—within a single program of research. It moves the discussion beyond simple association toward mechanism, highlighting the hippocampus as a convergent node and the affective pathway as a clinically actionable amplifier. The challenge paradigms themselves constitute methodological tools that other laboratories can adopt to phenotype risk or to evaluate novel compounds. The imaging findings open avenues for mid-life or late-life studies of residual synaptic deficits and for trials that test whether cognitive remediation or pharmacological agents can restore hippocampal integrity after cannabis cessation.

Societally, the work has direct implications for policy, clinical practice, and public education. Age-related elevations in CB1 availability furnish a biological rationale for restricting recreational cannabis access during adolescence and early adulthood—the developmental windows of greatest vulnerability. Recognition of sex differences in receptor density and of the narrowing of the usual age-of-onset sex gap among cannabis users argues for sex-sensitive messaging and for closer monitoring of young women who use cannabis heavily. The demonstration that affective symptoms both mediate and are amplified by cannabis use supports the integration of anxiety- and depression-focused interventions into early-psychosis and CHR services; treating affective dysregulation may interrupt a key feedback loop. Finally, the identification of hippocampal synaptic density as a measurable correlate of both chronic cannabis exposure and schizophrenia risk suggests that imaging or cognitive markers of hippocampal function could eventually be incorporated into stratified risk-assessment algorithms, allowing clinicians to move from generic "cannabis is a risk factor" warnings to individualized counseling.

In an era when many countries are legalizing or decriminalizing cannabis, the absence of mechanistic insight risks leaving policy makers and clinicians with only correlational evidence. By delineating developmental windows of biological vulnerability, circuit-level mechanisms, and interactive environmental–affective pathways, this thesis

supplies a more precise evidence base for harm-reduction strategies, for the design of secondary-prevention trials, and for the education of young people, parents, and healthcare providers. The ultimate impact will be measured by the extent to which these findings inform policies that protect the most vulnerable developmental stages, clinical practices that target modifiable mediators such as affective dysregulation, and research programs that continue to refine the multi-level model of cannabis-associated psychosis risk.

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This thesis is a testament to the collective efforts of all those who have supported me, and I am profoundly grateful for their contributions.

Curriculum Vitae

Curriculum Vitae

Rajiv Radhakrishnan was born on June 18th 1979 in Bangalore, India. He obtained his medical education at St. John's Medical College, Bangalore, India in 2004. He subsequently went on to complete psychiatry residency training at the same institution, receiving a MD degree in Psychiatry in 2008. Driven by the quest to understand the biological basis of psychiatric disorders, he undertook postdoctoral research training at Yale University in 2010. Subsequently he went on to complete psychiatry training at Yale University and established a research program at Yale University as a faculty member to understand the overlap between cannabis use and psychosis. He began his Ph.D. at the School for Mental Health and Neuroscience in the Department of Psychiatry and Neuropsychology at Maastricht University under the supervision of Professors Jim van Os and Sinan Guloksuz.

List of Publications

List of Publications

Peer-Reviewed Original Research

1. **Radhakrishnan R**, Duth M, Menon J, Calvin S, Jacob A, Janish A, Srinivasan K. Association between unconjugated bilirubin and schizophrenia. Psych Research 2011 Oct 30;189(3):480-2.

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Chapters, Books and Reviews

Book chapters

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Reviews

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Research Support

Agency: NIDA/NIH R01DA054314-01

Title: Do hippocampal synaptic density deficits in cannabis use disorder improve following abstinence? P.I: **Rajiv Radhakrishnan**, M.B.B.S., M.D., Deepak D'Souza
Project Period: 09/15/2021 - 06/30/2026

Agency: VA Central Office of Homelessness

Title: Neurobiology of chronic homelessness: A multimodal imaging study
P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 09/22/2020 - 09/21/2025

Agency: NIDA/NIH R21DA057540-01

Title: Impact of cannabis legalization on prescription drug use and health outcomes in Connecticut
P.I: **Rajiv Radhakrishnan**, M.B.B.S., M.D., Samuel T Wilkinson, M.D, Tahoe Rhee, Ph.D
Project Period: 04/01/2023- 03/31/2025

Agency: NIDA/NIH R21DA054314-01
Title: Effect of Cannabis Use on Synaptic Density in Older Adults P.I: **Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 9/30/2021 - 08/31/2023

Agency: NCCIH/NIH 1R21AT010763

Title: Effect of Cannabidiol of microglial activation and central pain-sensitization P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D., Mohini Ranganathan, M.B.B.S. Role: Co- P.I.
Project Period: 09/27/2019 - 08/31/2024

Agency: Wallace Research Foundation

Title: Neurobiology of psychedelics: Dose occupancy of psilocybin at 5HT2A receptors in Major Depressive Disorder
P.I: **Rajiv Radhakrishnan**, M.B.B.S., M.D., Mohini Ranganathan, M.B.B.S., Schindler E, D'Souza DC
Project Period: 12/01/2020- 11/30/2023

Agency: NIMH/NIH 1R21MH123870-01A1 (NCE)

Title: Preliminary studies of muscarinic M1 receptor availability and cognition in schizophrenia
P.I: **Rajiv Radhakrishnan**, M.B.B.S., M.D., Deepak D'Souza M.D.
Project Period: 12/01/2020 - 10/31/2023

Agency: Columbia University (Stavros Niarchos Foundation)

Title: Multimodal imaging for Precision Psychiatry
P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 10/01/2022 - 09/30/2023

Agency: NIMH/NIH 5R21MH115316-02
Title: Imaging complement component 4 gene effects on human synaptic density in vivo using 11C-UCB-J PET
P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D., *(transferred from Dr. Robert Malison posthumously)*
Role: P.I.
Project Period: 09/18/2017 - 08/31/2022

Agency: Neurocrine Biosciences

Title: Effect of VMAT2 inhibition on microglial activation P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 03/01/2019 - 02/28/2022

Agency: Yale Center for Clinical Investigations (YCCI) Community-Engaged Research Pilot Award 2019-2021

Title: Effect of feedback from self-monitored cognitive test performance on perceived risk of harm with cannabis use among teenagers
P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D. Project Period: 09/01/2019- 08/31/2021

Agency: Yale Center for Clinical Investigations (YCCI) Scholar Award

Title: Effect of antipsychotic medication on microglial activation
PI: Sherwin, R Role: YCCI Scholar
Project Period: 07/2018- 06/2020

Agency: Yale-VA Schizophrenia Advanced Research Fellowship

Title: In vivo measurement of synaptic density in patients with schizophrenia
This training grant provided research support to examine synaptic density in schizophrenia using [11C]
UCB-J Positron Emission Tomography. Role: Postdoctoral trainee
Project Period: 07/01/2017 – 02/28/2018

Agency: Dana Foundation: David Mahoney Neuroimaging Program 2016.

Title: In-vivo imaging of synaptic density in schizophrenia.
Co-P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D., Patrick Skosnik, Ph.D. Role: Co-P.I.
Project Period: 9/8/2016 – 9/8/2019

Agency: Thomas P. Detre Fellowship Awards in Translational Neuroscience Research in Psychiatry

Title: Imaging synaptic density in-vivo in schizophrenia P.I**.: Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 07/01/2015-06/30/2016

Agency: R25 MH071584: R25 IMPORT in Psychiatry

Title: Effect of delta-9-tetrahydrocannabinol (THC) on top-down information processing in individuals with family history of psychosis.
PI: Robert Malison, M.D. Role: Postdoctoral trainee
Project Period: 07/01/2014-06/30/2015

Agency: American Psychiatric Foundation/ Janssen Pharmaceutical Resident Psychiatric Research Scholar Fellowship

Title: Effect of delta-9-tetrahydrocannabinol (THC) on top-down information processing in individuals with family history of psychosis.
P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 07/01/2014-06/30/2015

Agency: Yale Global Mental Health Program/ Yale New Haven Hospital

Title: Improving treatment adherence using tele-mental health in rural India: An evaluation of feasibility, acceptability and barriers to implementation.
International Site: St. John's National Academy of Health Sciences, Community Health Training Center, Mugalur, Karnataka.
P.I.: **Rajiv Radhakrishnan**, M.B.B.S., M.D.
Project Period: 08/2014- 07/2015

Honors

2021: ISCTM/ASCP Advanced CNS Clinical Trials Travel Award
2020: College on Problems of Drug Dependence (CPDD) Travel Award for Early Career Investigators
2020: International Society for CNS Clinical Trials and Methodology (ISCTM) New Investigator Award
2019: Alavi-Mandell Award (from Society for Nuclear Medicine and Molecular Imaging)
2018: American College of Neuropsychopharmacology (ACNP) Travel Award 2018. 2017: American Society of Clinical Psychopharmacology (ASCP) New Investigator Award
2016: Dana Foundation: David Mahoney Neuroimaging Program 2016 Award 2015: American Psychiatric Association Diversity Leadership Fellowship 2015. 2015: Society of Biological Psychiatry 2015 Chairman's Choice Award.
2014: Group for Advancement of Psychiatry Fellowship (2015-2016).
2014: American Association of Directors of Psychiatric Residency Training (AADPRT) International Medical Graduate (IMG) Fellowship Program 2015
2014: Academy of Psychosomatic Medicine Travel Award.
2014: Indo-American Psychiatric Association (IAPA) Outstanding Resident Award 2014-2015.
2014: American Society of Clinical Psychopharmacology Clinical Trials Workshop Fellowship Award.
2012: Best Student Poster Award. 15th Annual Cognitive Remediation in Psychiatry Conference. 2012.

Medical School

2018: Seymour Lustman Research Award 2018.
2018: Award-winning Oral Presentation at Yale Clinical Neuroimaging Symposium
2016: Laughlin Family Foundation Outstanding Psychiatric Resident Award 2016
2014: Seymour Lustman Research Award 2014.