STATISTICAL ANALYSIS PLAN

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Forgetting Alcohol: A Double-blind, Randomized Controlled Trial Investigating Memory Inhibition Training in Young Binge Drinkers

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Forgetting alcohol: a double-blind, randomized controlled trial investigating memory inhibition training in binge drinkers

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Abstract

Background: Binge Drinking (BD), a pattern of alcohol misuse widely prevalent among youngsters, has been associated with altered inhibitory control and augmented reactivity to alcohol-related information. Memory inhibition (MI), the ability to voluntarily suppress unwanted thoughts or memories, has shown to lead to forgetting of memories in several psychiatric conditions. However, despite its potential clinical implications, no study to date has explored the MI abilities in populations with substance misuse, such as binge drinkers (BDs).

Method: This study protocol aims firstly to examine the behavioral and electroencephalographic (EEG) correlates of MI among college BDs. For this purpose, 45 BDs and 45 age-matched non/low-drinkers will be assessed by EEG while performing the Think/No-Think Alcohol task, a paradigm that evaluates alcohol-related MI. Additionally, this work aims to evaluate an alcohol-specific MI training protocol using cognitive and transcranial direct current stimulation (tDCS) while its effects on behavioral and EEG outcomes are assessed. For that, BDs will be randomly assigned to one of three training groups: *combined* (verum cognitive training [CT] and tDCS applied over the right dorsolateral prefrontal cortex [DLPFC]), *cognitive* (CT and sham tDCS), or *control* (sham CT and sham tDCS). Training will occur in three consecutive days, in three sessions. MI will be re-assessed in BDs through a post-training EEG assessment. Alcohol use and craving will be measured at the first EEG assessment, and both 10-days and 3-months post-training. In addition, behavioral and EEG data will be collected during the performance of an alcohol cue reactivity (ACR) task, which evaluates attentional bias towards alcoholic stimuli, before and after the MI training sessions.

Discussion: This protocol will provide the first behavioral and neurofunctional assessment of MI in BD. Along with poor MI abilities, BDs are expected to show alterations in the amplitude of several event-related potentials (ERPs) linked to MI (e.g., N2 and late parietal positivity) as well as abnormal functional connectivity (FC) patterns within/between regions associated with MI (e.g., DLPFC and hippocampal/parahippocampal regions). Results should also demonstrate the effectiveness of the training protocol, with BDs exhibiting an improved capacity to suppress alcohol-related memories after both *combined* and *cognitive* MI training, along with a significant reduction in alcohol use and craving in the short/medium-term. Furthermore, this protocol should also lead to significant modifications in the ERP and FC patterns, reflecting stronger MI capabilities and reduced alcohol cue reactivity in trained BD participants. Finally, in case the protocol provides significant results, these findings might have major implications for the understanding and treatment of alcohol misuse.

Statistical Analysis

The first step of the statistical analyses will be to examine the behavioral and EEG correlates of MI among college BDs and non/low-drinkers. For that purpose, the outcomes of the TNTA task collected during the pre-training assessment (T2) in BDs and non/low-drinkers will be compared and correlated with alcohol consumption (AUDIT) and alcohol craving measures (i.e., the PACS' and ACQ-SF-R's scores, and the ERPs and valence/arousal ratings recorded during the ACR task). Secondly, with the purpose of verifying the impact of the *combined, cognitive* and *control* training procedures applied to BDs on psychological, behavioral and neurofunctional measures, the pre-training data (T2) from the 45 BDs will be compared with the post-training data (T4) from the same subjects. Thirdly, to examine potential differences in the psychological, behavioral and neurofunctional effects of the training sessions both in terms of MI abilities and alcohol craving/consumption, the results of the post-training assessment (T4) of the three training groups will be compared.

With regard to the behavioral data, items learned during the learning phase and correctly recalled during the memory test phase will be considered correct responses. Accordingly, the percentage of correct responses (for Think, No-Think and Baseline items) will be computed according to the following formula:

$$\left(\frac{number\ of\ correctly\ recalled\ items}{number\ of\ previously\ learned\ items}\right) \times 100$$

A mixed-model analysis of variance (ANOVA) with one between-subject factor, Group (non/low-drinkers, BDs), and two within-subject factors, Condition (Think, NoThink, Baseline) and Content (Alcohol, Non-Alcohol) will be conducted on the recall accuracy rate to examine the participants' MI ability at T2. Afterwards, a repeated-measures ANOVA with three within-factors: Moment (T2 and T4), Condition (Think, NoThink, Baseline) and Content (Alcohol, Non-Alcohol) will be performed to explore the training effects on the MI ability of BDs only.

Furthermore, in order to examine the emotional response of participants to alcoholic cues (self-assessed during the ACR task using the Manikin test) at T2, two ANOVAs with Group (non/low-drinkers, BDs) as between-subject factor and with Content (Alcoholic, Non-Alcoholic) as within-subject factor will be conducted for valence and arousal ratings, separately. In addition, to evaluate possible variations in valence and arousal responses as a function of training sessions, new ANOVAs will be performed for each training group with two within-factors - Moment (T2 and T4) and Content (Alcoholic, Non-Alcoholic).

For the TNTA task, we will analyse the ERPs, specifically the mean amplitudes of N2, LPP and frontal slow wave (FSW). A mixed-model ANOVA with one between-subject factor Group (non/low-drinkers, BDs) and four within-subject factors: Condition (Think, NoThink), Content (Alcohol, Non-Alcohol), Region (Left, Midline, Right) and Electrode (2 electrodes) will be conducted on the mean amplitude of each component to explore the MI neural mechanisms during pre-training (T2). A repeated-measures ANOVA with five within-subject factors: Moment (T2 and T4), Condition (Think, NoThink), Content (Alcohol, Non-Alcohol), Region (Left, Midline, Right) and Electrode (two electrodes) will be conducted on the mean amplitude of each component separately, to explore the effects of MI training on the neural activity.

For the ACR task at T2, the mean amplitude of the P1, N1 and P2 ERP components will be analysed by means of separate mixed-model ANOVAs with one between-subject factor Group (non/low-drinkers, BDs) and three within-subject factors: Content (Alcoholic, Non-Alcoholic), Region (Left, Midline, Right) and Electrode (two electrodes of interest). Furthermore, in order to investigate potential MI training effects on the electrophysiological reactivity to alcoholic cues, the amplitude of the abovementioned components will be analysed through repeated-measures ANOVAs using within-subject factors: Moment (T2 and T4), Content (Alcoholic, Non-Alcoholic),

Region (Left, Midline, Right) and Electrode (two electrodes). The behavioral and ERP correlates of MI and alcohol craving/reactivity will be correlated with the scores obtained from AUDIT, ACQ-SF-R and PACS.

In addition, analysis of the brain FC will also be conducted. FC will be calculated in the four classical bands –i.e., theta (4 – 8 Hz), alpha (8 – 12 Hz), beta (12 – 30 Hz), and low gamma (30 – 45 Hz)- using the Phase Locking Value (PLV) [70]. In a first step, PLV will be calculated separately for each pair of source positions, generating a 1489 x 1489 FC matrix. Secondly, we will average the PLV values of all the links connecting each pair of 58 cortical regions of interest (ROI) according to the Harvard-Oxford atlas, obtaining a 58 x 58 whole-brain FC matrix. For the whole-brain analysis, the PLV value between each pair of ROIs will be compared between groups by an ANCOVA test using Beamformer filter correlation (in order to correct potential source leakage differences between groups) as covariate and gender as factor. The resulting *p*-values will be corrected for multiple comparisons (number of pair comparisons and number of frequency bands) with a False Discovery Rate (FDR) of 0.1. Only links that survived the FDR-corrected threshold will be reported as significant. Once the significant links for each condition have been determined, the FC values will be correlated with the scores obtained from AUDIT, ACQ-SF-R and PACS.