

## **Statistical Analysis Cover Page**

**Official Title: Neurobehavioral Plasticity to Regular Sugar-Sweetened Beverage Intake: An fMRI Experiment**

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## **Statistical Analysis Plan**

### **A. Sample Size and Power**

Data collection was ended early due to COVID-19 (ending March 2020), resulting in a lower than anticipated sample size (160 vs. 230), which may impact results, specifically, measures of individual differences. As our sample was smaller than anticipated we performed a sensitivity analysis using Gpower (ver. 3.1.9.7, Heinrich Heine University Düsseldorf) for the primary hypothesis using alpha = 0.05, power of 0.80 and a total sample of 123 (accounting for missing data, incomplete data collection, etc.), and we are still able to detect effect of 0.25. Inclusion Criteria: Potential participants were eligible for the study if they had BMI scores between 18 and 34 at baseline. Exclusion Criteria: Individuals with probable current Axis I psychopathology or any fMRI contra-indicators (e.g., metal implants, braces) were excluded. Those with a probable Axis I diagnosis will be provided with treatment referral information and encouraged to seek treatment, current regular use (3 or more times a week) of psychoactive drugs (e.g., cocaine, marijuana, nicotine), regular smoking, serious medical problems (e.g., cancer, diabetes), dietary practices that do not allow for intake of intervention beverages.

### **B. Analysis Plan**

#### *1. Primary and Secondary Outcomes*

Behavioral data analysis (stop-signal reaction time & perceptual ratings of beverages): Continuous variables from the stop signal task and the visual analogue scale (VAS) ratings were summarized by descriptive statistics (e.g., mean, SD, range, median), examined via graphical analyses (e.g. histogram, Q-Q plot) and analyzed to assess the normality of the data. Data transformation of continuous variables were used as necessary. Model assumptions and potential outliers were analyzed with various modeling diagnostics and plotting techniques. Two analytic datasets are used. Participants that provided data at baseline and follow-up are used in a completers analysis (CA). An intent-to-treat (ITT) dataset, including all participants that were consented and completed one assessment, are also used. In that analysis, missing data are addressed via multiple imputation methods. Of note, in both the CA and ITT all subjects are included independent of their adherence to the intervention.

For **Aim 3**, reaction times from the stop signal task (SST) are analyzed. Following previous standards, reaction times <50 milliseconds are excluded (occurred prior to imputation). As we are not analyzing inhibition (inhibition accuracy/SSRT), no other exclusion criteria were used. Data from pre- and post-intervention SST measures are analyzed using PROC MIXED implemented in SAS (Version 9.4, Cary, NC). All available data from those completing the pre- and post-intervention behavioral visits (separate from the scan assessments) are used in the completers analysis. All those who consented are included in the ITT analysis with missing data imputed. Outcome variables assessed via the SST task are: mean reaction time, standard deviation, go accuracy of the assigned beverage (pre-/post-intervention). Each of the outcome variables is entered into 2x2 repeated measure mixed models, including group-by-time interactions. Control variables of no interest to be included are BMI, assigned flavor, and gender. Interactions are probed for pairwise comparisons using least-squared mean comparisons, specifically of a priori contrasts of interest: between-group baseline differences and follow-up differences, and within-assignment change from pre- to post-intervention. Bonferroni-adjusted two-tailed *p*-values less than 0.05 are considered significant.

For **Aim 4**, perceptual hedonic, taste and internal state ratings (assessed using visual analog scales (VAS)) are analyzed. Data from pre- and post-intervention perceptual ratings are

analyzed using PROC MIXED implemented in SAS (Version 9.4, Cary, NC). Pleasantness and Desire of the assigned beverage (pre-/post-intervention) are each entered into 2x2 repeated measure mixed models, including group-by-time interactions. Control variables of no interest to be included are BMI, assigned flavor, gender, and thirst. Four pairwise comparisons of interest (pre- and post-intervention differences by intervention assignment and within-assignment change over time) are probed. Tukey-adjusted two-tailed  $p$ -values less than 0.05 are considered significant.

**fMRI data preprocessing:** Neuroimaging data were preprocessed using the fMRIPrep pipeline. DICOMS were converted to the Brain Imaging Data Structure (BIDS file structure), then preprocessed using fMRIPrep. Prior to preprocessing, the first four volumes of each functional run were trimmed. fMRIPrep preprocessing included skull stripping using Advanced Normalization Tools (ANTs); tissue segmentation using FSL's Automated Segmentation Tool (FAST); and spatial normalization to Montreal Neurological Institute (MNI) 152-Asymmetrical space using ANTs' registration option. Functional data were co-registered to anatomical data using FSL's FMRIB's Linear Image Registration Tool (FLIRT) with boundary-based registration. Functional data were additionally motion corrected using FSL's MCFLIRT and corrected for fieldmap distortion. In FSL, final preprocessing included spatial smoothing using a 5mm full width half maximum isotropic Gaussian kernel, high-pass filtering, adding temporal derivatives, adjusting for autocorrelation, and adjusting for nuisance regressors, including the 6 motion parameters, 6 anatomical component-based noise correction regressors (aCompCor), and high motion time points (FD>0.5mm). A functional run for a subject was excluded if >30% of total volumes were flagged as high motion points.

**fMRI primary analyses:** Neuroimaging analyses were primarily completed in FSL (FMRIB Software Library, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Individual (level 1), within-subject (level 2), and group level (level 3) analyses were carried out in FSL's fMRI Expert Analysis Tool (FEAT). At the 1st (individual) level, SSB, USB, SSB logo, USB logo vs. water logo contrasts were created. These were carried forward to a within-subject (level 2) model testing for the effect of time.

At the 2nd (within-subject) level analyses, the above SSB, USB, SSB logo, USB logo vs. water logo data were put into a within-subject t-test testing individual-level differences as a function of time, i.e., post-intervention > pre-intervention (testing for increases in BOLD response as a function of the intervention) and pre-intervention > post-intervention (testing for decreases in BOLD response as a function of the intervention).

Level 3 (group-level) analysis used the resulting level 2 data (post- > pre-intervention; SSB/USB > water taste/logo to test for increases in response and, separately, pre- > post-intervention; SSB/USB > water taste/logo for decreases in response) to test for the main effect of the intervention and the interaction of BMI (**Aims 1 and 2**).

- a. First, the assignment\*BMI interaction (**Aim 2**) is tested with flavor assignment, gender, thirst, and beverage pleasantness as covariates of no interest. By using the level 2 within-subject data that tested for the effect of time, this results in a time\*group\*BMI interaction. Contrasts of interest for the group model are SSB > water and SSB logo > water logo. This is tested with the pre- > post-intervention and the post- > pre-intervention level 2 data. Parameter estimates of significant BOLD response are plotted for visualization purposes.
- b. **Aim 1** is tested in a separate model controlling for BMI (no interaction), flavor assignment, gender, thirst, and beverage pleasantness. Contrasts of interest for the group model are SSB > water and SSB logo > water logo. This is tested with the pre- > post-intervention and the post- > pre-intervention level 2 data. Mean

cluster parameter estimates of significant BOLD response are plotted for visualization purposes.

All group-level analyses were corrected for multiple comparisons by using the threshold free cluster enhancement (TFCE), nonparametric thresholding algorithm in FSL's RANDOMISE (n permutations = 10000), resulting in a family-wise error rate corrected significance threshold of  $p_{FWE} < 0.05$ . At the group level, functional data were masked via an inclusive grey matter mask to reduce the number multiple comparisons, resulting in whole brain, grey matter analyses. The mask was created via the ICBM 2009c Nonlinear Asymmetric template that was binarized at a threshold of 0.04 using FSLmaths. Localization of significant clusters will be used and determined using Mango's MNI atlas tool.

## 2. *Exploratory Outcomes*

Behavioral data analysis (questionnaire scores): Questionnaires administered during the study assessments (FFQ, BIS-15, FCI, DEBQ, IPAQ, Handedness, SPSRQ, YFAS, BIS/BAS, PFS) were scored per each questionnaire's instructions and different subscales were created for each questionnaire (e.g. DEBQ restrained eating subscale). Subscale scores were examined via graphical analyses (e.g. histogram, Q-Q plot) and analyzed to assess the normality of the data. Data transformation of continuous variables were used as necessary. Model assumptions and potential outliers were analyzed with various modeling diagnostics and plotting techniques. Data from pre- and post-intervention subscale scores are analyzed using PROC MIXED implemented in SAS (Version 9.4, Cary, NC). All available data from those completing the pre- and post-intervention behavioral assessments (separate from the scan assessments) are used in the analysis. Outcome variables assessed via the different questionnaires and included in the analysis are: DEBQ restrained eating, FFQ average daily total kcal consumed, FFQ percent of daily kcal from fat, FFQ percent for daily kcal from sugar, BIS-15 total impulsivity, FCI total food craving score, SPSRQ sensitivity to punishment, SPSRQ sensitivity to reward, BIS/BAS behavioral approach score, BIS/BAS behavioral inhibition score, PFS total score, YFAS total score, IPAQ total physical activity MET. Each of the outcome variables is entered into 2x2 repeated measure mixed models, including group-by-time interactions. Control variables of no interest to be included are BMI, assigned flavor, and gender. Interactions are probed for pairwise comparisons using least-squared mean comparisons, specifically of a priori contrasts of interest: between-group baseline differences and follow-up differences, and within-assignment change from pre- to post-intervention. Bonferroni-adjusted two-tailed  $p$ -values less than 0.05 are considered significant.