The Effect of Cannabidiol on Human Learning and Memory

Protocol and Statistical Analysis Plan (SAP)

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V2.0

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1. Protocol

The broad objective of our study was to examine the potential effect of Cannabidiol (CBD) on human learning and memory (L&M) in relatively small scale surveys, and with consideration of other confounding factors (i.e., other drugs being specifically combined with CBD or particular preexisting medical conditions). Our study design aimed to determine whether CBD, when administered alone to healthy human subjects is a modulator of human L&M. We also aimed to determine whether particular demographic factors present in the general population (e.g., age, sex, history of CBD use, and urine THC) would affect the modulatory effect of CBD on L&M.

a. Design and Participants

57 human volunteers were recruited from the Front Range communities in Colorado (Pueblo, Colorado Springs and Denver). Subjects were randomized to two treatment arms: 1) to receive CBD First; 2) to receive placebo first. Each applicant signed an informed consent form and filled out a demographics questionnaire. The L&M for each subject was tested after administration of 246 mg CBD absolutely or placebo. Testers were blinded as to whether someone is getting CBD or Placebo. The principal investigator remained unblinded, and was not be involved in data collection nor analysis to prevent any bias. Generation of the randomization list was performed by the principal investigator.

After administration of CBD or placebo the participants waited 2hrs before taking the learning and memory tests. The experiment was repeated one week later, by reversing the CBD and control groups, in order to eliminate (or minimize) the placebo effect.

b. Cognitive Assessments

Each subject will be required to take two 30-min tests (Version 1 L&M test and Version 2 L&M test). Each test compromised of two sessions: session A, session B, and session C. Test session A was an assessment of basal cognitive function, session B was a verbal declarative memory test, and session C was a prose recall-cognitive L&M test (Schoeler and Bhattacharyya 2013).

First, participants were asked to complete the Montreal Cognitive Assessment (MOCA) as part of session A. The MOCA was given by a tester and took 5-10 minutes. This was a written non-invasive assessment of basal cognitive function. The purpose of the prose test was to evaluate logical memory in study subjects (Curran et al. 2002, Morgan et al. 2010, Hindocha et al. 2018).

For the verbal declarative memory test (session B), we used the Rey-Auditory Verbal Learning Test-Revised (RAVLT-R) test (Solowij et al. 2011, Meier et al. 2012, Schoeler and Bhattacharyya 2013, Becker et al. 2014, Khosravi Fard et al. 2016). Participants were instructed to listen to a list of 12 words (List A) read to them. They were asked to recall List A in four trials, with the words repeated to them during each trial. The number of words the participants remember was expected to increase between trails 1 and 4. The purpose of the multiple trails was to check if list learning is enhanced. Participants were scored for the number of correctly repeated words for each trail and for the number of intrusions (number of words not on list). Participants were also be scored for clusters (number of words said consecutively that were in the same semantic category). Then, the participants were instructed to listen to another list of 12 words (List B), asked to recall List B once, and scored

for the number of correctly repeated words. Afterward, participants were asked to recall List A and were scored for the number of correctly repeated words.

Participants were instructed to listen to a short prose story (Passage I) and asked to immediately recall the passage as part of session C. Then, the participants were asked a series of questions about Passage I and given a score of 0 if they said the incorrect answer and a score of 1 if they got the right answer. Scores were added. Participants were distracted with delayed recall of list A. Afterward, participants were asked to recall passage I.

The test sessions were conducted at CSU-Pueblo in Pueblo.

c. L&M data collection from participants

The learning and memory scores collected from participants included MOCA performance, RAVLT-R performance (List A trials: A1, A2, A3, A4, A5, A6, A7, sum of list A trials; List B trial: B), and prose recall performance (immediate, delayed, and total). The effects of two types of interference from the RAVLT-R were quantified as:

- 1) Proactive Interference: (PI) $= \frac{B}{A1}$, where B is the number of words recalled from List B (distractor list), and A1 is the number of words recalled from list A on the 1st trial
- 2) Retroactive Interference (RI) $= \frac{A6}{A5}$, where A6 is the number of words recalled from List A after the distractor List B, and A5 is the number of words recalled from List A on the 5th trial.

Assessing Proactive Interference allowed for the determination of how learning old material impacts learning new information. In this study, effects of proactive interference were determined

by seeing how List B recall was impacted after List A recall. Assessing Retroactive Interference allowed for determination of how newly acquired information impacts the recall of previously learned information. In this study, effects of retroactive interference were determined by seeing how immediate recall is impacted after List B recall by comparing trial 5 recall (before List B) and immediate recall (after List B). These equations were utilized by Magalhães (et al., 2012) to evaluate the PI, RI, and FS Ratios and the ratios have been used in other L&M studies (Geffen et al., 1990; Vanderploeg et al., 2001; Kramer et al., 1991; Numan et al., 2000; Torres et al., 2001; Malloy-Diniz et al., 2007; Magalhães et al., 2012; Frith et al., 2018).

2. Statistical Analysis Plan

All statistical analyses were performed using SPSS. Linear Mixed Models (LMM) with Bonferroni Corrections were to examine L&M scores of placebo and CBD recipients, while also controlling for demographic factors. Two-tailed $P < 0.05$ will be considered statistically significant. Subject IDs were included as random effects. Treatment (CBD vs placebo) was included as a factor. The following were included as covariates: age, history of CBD use (user or non-user), sex (male or female), and urine THC result (THC positive or negative). A Linear Mixed model was created for each of the following dependent variables: MOCA, Sum of List A trials, List B, PI Ratio, RI Ratio, FS Ratio, and Total Prose Recall.

COVID as a factor was removed from the Linear Mixed Models. Data related to COVID will be independently analyzed in a separate statistical model and reported later.