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Appendix S1

Introduction

Stages of Sleep

According to the American Academy of Sleep Medicine scoring manual, sleep divides into nonrapid eye movement (non-REM, NREM) sleep and REM sleep. NREM sleep further divides into stages N1, N2, and N3. NREM sleep stage N1 is a transitional sleep state following the stage of wake. It shows low-voltage, fast electroencephalography (EEG) activity. A person in stage N1 exhibits shallow breathing, fallen blood pressure, regular heart rate, and little body movement, and can be easily awakened. The duration of stage N1 generally increases with aging. NREM sleep stage N2 is an intermediate sleep state following the stage N1. On EEG, it shows predominant theta activity and occasional quick bursts of faster activity. In addition, K complexes and sleep spindles can occur episodically. It is accompanied with a relative decrease of the physiologic functions including blood pressure, cardiac activity, cerebral metabolism, and gastrointestinal secretions. A person in stage N2 falls into deeper sleep and becomes more difficult to arouse. Stage N2 may last about 20 minutes. NREM sleep stage N3 is a slow-wave sleep. It is characterized by high amplitude slow waves on EEG. It is the deepest and restorative sleep stage, creating cerebrospinal fluid flux into the interstitial space of the brain, which leads to the increase in the glymphatic clearance. Stage N3 can be associated with further decrease in the muscle tone and progressive decrease and cessation of eye movement, and links with the highest threshold for arousal. The duration of stage N3 decreases with aging, and also decreases in a certain type of sleep disorder such as REM sleep behavior disorder (RBD). REM sleep is called paradoxical or active sleep. In adults, it typically occurs about 90–120 minutes after sleep onset, and occupies 20%–25% of the major period of sleep. On EEG, it shows relatively low amplitude and mixed frequency theta waves. Unlike the progressive decrease of the physiologic functions during NREM sleep, physiologic activity gets higher during REM sleep. Blood pressure and heart rate may increase, breathing gets irregular, and oxygen consumption of the brain increases.

Materials and Methods

Study Participants

A clinical diagnosis of rapid eye movement (REM) sleep behavior disorder (RBD) was made based on the standard guidelines of the International Classification of Sleep Disorders, third edition (27): (a) presence of REM sleep without atonia at polysomnography; (b) presence of sleep-related injurious, potentially injurious, or disruptive behaviors as indicated by a patient's history or as documented during polysomnography monitoring; (c) absence of epileptiform activity on electroencephalography during REM sleep, unless RBD could clearly be distinguished from any concurrent REM sleep-related seizure disorder; and (d) no other sleep, medical, or neurologic disorders, medications, or substance use disorders that could better explain the behavior.

To define REM sleep without atonia on polysomnography, we used the criteria of the American Academy of Sleep Medicine manual (27): (a) sustained muscle activity in REM sleep in chin electromyography (EMG), an epoch of REM sleep with at least 50% of the duration having a chin EMG amplitude greater than the minimum amplitude in non-REM sleep; (b) excessive transient muscle activity during REM sleep on chin or limb EMG, in a 30-second epoch of REM sleep divided into 10 sequential 3-second mini-epochs, at least five mini-epochs (50%) contain bursts of transient muscle activity of 0.1 seconds to approximately 5.0 seconds in duration and at least four times as high in amplitude as the background EMG activity. For polysomnographic recordings, we used Embla N7000 (Embla), and a video was simultaneously recorded. Electroencephalography electrodes were applied at C3/A2, O1/A2, and O2/A1, and two electrooculography electrodes were applied on the sides of both eyes. EMG electrodes were applied to the submentalis and anterior tibialis muscles. Strain gauges were used to record chest and abdominal respiratory movements, and nasal pressure cannulas were used to record airflow. A pulse oximeter was applied to the index finger to measure arterial oxygen saturation. Using Rechtschaffen and Kales' criteria, every 30-second epoch of video-polysomnography was scored. At the time of video-polysomnography, participants with RBD were not taking any medications, including clonazepam, melatonin, or antidepressants, which may have influenced the polysomnography results.

Neurologic Examination for Study Participants

All participants underwent neurologic examination including consciousness and orientation, cranial nerve function, motor, deep tendon reflex, sensory functions, cerebellar function, and gait items at every visit to find any signs of neurodegenerative disorders. Unified Parkinson's Disease Rating Scale Part III (UPDRS-III), mini-mental state examination (MMSE), and Montreal cognitive assessment (MoCA) were checked yearly for control participants and participants with PD, and at every visit for participants with RBD.

MRI Protocol

Noncontrast MRI scans were performed using a 3T scanner (Ingenia and Ingenia CX, Philips) with a 32-channel SENSE head coil (Philips Healthcare). The Parkinson-dedicated MRI sequences in our institution include 3D T1-weighted imaging, 3D fluid-attenuated inversion recovery imaging, axial diffusion-tensor imaging (DTI), axial T2-weighted imaging, axial susceptibility-weighted imaging (SWI), and neuromelanin imaging. Among them, DTI and SWI were required for the diffusion-tensor imaging-analysis along the perivascular space (DTI-ALPS). DTI was obtained based on a single-shot echo-planar imaging sequence in the axial plane with b-values of 0 and 1,000 s/mm^2 (19–25). We applied 32 directions of motion probing gradients, and other imaging parameters for DTI were as follows: repetition time (TR), 9900 ms; echo time (TE), 77 ms; section thickness, 2 mm; flip angle, 90°; field-of-view, 224 × 224 mm^2; acquisition matrix, 112 × 112; scan time, 5 minutes and 50 seconds. SWI was obtained using multiecho fast-field-echo sequence with the following parameters: TR, 41 ms; total 4 echoes; first TE, 7.2 ms; echo interval, 6.2 ms; section thickness, 2 mm; flip angle, 20°; field-of-view, 200 × 220 mm^2; acquisition matrix, 384 × 384.

^{123}I-FP-CIT SPECT Protocol

Participants were administered oral Lugol's solution (6 mL) 60 minutes before ^{123}I-FP-CIT injection. After injection of 185 MBq of ^{123}I-FP-CIT (DATrace-123, Samyoung Unitech)

intravenously, scans were acquired 3 hours later. Scans were performed with a triple-headed rotating gamma camera system (Trionix XLT; Trionix Research Laboratory, Inc) with low-energy, ultra-resolution, parallel hole collimators. Scans were acquired for 40 seconds per step, with 40 steps for 120° via the step-and-shoot method. Image reconstruction was done by filtered back-projection with a Butterworth filter (cutoff frequency 0.6 cycle/cm; order, 13), and attenuation correction with Chang's method (coefficient of 0.12/cm).

DTI-ALPS Processing

DTI-ALPS method can isolate perivascular water diffusivity at the level of lateral ventricular body where the direction of perivascular water flow is along the medullary arterioles and veins, parallel to the x-axis on DTI. At this axial level, the direction of the perivascular water flow around the medullary vessels is perpendicular to the ventricular wall, mostly along the x-axis (19–25) (Fig S1). On this axial section, the direction of the perivascular water flow is also perpendicular to the direction of the projection neural fibers which are mostly lined up parallel to the z-axis, and perpendicular to the association neural fibers which are mostly lined up parallel to the y-axis. Therefore, the diffusivity along the x-axis (D*x*) in the regions of projection and/or association neural fibers (D*xproj* and/or D*xassoc*) can represent the water diffusivity along the perivascular space without the interference of diffusivity from the neural fibers, thereby reflecting glymphatic activity. However, D*x* measured in the regions of subcortical neural fibers (D*xsubc*) is not considered to represent the pure glymphatic diffusivity, since the subcortical neural fibers are parallel to the x-axis, thus the diffusivity from the subcortical neural fibers will obscure the water diffusivity of the D*x*. On the other hand, D*y* (ie, the diffusivity along the y-axis) and D*z* (ie, the diffusivity along the z-axis) cannot reflect perivascular glymphatic flow at the chosen axial level, since the directions of D*y* and D*z* are not parallel to the perivascular water flow along the medullary arterioles and veins, but mostly perpendicular to them. Consequently, among the diffusivity parameters, D*xproj* and/or D*xassoc* are known to reflect glymphatic activity by reflecting perivascular water flow (19–25).

For DTI-ALPS processing and measurement, we used the same methodology as that in previous studies (19–25). For DTI-ALPS calculations, we used an inhouse program, which is named DTI-ALPS Analyzer, based on MATLAB 9.6 (version 2019a, The Mathworks, Inc). Diffusion coefficients along three orthogonal directions (D*xx*, D*yy* and D*zz*) were extracted from the calculated diffusion tensor, pixel by pixel, for the generation of the diffusion coefficient maps along x, y and z directions (in other words, right-left, anterior-posterior, and cranio-caudal direction along head orientation). These three orthogonal diffusion coefficient maps were used for the DTI-ALPS. Calculated tensors were diagonalized based on singular value decomposition (SVD) method, for eigen values and eigen vectors calculation. Using these information, a color-coded fractional anisotropy (FA) map was postprocessed and employed for drawing regions-of-interest (ROIs) on projection, association and subcortical neural fiber regions of white matter. On the extracted diffusivity maps, D*x*, D*y*, and D*z* were measured by the unit of apparent diffusion coefficient (ADC, $\times 10^{-3}$ mm^2/s) values.

Two neuro-radiologists (Y.J.B. and B.S.C. with 11 and 21 years of experience, respectively) independently measured the DTI-ALPS values, blinded to the clinical diagnosis. First, they identified the horizontal parenchymal vessels at the lateral ventricular body level with reference to SWI. Next, the axial section was selected on the color-coded FA map at the same level as on SWI. On this axial section of the FA map, two readers independently allocated three

5-mm-diameter spherical ROIs in the areas of the projection, association, and subcortical neural fibers (Fig S1). From inhouse software, a total of nine separate DTI-ALPS measurements from nine ROIs were automatically generated: (a) D*x* in the projection (D*xproj*), in the association (D*xassoc*), and in the subcortical (D*xsubc*) neural fiber areas, (b) D*y* in three neural fiber areas (D*yproj*, D*yassoc*, D*ysubc*), and (c) D*z* in three neural fiber areas (D*zproj*, D*zassoc*, D*zsubc*). Lastly, the ALPS index was calculated by the following equation to mitigate the influence of the periventricular white matter lesions and to standardize measurements across individuals (19–25):

$$\text{ALPS index} = \text{mean}\left(\text{D}xproj,\ \text{D}xassoc\right) / \text{mean}\left(\text{D}yproj,\ \text{D}zassoc\right).$$

In the calculation of ALPS index, the D*x* components, D*xproj* and D*xassoc*, are included, but D*xsubc* is excluded to rule out the diffusivity component from the subcortical neural fiber tracts which are parallel to the x-axis. Furthermore, ALPS index can minimize the effect from the individual white matter degeneration by dividing D*x* values by the values of the diffusivities perpendicular to them–D*yproj* and D*zassoc*. D*yproj* is measured at the same ROI as D*xproj,* but it measures the diffusivity along the y-axis, which can include underlying white matter diffusivity in the direction of other than x-axis. However, D*zproj* is not considered here, because this reflects the large diffusivity from neural fiber tracts. Similarly, D*zassoc* can include the underlying white matter diffusivity at the location where D*xassoc* is measured. But, D*yassoc* has no impact on the cancellation of the white matter effect, since it reflects the large diffusivity of major association neural fiber tracts. Therefore, dividing mean (D*xproj*, D*xassoc*) by mean (D*yproj*, D*zassoc*) will give us the cancellation of the underlying white matter diffusivity. This ALPS index can isolate the contribution of perivascular diffusivity from the water flow to the diffusion signal. The ALPS index is known to be close to 1 if the influence of water diffusion along the perivascular space is minimal, but a larger ALPS index will represent larger water diffusivity along the perivascular space, meaning better glymphatic function (19–25).

Table S1

Interobserver Agreement on Diffusivities and ALPS indexes

Value	Reader 1	Reader 2	ICC*	95% CI
D*xproj (ADC, × 10^{-3}mm^2/s)*	0.62 ± 0.09	0.62 ± 0.09	0.87	0.78–0.92
D*xassoc (ADC, × 10^{-3}mm^2/s)*	0.61 ± 0.13	0.59 ± 0.12	0.87	0.78–0.92
D*xsubc (ADC, × 10^{-3}mm^2/s)*	1.08 ± 0.2	1.05 ± 0.19	0.53	0.21–0.72
D*yproj (ADC, × 10^{-3}mm^2/s)*	0.44 ± 0.07	0.43 ± 0.07	0.77	0.61–0.86
D*yassoc (ADC, × 10^{-3}mm^2/s)*	0.97 ± 0.12	1 ± 0.14	0.57	0.29–0.75
D*ysubc (ADC, × 10^{-3}mm^2/s)*	0.7 ± 0.22	0.71 ± 0.19	0.86	0.77–0.92
D*zproj (ADC, × 10^{-3}mm^2/s)*	1.69 ± 0.18	1.19 ± 0.19	0.81	0.68–0.89
D*zassoc (ADC, × 10^{-3}mm^2/s)*	0.35 ± 0.07	0.36 ± 0.09	0.76	0.6–0.86
D*zsubc (ADC, × 10^{-3}mm^2/s)*	0.59 ± 0.2	0.63 ± 0.16	0.58	0.3–0.75
ALPS index	1.57 ± 0.24	1.55 ± 0.22	0.85	0.75–0.91

Note.—Values are shown as mean ± SD. ALPS = analysis along the perivascular space, ADC = apparent diffusion coefficient, ICC = interclass correlation coefficient, D*xproj/xassoc/xsubc* = diffusivity along x-axis in projection/association/subcortical fiber area, D*yproj/yassoc/ysubc* = diffusivity along y-axis in projection/association/subcortical fiber area, D*zproj/zassoc/zsubc* = diffusivity along z-axis in projection/association/subcortical fiber area.

* ICC greater than or equal to 0.75, excellent agreement; 0.60–0.74, good agreement; 0.40–0.59, fair agreement; and less than 0.40, poor agreement.

Table S2

Results on SMWI and ^{123}I-FP-CIT SPECT in Participants with RBD

Imaging Findings	Normal Result of ^{123}I-FP-CIT SPECT	Abnormal Result of ^{123}I-FP-CIT SPECT*	Total
Intact bilateral nigral hyperintensity on SMWI	6	1	7
Loss of corresponding nigral hyperintensity on SMWI	0	8	8
Total	6	9	15

Note.—Values are shown as numbers. *P* value derived from χ^2 test was 0.001. SMWI = susceptibility map-weighted imaging, ^{123}I-FP-CIT SPECT = iodine 123 2β-carbomethoxy-3β-(4-iodophenyl)-*N*-(3-fluoropropyl)-nortropane SPECT, RBD = rapid eye movement sleep behavior disorder.

* Abnormal ^{123}I-FP-CIT SPECT result refers to the visibly defined reduced dopamine transporter uptake in the unilateral or bilateral striata, suggesting the presence of nigrostriatal dopaminergic degeneration.

Table S3

SMWI and ^{123}I-FP-CIT SPECT Results According to Phenoconversion Status in Participants with RBD

SMWI Findings (*n* = 20)	Conversion	Nonconversion	Total
Intact bilateral nigral hyperintensity	2	9	11
Loss of corresponding nigral hyperintensity	5	4	9
Total	7	13	20
^{123}I-FP-CIT SPECT findings (*n* = 15)	Conversion	Nonconversion	Total
Normal result	2	4	6
Abnormal result*	5	4	9
Total	7	8	15

Note.—Values are shown as numbers. *P* values derived from χ^2 test were 0.02 and 0.6, respectively. SMWI = susceptibility map-weighted imaging, ^{123}I-FP-CIT SPECT = iodine 123 2β-carbomethoxy-3β-(4-iodophenyl)-*N*-(3-fluoropropyl)-nortropane SPECT, RBD = rapid eye movement sleep behavior disorder.

* Abnormal ^{123}I-FP-CIT SPECT result refers to the visibly defined reduced dopamine transporter uptake in the unilateral or bilateral striata, suggesting the presence of nigrostriatal dopam