

**Title: Mechanistic Studies on Video-guided Acupuncture Imagery Treatment of Knee Pain**

**Clinical trial ID: NCT03261505**

**Date: 11/8/2023**

## Statistical plan for primary outcome

**Primary outcome:** fMRI signal changes evoked by the different interventions.

**fMRI data acquisition** All brain imaging was performed with a 3-axis gradient head coil in a 3 Tesla Siemens MRI System equipped for echo planar imaging. A high-resolution 3D MPRAGE sequence was collected for anatomic localization of significant signal changes. Functional MRI images were acquired using a gradient echo T2\*-weighted pulse sequence (TR/TE = 2000/30ms, flip angle = 90°, FOV = 192x192 mm, 48 AC-PC aligned slices, slice thickness = 3.0 mm with 0.6 mm inter-slice gap, 90 image volumes per slice, matrix = 96x96).

**Data analysis:** SPM 12 was applied to investigate the brain responses to different interactions. Preprocessing included co-registration, motion correction, normalization to MNI stereotactic space, and spatial smoothing with an 8 mm Gaussian kernel. For each subject, the contrast between manipulation and no manipulation during acupuncture and sham acupuncture was calculated using a general linear model. The same procedure was followed for V-GAIT and control touching. For V-GAIT, the contrast was between watching/imagining needle manipulation vs. no manipulation, and for the control, contrast was between cotton swab touching vs. no manipulation.

Group analysis was performed using a random-effects model. A one-sample t-test was performed to compare the fMRI signal changes during manipulation vs. no manipulation within each treatment. Then, ANOVA was used to compare the differences among treatment groups. Thresholds of  $p < 0.005$  and  $p < 0.05$  small volume corrected were applied for the region of interest (ROI). Thresholds of  $p < 0.005$  uncorrected and  $p < 0.05$  Family-wise Error (FWE) corrected were used for non-ROI. Based on previous studies and our hypothesis, the ROIs included: the cingulate cortex, insula, primary and secondary somatosensory cortex, dorsal lateral prefrontal cortex, medial prefrontal cortex, inferior parietal lobule, and periaqueductal grey (PAG).

Some subjects were excluded from the MRI data analysis due to the poor quality of the MRI signal due to head movement during the MRI scan. The number of subjects used in this analysis was: 24 for real acupuncture, 25 for sham acupuncture, 23 V-GAIT, 26 V-GAIT control.