Therapeutic Potential of Chronic Administration of Cannabidiol for Treatment of Anxiety

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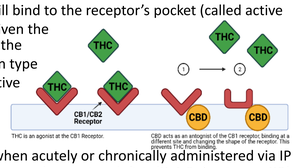
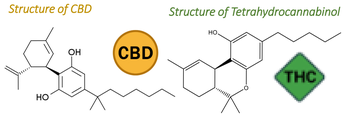
Introduction

Cannabis sativa contains more than 100 phytocannabinoids, the most popular being Δ9-tetrahydrocannabinol (Δ9-THC), the psychoactive component of marijuana, and **cannabidiol** (CBD), the major non-psychoactive component [2]. As the medicalization of marijuana occurs across the United States, the psychoactive component of the cannabis plant, **Delta9-Tetrahydrocannabinol** (THC), has gained recognition for its therapeutic potential. Meanwhile, its non-psychoactive counterpart, cannabidiol (CBD), has been marketed to treat Parkinson's disease, Chron's disease, dystonia, attention-deficit hyperactivity disorder (ADHD), inflammation, depression, fibromyalgia, epilepsy, and most commonly, anxiety. Cannabinoids are a group of naturally occurring chemical substances that are present in humans and mammals that bind to the cannabinoid receptors and activate them. Thus, sending signals throughout the body in order to maintain proper homeostasis. Phytocannabinoids, on the other hand, are synthesized from plants, but also have as an effect to interact with the cannabinoid receptors in our brain. To understand how these cannabinoids act on the body, a general understanding of cannabinoid receptors is necessary. The two main receptors that are pertinent to this project are CB1 and CB2, found mainly in the brain and peripheral organs, respectively [1]. The CB1 receptor is mainly responsible for inhibiting the release of neurotransmitters in the neurons in which it is present [4]. Thus, activation of CB1 will decrease either the inhibitory or excitatory drive of the neuron, resulting in physiological changes involved in regulating homeostasis (depending on the presynaptic terminals in which the receptor is located) [3]. In our study, CBD will bind to the side of the CB1 receptor and act as a non-competitive antagonist; that is, it will prevent other molecules from activating the receptor (this process is also called allosteric inhibition). THC, on the other hand, will bind to the receptor's pocket (called active site) and activate it, thus acting as an agonist. Given the over-the-counter (OTC) accessibility of CBD and the fact that anxiety disorders are the most common type of mental illness in the United States, our objective was to access the therapeutic potential of cannabinoid receptor agonists and antagonists for treatment of anxiety and attention deficit. We hypothesized that CBD might be anxiolytic when acutely or chronically administered via IP injection to a newly found mouse model of anxiety and attention deficit (Kv1.3-/- mice, or KO). Kv1.3 -/- mice lack potassium (Kv) channels in cells that can be found in the olfactory bulb, hippocampus and the pyriform cortex. This causes a decreased repolarization, resulting in hyperexcitability, and usually, heightened anxiety. Herein, we were particularly interested in chronic effects of CBD (greater than 3 weeks use) as well as the concentration of drug (dose responsivity).

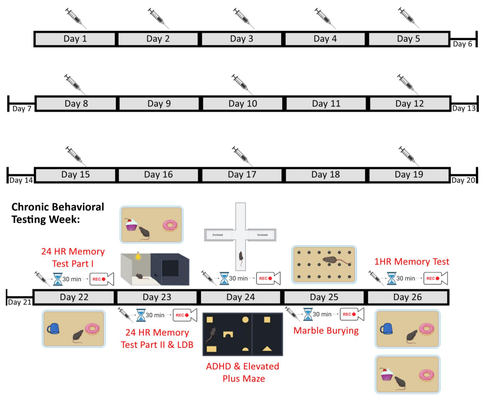


Structure of CBD *Structure of Tetrahydrocannabinol*



Methods

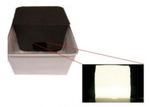
Chronic **intraperitoneal** (IP) injections started three weeks prior to the behavioral testing period, and during the behavioral period, the injections were made 30 minutes prior to testing. The following scheme shows the behavioral testing period and order of performance of each test.

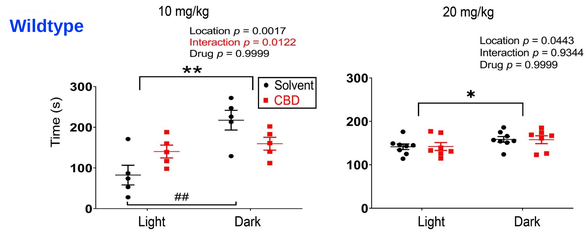


All mice used in this study were housed in *vivaria* with a reverse 12/12-hour light/dark cycle (lights off at 8:00 A.M. and on at 8:00 P.M.). Experiments were approved under protocol number #202000036 by the Florida State University Institutional Animal Care and Use Committee (IACUC). Experiments were performed on approximately 3-month-old male and female C57BL6/J mice **(WT and Kv1.3−/−)**. Behaviors were digitally recorded and analyzed post-hoc.

1. WT mice preferred the dark over the light compartment (state anxiety, stars). Longer-term CBD treatment reduced anxiety when administered at low dose (10 mg/kg, location x drug interaction, #) but not at a higher dose (20 mg/kg).

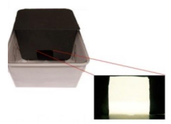
Light-Dark Box: WT

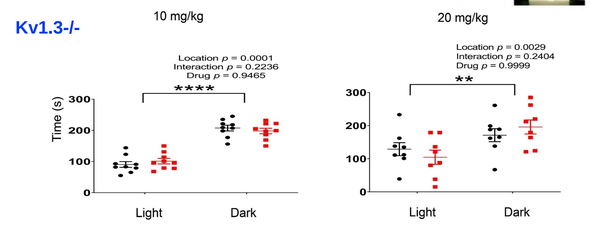




2. Mice with trait anxiety (Kv1.3-/-, KO) preferred the dark compartment over the light (state anxiety), but neither CBD dose had an effect on anxiety-like behaviors.

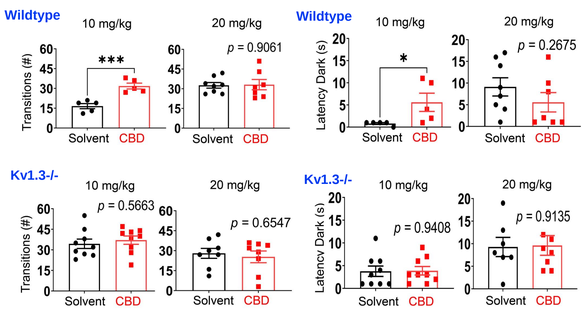
Light-Dark Box: KO





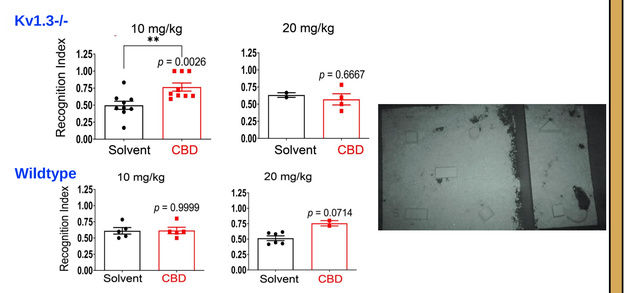
3. Longer-term, low dose CBD treatment increased ambulatory activity and increased 1st latency to the dark in WT mice that was consistent with their decrease in state anxiety.

LDB: Locomotor Activity



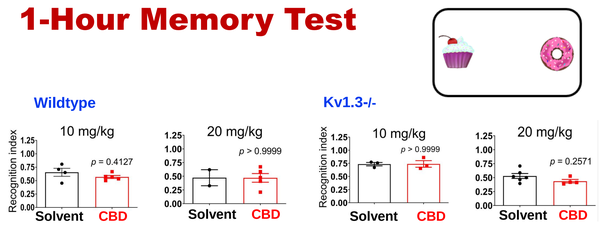
4. Longer-term CBD treatment was effective at the low dose to decrease ADHD-like behaviors in Kv1.3-/- mice, however, not in WT mice.

Object Attention (ADHD)



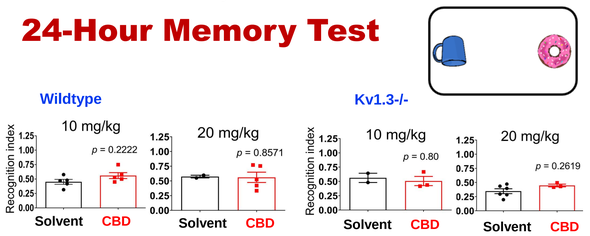
5. Chronic CBD did not increase object memory recognition in either WT or Kv1.3-/- mice.

1-Hour Memory Test



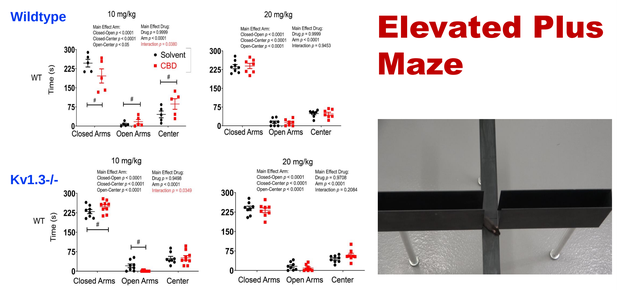
6. Chronic CBD treatment had no significant effect on longer-term object memory recognition for either WT or Kv1.3-/- mice.

24-Hour Memory Test



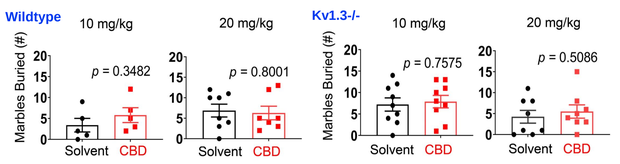
7. Mice prefer the closed arm location in an elevated plus maze (causes state anxiety; strong arm effect in all trials). At low dose, CBD decreases anxiety for WT mice but is anxiogenic for Kv1.3-/- mice (drug x location interaction, #).

Elevated Plus Maze



8. Chronic CBD treatment did not reduce obsessive compulsive-like behaviors in mice, whether given at low or high dose, in either WT or Kv1.3-/- mice.

Marble Burying Test



Conclusions

1. Ten mg/kg dose of CBD reduces anxiety-like behaviors in WT mice but is ineffective in reducing anxiety in Kv1.3-/- mice with trait anxiety.
2. Concerning ambulatory activity, WT mice were found to have a higher number of transitions, and a longer first latency period towards the dark at 10 mg/kg dose of CBD. This is consistent with their reduced anxiety-like behaviors. Kv1.3-/- exhibited no drug dependent effects on locomotor or first latency to the dark activity.
3. At low dose chronic administration, object recognition increased for the Kv1.3-/- mice, suggesting a decrease in ADHD-like behaviors. Kv1.3-/- mice are known to have attention deficit behaviors and were responsive to CBD at the 10 mg/kg dose whereas WT mice showed no change in behavior following drug treatment.
4. In the elevated plus maze, all mice preferred the closed arm location. At low dose, CBD was anxiolytic for WT mice but was anxiogenic for Kv1.3-/- as demonstrated in the significant location x drug interaction.
5. Chronic or longer-term CBD treatment does not reduce obsessive compulsive-like behaviors in either WT or Kv1.3-/- mice.
6. Chronic CBD treatment did not affect short- or long-term object memory.
7. Overall, longer-term CBD has no effect on obsessive compulsive-like behaviors in WT mice but is effective in reducing anxiety-like behaviors. For mice with trait anxiety (Kv1.3-/-), longer-term CBD is effective in reducing ADHD-like behaviors but not effective in reducing state anxiety in the LDB. When tested for extreme anxiety producing situations in the EPM, CBD lessens anxiety in WT but enhances anxiety in Kv1.3-/- mice.

References

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Consortium for Medical Marijuana Clinical Outcomes Research

Future Considerations

According to the acute administration results, there seem to be sex dependent effects regarding drug treatment. Our next objective is to increase our sample size so we can divide our current analyses by sex.