Title: Auricular Acupuncture Stimulation for Chronic Pain

Identifiers: NCT03959111

Date: 10/17/2019

Statistical Analysis Plan

Data analysis on clinical and behavioral assessment For clinical outcomes, a two sample t-tests were applied to compare the pre-post treatment difference between the two groups.

Resting state functional connectivity analysis Seed-based resting state functional connectivity analysis Seed based rsFC analysis were calculated in MATLAB by applying the CONN toolbox. Pre-processing of fMRI data include slice timing, head-motion correction, and co-registration to respective structural images for each subject. In addition, 6 rigid body motion parameters, as well as white matter and cerebrospinal fluid (CSF) signals were regressed out, and images were normalized using structural image unified segmentation and then re-sampled to 2-mm cubic voxels. After linear de-trending, data were filtered using a temporal band pass (0.01-0.08 Hz) to remove low frequency noise and influences of higher frequencies reflecting cardiac and respiratory signals and finally smoothed using a full width half maximum of 6 mm. To eliminate head motion and artifacts, we identified outlier time points in the motion parameters and global signal intensity using ART. For each subject, we treatted images (time points) as outliers if composite movement from a preceding image exceeds 0.5 mm, or if the global mean intensity is greater than 3 standard deviations from the mean image intensity for the entire resting state scan.

In this study, we used two seed regions. The first seed region will be the bilateral PAG (spheres of 2 mm radius on the left and right sides, peaks of MNI coordinates 4 -26 -14 and -4 -26 -14). This allows us to elucidate the involvement of the descending pain modulation system. The second seed regions were the bilateral medial hypothalamus (MH) (MNI coordinates:x = ± 4; y=-2; z=-12 plus 2mm sphere) and lateral hypothalamus (LH) (MNI coordinates: x = ±6; y=-9; z=-10 plus 2mm sphere). The reason we chose this location is due to its important role in stress, immune, and inflammation. Then, the averaged time course will be obtained from the seeds, and correlation analysis were performed in a voxel-wise way to generate a functional connectivity map. The correlation coefficient map was converted into a Fisher-Z map using Fisher's r-to-z transformation to improve normality.

Group analysis will be applied using a random effects model. A flexible factorial design module in SPM12 were applied with two treatments (real and sham tVNS) and two time points (before and after treatment). Multiple regression analyses will be applied between PAG rsFC changes (pre- minus post-treatments) and the corresponding clinical assessments and QST changes. A threshold of $p < 0.001$ and $p < 0.05$ small volume corrected were applied for the regions of interest (ROI). A threshold of $p < 0.001$

uncorrected and $p < 0.05$ Family-wise Error (FWE) corrected threshold were used for non-ROIs. The ROIs include ACC, MPFC, MCC, insula, amygdala, NAc, S1, S2, DLPFC, hippocampus, and parietal lobule.