Study Protocol

Official Title of the Study:
Forgetting Alcohol: A Double-blind, Randomized Controlled Trial Investigating Memory Inhibition Training in Young Binge Drinkers

NCT ID: not yet assigned

Date: February 5, 2019

Study Design and Setting

The present study will be composed of three different phases: *1)* behavioral and electrophysiological analyses of MI abilities in young BDs as compared to age-matched non/low-drinkers; *2)* a double-blind, randomized controlled trial (RCT) aiming at assessing the effects of a MI training protocol at the behavioral and electrophysiological level; and *3)* monitoring of self-reported alcohol consumption and alcohol craving 10 days and 3 months after the MI training.

Target population

The volunteers will be ninety college students (~50% female), aged between 18 and 23 years: 45 non/low-drinkers and 45 BDs matched for age and gender. All participants will be recruited through a screening questionnaire administered in the classroom of several courses taught at the University of Minho (UM). The screening will include the Alcohol Use Disorder Identification Test (AUDIT) [1] along with other questions concerning alcohol and drugs use.

Inclusion and exclusion criteria

To participate in the study, college students must meet the following eligibility criteria: report (i) drinking 5 or more drinks on one occasion at least once a month, and (ii) drinking at a speed of at least two drinks per hour during these episodes (which brings blood alcohol concentration to 0.08 gram percent or above [2], in order to be classified as BDs; or report (i) never drinking 5 or more drinks on each occasion and (ii) having an AUDIT score ≤ 4, to be considered as non/low-drinkers. The students who fulfilled the inclusion criteria will perform a clinical interview that will assess the following exclusion

criteria: a) use of illegal drugs except cannabis (as determined by the Drug Use Disorders Identification Test-Extended [DUDIT-E], [3]); b) alcohol abuse (i.e., AUDIT ≥ 20); consumption of medical drugs with psychoactive effects (e.g., sedatives or anxiolytics) during the two weeks before the experiment; c) personal history of psychopathological disorders (according to DSM-V criteria); d) history of traumatic brain injury or neurological disorder; e) family history of alcoholism or diagnosis of other substance abuse; f) occurrence of one or more episodes of loss of consciousness for more than 20 minutes; g) non-corrected sensory deficits; h) Global Severity Index (GSI) > 90 (Symptom Checklist-90-Revised questionnaire [SCL-90-R], [4]) or a score above 90 in at least two of the symptomatic dimensions.

Quality assurance and randomization

The protocol will be implemented by three skilled researchers with expertise in behavioral and EEG assessments and tDCS interventions. Researchers will not be aware of the results of the pre-training EEG assessment and will be blind to the randomization procedure that will follow. The randomization of the BD groups will be performed by an independent researcher using Microsoft Excel. BDs will receive one of the following interventions: 1) Combined training (verum CT and tDCS applied over the right dorsolateral prefrontal cortex); 2) Cognitive training (active CT and sham tDCS); or 3) Control (sham CT and sham tDCS). Based on this experimental procedure, it is expected that researchers will be able to answer the following research questions:

1. How does BD affect alcohol-related MI in young adults? Namely, are the behavioral and electrophysiological MI mechanisms - specifically those related to the suppression of alcohol-related memories - altered in BDs when compared to non/low-drinkers?

2. What is the effect of a MI training on behavioral TNTA task performance? Specifically, will the BDs show a reduced recollection of no-think images - mainly for alcoholic no-think images – after training?

3. Are the electrophysiological correlates underlying MI mechanisms and alcohol cue reactivity changed by MI training?

4. Will the MI training reduce alcohol consumption and craving levels at 10-day and 3-month follow-ups?

Experimental tasks

Alcohol Cue Reactivity (ACR) task

Firstly, the participants will perform the ACR task. In this task, each trial starts with a white fixation cross in a grey background for a variable duration ranging from 1000 to 1500 ms. Subsequently, an alcoholic or non-alcoholic image is randomly presented at the center of the screen for 3000 ms. Participants are asked to be focused on the fixation cross and then to look at the image whenever it appears. After the visualization of each image, participants have to register their emotional responses in terms of valence and arousal using the Self-Assessment Manikin [5]. The full task includes a total of 80 trials with 40 alcoholic and 40 non-alcoholic images obtained from the Amsterdam Beverage Picture Set [6]. This task has the purpose of assessing the emotional response and electrophysiological reactivity of participants to alcohol cues.

TNTA task

The TNTA task is a version of the classical Think/No-Think paradigm [7], which was specially developed to examine MI mechanisms in alcohol-related contexts [8]. This task includes thirty-six pictures (18 related to alcohol and 18 non-related to alcohol) from

the Galician Beverage Picture Set (GBPS) [9] and 36 images of neutral objects obtained from the POPORO database [10]. The GBPS is a database of alcohol and non-alcohol pictures embedded in real-life scenarios which comprises 6 types of beverages: beer, wine, and liquor (alcoholic drinks), and water, juice, and milk (non-alcoholic drinks). The TNTA task includes 6 images from each of the 6 beverages. The pictures also differ in terms of orientation (vertical or horizontal) and number of people (no people, 1 person, 2 or more people). As such, within each type of beverage, 3 are vertical (each one with a different number of people: 0 people, 1 person, 2 or more people) and the other 3 are horizontal (also with 0 people, 1 person, 2 or more people). The task is divided into three phases, i.e., 1) the Learning phase, 2) the Think/No-Think (TNT) phase and 3) the Memory-Test phase, which are carefully described below.

Learning Phase

During the Learning phase, participants are asked to memorize 36 image-pairs (i.e., a neutral image paired with an image including alcoholic or non-alcoholic drinks) divided into three blocks of 12 pairs. Each block starts with the presentation of the 12 image-pairs at the center of the screen, each for 4000 ms, in a randomized order, and with an inter-stimuli interval (ISI) ranging from 1000 to 2000 ms. When the presentation of the 12 pairs finished, each of the neutral images are presented for 2000 ms, and the participants must try to remember the image that was associated with the neutral object through three questions: Q1. “Which beverage was associated with this picture?”; Q2. “How was the picture oriented?”; Q3. “How many people were there in the picture?”. In each block, the 12 pairs and the questions are repeated three times. At the end of each block, feedback with the total number of correct responses will be provided. Correct recall will only be considered when participants provide the right answer to the 3 questions.

Thus, the combination of the potential answers to the 3 questions (6 x 2 x 3 = 36) ensured that each target image displayed a unique combination.

TNT Phase

In this phase, there are two possible actions: to Think or to No-Think on the alcoholic/non-alcoholic image paired with the neutral object previously. Specifically, in the Think condition – determined by a green frame that circumvents the neutral image -, participants will be asked to focus on the neutral image and think in the alcoholic/non-alcoholic image that was associated with it (i.e., the one that was paired with this specific neutral image). In the No-Think condition - determined by a red frame that circumvents the neutral image -, participants are instructed to focus on the image and to not let the previously associated alcoholic/non-alcoholic picture enter their consciousness. All the images will be presented for 3000 ms and randomly repeated 10 times and presented for four seconds at the center of the screen with an offset-onset ISI ranging from 1100-1300 ms. From the initial set of 36 neutral images shown in the learning phase only 24 will be depicted during the TNT phase, 12 in each condition (i.e., Think vs No Think). The remaining 12 neutral images not depicted in this phase will serve as a baseline condition for the following Memory-Test phase of the task.

Memory -Test Phase

During this phase, the 36 neutral images from each pair are again presented, including the 12 images of the baseline condition which are not presented during the TNT phase. Participants are asked to remember the image (alcoholic or non-alcoholic) that was initially associated with the neutral object through the same three questions of the Learning phase. Three different versions of the task (where all the pictures were part of

the three conditions: Think, No-Think and baseline) will be created and counterbalanced across participants.

TNTA task variations for active and false cognitive training

Two variations of the TNTA task were developed for the active cognitive training and for the false cognitive training. The stimuli employed in these variations of the TNTA task were also obtained from the GBPS and POPORO databases; however, these stimuli differ from those used in the original TNTA task. The structure of the task for the MI active cognitive training is the same as the original TNTA task. However, in this variation, the Learning phase is only composed of two blocks of 12-image pairs, and in the TNT phase, all the stimuli to be inhibited are alcohol-related images.

In the false cognitive training version, the Learning phase also have only two blocks of 12-image pairs. However, the TNT phase is replaced by a Forced-Choice Reaction Time (FCRT) task, during which the participants only must categorize alcoholic and non-alcoholic images answering to the question "What type of beverage was there in the image?" (answer: "Alcoholic drink" or "Non-alcoholic drink").

All the computerized tasks will be programmed in open-source software Psychopy [11].

Procedure

T1: Clinical Interview

During this interview, we will verify the fulfillment of the exclusion/inclusion criteria and assess the baseline levels of some constructs (e.g., craving levels, impulsivity). Consequently, we will use the following instruments: *1)* the AUDIT along with five additional alcohol use questions (i.e., speed of drinking [number of drinks

consumed per hour], number and type of drinks consumed in a standard week, percentage of times getting drunk when going out, age of onset of regular and BD) to assess participants' drinking patterns; *2)* the DUDIT-E to examine the consumption of other drugs; *3)* the Penn Alcohol Craving Scale (PACS) and the Alcohol Craving Questionnaire-Short Form Revised (ACQ-SF-R) to assess the alcohol craving; *4)* the Barratt Impulsivity Scale-11 (BIS-11) and the short form of the Urgency-Premeditation-Perseverance-Sensation Seeking-Positive Urgency (UPPS-P) impulsive behavior scale to assess participants' impulsiveness; *5)* the Symptom Checklist-90-Revised questionnaire (SCL-90-R) to evaluate the presence of psychopathological traits; *6)* a clinical history interview to explore the personal/family history of psychopathological and neurological disorders as well as the overall medical history of the participants; and *7)* the Edinburgh Handedness Inventory (EHI) to evaluate the participants' handedness.

T2: Pre-training EEG Assessment

During the pre-training EEG assessment, psychological (i.e., craving levels), behavioral (i.e., alcohol consumption levels and task performance) and neurofunctional (e.g., ERPs, brain FC) outcomes will be assessed. EEG data will be collected while participants perform the ACR and the TNTA task. Before the EEG recording, participants will be asked to perform a breathalyzer test to ensure that blood alcohol concentration value is 0.0% (Alcoscan ALC-1). Along with the AUDIT, and in order to determine alcohol consumption levels during the previous week and the previous three months, we will administer the Alcohol Timeline Followback (TLFB) and the Typical and Atypical Drinking Diary (TADD), respectively. After filling in the questionnaires, participants will perform the ACR task. Participants' resting brain activity will be recorded for three minutes during eyes-closed prior to the TNTA task. Finally, they will perform the TNTA

task. The total duration of the pre-training EEG assessment will be approximately two and a half hours.

EEG data will be recorded using the ActiveTwo Biosemi System (Biosemi, Inc.) from 64 electrodes placed according the 10-10 system [12] and digitized at a 512 Hz rate. Vertical and horizontal electrooculogram activity will be recorded to control for eye movements and blinks. Two additional electrodes will be placed on the mastoids, bilaterally, to provide the signal offline reference. Electrode impedances will be kept below 20 kΩ and the EEG signal will be filtered on-line with a 0.01–100 Hz band pass filter.

T3: MI Training Sessions

After the pre-training EEG assessment (T2), BDs will be randomly assigned to one of the three training subgroups: *a) Combined training*; *b) Cognitive training*; or *c) Control*. The participants' allocation to the training group will be done by an independent researcher who will be responsible to program the tDCS parameters. Thus, both participants and research team will be blind to the randomization procedure.

During T3, subjects will perform the variation of the TNTA task corresponding to the group to which they will be assigned. After the first phase of the task (i.e., Learning Phase), neuromodulation (sham or active) will be performed using tDCS - a non-invasive, painless and safe brain stimulation method capable of modulating cortical excitability through a weak constant electric current induced from two surface electrodes placed on the scalp [13]. Twenty minutes of 2 mA direct current will be applied to the scalp using a saline-soaked pair of 35 cm^2 surface sponge electrodes, through an Eldith DC Stimulator Plus (Neuroconn, Germany). To stimulate the right DLPFC, the anodal electrode will be placed over F4 according to the 10-20 international system for EEG electrode placement.

The cathode electrode will be placed over the contralateral supraorbital area. During the active simulation, the current will fade in for 15 seconds, will be constant at 2 mA for 20 minutes, and then will fade out for 15 seconds. During the sham stimulation, the electric current will fade in during 15 seconds until reaching 2 mA, then will be constant at 2 mA for 15 seconds and will fade out for 15 seconds. This procedure makes both active and sham stimulation indistinguishable for the participants. Before and after the training sessions, participants will answer to a continuous Visual Analog Scale that allows checking for possible secondary effects of the electrical stimulation.

T4: Post-training EEG Assessment

At T4, participants will perform the ACR and TNTA tasks under the same procedure to the one undertaken during the T2.

T5: Monitoring of alcohol consumption and alcohol craving.

Ten days after the post-training EEG assessment, the primary craving and alcohol consumption outcomes will be assessed. For this purpose, we will administer the PACS, the ACQ-SF-R and the AUDIT. Additionally, three months post-intervention, BDs will answer the same questionnaires and also the TADD aiming at assessing medium-term effects of the MI training in the alcohol craving and consumption levels.

Management and Ethics

Ethical requirements for human research will be followed in full accordance with the Code of Ethical Principles for Medical Research Involving Humans Subjects outlined in the Declaration of Helsinki (64th World Medical Association General Assembly, Brazil, 2013). The Ethics Committee for Social and Human Sciences of University of

Minho approved the present protocol in December 12, 2018 (approval reference: CE.CSH 078/2018). Prior to enrolling in the study, subjects will be informed about the aims, conditions and procedure of the study. Two copies of the informed consent forms will be provided, both of which will be signed by the researchers and participants. It is researchers' responsibility to guarantee the anonymity of the participants and the confidentiality of the data. The files containing data from the participants will be identified by the participant's code, not the name. The document containing the records of participants' personal information (e.g., names, contacts, code) will be stored in a different location from the data files and will only be accessible to authorized researchers. All files will be password protected and stored by the researchers in secure locations. Participants will be able to cease their participation in the study at any time without any consequences related to the relationship between the research team, the participant and the university. If participants want to terminate their enrollment in the study or ask for their data to be deleted later, researchers will promptly erase all data from files and eliminate documents using a paper shredder. There are no risks associated to the research protocol. College students will receive gift vouchers in order to compensate for their participation.

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