The Effect of Venlafaxine on Language Function in Patients With Subcortical Aphasia: A fMRI Study

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Statistical Analysis Plan

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1. Functional magnetic resonance data processing

1.1 Task-state fMRI processing

DPARSF software package (http://www.rfmri.org) and SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm) will be use to process the Task-state fMRI data on the Matlab R2013b platform(https://cn.mathworks.com/). we will transform the DICOM format into NIFTI format firstly. And then, the first 10 time points are removed to eliminate the influence on the data acquisition due to the uneven magnetic field or the patient's inadaptation to the data acquisition. Then the time correction and head motion correction (the translation which is greater than the 2mm or more than 2 degrees on the X, Y, Z axis will be eliminated) will be perform. Next, Normalization and Smoothing (6 mm x 6 mm x 6 mm FWHM Gauss kernel) will be performed. The Normalization is carried out by the DARTEL method, which is divided into three parts: first registration, matching the structure image with the functional image; then dividing, dividing the transformed structure into gray matter, white matter and cerebrospinal fluid with a uniform segmentation rule, and obtaining a conversion matrix from functional image to standard space; finally, standardize the transformation matrix to write the function.

The first level analysis of the task-state fMRI image is used by the SPM12 software(http://www.fil.ion.ucl.ac.uk/spm). In order to produce a single contrast image corresponding to each task and each of the subjects, FWE correction will be used ($p<0.05$, $ke>10$). Then the activation files of each group will be extracted, and the group analysis will be carried out by one-sample t test ($p<0.05$, FDR correction, $ke>10$). The fMRI activation map of V1, V2 and V3 in each group will be obtained. The comparison between the venlafaxine group and the control group will take the age, the volume of lesion and the education as covariate to eliminate the interference factors outside the activation level, and using the two-sample t test ($p<0.001$, uncorrected, activation range $ke>10$). The average activation brain map will be presented by xjview software (http://www.alivelearn.net/xjview). And then, the map

will be superimposed on the standard three-dimensional meninges, and the region of interest (ROI) , MNI coordinates, activation volume (voxel) and activation intensity (T) will obtain.

1.2 Rest-state fMRI processing

According to the seed point method, the DPARSF software package and the SPM8 software package are adopted on the Matlab R2013b platform, which mainly include the following steps：

1.2.1 Data pre-processing

(1)Format conversion: converting files from DICOM format to NIFTI format.

(2)The data of the first 10 time points should be removed to eliminate the influence of magnetic field inhomogeneity or patient's inadaptability on data acquisition.

(3)Time correction.

(4)Head motion correction: data that moves more than 2mm or rotates greater than 2° on X, Y and Z axes is eliminated.

(5)Normalization: registration firstly, matching the structure image with the functional image; then dividing, the transformed structure is segmented into gray matter, white matter and cerebrospinal fluid by a uniform segmentation rule, and a conversion matrix from functional image to standard space is obtained; finally, standardize the transformation matrix to write the function.

(6)Smoothing: using 4 mm x 4 mm x 4 mm FWHM Gauss kernel.

(7)Linear drift and low frequency filtering processing: to eliminate the influence of low frequency drift and high frequency noise.

(8)Removal of covariates: including removal of six head motion parameters, whole brain mean signal, white matter and cerebrospinal fluid signals.

1.2.2 Selection of seed point

According to the activation maps of the main brain regions in task-state fMRI, and referring the language function area of previous the literature, we select the left Broca’s area (equivalent to the posterior part of left inferior frontal gyrus) , and the left Wernick’s area (equivalent to the posterior part of left superior temporal gyrus)

and the opposite side mirror area as seed point. In total four seed points which the areas consist with the MIN coordinate(as the center of the sphere) and 6 mm as the radius, see table 1.

Tab.1 MNI coordinates and range of 4 seed points

Seed Point	Anatomical location	MNI Coordinate of Sphere			Radius（mm）
		x	y	z	
Broca_L	posterior part of left IFG	-45	26	13	6
Broca_R	posterior part of right IFG	52	28	18	6
Wernicke_L	posterior part of left STG	-48	-53	36	6
Wernicke_R	posterior part of right STG	51	-48	37	6

IFG：Interior Frontal Gyrus; STG：superior Temporal Gyrus; MNI：Montreal Neuro logical Institute

1.2.3 Functional connection analysis

DPARSF software is used to extract the time series of voxels defined in the template, and then the time series are averaged to get the reference time series of four seed points. The correlation analysis between the time series of the reference time series and the other voxels of the whole brain is made by Pearson correlation analysis. And the correlation diagram is obtained. The relative intensity of each element in the correlation diagram represents the correlation between the region of interest and the low frequency oscillation signal of the voxel. Finally, the correlation map is transformed into Z diagram by Fisher-Z transformation, so that the correlation coefficient is distributed as normal as possible. Based on the SPM12 software, the one-sample t test will be used to show the brain regions associated with the function of 4 seed points ($p<0.05$, FDR correction,$ke>10$). Next, the age, the volume of lesion and the education will be used as the covariate, and the changes of the corresponding functional connections of the two groups will be compared with the two-sample t test ($p<0.001$, uncorrected, $ke>10$). The function connection diagram is presented by

xjview software, then superimposed on the standard three-dimensional meninges, and positioning the activated brain area to obtain the MNI coordinates of the region of interest, the volume (voxel representation) and the maximum activation intensity (T value).

1.3 DTI data processing

1.3.1 Pre-processing

Tools from the FSL library (FMRIB Software Library, (http://www．fmrib.ox.ac．uk/fs1) will be used to process the DTI data and for probabilistic tractography analysis. we extract the diffusion gradients and the corresponding b-values from the DICOM files, Head motion and image distortions (stretches and shears) due to eddy currents are corrected with affine transformation in FDT. A binary brain mask will be created from the B0 image using Brain Extraction Tool(BET) with fractional intensity set to 0.1. Then a diffusion tensor model is fit at each voxel to the 4D DWI data series (with additional inputs of the binary brain mask and gradient table) using DTIFit. The outputs from this process included fractional anisotropy (FA) and mean diffusivity (MD) maps as well as 1st-3rd eigenvector and eigenvalue maps, which are visually inspected as a quality control measure. Diffusion parameters will be then modeled using FSL's Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques and modeling crossing fibers (BEDPOSTX). BEDPOSTX models diffusion signal as ball (isotropic) and stick (anisotropic) components to generate a distribution of likely fiber orientations within each voxel as well as an estimate of the uncertainty on these orientations.

1.3.2 Region of interest selection

Regions of interest (ROIs) will be selected at template of ALL_116_2MM. ROIs are drawn on T1 in register with each subject's FA map generated from native DWI space.

1.3.3 Probabilistic tractography

Probabilistic tractography will be performed using FSL's Probtrackx 2.0, default settings of 5000 samples/voxel, step length (0.5 mm), curvature threshold (0.2).

1.3.4 Statistical analyses

Statistical analyses will be conducted in SPSS (23.0). Bivariate (Pearson) correlations were initially used to explore the relationship between age, gender, education, ROI-derived, and tractography variables. In group analysis, ANOVA analysis of single factor repeated measurement will be used, and two samples t-test will be used between group analysis, and correlation analysis will be done with language test results. P value of< 0.05 areconsidered significant.

1.5 Statistical analysis of quantitative data

The general quantitative data will be represented by mean number±standard deviation, Levenne variance homogeneity test (P>0.05, which can be considered homogeneity of variance) and normal distribution test will be used firstly, and two-samples t-test will be used for quantitative data which satisfied Levenne's homogeneity of variance test and normal distribution, and the rates of two group will be compared by Fisher exact probabilities. The repeated measurement data will be analyzed by repeated measures analysis of variance. Comparisons inter-group will be performed by using a two-samples t-test, and pair-samples t-test will be used to compare pairs of time points. All the above analyses used SPSS23.0 software package, P<0.05 (two-tailed) will be considered statistically significant. And the bivariate correlation option in this software package will be used to analyze the correlation between the improvement of Aphasia Quotient and the change of activation of task-state fMRI.