Cover Page for Statistical Analysis Plan

Official study title	Project 1 Aim 2, Adaptations of the Brain in Chronic Pain With Opioid Exposure
NCT #	NCT05463367
Protocol ID	STU00209670
Document date	November 21, 2024

Statistical analysis plan for multi-receptor mapping of brain activity

The main objective of the study is to investigate behavioral and cortical multi-receptor brain activity changes following the administration of 3 drugs (Placebo, opioids, dopamine + NSAID) in 23 chronic back pain (CBP) patients on opioid therapy for at least 6 months (CBP+O) following 24 hours abstinence.

Participants and study design

A total of 34 CBP+O patients were enrolled in the study, 23 completed all parts of the study, and they will be used in this analysis. All patients participated in three sessions in which they received opioid, placebo or dopamine following 24 hours abstinence. For any given session, patients were first instructed to abstain from taking their opioids for 24 hours. Following abstinence, patients reported their pain intensity and craving and participated in brain scanning session in which we collected resting state fMRI (Scan 1). patients were given either placebo, dopamine or their opioid and scanned 4-5 hours later (Scan2). This sequence was repeated 3 times for each patient in a randomized order. All researchers and patients were blinded to the type of drug used for any given session.

Outcomes measures

Behavioral outcome measures include pain intensity (0-10) and drug craving index (0 -10) with higher scores corresponding to higher pain and higher craving. Brain functional measure is resting state fMRI. We will use the resting-state fMRI to generate whole-brain voxel-wise amplitude of low-frequency fluctuations (ALFF) maps that reflect the low-frequency energy of spontaneous

BOLD activity. Cortex-wide receptor-related activity for each subject will be computed as the normalized dot product between ALFF maps and the standardized receptor density distribution maps obtained from Hensen et al 2022. This will result in one value per subject representing cortical receptor-related activity for any given receptor type. For this analysis we will focus on 3 receptors that we have shown to be modulated in long-term opioid use including the serotonin (5-HT_{1A} and 5-HT_{1B}) receptors and the μ-opioid (MOR) receptor. We will also investigate both dopamine (D1 and D2) receptors to test their modulation with administering dopamine+NSAID.

Analysis and Hypothesis

Differences in pain intensity and craving following treatment will be investigated using repeated measure ANOVA, for the average rating before and after drug ingestion. We will examine both treatment (scan1 vs scan2) and drug effects (Placebo, dopamine and opioid) as well as their interaction. We hypothesize that opioid ingestion will show significantly better pain and craving reduction compared to placebo. Changes in receptor-specific cortical activity will be examined using a similar analysis. We hypothesize the serotonin and opioid-related activity to be modulated more with opioid treatment, compared to placebo. Specifically, opioid use will enhance 5ht1a-related positive activity and reduce mu opioid (MOR)-related activity (increased negative activity), as compared to placebo. We also expect dopamine treatment to influence D1 and D2 related activity compared to placebo.

All preprocessing and post-processing analyses will be performed blinded to group assignments. Group information will only be unblinded after the final results are obtained, which are done based on an arbitrary group assignment (A, B, C, where actual treatment remains unknown, until final result).

The details of preprocessing, post-processing, and calculating receptor-related whole-cortex activity (ALFF*R) are all outlined in an archived paper: .