STATISTICAL ANALYSIS PLAN

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IND/FDA: 135570, oxytocin and yohimbine (Holder: Haass-Koffler)

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Study design, setting and approval

This was a Phase 1, outpatient, randomized, double-blind, cross-over (2x2 design with oxytocin and yohimbine), placebo-controlled, human laboratory study (**Figure 1**). The trial took place at the Center for Alcohol and Addiction Studies, Brown University, Providence, RI, USA from 2019-2023. The clinical protocol was approved by the Brown University Institutional Review Board (IRB), received an FDA Investigational New Drug (IND135570), and was registered on clinicaltrials.gov (NCT04051619). The study was initiated as a between-subjects design. However, following the COVID-19 pandemic, in order to speed up recruitment, the study was changed to a within-subjects crossover design. This modification in the clinical protocol was executed following the FDA Guidance Document on Changes or Modification during conduct of Clinical Investigations [1]. After receiving approval from the Brown University IRB in November 2021, the FDA was notified in an annual progress report and the study design was updated on clinicaltrials.gov in February 2022.

Statistical analysis

For all outcomes, we utilized an intention-to-treat (ITT) approach, where participants were examined based on their *a priori* randomized protocol and received at least one dose of the study medication (oxytocin or oxytocin-matched placebo) [2]

The ITT analysis was suitable for this crossover design [3] as the placebo condition was treated identically to the active drug condition (route, administration and laboratory procedures). Distributional characteristics of outcome measures were examined to evaluate similarity to the normal distribution, detailed descriptive analysis of demographics, substance use, and clinical characteristics. The cortisol had a kurtosis in excess of four; consequently, an outlier analysis was performed and a total of 5 outliers falling outside of ±3 interquartile range were Winsorized as recommended [4]. Comparisons with these characteristics, in relation to enrolled versus completer status, were performed using *t*-tests to analyze continuous variables (age) and χ^2 for categorical variables (sex, race, medication status). Attrition rates between the screening visit and follow-up visit were examined descriptively to assess for potential bias. In addition, a logistic regression was performed to test for possible bias due to period (placebo first, then oxytocin and oxytocin first, then placebo) or medication carryover (placebo and oxytocin), as done in our prior cross-over trial [5]. This study tested the feasibility of the combined study design, the safety and tolerability of IN oxytocin and yohimbine, and the potential value of testing IN oxytocin as adjunct therapy in an appropriately-powered larger RCT. In selecting a target sample size, we balanced power considerations and feasibility given the preliminary nature of this trial. Because of the within-subjects design, power to test the effects of the study drug was optimized for this modest sample size. For the safety and tolerability outcomes (primary) adverse events, difference was detected based on a

judgement concerning the minimal effect, which has clinical relevance in the management of patients. In a noninferiority trial, the exact sample size could not be fixed in advance because it depends upon the chosen stopping guidelines [6].

Outcomes: Primary (craving) and secondary (safety and tolerability) outcomes were assessed in real-time in the laboratory. Seven-day oxytocin administration was compared to the oxytocin-matched placebo condition during the CR procedure, in which noradrenergic activation produced by yohimbine or yohimbine-matched placebo condition (2X2 design) was measured. We used Generalized Linear Model (GLM) with model-based estimator in the covariance matrix, with medication (oxytocin/placebo) and noradrenergic activation (yohimbine/placebo) as within-subject factors. The model was specified to evaluate the effect of drug (oxytocin/placebo) by noradrenergic (yohimbine/placebo) interaction, main effect of the drug, and main effect of noradrenergic activation. Craving was assessed using the DDQ with time coded as: t_0=relaxation, t_1=interaction with drug paraphernalia/opioid auditory cues and t_2=opioid use video. Safety and tolerability were assessed using: SBP, DBP, HR (vital signs), HAM-A and STAI (anxiety). The STAI y1 (state) was included as an additional safety measure for anxiety to ensure participants were not experiencing acute symptoms of anxiety within the laboratory. The STAI y2 (trait) was inserted as a covariate in the model to control for baseline anxiety level, along with stress (PSS), HPA (cortisol), and noradrenergic response (α-amylase). Time at laboratory sessions was coded as: t_0=baseline, t_{45min}=45-min after yohimbine administration, and t_{90min}=post cue-exposure to specifically evaluate the contribution of each laboratory procedure (yohimbine and CR). Withdrawal symptoms (COWS) and AEs were assessed at specific timepoints: t_0=pre-laboratory and t_1=post-laboratory procedures.

Other outcomes (safety and tolerability) were measured retrospectively during the one-week outpatient setting and included the: COWS (withdrawal), anxiety (HAM-A and STAI), stress (PSS), depression (HAM-D) and AEs. The other outcomes were analyzed using ANOVA test and the number of AEs via a χ^2 test. For missing data approach, we first categorized missing data as missing completely at random (MCAR), missing at random (MAR) or missing not at random (MNAR) [7]. Analysis with GLM (using all available pairs of data to model missing values with maximum likelihood estimation) was deemed suitable for our analyses because no systematic differences existed between participants with missing data and those with complete data (MCAR) [8].

Missing data reporting [9]

1. *Amount of missing data in the dataset*:
    a. Oxytocin condition: 10% did not receive either oxytocin or placebo condition (within subject, *n*=16; between subject, *n*=4)
    b. Yohimbine condition: 11.12% did not receive yohimbine condition (yohimbine, *n*=14; placebo, *n*=16; no yohimbine, *n*=4):

2. *Reason for missing*:
    a. Oxytocin condition: change from between subject to within subject design
    b. Yohimbine condition: safety concerns due to past history of cardiovascular disease and/or current hypertension (*n*=3), stroke (*n*=1)
3. *Consequences*:
    a. *Oxytocin condition*: no differences between participants with and without missing values except there were no subject taking methadone in the subjects with missing values.
    b. *Yohimbine condition*: no differences between participants with and without missing values except there were cardiovascular concerns in the subjects with missing values.
4. *Method*: GLM with MNAR using all available pairs of data to model missing values with maximum likelihood estimation
5. *Software*: IBM SPSS Statistics for Windows, version 29 (IBM Corp., Armonk, NY, USA)

All statistical analyses were performed after participants had completed their follow-up visits and the study database had been locked. The results were presented using summary statistics: number of subjects (*n*); mean (*M*); standard deviation (*SD*) or frequency distributions (%) and effect size was reported as Cohen's *d*.

All statistical procedures were performed by IBM SPSS Statistics for Windows, version 29 (IBM Corp., Armonk, NY, USA), and GraphPad Prism (v.10) was used to generate figures (La Jolla, CA, USA). All statistical tests were two-tailed, and statistical significance was accepted if an alpha value $p<.05$ was obtained.

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