Using Imaging to Assess Effects of THC on Brain Activity (fNIRS)

Statistical Analysis Plan

The fNIRS Team

Version 1

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Glossary

Abbreviation	Label
ETOH	Alcohol
FDA	Federal Drug Administration
fNIRS	Functional near-infrared spectroscopy
HbO	Oxygenated hemoglobin
PFC	Pre-frontal cortex
THC	Tetrahydrocannabinol

Study Design

This study will assess the effects of THC intoxication using dronabinol (synthetic THC) on the HbO signal during resting state and task-based activation in the prefrontal cortex (PFC), resting state connectivity, neurocognitive task performance, clinical signs of intoxication, and correlations between these measurements. Participants will be given up to 80 mg of dronabinol, an FDA-approved synthetic form of THC that is used to treat loss of appetite that causes weight loss in people with AIDS. THC is the principle psychoactive drug in marijuana. The study will be conducted in regular cannabis users who present at the screening visit with a positive urine screen for THC metabolites. Data will be collected over two phases:

- Phase 2A is a randomized, double-blind, placebo-controlled, study of effect of dronabinol vs placebo. This phase will investigate the effect of THC on fNIRS brain signature and its association with self-reported intoxication, laboratory measures of impairment, and the gold-standard behavioral field test of driving impairment used by law enforcement.
- Phase 2B is a randomized, double-blind, placebo-controlled, 2 by 2 crossover study of effect of dronabinol, ethanol, and combined dronabinol and ethanol on brain activation and connectivity as measured by fNIRS. Phase 2B will examine potential interaction between THC and alcohol following co-administration of THC with oral ethanol exposure in healthy volunteers.

Participants

Participants will be 150 adults who use marijuana at least monthly (aged 18-55) will be recruited to participate in this study.

Analytic Approach

Primary Outcomes

The primary outcomes for the study will be…

1. The mean HbO level averaged over the duration of the N-back task scan (approximately 2938 samples taken over 376 seconds in 128 millisecond intervals).
2. The mean HbO level averaged over the duration of the resting state scan (2814 samples taken over 360 seconds in 128 millisecond intervals).

Mean HbO levels for both outcomes will be determined over (a) four conditions administered over separate visits about a week apart (where participants could be dosed with placebo, THC only, ETOH only, or both THC and ETOH), and (b) two scans per condition: A scan prior to and approximately 100 minutes after being dosed. Higher HbO levels indicate a greater degree of neural activity in the PFC regions of the brain.

Statistical Model

We will analyze both primary outcomes using a multi-level linear regression model with subject-varying intercepts estimated via maximum likelihood. The key exposures are whether a person was dosed with placebo, THC, ETOH, or the combination of THC and ETOH. Table 1 lists the numeric coding used to test our planned contrasts. All terms will be coded as 0 for pre-dose scans, as no combination of drugs will have been administered.

Table 1: Design matrix for testing key exposures

Scan	Dose	Main effects		Interaction
		THC	ETOH	THC x ETOH
Post-dose	Placebo	0	0	0
	THC	1	0	0
	ETOH	0	1	0
	THC + ETOH	1	1	1

Statistical significance for each contrast will be determined via a non-parametric bootstrap procedure for robustness. First, each subject's set of completed condition (placebo, THC, ETOH, THC + ETOH) will be randomly shuffled across their set of visits. Second, the shuffled data will be refit with the multi-level model, and coefficient estimates will be obtained for the key exposures. Third, the prior two steps will be repeated 10,000 times, providing a null distribution to compare the observed coefficient estimates against. The p-values for the key exposures will be estimated as $P(|o| < |s_i|)$, where o is the observed coefficient, and s_i is the ith coefficient from the shuffled data. For each outcome there will be 3 p-values (the main effects of THC and ETOH and their interaction). The total set of 6 p-values will be adjusted for multiple comparisons using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). An adjusted p-value < 0.05 will indicate a statistically significant effect of either THC, ETOH, or their interaction on neural activity in the PFC.

Covariates

We will include two sets of covariates in our model. First, we will include a dummy-coded contrast comparing pre-dose scans (coded as 0) to post-dose scans (coded as 1). Second, we will include covariates adjusting for visit order. The referent will be the first visit (coded as -1), and we will include three terms for the second, third, and fourth visit (coded as 1 for the given visit and 0 otherwise). All terms will then be converted to z-scores.

Missing Data

We will only include data from complete visits with both pre-dose and post-dose scans. Participants with no complete pairs of pre and post-dose scans for any conditions will be excluded from the analyses. Missingness will be naturally handled by the multi-level modeling approach, as the higher order terms governing the random effects provide shrinkage and information even in the presence of missing cases (Baayen, Davidson, & Bates, 2008).

Sample Size Determination

Sample size was pre-specified in our grant application. Since no prior studies have attempted to predict impairment using fNIRS measurements, we did not have an effect estimate with which to determine sample size. In order to maximize the chance that standard methods could detect an effect of intoxication on PFC activation patterns with the N-Back task, we collected as many scans as possible with the R42 grant funds allotted.

Software

All analyses will be done using the statistical software R (version 4.1.1; R Core Team, 2021) and integrated development environment RStudio (version 2020.9.0.351; RStudio Team, 2021). Data will be prepared using the R packages ‘dplyr’ (version 1.0.7; Wickham, François, Henry, & Müller, 2021. Models will be fit using the R package ‘lme4 (version 1.1-27.1; Bates, Maechler, Bolker, & Walker, 2015).

References

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