UNDERGRADUATE RESEARCH IN NATURAL AND CLINICAL SCIENCE AND TECHNOLOGY (URNCST) JOURNAL
Read more URNCST Journal articles and submit your own today at: https://www.urncst.com

RESEARCH PROTOCOL OPEN ACCESS

Using Knockout Mice Models to Explore Links between THC and Schizophrenia: A Research Protocol

Sayed Azher, HBSc Student [1]*, Keerat Grewal, HBSc Student [1], Harmy Thakar, HBSc Student [1], Shaun Jim, HBSc Student [1]

[1] Department of Biochemistry, McMaster University

*Corresponding Author: azhers1@mcmaster.ca

Abstract

The legalization of marijuana in Canada raises many concerns regarding its consequences on the central nervous system. From being widely used as a medicinal remedy to now being used recreationally, this proposal aims to focus on an experiment that examines THC and its potentially detrimental effects on the brain, specifically in mice at-risk for schizophrenia. This proposal outlines a mouse model analysis of an analogue of THC that will be constructed using a knockout mice lineage in which the mice have a genetic predisposition to schizophrenia and lack the CB1 receptors responsible for binding THC. The resultant mice will undergo a grooming behaviour test and an elevated zero maze test which will then be compared with other experimental and control groups. This will allow for the direct observation of THC's effects on the brain, providing insight on the consequences of the legalization of marijuana and its recreational use.

Keywords: biology; biochemistry; cannabis; cannabinoids; genomics; knockout; mice; protocol; schizophrenia; THC

Introduction

Cannabis is a plant that contains 421 compounds; 61 of which are cannabinoids [1]. Cannabinoids bind to the cannabinoid receptors within our bodies and can lead to a variety of implications [1]. Δ-9-tetrahydrocannibinol (THC) is the main psychoactive constituent of cannabis that binds to CB1 and CB2 endocannabinoid receptors, found in the brain and immune system, respectively [1]. Scientists have explored the use of cannabis in various medical practices, primarily when symptoms are untreatable by other means, such as severe chronic pain which does not improve by traditional medication [2]. Studies have shown that cannabis as a whole and the majority of its 61 cannabinoid by-products are safe to use and are effective in relieving neuropathic pain with some therapeutic efficacy [2]. Specifically, THC has shown to be useful in treating anorexia and dementia-induced behavioral symptoms [2]. The term cannabis and THC will be used interchangeably throughout this proposal.

On the other hand, numerous studies have showcased the adverse effects on patient health with the use of cannabis as a treatment. Among patients treated with various cannabinoids, the following symptoms appeared in at least two studies: mood changes, suicidal ideation, and hallucinations. Psychosis, dysphoria, and anxiety also appeared to be associated with higher concentrations of THC, specifically [3]. Additionally, several correlations are found between cannabis usage and mental illness prevalence. The findings thus far indicate that there may be some relationship between cannabis use and psychotic disorders, including schizophrenia, a neurologic disorder that is characterized by positive and negative symptoms, such as hallucinations, delusions, disordered thinking, and a lack of emotions [4]. This correlation exists especially for those that are already at an increased risk for psychotic disorders [4]. Cannabinoids can trigger a relapse or aggravate pre-existing symptoms for those with these illnesses [4]. However, minimal research has been focused on specific cannabinoids and their linkage to mental health.

Little is known about how the legalization of marijuana will impact the mental health of consumers. Available for medical, and now recreational use, there is greater need for research into the relationship between cannabis use and schizophrenia, to better understand the consequences of THC on the mental health conditions of those already at-risk for schizophrenia.

This research proposal presents an experiment with the goal of assessing a direct effect between THC and the onset of schizophrenia-like behavior in mice that have mutated catechol-O-methyl transferase (COMT) and protein kinase b (AKT1) genes making them genetically predisposed to schizophrenia [4]. Through the use of knockout mice models, it is hypothesized that those mice which cannot bind THC, due to CB1 receptor knockout, will not show schizophrenia-like symptoms.

Purpose

This experiment is designed to test if there is a link between the binding of THC to cannabinoid receptors and the onset of schizophrenia disorders in those with risk genes. Two types of cannabinoid receptors, the CB1 and CB2 receptors, have an affinity for the THC molecule. CB2 expression is largely restricted to the immune system, while

CB1 is primarily expressed in the brain. Therefore, the focus will be on the binding of THC to CB1 receptors as these receptors, being in the brain, are more likely to promote mental disorder symptoms such as those of schizophrenia [5].

Research Methods

Control & Experimental Groups

To determine the role of the binding of THC to CB1 in producing symptoms of schizophrenia, C57BL/6 mice that possess risk genes for schizophrenia will be obtained. Using the gene editing tool, CRISPR, a group of these predisposed schizophrenic mice will have a knocked out CB1 gene with an insertion of a premature termination codon in the CB1 gene. This will create a knockout strain of mice predisposed to schizophrenia that lacks functional CB1 receptors, and can be compared to the predisposed schizophrenic mice with functional CB1 receptors. Additionally, wild-type mice will be used as a secondary control group to determine if CB1 is required in developmental pathways. Two groups of wild-type mice, one with CB1 receptors and one without, will be exposed to THC to determine if the knockout of CB1 receptors has secondary effects (Figure 1). Each group will consist of 30 mice.

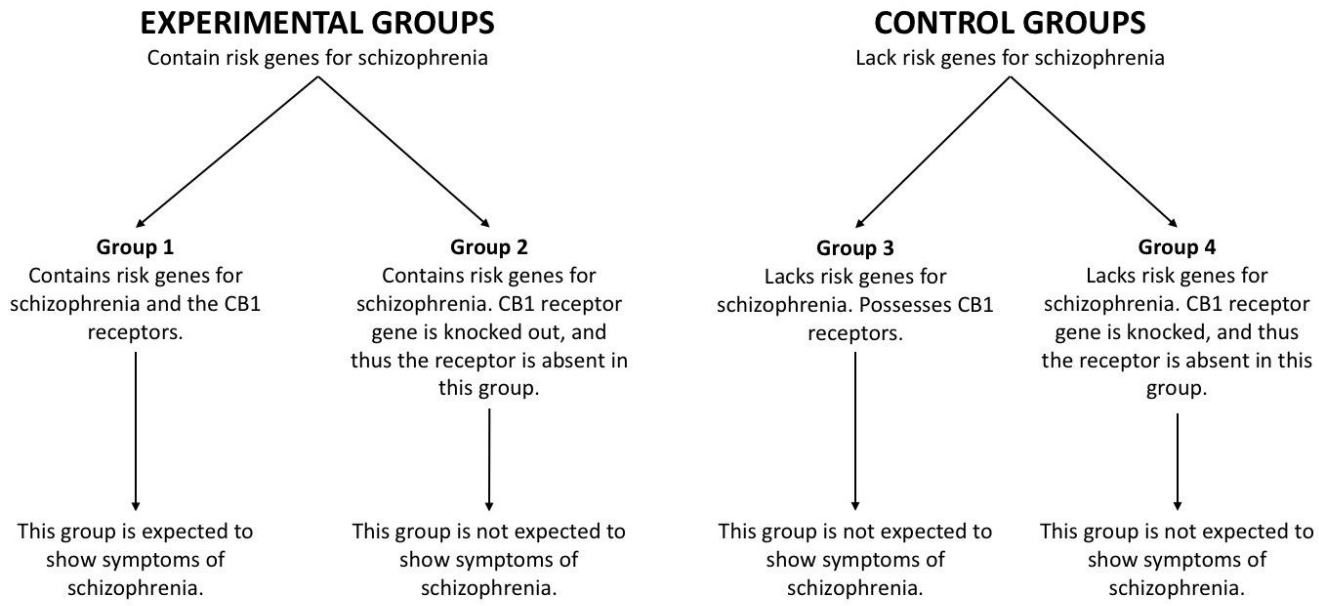

Figure 1: This figure outlines the differences between each of the control and experimental groups, and their expected results. Group 1 has risk genes for schizophrenia and CB1 receptors. Group 2 has risk genes for schizophrenia but does not have CB1 receptors. Group 3 does not have risk genes for schizophrenia but does have CB1 receptors. Group 4 does not have risk genes for schizophrenia or CB1 receptors. It is expected that Group 1 will be the only group to display symptoms of schizophrenia in the behavioural tests.

Experiment

CP-55940 will be introduced into both experimental groups 1 and 2 and control groups 3 and 4 (Figure 2). CP-55940, an analogue of THC, is an agonist for the CB1 receptor [6]. Previous studies that have performed knockout CB1 experiments have shown that CB1-/- mutant mice do not respond to the cannabinoids, indicating that the CB1 receptor has a role in the effects of cannabis use [7]. From studies showing the onset of schizophrenia from cannabis use, it is hypothesized that the group possessing CB1 receptors will display symptoms of schizophrenia after being exposed to CP-55940 after a period of time, due to the binding of the cannabinoid to a CB1 receptor [4]. This would be further supported if the group that lacks CB1 receptors, group 2, does not exhibit symptoms of schizophrenia to the same degree as control group 4. This alludes to the possibility that the binding of the cannabinoids to CB1 causes the onset of schizophrenia in those that are genetically predisposed to the disorder.

Measurement

To assess whether the treated mice display symptoms of schizophrenia such as excessive allogrooming, two behaviour tests will be conducted: the grooming behaviour test and the elevated zero maze test [8]. These tests will allow for the direct observation of positive and negative symptoms

of schizophrenia, namely observations of social interaction in the grooming behaviour test and hyperactive stimulant sensitivity in the elevated zero maze test, which can be attributed to the effect of THC (Figure 3). The grooming behaviour test observes the grooming behaviour of mice on themselves and other mice [8]. Mice with schizophrenia tend to exhibit excessive allogrooming behavior, behaving as barber mice, which can be detected by the grooming behaviour test by both direct observation and a comparison of time spent grooming [8]. The elevated zero maze test consists of a cross-shaped platform with two open arms and two closed arms. The amount of time spent in the open arms will aid in interpreting anxiety-like behaviours, with highly anxious mice spending more time in the closed arms [8]. Anxiety-like behaviours are also considered indicators of schizophrenia in mice models [8].

Statistical Analysis

To determine if the results from the grooming behaviour test are statistically significant, a Kruskal-Wallis test followed by a Dunn's test will be performed on the average times spent grooming for the two experimental groups and group 3 normalized with control group 4. For the elevated zero maze test, a one-way ANOVA will be conducted on the mean times spent in the open arms, followed by a Bonferroni test and normalized to control group 4. Results with $p < 0.05$ will be considered statistically significant.

Results

It is expected that experimental group 1 will show schizophrenic behavior by behaving as barbers while spending longer periods of time grooming in the grooming behaviour test. They will also prefer to stay in the enclosed, dark region of the elevated zero maze test. We subsequently expect experimental group 2 and control group 4 to not show schizophrenia-like symptoms by behaving as non-barbers, spending less time grooming, and having no preference for the closed or open arms in the elevated zero maze test. Mice in control group 3 are expected to show very moderate deviations from normal behavior due to the presence of the CB1 receptors being able to bind the CP-55940 analogue, but overall not show schizophrenic-like behaviour.

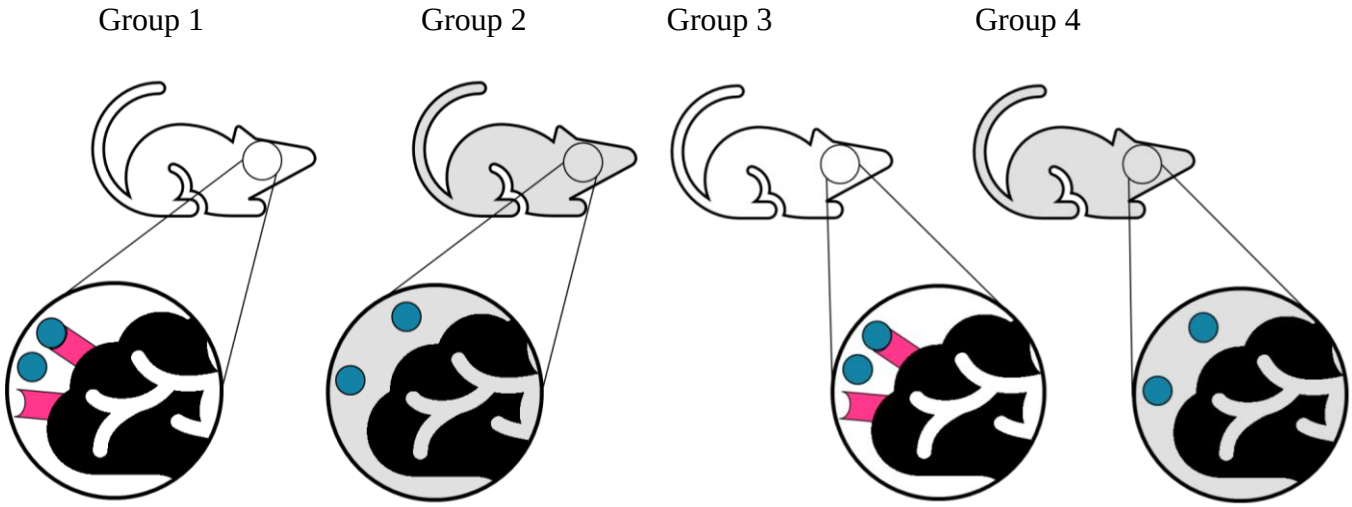

Figure 2: This figure provides a visual representation of how the THC analogue, CP-55940, is expected to interact with the CB1 receptors of the experimental and control groups of mice. In the experimental group 1 and control group 3, CP-55940 is expected to bind to the CB1 receptors. In experimental group 2 and control group 4, there are no CB1 receptors resulting in CP-55940 not binding to the receptors. A close-up visualization of the brains of the mice groups are shown to better understand the relationship between the CB1 receptors and CP-55940. The CB1 receptors are depicted in pink. The CP-55940 molecules are shown as blue circles.

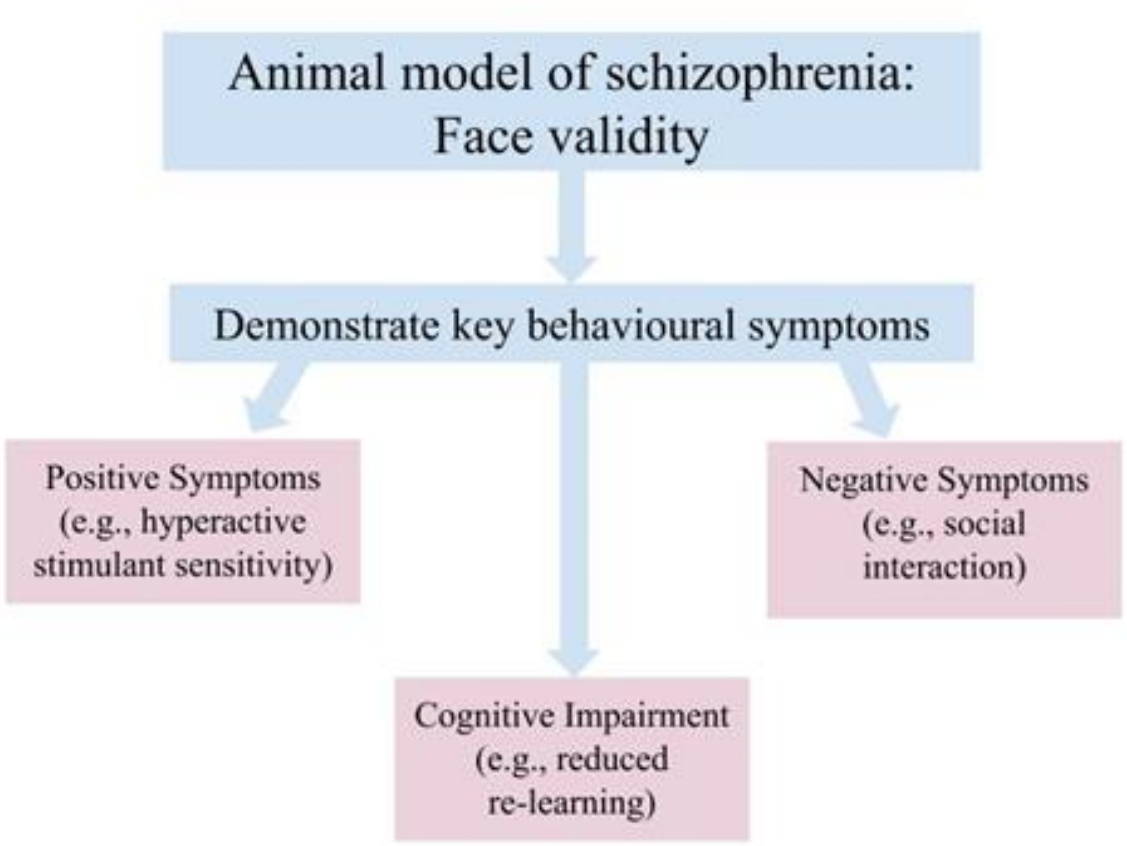

Figure 3: This figure, adapted from [9], illustrates the key behavioural symptoms that will be observed within our research design. The face validity of schizophrenia will be measured to determine if the mice are displaying symptoms of schizophrenia.

Conclusion

This research design aims to provide a direct effect between high levels of THC binding and mental health and consequential behavioural symptoms. By knocking out the CB1 receptor, alongside two control groups, the effects of THC on those at-risk for schizophrenia can be observed, a feat which previous research lacks. Prior research does not account for confounding effects as an observational approach was taken and did not knock out specific neurological variables. This prevents the direct association of mental health with cannabis use, making the research design offered in this proposal optimal. Despite this, this protocol has limitations including the mice model not being an exact replica of the human genome as well as analyses of behaviour being difficult to quantify. While the limitations of most mice model protocols include the aforementioned, the benefits of this research protocol are paramount to uncovering the effects of cannabis on mental health.

List of Definitions and Abbreviations Used

Δ9 THC: delta-9-tetrahydrocannibinol is the main psychoactive constituent of cannabis.
Allogrooming: grooming of other mice.
CB1 Receptor: a receptor which binds cannabinoids primarily in the brain.
CB2 Receptor: a receptor which binds cannabinoids primarily in the immune system.
CP-55940: an analogue of THC; an agonist for CB1 receptors.
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats are used in conjunction with Cas9 endonuclease proteins to edit genomes.
Dysphoria: an uncomfortable state in which one is displeased with life.
Wild-Type: a phenotype for typical forms of the species (i.e., mice); a phenotype that does not display any mutations (i.e., schizophrenia).

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Ethics Approval
No ethics approval or participant consent was required as this is only a proposed research protocol.

Authors' Contributions
SA: made substantial contributions to study design and planning, the collection and analysis of data, drafted the manuscript, revised the manuscript critically and gave final approval of the version to be published.
KG: made substantial contributions to study design and planning, the collection and analysis of data, drafted the manuscript, revised the manuscript critically and gave final approval of the version to be published.
HT: made substantial contributions to study design and planning, the collection and analysis of data, drafted the manuscript, revised the manuscript critically and gave final approval of the version to be published.
SJ: made substantial contributions to study design and planning, the collection and analysis of data, drafted the manuscript, revised the manuscript critically and gave final approval of the version to be published.

Acknowledgements
None.

Funding
This study was not funded.

References
[1] Sharma P, Murthy P, Bharath MM. Chemistry, Metabolism, and Toxicology of Cannabis: Clinical Implications. *Iran J Psychiatry*. 2012;7(4):149–56. PubMed Central PMCID: PMC3570572.
[2] Bridgeman MB, Abazia DT. Medicinal Cannabis: History, Pharmacology, And Implications for the Acute Care Setting. *Pharm. Ther.* 2017;42(3):180–8. PubMed Central PMCID: PMC5312634.
[3] Koppel BS, Brust JC, Fife T, Bronstein J, Youssof S, Gronseth et al. Systematic review: Efficacy and safety of medical marijuana in selected neurologic disorders. *Neurology.* 2014;82(17):1556–63. https://doi.org/10.1212/WNL.0000000000000363.
[4] Radhakrishnan R, Wilkinson ST, D'Souza DC. Gone to Pot - A Review of the Association between Cannabis and Psychosis. *Front. Psychiatry.* 2014;5. https://doi.org/10.3389/fpsyt.2014.00054.
[5] Zimmer A, Zimmer AM, Hohmann A, Herkenham M, Bonner T. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 1999;96(10):5780–85. https://doi.org/10.1073/pnas.96.10.5780.
[6] Braida D, Pozzi M, Cavallini R, Sala M. Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. *Neuroscience.* 2001;104(4):923–6. https://doi.org/10.1016/S0306-4522(01)00210-X.
[7] Ledent C, Valverde O, Cossu G, Petitet F, Aubert J, Beslot F et al. Unresponsiveness to Cannabinoids and Reduced Addictive Effects of Opiates in CB1 Receptor Knockout Mice. *Science.* 1999;283(5400):401–4. https://doi.org/10.1126/science.283.5400.401.
[8] Zhou Y, Kaiser T, Monteiro P, Zhang X, Van der Goes M, Wang D et al. Mice with Shank3 Mutations Associated with ASD and Schizophrenia Display Both Shared and Distinct Defects. *Neuron.* 2016;89(1):147–62. https://doi.org/10.1016/j.neuron.2015.11.023.
[9] Jones CA, Watson DJ, Fone KC. Animal models of schizophrenia. *Br. J. Pharmacol.* 2011;164(4):1162–94. http://doi.org/10.1111/j.1476-5381.2011.01386.x.

Article Information
Managing Editor: Jeremy Y. Ng
Peer Reviewers: Jennifer Williams, Bi-ru Amy Yeung
Article Dates: Received Oct 27 19; Accepted Dec 16 19; Published Dec 30 19

Citation
Please cite this article as follows:
Azher S, Grewal K, Thakar H, Jim S. Using knockout mice models to explore links between THC and schizophrenia: A research protocol. URNCST Journal. 2019 Dec 20: 3(11). https://urncst.com/index.php/urncst/article/view/169
DOI Link: https://doi.org/10.26685/urncst.169

URNCST Journal
"Research in Earnest"

Funded by the Government of Canada | Canada