Case Report

Magnetic Resonance Study on Restoration of the Glymphatic System and Brain Network in Insomnia Patients with TCM Physiotherapy: A Case Report

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**Magnetic resonance data collection:**

The 3D-T1 sequence was scanned from the top of the skull to the foramen magnum of the occipital bone, with the line connecting the anterior and posterior commissure as the scan baseline, based on the Gradient Echo (GRE) sequence, the imaging parameters were set as follows: slice thickness = 1.2 mm, slice gap = 0 mm, TR/TE = 7.8/3 ms, FOV = 24 cm × 24 cm, flip angle= 15°, matrix = 256 × 256, 248 slices.

Diffusion tensor imaging (DTI) based on the Gradient-echo echo-planar imaging sequences, the imaging parameters were set as follows: slice thickness = 5 mm, slice gap = 0 mm, TR/TE = 8000/87.6 ms, FOV = 24 cm × 24 cm, flip angle= 90°, matrix = 130 × 130, 30 slices, NEX 1, b = 1000 s/mm2, 15 directions.

**ALPS index calculation**1,2**:** DTI data were preprocessed to remove eddy current and phase distortion artifacts, masks were created (thresholding, smoothing, and defragmentation), and reconstruction was done with DTI Studio software (https://www.mristudio.org/), which includes open-source pictures. A 5 mm diameter ROI was created in the projection and association fiber region of the left cerebral hemisphere (Figure S1), and the diffusivity was extracted in three directions along the x-, y-, and z-axes, and the ALPS index were calculated using the formula (Formul S1). ROIs were separately set by two neuroimaging physicians with substantial data processing experience, who were requested to try to maintain the ROIs set for data at different time periods all in the same place. To eliminate the impact of subjective awareness on the measures, we did not tell the physicians of the time points to which the measurements related. Both were requested to measure the ALPS index three times, with the average of their results used as the final output.

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**Figure S1.** Circles with a diameter of 5 mm are set as regions of interest in the projection fiber (blue region) and association fiber (green region).



**Formul S1.** **Calculation formula of ALPS index.** *Dxx, proj*, diffusivity along the xaxis in projection fiber area; *Dxx, assoc*, diffusivity along the x-axis in association fiber area; *Dyy, proj*, diffusivity along the y-axis in projection fiber area; *Dzz, assoc*, diffusivity along the z-axis in association fiber area; Diffusivity was measured with apparent diffusion coefficient values (×10−3 mm2/s).

**Small-worldness calculation**3**:** Panda software (https://www.nitrc.org/projects/panda/) was used to do DTI data preprocessing and white matter network building. Brain extraction, eddy current correction, and head movement correction were among the preprocessing procedures. Whole-brain structural networks were constructed using a deterministic fiber bundle tracking imaging method, where natural diffusion space was assigned via the Fiber Assignment by Continuous Tracking (FACT) algorithm and Diffusion Toolkit were performed, and all regions were computed by tracking voxels with FA greater than 0.2. Fiber bundle imaging was terminated if the angle was greater than 45° or if voxels with FA less than 0.2 were observed. The subjects' nodes were defined using Automatic anatomical labeling (AAL) 116 atlas, and the diffusion picture of each data set was first co-aligned with the matching T1 structural images using a linear transformation. The T1 images were then nonlinearly translated in Montreal Neurological Institute (MNI) space to the AAL116 atlas' standard T1 structural images. The inverse transformation parameters were then inverted and utilized to warp the AAL atlas from MNI space to local diffusion space, with discrete marker values kept through a nearest neighbor interpolation method. Finally, the b0 images and modified AAL atlas were examined to confirm that there were no severe mismatch errors. To determine the edges of the white matter network, structural connections were defined as the presence of at least three fiber bundles connecting two brain regions. For each data set, a 116×116 matrix of the entire brain structural network will be produced.

The GRETNA toolbox (http://www.nitrc.org/projects/gretna/) combined with graph theoretic methods was used to calculate the small-worldness of each structural matrix. The calculation formula is as follows (Table S1)

**Table S1. Equations for calculating network topology parameters**

|  |  |  |
| --- | --- | --- |
| Parameters | Formula | Basic concepts and notation |
| Nodal degree, *k*4 |  | 1. N is the set of all nodes in the network, and n is the number of nodes. 2. (i, j) is a link between nodes i and j, (i, j∈ N).。 3. aij is the connection status between i and j: aij = 1 when link (i, j) exists (when i and j are neighbors); aij = 0 otherwise (aii = 0 for all i). 4. dij is the length of the shortest path between i and j; Ci is the clustering coefficient of node i (Ci = 0 for ki < 2). 5. C and Crand are the clustering coefficients, and L and Lrand are the characteristic path lengths of the respective tested network and a random network. Small-world networks often have S> 1. |
| Clustering coefficient, Cp5 |  |
| Characteristic path length, Lp5 |  |
| Normalized clustering coefficient, γ |  |
| Normalized characteristic path length, λ |  |
| Small-worldnes, σ3 |  |

**References**

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