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Document:

Statistical Analysis Plan

Official Study Title:

Mind-body Awareness Training and Brain-computer Interface

NCT03221374

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Introduction

The purpose of this statistical analysis plan (SAP) is to document technical details for the proposed statistical analysis involved in this study, NCT03221374, Mind-body Awareness Training and Brain-computer Interface. The proposed data analysis will also be included in manuscripts submitted to scientific journals and/or conferences.

Objectives and hypothesis

This study is proposed to assess the effect of mindfulness meditation on the brain computer interface learning. The following two sub-studies will be conducted: the first is to investigate if an 8-week MBSR (Mindfulness Based Stress Reduction) class has an effect on the BCI learning of participants without any prior meditation experience. The second is to investigate if long-term meditators exhibit better BCI learning abilities than meditation naïve participants.

Given the prior literature and experiments conducted in the PI's lab (Cassady et al., 2014), the primary hypothesis is that an participant taking an 8 week MBSR class will exhibit better BCI learning, and participants with long term meditation experiences will have higher BCI performance and learning.

In the following sections, details of the planned statistical analysis for these two sub-studies will be described.

Sub-study 1: Effect of 8-week MBSR on BCI learning

In this sub-study, participants will visit the lab for an initial assessment of BCI proficiency followed by random assignment to either the MBSR course or an 8-week waitlist condition, which are called intervention. After the intervention, BCI learning was assessed through a series of up to 10 training sessions occurring three times per week.

Participants will wear a 64-channel EEG cap, which was set up according to the international 10-10 system. EEG will be acquired using SynAmps RT amplifiers and Neuroscan acquisition software. Each BCI session consisted of 6 runs of LR, UD, and 2D conditions for a total of 18 runs per day. As sessions progressed, participants are encouraged to find their own strategies. The control signal will be extracted as different combinations of the autoregressive (AR) spectral amplitudes of the small Laplacian filtered electrodes C3 and C4 in a 3 Hz bin surrounding 12 Hz normalized to zero mean and unit variance. The magnitude of the cursor movement will be determined by the normalized AR amplitude difference, updated every 40 ms. Horizontal motion will be controlled by lateralized alpha power (C4—C3) and vertical motion will be controlled by up and down regulating total alpha power (C4 + C3). BCI accuracy are quantified by a percent valid correct metric, calculated as the number of hits divided by the total number of non-timeout trials.

To evaluate the learning of BCI control, analysis will be focused on participants that did not demonstrate ceiling performance in the baseline BCI assessment (accuracy above 90% in 1D control). Linear mixed-effects models will be fit using the lme4 package (1.1–21) in R (3.5.0) and *P*-values will be computed with the lmerTest package (3.1–0), using Satterthwaite approximation for degrees of freedom (Bates et al., 2015; Kuznetsova et al., 2017). For between session comparisons of BCI performance, BCI accuracy will be modeled over time with fixed effects of session (levels: 11), group (levels: MBSR, control), and task (levels: LR, UD, 2D). Random effects included within subject factors of session and task. The average BCI control signal over time will be modeled with fixed effects of session, levels, and task (levels: LR, UD). Models will be initially fitted with three-way interactions of group, task, and session. Fixed effects structures of the mixed-effects models will be reduced stepwise by excluding nonsignificant interaction terms/predictors and compared using ANOVA ratio tests until the respectively smaller model explained the data significantly worse than the larger model (significant χ^2-test) (Kuznetsova et al., 2017). Cubic polynomial contrasts will be computed for the session factor, however, only linear and quadratic terms (in addition to intercepts for task) will be used for the random effects. Residual plots will be examined for approximate normality and homoscedasticity. A two parameter box–cox transformation are applied to the dependent variable of Fisher score.

A cluster-based permutation test (CBPT) statistic will be used to assess the group differences in the changes of Fisher discriminant score recorded across the cortex during BCI training as well as for all source analysis (Oostenveld et al., 2010). A similar procedure will be used for investigating the relationship between source space rest ERS and meditation experience, except that a *t*-transformation of the Pearson correlation will be used as the statistical metric.

Repeated measures ANOVAs will be used to test group by time interactions, if data are found to be normally distributed using the Shapiro–Wilks test. If the breath counting data displayed significant deviations from normality, it will be fit with a rank-based analysis of linear models using the Rfit package (0.23.0) as a robust alternative to least squares (Kloke & McKean, 2012). Significant findings will be followed by independent post hoc tests to aid inference. Nonparametric tests will be used when group data if data are found to be non-Gaussian using the Shapiro–Wilks test. Confidence intervals and Cohen’s *d* (difference in means/pooled standard deviation) will be calculated if the parametric tests’ assumptions are met. All post hoc inferences will be two-sided at a Holm–Bonferroni corrected alpha level of 0.05 (0.025 per side). Outliers, defined as three-scaled median absolute deviations away from the median, will be removed for post hoc statistical testing and are marked with an *x* in all plots. Source imaging will be used as a post hoc inference to supplement the sensor level results, and control subjects with outlying electrophysiological data will be removed for these plots and tests.

Sub-study 2: Effect of long-term meditation on BCI performance and learning

In this sub-study, a single-blind two-group experimental design will be adopted, with a meditation group and a control group. The experimenters did not know the identity of the subject in relation to their meditation experience throughout the whole experiment. This will be achieved through the following: (1) we will ask two other researchers at our lab to refer potential subjects (both meditators and controls) to an unblinded research assistant (screener) and not to the blinded experimenters; (2) these potential subjects will be screened for inclusion/exclusion by this screener whose only involvement in the study was to conduct screening; (3) during the consent process and survey, the experimenter will ask the subject to cover any information related to meditation experience when submitting the paperwork; thus, the experimenter only knows information unrelated to meditation (age, sex, name, etc.) about this subject after collecting these documents. (4) During the experiment, the experimenter will remain unaware of the subjects' meditation status and avoided any conversation related to meditation with the subject throughout the entire six sessions.

The meditation group will consist of healthy subjects with a history of meditation practice, as evaluated by a questionnaire regarding personal meditation practice completed before experimentation. To be accepted into the meditator group, individuals have to cite at least a year of frequent and consistent practice, with most subjects having 2 or more years.

In the first session, subjects are asked to fill out two surveys before the BCI experiment. Both surveys aim to measure one's level of mindfulness. The first survey is called the Freiburg Mindfulness Inventory (FMI) (Walach et al., 2006), which has 14 statements, such as "I am open to the experience of the present moment." The subjects will be asked to use a 1–4 scale to indicate how often they have such experience. The FMI score are calculated by summing up the answers to each question with a proper recode of one question (Walach et al., 2006). The second survey is called Day-to-Day Experiences (Brown & Ryan, 2003), which has 15 questions, such as "I find it difficult to stay focused on what's happening in the present;" the subjects are asked to use a 1–6 scale to indicate how often they have such experience. The Mindful Attention Awareness Scale (MAAS) will be calculated by averaging answers to each question in this Day-to-Day Experiences survey. In both surveys, a higher score indicates a higher level of mindfulness.

Subjects in both groups will go through six sessions of BCI training within 4–6 weeks, with at least 1 session per week. Each experimental session will last about 2 h, with a 9 min break in the middle. EEG data will be recorded throughout the session using the Neuroscan SynAmps system with 64-channel EEG QuikCap (Neuroscan Inc., Charlotte, NC). The sampling frequency will be set to 1,000 Hz, and the impedance was kept below 5 kOhm during the preparation. In addition, to minimize the influence ofartifact on the EEG data, we will monitor the behavior of the subject and the recorded waveform. This run will restart if we find the subject moved a lot or the real-time EEG signal became noisy.

We will perform linear mixed-effects models per type of performance and neurophysiological measures to investigate the session, group, and interaction effect. lme4 package (1.1-25) in R (4.0.3) will be used to generate the linear mixed-effects models, and p-values will be computed using lmerTest package (3.1-3), using Satterthwaite approximation for degrees of freedom (Kuznetsova et al., 2017). Each BCI performance and neurophysiological measure will be modeled over time with a fixed effect of session (six levels) and group (two levels, meditator and control). Random effects include within-subject factors of the session. Models will be initially fit with the interaction of group and time, and then, fixed effects will be reduced stepwise by excluding non-significant interaction terms/predictors and compared using ANOVA ratio tests until this smaller model explained the data significantly worse than the larger model (significant Chi-squared test) (Kuznetsova et al., 2017).

To analyze the difference between survey results of two groups, Wilcoxon rank-sum test will be used. In addition, the linear regression model and the corresponding r value will be used to assess the linear correlation between the BCI performance and the survey score.

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