

**Project title of main study: Pre-treatment biomarker for clinical response to neuronavigated repetitive transcranial magnetic stimulation (rTMS) in the acute phase treatment of refractory major depressive episode- role of intrinsic functional connectivity**

**Project title of sub-study: Clinical utility of Functional near infrared spectroscopy (FNIRS) to define biomarkers for predicting and monitoring treatment response to neuro-navigated rTMS in Major Depressive Disorder**

### **Research Plan and Methodology**

#### **i. Sampling**

Participants will be referred by psychiatrists from specialist outpatient clinics based in the regional hospitals of New Territories East Cluster under the Hospital Authority of Hong Kong. Written informed consents will be obtained from all participants according to the Declaration of Helsinki.

**Inclusion criteria:** 1) Ages **18-64 years** with DSM-IV major depressive disorder (MDD), currently in a moderate to severe depressive episode, scoring > 20 on Montgomery-Asberg Depression Rating Scale (MADRS)<sup>1</sup>, >18 on Hamilton Depression Rating Scale (HDRS) 17-item<sup>2</sup>; 2) failed at least one full course of antidepressant medication given at adequate dosage for at least six weeks in the current episode, 3) right-handed

**Exclusion criteria:** 1) History of significant head trauma; 2) active abuse of alcohol or illegal substances; 3) current psychotic symptoms and suicide ideation; 4) recent suicide attempt, 5) any other DSM-IV Axis I and Axis II psychiatric diagnosis other than Major Depressive Disorder; 6) significant neurologic history such as dementia, stroke, seizure, Parkinson's disease, multiple sclerosis; 7) contraindications to fMRI (e.g. pace makers, metal implants, pregnancy etc).

#### **ii. Pre-treatment assessment**

##### **A) Baseline clinical assessment**

At baseline (before treatment), **subjects' age, level of education, handedness** will be noted by research assistant. A research psychiatrist will administer **MADRS<sub>1</sub>, HDRS<sub>2</sub>**, DSM-IV SCID-I/II <sup>3-4</sup> to ascertain current/ lifetime Axis I and II psychiatric diagnosis and score on Global Assessment of Functioning, age at onset of MDD and number of major depressive episodes, number of failed antidepressants, current life stressor(s) and the perceived severity on a visual analog scale of 0-10, and **clinical global impression scale (CGI)<sub>5</sub>**.

Subjects are asked to complete self-administered questionnaires including 1) **Beck Depression Inventory II** <sup>6</sup> that has been validated in local Chinese dialect <sup>7</sup>; 2) a 60-item **NEO Five Factor Inventory** that measures five personality traits on neuroticism, extraversion, openness to experience, agreeableness and conscientiousness;<sup>8</sup> 3) **ruminative response subscale (Chinese version) (RRS) of the response style**

**questionnaire**, a 22-item questionnaire comprising of two distinct factors on brooding and reflection.<sup>9</sup> Published data suggest rumination may be correlated positively with duration of depressive episode and predict onset of depressive episode<sup>10</sup>.

### **B1) Structural Magnetic Resonance Imaging (MRI) of brain for neuro-navigated rTMS and resting state functional MRI (rs-fMRI) to map intrinsic functional connectivity (baseline, 2 weeks and 8 weeks post-treatment)**

A whole brain anatomical data set is acquired with T1-weighted sequence (repetition time(TR)/echo time(TE): 7.6/3.5ms, field of view 230mm, 250 contiguous slices, 0.6mm thickness, reconstruction matrix 224 x 224) for voxel-based morphometry (VBM) analysis and functional to anatomical image co-registration.

A resting-state functional MRI will be taken for either six (phase I) or twelve minutes (phase II). Subjects will be instructed with written cues projected (for 5 seconds) on the screen to stay relaxed and look at the fixation cross hair. Stimuli are projected on a TV monitor equipped by MRI-compatible Esys (Invivo) system. Subjects view the screen through mirror glasses. The functional scans are acquired on a 3.0-T whole-body scanner (Achieva TX, Philips Healthcare, Best, the Netherlands) using PRESTO (principles of echo shifting with a train of observation) sequence (TR28ms, TE 12ms, flip angle 7°. Slab thickness 125mm, 230mm field of view, data matrix 80 x 51 x 25) with a nominal in-plane resolution of 2.8 x 2.8mm and a temporal resolution of 2.7 sec/scan. Slices will be tilted about 30° clockwise along the AC-PC plane to obtain better signal in the orbitofrontal cortex.

All the functional and anatomical MRI data are transferred to an offline workstation for image analysis. MRI data will be preprocessed using the default pipeline of the CONN toolbox v18.b11, which is an open-source MATLAB (2019B, Mathworks, Natick, MA, USA) and SPM-based (Statistical Parametric Mapping, [www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)) cross-platform software<sup>1</sup>. The preprocessing steps included realignment and unwarping, temporal slice time correction, functional outlier detection (ART-based identification of outlier scans), segmentation, normalization and smoothing (8 mm Gaussian kernel). The best way to denoise resting-state fMRI data is currently still a controversial topic<sup>12</sup>. Therefore, component based noise correction with and without global signal regression<sup>11,13</sup>, will be applied. For denoising with component based noise correction a principal component analysis was used to identify the time series data from the noise regions, including white matter and cerebrospinal fluid. Significant principal components are then introduced as covariates in the general linear model as an estimate of the physiological noise signal space. Regressors: white matter (10 dimensions), cerebrospinal fluid (5 dimensions), realignment parameters (6 dimensions), scrubbing (61 dimensions). Further, a default temporal band-pass filter (.008-.09 Hz) and detrending. For global signal regression the same procedure was performed, but an additional regressor of the global signal (1 dimension) was added. The Subject-level correlation coefficients of the regions of interest and seed maps were converted to normally distributed Fisher-transformed Z scores to conform to the assumptions of generalised linear models. These connectivity values and statistical seed maps were subsequently used for the second-level analyses to examine group differences (responders versus nonresponders) and perform correlational

analyses to examine whether either pretreatment connectivity or change in functional connectivity is associated with TMS treatment response.

## **B2) Offline resting state fNIRS (baseline and serial measures)**

In a **sub-sample** of subjects who agree for extra brain mapping sessions, offline resting state fNIRS measures will be taken in pre-treatment phase (within 7-10 days before first rTMS session), week 2 and week 4 in the course of rTMS treatment, 2 weeks and 8 weeks post-treatment.

The fNIRS measurements will be taken in a specialized laboratory situated in the Center for Cognition and Brain Studies, Department of Psychology of CUHK (Sino Building of Chung Chi College), which is in close proximity to University MTR station and inside the same amenity as the CU Clinical Psychology Department.

All subjects will be instructed to keep still with eyes closed, relax their mind and remain motionless for two blocks of 15-minute resting state session (totaling 30 minutes). The target mapping areas are bilateral fronto-parietal areas for their roles in default mode network (DMN). Optical signals will be recorded on a two-wavelength (690 and 830 nm, 110MHz) frequency-domain oximeter (Imagent, ISS Inc., Champaign, IL, USA).

Offline mode is opted in this study as clinical applicability is our main concern. Simultaneous fNIRS-rTMS is theoretically possible but the set-up will substantially increase session time, compromising the tolerability of rTMS session and likely compliance. Aside, previous studies have shown the fNIRS signal changes are sustainable and detectable with offline measures after TMS.

The fNIRS are measured with source fibers emitting light at the two different wavelengths that are co-localized as a pair, giving rise to 24 source locations. Photons that scatter through the head are collected by 16 detector fiber bundles connected to photomultiplier tubes (PMTs). These sources and detectors are held on the participants' scalp with a rigid custom-made head-mount system (montage).

Sources and detectors are arranged in groups of two parallel rows (1.7 cm to 2.5 cm apart from each other) to create overlapping light channels so as to increase the spatial resolution and signal-to-noise ratio.

Source-detector placement in each of the four mapping areas (left and right frontal, left and right parietal) is opted. After the montage is positioned, coordinates of 230 points on the scalp, including the locations of source-detector pairs and three fiducial points (nasion, left and right pre-auricular points), are digitized using ASA 4.5 software (ANT, Netherlands) in conjunction with an infrared camera and 3-dimensional digitization system (Visor, ANT BV, 1mm spatial resolution). These points are used for calculation of the exact source-detector distance for data analysis and, more importantly, for co-registration of functional optical data with each participant's structural MRI.

The individually co-registered data are then Talairach-transformed to permit comparison across subjects. The ISS Imagent control box is connected to a computer that controls and records the intensity of emitted and recorded signals. The sampling rate used in this study

is 19.5Hz, giving rise to a 51.3ms sampling interval. During this interval, light sources are time multiplexed to allow different sources to be distinguished from each other. Light intensity and phase-delay data are computed and stored in ASCII format in the computer during the recording. Similar to fMRI analyses, predicted time series are generated by convolving the hemodynamic response function (SPM 8) with the impulse function for each event, i.e., from the onset to the offset of one sequence trial.

The pulse-corrected bandpass-filtered oxy-haemoglobin concentration change from each channel is then correlated with the predicted time series for repeated and random conditions separately for each recording block and each participant. The Beta weight for each condition is estimated, Fisher-transformed and averaged across each recording block for each participant in each session. The averaged data are analyzed using Opt-3 for generation of statistical maps. In Opt-3D, the optical signal, in this case hemoglobin concentration change, for a given voxel is defined as the mean value of the channels that overlap at that particular voxel. Channels with source-detector distances shorter than 20mm and longer than 60mm are excluded from statistical map analysis as the light path would be too short to have reached the cortical surface or too long to have enough light reaching the detector, respectively. Channels will be removed if the standard deviation of the phase delay data is greater than 230 picoseconds (i.e. the channel is excessively noisy).

### C) Neuro-navigated rTMS protocol

**rTMS treatment suite and personnel:** the treatment facilities are based in Chen Wai Wai Vivien Foundation Therapeutic Physical Mental Exercise Centre, which is a CU clinical and research centre sponsored by private fund where the physicians delivering the treatment (PI and co-PI) are serving on site. The treatment centre is equipped with resuscitation facilities. Full-time research staff and physiotherapist are available to handle medical and behavioral emergencies.

**Stimulator set-up:** Neuro-navigated high-frequency rTMS over a specific area on left DLPFC (between BA9 and BA46, junction of middle and anterior two-thirds of the middle frontal gyrus) will be provided using Magstim Super Rapid2 (Phase I) or Neurosoft Neuro-MS/D Advanced Therapeutic Transcranial Magnetic Stimulator (Phase II) that both generate short duration biphasic pulses. A figure-of-eight coil will be held in place with a custom-made stand tangential to the scalp with the handle pointing back and away from the midline at 45 degrees. The resting motor threshold will be determined Motor Evoked Potential or the standard visual method (whichever revealed the lowest motor threshold).

**Stimulation parameters Phase I:** 10 hertz, 120% motor threshold, thirty trains of 5 seconds with 25 seconds between trains, 1500 pulses per day delivered 5 days per week, totaling 30000 pulses over 4 weeks.

**Stimulation parameters Phase II:** triplet 50 hertz, repeated at 5 hertz, 120% motor threshold, twenty trains of 2 seconds with 8 seconds between trains, 600 pulses per day delivered 5 days per week, totaling 12000 pulses over 4 weeks.

**Stimulation localization:** The DLPFC coordinates of stimulation target site is determined individually for each participant using a structural T1-weighted MRI scan. This area is

defined around the junction of Brodmann area 9 and 46. To coregister the scan with the patient's head, advanced stereotactic techniques incorporated into Brainsight TMS (Rogue Research Inc., Canada) for phase I of the study and the Neural Navigator (Brain Science Tools BV, Netherlands) for phase II of the study were used.

### **Clinical follow-up and outcome assessments**

All participants are evaluated by a research psychiatrist who is blinded to the functional connectivity maps of the participants and other potential clinical predictors of response measured at baseline. Follow-up assessments are scheduled at the end of week 2, week 4, week 6, week 8, and week 12.

Primary outcome measures are the percentage change in symptom scores on MADRS and CGI at week 4, week 6, week 8, and week 12 compared to baseline. Secondary outcome measures include scores on GAF, BDI and RRS.

Clinical response is defined as CGI= 2 and 50% reduction of MADRS score from baseline, respectively at the end of six weeks and twelve weeks.

Clinical remission criteria is MADRS <7 and CGI of 1, respectively at the end of six weeks and twelve weeks.

#### **iv. Management of decompensated mental states, serious adverse somatic reactions to rTMS and drop-outs**

The research psychiatrists (PI and co-I) will be responsible for the detection of high-risk mental states (emergence of psychotic symptoms, catatonic state, suicide ideation and para-suicide state) at the regular follow-up assessments. Physical adverse effects will be checked at each follow-up and evaluated on the severity. In times of emergent risky mental state and adverse somatic reactions, the research psychiatrists will work collaboratively with the participant and if necessary, the treating psychiatrists on the necessary psychiatric and medical management plans. All adverse effects will be reported to the "serious adverse effect monitoring sub-committee" under the local ethics committee. All drop-out participants will be assessed by phone followed by clinic-based interviews to work out the care plan as indicated clinically. At the end of the study, the subjects will receive the generic psychiatric service as they are originally entitled to. The research team will ensure a seamless aftercare will be arranged for all participants.

#### **v. Sample size estimation**

Sample size estimation is based on the parameter estimate that addresses the main objective. In our study it is the expected Pearson correlation coefficient between functional connectivity of the ROIs and quantitative measure of clinical efficacy (percentage drop in MADRS score). In Fox et al's study (2012)<sup>14</sup>, the correlation coefficient is -0.355 ( $p < 0.05$  one-tailed). According to Friedman's partial power table, the required sample size is at least 58 at  $\alpha = 0.8$  and Pearson  $r = 0.35$ . Taking into account the drop-out rate of 20% reported in the active rTMS group of a prior randomized controlled trial comparing efficacy

of neuro-navigated rTMS and conventional targeting protocol, the required sample size that is sufficiently powered to examine the main objective is adjusted to **128**.

#### **vi. Data analysis**

Intention-to-treat analysis will be adopted with the last observation carried forward for participants with at least one post-baseline observation. To describe the trajectory of clinical response over the course of follow-up, survival analysis will be used to track the proportions of responders and remitters respectively at 2, 4, 6, 8, 10, 12 weeks.

To test the main objectives, we plan to perform:

- 1) Group comparison of functional connectivity between ROIs between clinically defined responders and non-responders at 4 weeks, 6 weeks and 12 weeks, respectively. We hypothesize pre-treatment anti-correlation between the left DLPFC target and subgenual cingulate is stronger in responders than non-responders at the respective time points.
- 2) Group comparison of functional connectivity between ROIs in clinically defined remitters and non-remitters at 4 weeks, 6 weeks and 12 weeks, respectively. We hypothesize pre-treatment anti-correlation between the left DLPFC target and subgenual cingulate is stronger in remitters than non-remitters at the respective time points.
- 3) Correlations analyses of the functional connectivity between ROIs and percentage drop in MADRS scores at 4 weeks, 6 weeks and 12 weeks compared to baseline, respectively. We hypothesize larger percentage drop in MADRS from baseline correlates with greater anti-correlation between the left DLPFC target and subgenual cingulate, at the respective time points.
- 4) multiple linear regression of functional connectivity between ROIs on percentage drop in MADRS score from baseline at 4 weeks, 6 weeks and 12 weeks respectively, in the presence of other baseline demographic and clinical characteristics. We hypothesize higher percentage drop in MADRS from baseline correlates with greater anti-correlation between the left DLPFC target and subgenual cingulate, at the respective time points after adjusting for baseline demographic and clinical characteristics.
- 5) Correlate FNIRS signals in left DLPFC detected in week 1 and 2 with depressive symptom scores (MADRS) in week 6, 8, and 12 in order to look for early biomarkers for treatment response.
- 6) Group comparison of FNIRS signals in left DLPFC detected 7-10 days before rTMS treatment in clinically defined responders and non-responders at 6 weeks and 12 weeks, respectively.

#### **Declaration:**

The research protocol fully complies with the Declaration of Helsinki and guidelines of ICH-GCP

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