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# Advances in neuromodulation treatment of Parkinson's disease and aging-related movement disorders

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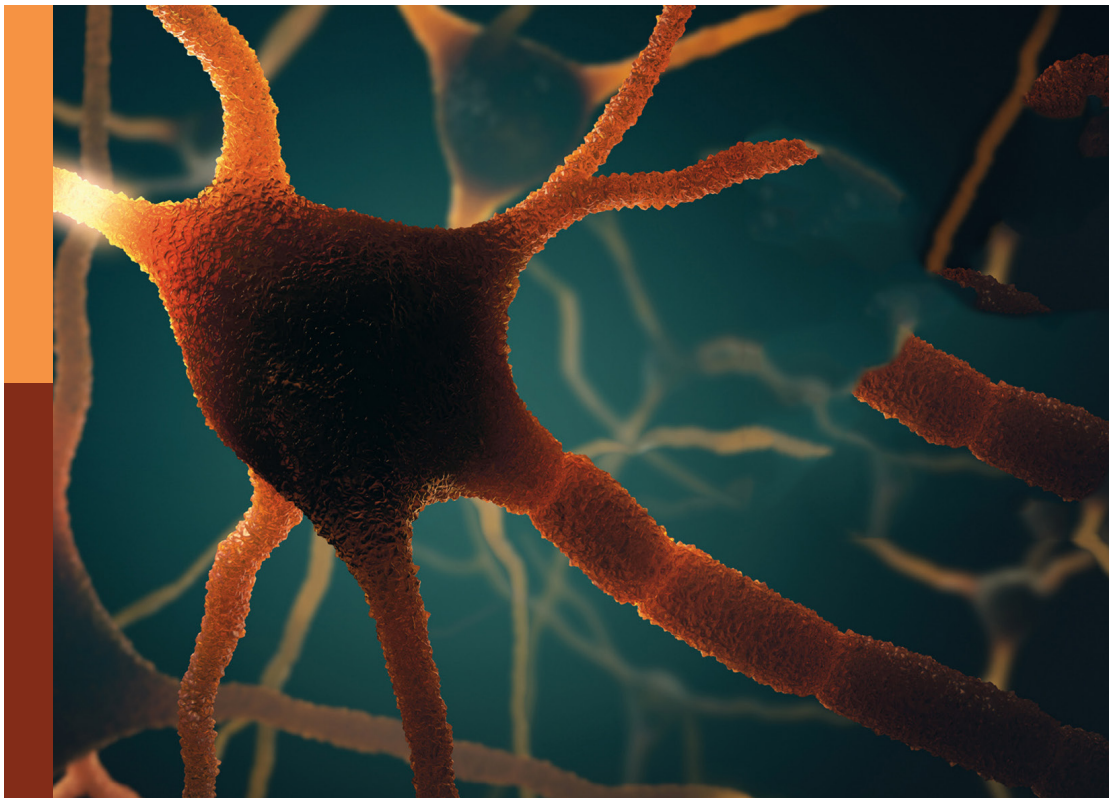

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# Advances in neuromodulation treatment of Parkinson's disease and aging-related movement disorders

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# Editorial: Advances in neuromodulation treatment of Parkinson's disease and aging-related movement disorders

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KEYWORDS

**Parkinson's disease, neuromodulation, deep brain stimulation (DBS), movement disorder, biomarkers**

Editorial on the Research Topic
Advances in neuromodulation treatment of Parkinson's disease and aging-related movement disorders

In recent years, significant strides have been made in the field of neuromodulation for the treatment of Parkinson's Disease (PD) and aging-related movement disorders. The intersection of neuroscience, technology, and medical innovation has paved the way for novel approaches to patients facing these debilitating conditions. From deep brain stimulation (DBS) to non-invasive neuromodulation techniques, the landscape of treatment options is rapidly evolving, promising improved symptom management and quality of life for individuals affected by these neurological disorders. This Research Topic "*Advances in neuromodulation treatment of Parkinson's disease and aging-related movement disorders*" explores the latest advances in neuromodulation therapies, related reviews summarizing, and assessment of disease progression, highlighting the potential prospect for managing aging-related movement disorders.

The topic is comprised of 12 articles including nine original articles and three review papers. These articles mainly focused on the effect of brain stimulation and physical activity on PD, the role of metabolites and receptors in PD, predictive factors, and the application of new approaches to PD progression.

The first reported randomized, double-blind, sham-controlled study to investigate the effectiveness of non-invasive vagus nerve stimulation (nVNS) in improving gait and other motor symptoms of PD patients was performed by Mondal et al.. Thirty-three PD patients experiencing freezing of gait (FOG) received either active nVNS or a placebo treatment. Remarkable enhancements in walking factors were observed with the use of active nVNS. Moreover, there were significant changes in serum tumor necrosis factor-α,

glutathione, and brain-derived neurotrophic factor levels following the active nVNS treatment. The results suggested that nVNS could serve as a supplementary treatment in managing PD symptoms, particularly for FOG. Furthermore, Liu et al. performed a meta-analysis involving 16 randomized controlled studies with 408 patients to explore the impact of transcranial magnetic stimulation (TMS) on FOG in PD. The result showed that TMS was beneficial in enhancing gait and motor performance, while further researches are needed to explore the most effective stimulation parameters for TMS.

Except for applied stimulations, interactive interventions like action observation training (AOT) and physical activity, have proven to be effective in improving both cognitive abilities and motor skills for PD. Meng et al. conducted a study involving 30 early-stage PD patients, aiming to examine changes in brain functional connectivity (FC) and clinical outcomes after 12 weeks of Tai Chi-based action observation training (TC-AOT) in comparison to traditional physical therapy (TPT). Patients with TC-AOT displayed significantly higher FCs between specific brain regions. Furthermore, these changes in FCs were positively correlated with improvements in both motor and cognitive abilities. The result indicated that the TC-AOT enhanced the early-stage rehabilitation outcomes of PD by fostering brain neuroplasticity. Meanwhile, a study conducted by Lin et al. observed that the consumption of fish oil supplements and physical activity have both been linked to a lower risk of developing PD. Furthermore, the protective effect of physical activity against PD appears to be even stronger when combined with the use of fish oil supplements.

The study by Zeng et al. illustrated the progression of research focused on surgical interventions for tremors in PD patients between 2002 and 2022. DBS for PD tremor is still a research hotspot. Several concerns regarding DBS like operative indications, targets, and protocols associated should also be considered. Furthermore, magnetic resonance-guided focused ultrasound (MRgFUS) has become a hopeful treatment option for PD tremors. Consistent with Zeng et al. and Hong et al. also found that DBS was a reliable therapy for Young Onset Parkinson's Disease (YOPD). A total of 27 YOPD patients who underwent STN-DBS experienced obvious enhancements in their emotional wellbeing, with no negative impact on their cognitive abilities after a follow-up of 2 years. Honma et al. also investigated the contribution of the STN-DBS in temporal processing. The result suggested STN participated in the encoding of time duration and the role of time perception might be restricted to the externalization of memories acquired through experiences. STN-DBS may potentially enhance the functioning of the prefrontal cortex by modulating the basal ganglia-thalamo-cortical circuit.

Multiple research studies have found a connection between PD and reduced levels of uric acid (UA). Low UA levels have been linked to an increased risk of developing PD, as well as the progression and severity of the disease. Using resting-state functional MRI, Chang et al. investigated the connection between FC related to UA and outcomes of STN-DBS in PD patients. They found that PD patients with abnormal brain connections related to UA are strongly linked to the effectiveness of STN-DBS. Combining the two biomarkers of UA and FC provides neurosurgeons with valuable tools to identify the most suitable candidates and forecast the prognosis of PD patients. Another study by Shin et al. explored the effects of UA on the transfer of extracellular α-synuclein (α-syn) between cells and the protection of dopaminergic neurons in a model enriched with α-syn. The result indicated that UA could potentially manage the progression of PD by targeting multiple pathways that regulate the spread of α-synuclein. Yu et al. summarized the development of the cannabinoid type-2 receptor ($CB_2$ receptor). The $CB_2$ receptor was reported to have a potential impact on iron transport, oxidative stress, neuroinflammation, and neuronal cell death, which may play a role in the treatment of PD.

Early detection and early treatment of PD symptoms are essential. New approaches are emerging to play a role in predicting the advancement of PD. Hu et al. clarified the connection between the development of impulse control disorder (ICD) and the advancement of PD. Patients with different patterns of ICD evolution had varying changes in white matter microstructure at the onset of PD. The brain regions affected by these changes are known to play a role in both ICD and non-motor Functions. These patterns may also serve as predictive markers for the progression of motor symptoms and cognitive decline in PD patients. Visuospatial and cognitive dysfunction are prevalent among PD patients, which was also proven by the study of Shao et al.. The assessments conducted through the application (APP) demonstrated increased sensitivity and specificity, aiding clinicians in the swift and accurate diagnosis of PD patients with visuospatial disorders. The result indicated the potential of APP to provide early rehabilitation strategies and pharmacological interventions.

In conclusion, with the rapid development of neuromodulation, innovative findings continue to emerge. There is great hope for promising treatments for PD and aging-related movement disorders.

## Author contributions

K-LW: Writing – original draft, Writing – review & editing. FM: Conceptualization, Project administration, Resources, Supervision, Writing – review & editing. SC: Project administration, Resources, Supervision, Writing – review & editing. AR-Z: Resources, Supervision, Writing – review & editing. Y-QZ: Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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# Prediction of STN-DBS for Parkinson's disease by uric acid-related brain function connectivity: A machine learning study based on resting state function MRI

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**Introduction:** Parkinson's disease (PD) is a neurodegenerative disorder characterized by dyskinesia and is closely related to oxidative stress. Uric acid (UA) is a natural antioxidant found in the body. Previous studies have shown that UA has played an important role in the development and development of PD and is an important biomarker. Subthalamic nucleus deep brain stimulation (STN-DBS) is a common treatment for PD.

**Methods:** Based on resting state function MRI (rs-fMRI), the relationship between UA-related brain function connectivity (FC) and STN-DBS outcomes in PD patients was studied. We use UA and DC values from different brain regions to build the FC characteristics and then use the SVR model to predict the outcome of the operation.

**Results:** The results show that PD patients with UA-related FCs are closely related to STN-DBS efficacy and can be used to predict prognosis. A machine learning model based on UA-related FC was successfully developed for PD patients.

**Discussion:** The two biomarkers, UA and rs-fMRI, were combined to predict the prognosis of STN-DBS in treating PD. Neurosurgeons are provided with effective tools to screen the best candidate and predict the prognosis of the patient.

KEYWORDS

**Parkinson's disease, deep brain stimulation, functional connectivity, uric acid, machine learning**

## 1. Introduction

Parkinson's disease (PD) generally develops between 55 and 65 years of age, affecting 1–2% of people over 60 years of age, or about 0.3% of the total population (Ascherio and Schwarzschild, 2016; Cerri et al., 2019). (UA) is the final product of purine metabolism and is considered an antioxidant in the body. Previous studies have shown that UA inhibits free radical-induced lipid peroxidation and DNA damage, thus acting to protect nerve cells (Narendra et al., 2018; Ya et al., 2018; Mahoney-Sánchez et al., 2021).

Changes in UA levels are associated with several disease states. Abnormally high levels of UA are associated with gout, high blood pressure, cardiovascular disease (Kleber et al., 2015; Gherghina et al., 2022; Méndez-Salazar and Martínez-Nava, 2022). In contrast, lower levels of UA have been confirmed with PD, Alzheimer's disease (AD), multiple sclerosis (MS) and development of Meg syndrome are associated with (Koch and De Keyser, 2006; Du et al., 2016; Chang and Chen, 2020; Ellmore et al., 2020; van Wamelen et al., 2020; Guan et al., 2021; Seifar et al., 2022). In addition, UA affects the brain structure of PD patients. An MRI using a stationary state function in PD patients (rs-fMRI) found UA levels and broad white matter The integrity of (WM) has a significant correlation (Lee et al., 2020). At the same time, some researchers have shown cortical functional connectivity between UA and PD patients (FC) is closely correlated with high levels of FC in patients with high PD UA and negative correlation with motor symptoms (Lee et al., 2018). These results show that UA is an important biomarker for patients with PD and can be analyzed in combination with rs-fMRI.

Deep brain stimulation (DBS) is becoming one of the most effective treatments for patients with advanced PD, and many previous studies have shown that DBS can significantly improve motor symptoms in patients with PD (Chang et al., 2022; Mei et al., 2022). Interestingly, in PD patients with bilateral subthalamic nucleus (STN) DBS, we observed a positive correlation between UA and postoperative motor symptom improvement. So we guess whether UA can be analyzed in conjunction with rs-fMRI, two biomarkers, to predict the outcome of STN-DBS treatment of PD. The mechanism by which STN-DBS improves motor symptoms in patients is unclear. Some researchers compared the rs-fMRI before and after STN-DBS and found that STN-DBS altered graph theoretical indicators, FC and WM integrity, resulting in significant improvement of motor and mental symptoms in PD patients (Prent et al., 2019; Huang et al., 2022). This suggests that the prognosis of DBS in PD may depend on connectivity between brain regions. Several researchers previously examined structural and functional brain connections associated with PD prognosis after STN-DBS and tested their ability to predict the efficacy of independent cohorts (Horn et al., 2017). Artificial intelligence and machine learning have become increasingly important in healthcare decision-making and prediction in recent years (Naik et al., 2022a,b). Based on the above, we aimed to explore whether FCs associated with UA in PD patients are associated with prognosis in PD treated with STN-DBS, and whether these FCs could be used to predict the improvement of motor symptoms in PD patients treated with STN-DBS. It is hoped that integrated analysis of UA and rs-fMRI can be combined with machine learning to predict the prognosis of STN-DBS treatment in PD patients, so as to provide help for neurosurgeons to predict patients' conditions and screen patients.

# 2. Participants and methods

## 2.1. Participants

Medical records and questionnaire results were retrospectively collected from patients with PD who underwent STN-DBS at the First Hospital of the University of Science and Technology of China from September 2019 to April 2020. The study protocol was approved by the Ethics Committee of our hospital (2022-RE-154). The included patients had intermediate-to-advanced PD, and the exclusion criteria were moderate/severe cognitive impairment, persistent severe psychiatric disorder, severe atrophy or diffuse ischemic lesions on MRI, and systemic diseases that prevented surgery. Moreover, the medical records of age- and sex-matched healthy participants who underwent annual physical check-ups at the same hospital were collected as healthy controls (HC).

## 2.2. Acquisition clinical assessment

Demographic and clinical variables, including age, sex, duration of illness, and levodopa equivalent dose, were collected from patients' medical records and questionnaires. Symptom severity was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS-III). The patients' motor symptoms were reassessed 2 years after surgery using the UPDRS-III scale during the stimulation and medication on period, and the patients' UPDRS-III score improvement rate was subsequently calculated. The Hamilton Anxiety (HAMA) and Hamilton Depression (HAMD) scales were used to assess the psychological status of patients. The Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Examination (MMSE) scales were used to assess cognitive status. UA values obtained 5 days before STN-DBS were included in the analysis. Each specimen was assayed by the Department of Clinical Laboratory in 2 h post-collection. To be specific, UA was examined according to liver tests. The above clinical variables were determined by the standard automatic counters.

## 2.3. MRI data and preprocessing

For PD patients and HC, a 3.0 T MR scanner (Discovery MR750; General Electric Healthcare, Chicago, IL, USA) with an eight-channel phased-array head coil was used. Prior to scanning, the researchers placed earplugs in the subjects' ears to isolate noise. The participants were then instructed to immobilize their heads with sponge pads to reduce unconscious activity. During the scans, the subjects were allowed to close their eyes, but remained awake to avoid specific, intense ideation activities. We explicitly instructed the participants not to fall asleep during the entire scan. We further confirmed that the participants were awake throughout the scan after completion. Functional and structural MRI data were acquired with a 3T GE (Achieva TX) MRI scanner in the OFF medication state before DBS surgery, following an 12-h period of medication withdrawal. Structural images were acquired using a sagittal magnetization prepared rapid gradient echo three-dimensional T1-weighted sequence [repetition time (TR) = 8.5 ms, echo time (TE) = 3.2 ms, inversion time (TI) = 450 ms, and flip angle (FA) = 12°]. Functional MRI images were obtained using the following SE-EPI sequence: repetition time [TR] = 2,000 ms, repetition time [TR] = 30 ms, slice thickness/gap = 3.6/0 mm, axial slices = 38 layers, flip angle [FA] = 90°, FOV = 256 × 256 mm, matrix size = 64 × 64, and scanning time = 484 s.

Data pre-processing was conducted with Resting-State fMRI Data Analysis Toolkit plus V1.25 (RESTplus V1.25),[1] which is based on Statistical Parametric Mapping (SPM).[2] Data from 242 volumes were separately acquired as functional scans of the subjects and healthy controls. The first 10 volumes of each functional scan were excluded

1 http://restfmri.net/forum/index.php

2 https://www.fil.ion.ucl.ac.uk/spm/

to correct for subject habituation to the scanning environment and for magnetization stability. Slice-timing correlation was performed to help compensate for differences in acquiring data across all slices with the FOV at any given time point; realignment for head-motion correction was also considered; one healthy control whose head motion exceeded 3.0 mm or involved rotation exceeding 3.0° during the fMRI scanning was excluded. Individual 3D T1-weighted anatomical images were co-registered to the functional images and spatially normalized to the Montreal Neurological Institute template. Each voxel was resampled to 3 mm× 3 mm× 3 mm. Subsequently, the resampled images were smoothed using a 6 mm full-width half-maximum (FWHM) isotropic Gaussian kernel. Subsequently, a linear trend and bandpass filter (0.01∼0.08 Hz) were used to remove the effect of high-frequency noise. Finally, Friston-24 head motion parameters, cerebrospinal fluid signal, white matter, and the Friston-24 head motion parameters model were considered as nuisance covariates and were regressed from fMRI signals. The resulting data were analyzed further. Subsequently, two voxel-wise whole-brain analytic methods were applied.

## 2.4. DC calculation

To identify functional hubs, The voxel-wise correlation matrix was performed by Pearson's correlation for whole brain time series. Then we set the correlation coefficients with $r \geq 0.25$. The threshold was used to eliminate counting voxels that had low temporal correlation. We took each voxel as a node, and the correlation value between any pair of voxels as the internodal edge weight. The weighted DC of each voxel was further divided by the global mean DC of every individual for group comparison.

## 2.5. Spatial correlation analysis of correlations with UA

In this study, the mALFF and mReHo values of each region of interest (ROI) in the AAL3-170 atlas were separately extracted as candidate features. The AAL3-170 atlas (accessed June 4, 2022)[3] is an improved version of the AAL2 atlas that divides the entire brain into 166 ROI (**Supplementary Table 1**). In addition, two small regions of the AAL3 atlas (nos. 133–134) were not defined because the original voxel size of 1 mm× 1 mm× 1 mm was resampled to 3 mm× 3 mm× 3 mm; thus, the number of remaining regions in the AAL3-170 atlas was 164. Serum UA values of PD patients were correlated with the mALFF and mReHo values of each of the 164 brain regions. False discovery rate (FDR) correction was not performed for the 164 correlated values, with the threshold set to 0.05.

## 2.6. Functional connectivity analysis

Using the AAL3 template, the DC values and UA significantly correlated with the ROIs were filtered. Following correlation analysis, 15 significantly correlated ROIs remained between DC values and UA. The average resting state blood oxygenation level-dependent (BOLD) time series for each ROI was extracted. The BOLD time series for each ROI was then correlated with the BOLD time series of every other ROI (Pearson's correlation) for each participant. A 15 × 15 correlation matrix was obtained for each subject. Fisher's Z transformation was applied to the FC maps for subsequent statistical analysis.

## 2.7. Statistical analyses

Correlations between the UPDRS-III score improvement rate and UA values were analyzed using Pearson's correlation coefficient test. A two-sample *t*-test was performed in the PD and HC groups to detect zFC differences with FDR correction ($p < 0.05$), with * representing significantly abnormal zFC values between the two groups.

## 2.8. Feature extraction and SVR model training

In the paper, we use SVR to investigate whether inter-group differences in functional connection values can predict the rate of improvement after STN-DBS. Radial Basis Kernel is used in SVR model to find a non-linear regression line and analysis steps. Are done using the LIBSVM software package.[4] The functional connectivity values of PD group come from differences between groups as features (These ROIs for functional connectivity were selected from significant correlation between DC and UA). The patient's improvement after STN-DBS as label. Each feature is normalized to between −1 and 1, so do as label. We applied a leave-one-out cross-validation (LOOCV) to train SVR model, and a "grid search" method was used to access parameter optimization. The adaptability of the model was assessed by the Pearson's correlation coefficient(r) and mean squared error (MSE) between the original and the predicted rate of improvement.

The optimal parameter settings of SVR:
Kernel name: RBF.
Parameter optimization algorithm: Gird search algorithm.
Cross-validation type: Leave one out.
K fold number: 32.
C: 8388608.
g: 2.7387e−07.
p: 0.4.

# 3. Results

## 3.1. Participants' characteristics

**Table 1** presents the characteristics of the study participants. Thirty-eight patients were consecutively enrolled, none of whom were lost to follow-up, comprising 17 (44.74%) men and 21 (55.26%) women aged 41–73. The mean improvement rate according to the UPDRS-III score 2 years postoperatively in the medicine-on-period was 66%. The mean preoperative UA level of the patients

3 https://www.oxcns.org/aal3.html

4 https://www.csie.ntu.edu.tw/~cjlin/libsvm/

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TABLE 1 Characteristics of the patients with Parkinson's disease (PD) and healthy controls (HC).

| Variables | PD | HCs | *P*-value |
|---|---|---|---|
| No | 38 | 32 | |
| Age (years, mean ± SD) | 58.87 ± 7.61 | 63.09 ± 1.38 | 0.225 |
| Gender | | | 0.860 |
| Male | 17 (44.74%) | 15 (46.87%) | |
| Female | 21 (55.26%) | 17 (53.13%) | |
| Uric acid (μmol/L) | 288.45 ± 87.05 | 327.36 ± 10.57 | 0.036 |
| LED | 642.11 ± 399.51 | | |
| Duration (years) | 8.84 ± 3.83 | | |
| Age of onset (years) | 50.03 ± 7.30 | | |
| cUPDRS III med off | 57.29 ± 12.16 | | |
| UPDRS III med on | 29.05 ± 10.43 | | |
| UPDRS III med off Postop | 50.79 ± 18.13 | | |
| UPDRS III med on Postop | 19.34 ± 11.36 | | |
| UPDRS III med on Postop improvement rate | 0.66 ± 0.21 | | |
| H–Y | | | |
| 2.5 | 3 (7.89%) | | |
| 3 | 18 (47.37%) | | |
| 4 | 13 (34.21%) | | |
| 5 | 4 (10.53%) | | |

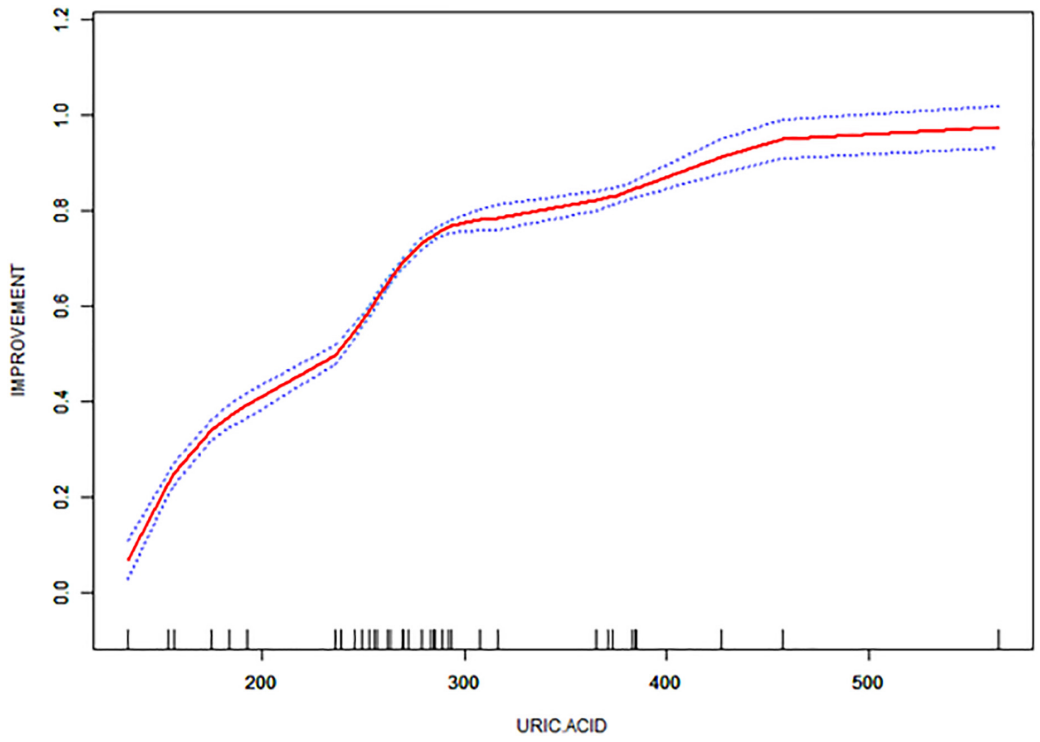


FIGURE 1
Correlation of UPDRS-III score improvement rate with uric acid (UA) values in the enrolled patients.

was 288.45 ± 87.05 μmol/L 5 days prior to STN-DBS. Thirty-two healthy participants were included in the analysis. The median age of the healthy participants was 63 years (range: 44–75 years), and the majority were also female (53.12%). The mean preoperative UA level of the healthy participants was 327.36 ± 10.57 μmol/L. UA values were positively correlated with the improvement rate

TABLE 2 Region of interests (ROIs) where DC values correlate with uric acid (UA) values.

| Index | ROIs | *r* | *P*-value |
|---|---|---|---|
| 3 | Frontal_Sup_2_L | 0.444* | 0.011 |
| 4 | Frontal_Sup_2_R | 0.425* | 0.015 |
| 5 | Frontal_Mid_2_L | 0.398* | 0.024 |
| 20 | Frontal_Sup_Medial_R | 0.394* | 0.026 |
| 83 | Heschl_L | −0.350* | 0.049 |
| 101 | Cerebellum_4_5_L | −0.364* | 0.041 |
| 109 | Cerebellum_9_L | −0.359* | 0.044 |
| 110 | Cerebellum_9_R | −0.431* | 0.014 |
| 111 | Cerebellum_10_L | −0.365* | 0.040 |
| 112 | Cerebellum_10_R | −0.415* | 0.018 |
| 119 | Vermis_9 | −0.423* | 0.016 |
| 120 | Vermis_10 | −0.368* | 0.038 |
| 123 | Thal_LP_L | −0.369* | 0.038 |
| 135 | Thal_MDm_L | −0.405* | 0.021 |
| 136 | Thal_MDm_R | −0.380* | 0.032 |

*P < 0.05.

of the UPDRS-III score two years after surgery (Figure 1, Supplementary Tables 2, 3).

## 3.2. Brain connectivity estimation

Six PD subjects were excluded because the structural image data dimensions were not consistent with those of the other subjects. In addition, one of the HC subjects was excluded because of head movement of more than 3.0 mm or 3°. A total of 32 subjects with PD and 31 HCs were included in the final fMRI data analysis.

The UA values of PD subjects were correlated with the DC values in 164 brain regions. The 164 correlated values were FDR corrected, DC values were correlated with UA values in 15 brain regions in the Table 2. The 15 brain regions in which the DC values were significantly correlated with UA values were used as ROIs (Figure 2). The zFC matrices were subsequently calculated for each participant by calculating the functional connectivity values of the ROI. A two-sample *t*-test was then performed using the zFC in the PD and HC groups, and the *t*-test results were FDR corrected to $p < 0.05$ to obtain the *t*-value matrix of functional connectivity between the two groups, as shown in Figure 3. 4 ROI-pair FC from the lower triangular part of the matrix were retained (redundant elements and diagonal elements were excluded) in a 15 × 15 matrix, namely, the ROIs with a significant correlation between DC and UA (Figure 3 and Table 3). Intergroup differences in FC between the PD and HC groups are shown in Figure 4.

## 3.3. Prediction and validation of SVR

The functional connectivity values of PD groups come from differences between groups as features (These ROIs for functional connectivity were selected from significant correlation between DC and UA), the Pearson's correlation coefficient was calculated between the actual improvement rate and the predicted improvement rate ($r = 0.487$, $p < 0.005$, MSE = 0.173) (Figure 5). This shows that using the SVR model, serum UA-related differential brain function connectivity in patients with PD can predict the improvement rate of motor symptoms following STN-DBS.

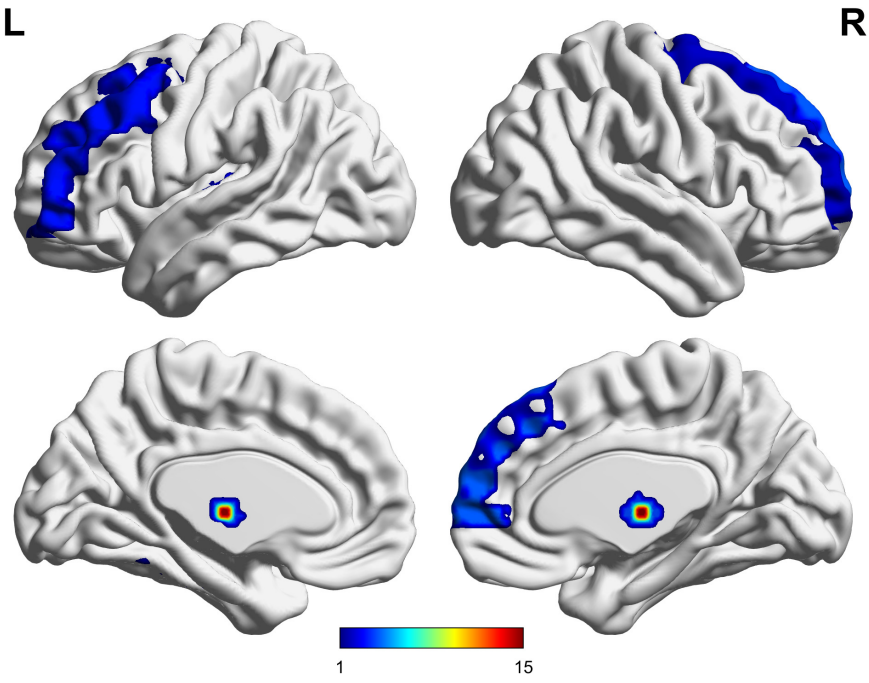


FIGURE 2
Functional region of interests (ROIs) used in the study.

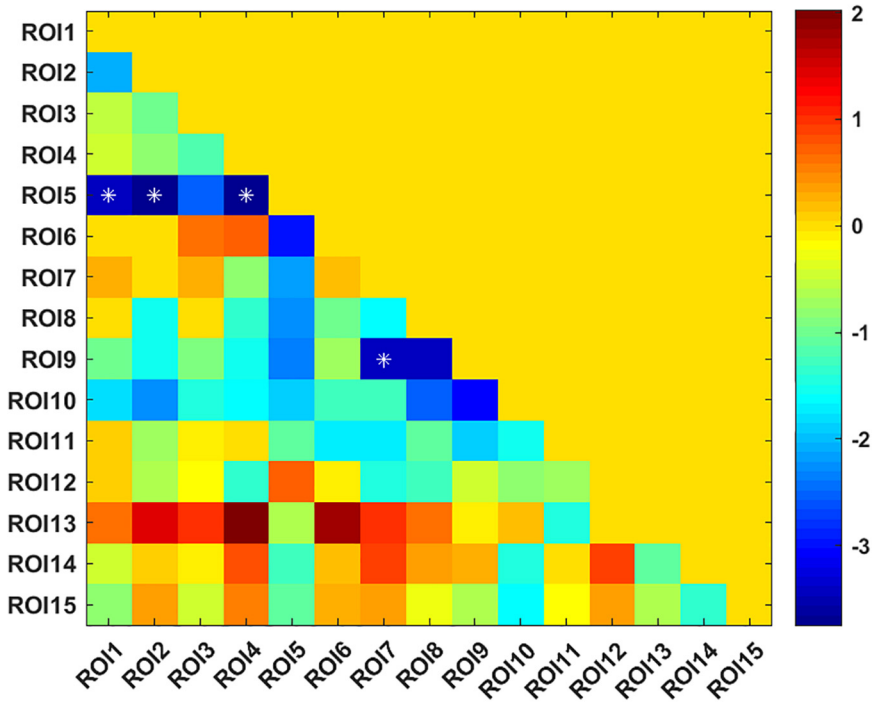


FIGURE 3
*T*-value matrix of functional connectivity between the Parkinson's disease (PD) and healthy controls (HC) groups. 4 ROI-pair significant FCs (marked as *) from the lower triangular part of the matrix were retained (redundant elements and diagonal elements were excluded) in a 15 × 15 matrix, namely, the ROIs with significant correlation between DC and uric acid (UA).

## 4. Discussion

STN-DBS is an accepted treatment for a variety of motor disorders, especially PD. STN-DBS has shown long-term efficacy and has been used in patients with advanced PD for many years (Ashkan et al., 2017). Although the effectiveness of STN-DBS in treating PD is satisfactory, its mechanism needs to be further clarified. Previous studies have found that the prognosis of STN-DBS is associated with brain connectivity. A previous study based on preoperative diffuse tensor imaging (DTI) in patients with PD found that the regions of the brain most associated with the efficacy of STN-DBS include the thalamus, nigra, brainstem and superior frontal gyrus (Vanegas-Arroyave et al., 2016). In addition, functional connectivity can be assessed by the blood oxygen level dependent sequence (BOLD) of rs-fMRI. Many rs-fMRI-based studies have shown that STN-DBS regulates all major components of the motor cortex-striatum-thalamus-cortex loop, including the cortex-striatum, thalamus-cortex, and direct and indirect basal ganglion pathways (Kahan et al., 2014). Also DBS is an expensive and complex treatment. Prior to STN-DBS, doctors conduct a detailed assessment of PD patients to select the most appropriate patient to ensure the best response to DBS. Therefore, some studies attempt to predict results on the basis of brain connections. Some studies have found that ultradirectional, direct and basal intake of STN can predict the clinical status and therapeutic response of DBS (Horn et al., 2017). However, DTI or fMRI images are not always available for various reasons; Therefore, some researchers try to predict the prognosis on the basis of the common connection group. Interestingly, studies have shown that structural and functional connectivity is a predictor of clinical improvement and estimated responses in individual patients, with significant errors (Schlesinger and Schlesinger, 2008). Therefore, predicting the prognosis of STN-DBS based on machine learning synthesis of blood biomarkers and rs-fMRI may be a new direction.

Uric acid has been shown to play an important role as a natural antioxidant in the development and progression of PD. However, the effect of UA on the efficacy of STN-DBS in treatment of PD remains unknown. Interestingly, we observed a positive correlation between UA levels and the rate of improvement in motor symptoms in patients with PD following STN-DBS. Previous studies have similarly demonstrated that low levels of serum UA are involved in the pathogenesis and progression of PD, although its sensitivity as a single biomarker for PD is low (Li et al., 2017; Koros et al., 2021). Similarly, previous studies based on rs-fMRI showed a close relationship between UA and WM and FC integrity in patients with PD. Based on this, we organically combine UA, a biomarker for PD patients, with rs-fMRI and predict the prognosis of STN-DBS by machine learning. DC describes the strength of the brain network connection between an individual protein and all voxels of the whole brain, indicating

TABLE 3 Compared with healthy controls (HC) group, there were significant differences in DC-ROI-pair function connectivity (FC).

| FC | *t*-value | *P*-value |
|---|---|---|
| Frontal_Sup_2_L–Heschl_L | −3.402 | 0.001 |
| Frontal_Sup_2_R–Heschl_L | −3.745 | <0.001 |
| Frontal_Sup_Medial_R–Heschl_L | −3.704 | <0.001 |
| Cerebellum_9_L–Cerebellum_10_L | −3.473 | <0.001 |

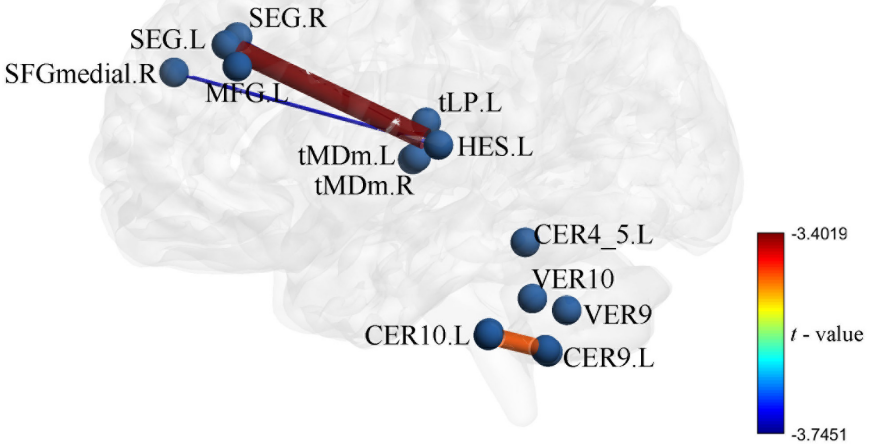


FIGURE 4
Intergroup differences in functional connectivity between Parkinson's disease (PD) and healthy controls (HC) groups the zFC pattern of 4 function connectivitys (FCs) between PD and HC, these FCs were significantly correlation in DC and uric acid (UA).

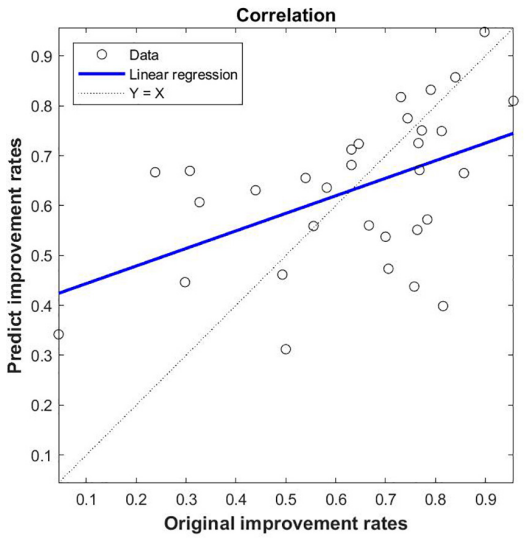


FIGURE 5
SVR-based prediction of uric acid (UA)-related function connectivity (FC) on the rate of improvement of motor symptoms in patients with Parkinson's disease (PD) following STN-DBS. The predicted value of SVR was correlated with the original value; the feature of SVR was that the zFC of PD was different between PD and HC, and the ROIs were significantly correlated with DC and UA.

the importance of this voxel as a network node. We selected DC and serum UA values as characteristics of ROI in PD patients to build FC, using the SVR model for machine learning. Through these explorations, we found that the FCs of Frontal_Sup_2_L–Heschl_L, Frontal_Sup_2_R–Heschl_L, Frontal_Sup_Medial_R–Heschl_L, Cerebellum_9_L–Cerebellum_10_L can predict the prognosis of STN-DBS.

Our research has several novel aspects. First, we identified a set of possible imaging biomarkers prior to STN-DBS treatment to predict the clinical response 1 year after treatment. Therefore, the establishment of pre-operative predictor of therapeutic response has important clinical value, which helps neurosurgeons to predict the efficacy and screen patients before DBS. Secondly, ROI method is widely used in previous research. This approach focuses on selected brain regions, but may omit other brain regions that are critical to the underlying pathophysiology of PD (Gong et al., 2014). In contrast, the DC analysis was used in our study, which used the strength of brain network connections between individual and all voxels of the whole brain. Third, we combine rs-fMRI with UA, a PD blood biomarker, to improve the predictive performance of neuroimaging. Fourth, most previous studies that attempted to determine predictors of therapeutic responses used univariate statistics, which applied to group level predictions (Koros et al., 2021); Instead, SVR analysis, the pattern classification technique used in our study, is a promising individual level prediction tool (Redlich et al., 2016).

In this study, global signal regression and scrubbing were not used to process the data. Previous research has shown that global signal regression can cause reductions in sensitivity and can introduce false deactivations in studies of task activation since the assumption of orthogonality can be violated when the experimentally induced activations contaminate the global signal (Murphy et al., 2009). In additional, discarding problematic volumes (scan "scrubbing"), or alternatively including spike regressors to act as catch-alls for non-linear and non-quadratic spin history effects at problematic time points provides further defense from motion-induced artifacts. However, results have been mixed as to whether any of these participant-level motion correction approaches completely remove inter-individual differences in motion-related MR signal changes (Muschelli et al., 2014).

The present study has several limitations. First, our current study is retrospective and lacks reproducibility analysis (testing the same individual under the same conditions at two different time points). Therefore, it may be a potential confounder of unknown significance. As mentioned above, our study was retrospective; therefore, the required sample size and statistical efficacy were not estimated at

this time, and predictive analyses were performed after collection of follow-up information. In addition, we did not collect postoperative fMRI data because of possible artifacts and MRI heating of the implant. Therefore, for safety reasons, we recommend performing long-term follow-up prior to postoperative data collection.

## 5. Conclusion

The results of the present rs-fMRI-based analysis showed that UA-related FCs in patients with PD are closely related to the prognosis of STN-DBS, and can predict the prognosis of STN-DBS by machine learning. Effective tools are provided for neurosurgeons to screen the best patient candidates and to predict patient outcomes.

## Data availability statement

The original contributions presented in this study are included in this article/**Supplementary material**, further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital of USTC. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

BC and CN jointly completed the experiment and the writing. CX, JM, PC, and MJ assisted in the writing and followed up patients. CSN took overall control of the whole study. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1105107/full#supplementary-material

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# Outcome of visuospatial dysfunction assessment in patients with Parkinson's disease using mobile application software

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**Background:** Visuospatial dysfunction and cognitive impairment are common in Parkinson's disease (PD), which draw increasing attention in the current literature. But clinicians still lack rapid, effective and unified cognitive battery for visuospatial assessment.

**Objective:** A new approach was studied to explore the feasibility of using mobile application software (APP) to evaluate visuospatial dysfunction in patients with PD and compared with traditional assessment tools. We aimed to verify the threshold score of the APP for early diagnosis.

**Materials and methods:** A total of 41 patients with PD underwent assessments using several test modules including Digit Symbol Test (DST), Visual Organization Test (VOT), Facial Recognition Test (FRT), Vocabulary Memory Test (VMT) of this APP, as well as Clock Drawing Test (CDT), Cube Copying Test (CCT) and the Mini-Mental State Examination (MMSE) for comparison. Among the 41 PD patients, 30 individuals were found to have visuospatial dysfunction based on CDT score<5 and CCT score of<18 while the remaining 11 patients served as control.

**Results:** There were statistically significant differences in DST, VOT, and FRT scores (all $p \leq 0.001$ for group comparisons). DST, VOT, and FRT-1 were significantly correlated with MMSE, CDT and CCT and the correlations were moderate or fairly strong. For visuospatial dysfunction diagnosis, all the areas under curves (AUC) of DST, VOT, and FRT-1 were statistically significant ($p<0.0001$, $p=0.0002$, and $p=0.0002$, respectively). The estimates and 95% confidence intervals of AUC were 0.8303 (0.6868, 0.9739), 0.8045 (0.6423, 0.9668), and 0.7833 (0.6344, 0.9322), respectively. Their cut-off points for visuospatial dysfunction were 26, 17, and 19, respectively. After dichotomization by the cut-off points, DST had high sensitivity of 96.67% while VOT and FRT-1 had high specificity of 81.82 and 90.91%.

**Conclusion:** This study demonstrated that visuospatial disorders was highly prevalent in PD patients, and the APP used in study could be a practical clinical screening tool for visuospatial ability assessment with high sensitivity and specificity.

KEYWORDS

**Parkinson's disease, cognitive impairment, visuospatial dysfunction, mobile application, clock drawing test, cube copying test**

# 1. Introduction

Parkinson's disease is a multisystem neurodegenerative disease with motor symptoms characterized by resting tremor, bradykinesia, muscle rigidity, and postural gait abnormality (Yang et al., 2016). In addition to motor impairments of patients with PD present, non-motor impairments manifest a variety of neuropsychiatric symptoms mainly including sleep, behavior and cognition. The impairments in cognitive functions, such as memory, executive function, visuospatial skills and language in PD, are drawing increasing attention in the current literature (Aarsland et al., 2021). Non-motor symptoms are predictive of decreased ability to perform daily living, especially visuospatial impairment, which is distinguished by its early appearance, divided into visuospatial functions impairment and visuospatial cognition impairment. Previous cross-sectional studies have shown that PD patients may have deficits in executive functioning, concentration, facial recognition, recent and working memory (Tachibana, 2013; Galtier et al., 2014). Various neuropsychological tests are available for diagnosing visuospatial impairment, however, motor symptoms including tremor and muscle rigidity can be challenging for the diagnostic procedure in PD patients. CDT, CCT, and MMSE are traditional screening instruments for dementia as a measure of visuospatial dysfunction, but requires fine motor ability. In order to reduce the bias of motor factors, mobile apps were developed as a screening tool for cognition impairment to investigate the characteristics, distribution and possible related factors of visuospatial impairment in PD patients.

# 2. Materials and methods

## 2.1. Study design and population

We consecutively enrolled patients who visited our study group for PD between November 2021 and September 2022. Eligible patients were those who were diagnosed with PD according to the International Parkinson and Movement Disorder Society (MDS) criteria (Postuma et al., 2015). Exclusion criteria were any neurological disorder other than PD including parkinsonism secondary to trauma or drugs, metabolic diseases, encephalitis, progressive supranuclear palsy, essential tremor, and hepatolenticular degeneration. All eligible patients underwent assessment *via* APP tests including DST, VOT, FRT, and VMT in the APP with raw scores recorded, at the same time, CDT, CCT, and MMSE were also evaluated as classic evaluation tools for comparison. Patients with the CDT score of 5 (Spenciere et al., 2017) and the CCT score $\geq$ 18 (Bu et al., 2013) were classified to no visuospatial disorder group, while patients with the CDT score $<$ 5 or CCT score $<$ 18 were classified to visuospatial disorder group. Information on patients' demographic characteristics and clinical profile were collected from medical records. This study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of China-Japan friendship Hospital (2020-129-K82). All participants gave their informed consent to participate in the study in written form.

## 2.2. Neuropsychological assessment

A cognitive assessment application developed by Dr. Xiaodong Pan, Department of Neurology, Fujian Medical University Union Hospital was used for tests, including DST, VOT, FRT, and VMT. These tests could assess visual acuity, visual speed of processing and attention, visual and verbal memory, visual constructional abilities and executive functions. The assessment application could be downloaded and used through the Android App Market and Apple App Market for free.

### 2.2.1. Digit symbol test

Digit symbol test (DST) was conducted to assess visual processing speed, visual shape judgment and motor coordination through the association of numbers and symbols. Numbers 1–9 each correspond to a symbol. The participant was required to select the symbol matching the number on the screen as soon as possible within 1 min according to the given list of numbers and symbols. The software automatically scored according to the number of correct selections and the full score was 54.

### 2.2.2. Visual organization test

Visual organization test (VOT) was conducted to assess visual constructional ability and mental rotation function. In this test, a complete object picture was divided into several parts by image segmentation and rotation. The participant was asked to identify local features or abstract combinations, and then select the appropriate answer. The software automatically scored based on the number of correct selections and the full score was 30.

### 2.2.3. Facial recognition test

Facial recognition test (FRT) was conducted to assess facial recognition and visual perception. In this study, the spatial structure cognitive ability and emotional perception experience ability of facial features as the main characteristics were evaluated through the recognition of facial expressions. The first stage (FRT-1) was to choose the appropriate expression option according to the photo of face, a total of 24 questions meaning full score was 24. In the second stage (FRT-2), according to the given emoticon instruction, two corresponding facial photos were selected from eight similar pictures, a total of 16 questions meaning full score was 16. The software automatically scored based on the number of correct selections.

### 2.2.4. Vocabulary memory test

Vocabulary memory test (VMT) was conducted to evaluate the ability of visual and verbal memory by memorizing a limited vocabulary through rapid browsing. The participant would read 12 words in 24 s in turn, each word appearing once on the screen, and then confirm which words have been read before in 24 words including 12 new words and 12 remembered words. The software automatically scored based on the number of correct answers and full score was 24.

### 2.2.5. Clock drawing test

Clock drawing test (CDT) which had more than one version with different scoring methods seemed to be impacted quite early in the decline process of cognitive in PD. The 5-item score Shulman system was considered as an accurate method for the general use in the diagnosis of PD, requiring substantial understanding of its scoring system (Park et al., 2018). The participant was required to draw a digital clock on a white paper, with the clock indicating "10 min past 11 h" with no concern about the speed. The Shulman system indicated that a score of 5 for a perfect clock, a score of 4 for a slight visuospatial error, a score of 3 for an inaccurate representation of 10 min past 11 h when the visuospatial organization was well done, a score of 2 for

moderate visuospatial disorganization of numbers such that accurate denotation of 10 min past 11 h was impossible, a score of 1 for a severe visuospatial disorganization, and a score of 0 for no reasonable representation of the clock could be made (Shulman, 2000).

#### 2.2.6. Cube copying test

The evaluation of the Cube copying test (CCT) was based on the cube assessment of Maeshima (Maeshima et al., 2004). In this test, the connections and lines in the cube were evaluated. A connection point was defined as a point where 3 lines intersect to form a vertex. Lines less than 3 mm from this point were considered accurate. Because a cube consists of 8 connections and 12 parallel lines, patients could get up to 20 points (8 + 12). The cube-copying task, which mainly measured visuospatial ability (Palmqvist et al., 2008) and motor dysfunction (Bu et al., 2013), had been shown to be deteriorated in PD.

#### 2.2.7. Mini-mental state examination

As a widely known cognitive assessment tool, MMSE was used to assess the cognitive status of people at high risk of dementia, such as AD and PD patients. But MMSE had been criticized for its lack of sensitivity, especially in mild cases of PD (Snyder et al., 2021). A normative study of Chinese elderly population showed that the optimal cut-off points for dementia screening were 16/17 for illiterate (sensitivity 87.6% and specificity 80.8%), 19/20 for individuals with 1–6 years of education (sensitivity 93.6% and specificity 92.7%), and 23/24 for individuals with 7 or more years of education (sensitivity 94.3% and specificity 94.3%; Li et al., 2016).

### 2.3. Statistical analysis

The statistical analyses of the patients were summarized in the tables and figures to provide detailed information. Data were represented as number (percentage) for categorical variables and mean ± SD for continuous variables where appropriate. To compare differences in demographic information, clinical profile, and visuospatial assessment scores, two independent *T*-test were conducted for normally distributed continuous variables while non-parametric Wilcoxon rank sum test was used for non-normally distributed ones. Chi-square or Fisher' exact test was utilized for categorical variables. Correlations between APP assessments and MMSE, CCT, and CDT were quantified using Pearson or Spearman correlation coefficients based on distribution of variables. Areas under the curve (AUC) of moderately or strongly correlated APP assessments for visuospatial disorder were tested and their cut-off points with optimal Youden index were determined. Sensitivity, specificity, positive and negative prediction were estimated. All statistical analyses were performed by using SAS V9.4 (SAS Institute, Cary, North Carolina, United States).

## 3. Results

### 3.1. Demographic data and cognitive evaluations

The demographic data, clinical profiles and cognitive assessment outcomes of the 41 patients were summarized in Table 1. Among the study patients, 30 individuals were grouped as visuospatial disorder while the resting 11 patients were classified as non-visuospatial disorder group. Age, gender, and education level did not differ statistically between the two groups. However, there were significant differences in the scores of classic assessments and APP assessments such as DST, VOT and FRT. Group comparison of VMT scores did not reveal significant difference, indicating that visuospatial dysfunction in PD patients was not accompanied by word transient memory disorders.

### 3.2. Exploratory correlation analysis between APP assessments and classic tests scores

DST, VOT, and FRT-1 were significantly correlated with MMSE, CCT and CDT with strong or moderate correlations (Table 2). The correlation coefficients of FRT-1 with MMSE, CCT and CDT were consistently higher than those of FRT-2 with those classic indicators, particularly with CDT. Thus, we analyzed FRT-1 in the following ROC analysis instead of FRT-2. Figures 1–3 illustrated ROC curves of the three metrics for visuospatial disorder diagnosis. All the AUCs of DST, VOT, and FRT-1 were significantly different from 0.5 ($p < 0.0001$, $p = 0.0002$, and $p = 0.0002$, respectively). Their estimates and 95% confidence intervals were 0.8303 (0.6868, 0.9739), 0.8045 (0.6423, 0.9668), and 0.7833 (0.6344, 0.9322) respectively. Comparison of AUCs between DST and FRT-1 demonstrated insignificant differences ($p = 0.5026$) and AUC of VOT did not differ from that of FRT-1, neither ($p = 0.8251$). The cut-off points of DST, VOT, and FRT-1 were 26, 17 and 19, respectively. Based on the thresholds (DST ≤ 26, VOT ≤ 17, FRT-1 ≤ 19), DST had high sensitivity with 0.9667 (0.8278, 0.9992) while VOT and FRT-1 had excellent specificity with 0.8182 (0.4822, 0.9772) and 0.9091 (0.5872, 0.9977). DST had both high positive and negative predictions while the latter two metrics had high positive prediction. It suggested the combination of APP assessments could obtain objective visuospatial ability assessment quantitative scores (Table 3).

## 4. Discussion

Although the mechanism of visuospatial dysfunction in PD patients remains unclear, relevant research results show that visuospatial processing division include dorsal and ventral streams. The dorsal stream starts from the occipital lobe and projects to the parietal lobe, called the occipitoparietal pathway, which is related to the spatial location of objects, and its structure includes bilateral superior parietal cortex and lateral occipital lobe. The ventral stream is the occipitotemporal pathway, which is related to face recognition. These structures include the middle occipital gyrus, the occipitotemporal junction area, the parahippocampal gyrus, etc. The two pathways send fibers directly or indirectly to the prefrontal cortex through the ventroposteromedial thalamic nucleus and the corticospinal tract. The prefrontal cortex plays a role in keeping spatial information updated in real time in visuospatial function (Kravitz et al., 2011).

Recently, symptoms about visuospatial dysfunction in PD patients, such as stumble and becoming lost, have attracted attention

TABLE 1 Demographic, clinical, and visuospatial profile of patients with PD.

| Variable[a] | Value | All *n*=41 | Visuospatial disorder group *n*=30 | None visuospatial disorder group *n*=11 | *p* value |
|---|---|---|---|---|---|
| Demographic characteristics | | | | | |
| Age | | 62.0 ± 9.5 | 63.3 ± 8.5 | 58.2 ± 11.2 | 0.1239 |
| Gender | Male | 20 (48.78%) | 13 (43.33%) | 7 (63.64%) | 0.2492 |
| | Female | 21 (51.22%) | 17 (56.67%) | 4 (36.36%) | |
| Education level | College or higher | 7 (17.07%) | 4 (13.33%) | 3 (27.27%) | 0.3608[b] |
| | High school or lower | 34 (82.93%) | 26 (86.67%) | 8 (72.73%) | |
| Clinical profile | | | | | |
| Disease duration | | 7.0 ± 5.1 | 7.7 ± 5.4 | 5.0 ± 3.4 | 0.1197 |
| Side of disease onset | Bilateral | 7 (17.07%) | 4 (13.33%) | 3 (27.27%) | 0.3681[b] |
| | Right | 18 (43.90%) | 15 (50.00%) | 3 (27.27%) | |
| | Left | 16 (39.02%) | 11 (36.67%) | 5 (45.45%) | |
| Hoehn-Yahr grade | 1 | 4 (9.76%) | 1 (3.33%) | 3 (27.27%) | 0.0466[b] |
| | 1.5 | 2 (4.88%) | 2 (6.67%) | 0 (0.00%) | |
| | 2 | 8 (19.51%) | 4 (13.33%) | 4 (36.36%) | |
| | 2.5 | 7 (17.07%) | 7 (23.33%) | 0 (0.00%) | |
| | 3 | 14 (34.15%) | 10 (33.33%) | 4 (36.36%) | |
| | 3.5 | 1 (2.44%) | 1 (3.33%) | 0 (0.00%) | |
| | 4 | 5 (12.20%) | 5 (16.67%) | 0 (0.00%) | |
| Visuospatial disorder assessment using APP | | | | | |
| Digit Symbol (DST) | | 19.5 ± 9.2 | 16.3 ± 6.7 | 28.3 ± 9.7 | <0.0001 |
| Visual Organization (VOT) | | 17.0 ± 5.2 | 15.5 ± 4.6 | 21.0 ± 4.9 | 0.0019 |
| Facial Recognition-1 (FRT-1) | | 18.9 ± 3.5 | 18.1 ± 3.6 | 21.1 ± 1.9 | 0.0058 |
| Facial Recognition-2 (FRT-2) | | 9.6 ± 3.1 | 8.9 ± 3.0 | 11.5 ± 2.4 | 0.0164 |
| Vocabulary Memory (VMT) | | 16.7 ± 4.2 | 16.2 ± 4.5 | 18.1 ± 2.8 | 0.3130 |
| Visuospatial disorder assessment by classic tools | | | | | |
| MMSE | | 23.9 ± 4.1 | 22.6 ± 4.1 | 27.4 ± 1.3 | <0.0001 |
| CCT | | 14.1 ± 4.0 | 12.3 ± 3.1 | 19.1 ± 0.8 | <0.0001 |
| CDT | 1 | 3 (7.32%) | 3 (10.00%) | 0 (0.00%) | <0.0001[b] |
| | 2 | 6 (14.63%) | 6 (20.00%) | 0 (0.00%) | |
| | 3 | 16 (39.02%) | 16 (53.33%) | 0 (0.00%) | |
| | 4 | 5 (12.20%) | 5 (16.67%) | 0 (0.00%) | |
| | 5 | 11 (26.83%) | 0 (0.00%) | 11 (100.0%) | |

[a]Continuous variables were expressed as mean ± SD. Categorical variables were expressed as number (percentage). [b]Fisher exact test.

as serious social problems. Visuospatial function is impaired from the early phase (Zhang et al., 2020). It is known that the focus of route finding is associated with the striatal dopamine depletion, dopamine transporter availability in the caudate, anterior putamen, and ventral striatum was directly associated with attention/working memory, frontal/executive, and visuospatial functions (Chung et al., 2018). Berlot et al. (2022) evaluated relationships between structure of the cholinergic basal forebrain, medial temporal lobe and cognition by measuring volumes of the cholinergic basal forebrain nuclei, the entorhinal cortex, the hippocampus and its subfields in PD patients and controls. Their data implied that the integrity of the cholinergic basal forebrain was associated with subregional hippocampal volume, and influencing visuospatial function.

It is difficult to find a common cognitive assessment battery across studies. In addition, some instruments traditionally used in PD may not be adequate for use in visuospatial function assessment. More than 53 assessment tools can be used in Parkinson's cognitive impairment including the Montreal Cognitive Assessment (MoCA), the Digit Span, the Trail Making Test, the Semantic Fluency test, the Rey Auditory Verbal Learning Test, the Brief Visuospatial Memory Test-Revised, the Boston Naming Test and the CDT, etc. (SeverianoE Sousa et al., 2022). MMSE and MoCA which including CCT and CDT are practical and efficient screening tools for PD dementia with visuospatial dysfunction (Ohta et al., 2014). In addition, the Pentagon Copying Test, Judgment of Line Orientation Test, Visual Form Discrimination Test, Facial Recognition Test, Symbol Digit Modalities

TABLE 2 Correlation analysis between APP assessments and classic tests scores.

| APP assessments | | Classic tools | | |
|---|---|---|---|---|
| | | MMSE | CCT | CDT |
| DST | Correlation | 0.31807 | 0.56972[a] | 0.56558 |
| | *p* value | 0.0427 | 0.0001 | 0.0001 |
| VOT | Correlation | 0.70403 | 0.59044[a] | 0.58703 |
| | *p* value | <0.0001 | <0.0001 | <0.0001 |
| FRT-1 | Correlation | 0.54962 | 0.43964 | 0.55016 |
| | *p* value | 0.0002 | 0.004 | 0.0002 |
| FRT-2 | Correlation | 0.51772 | 0.42436[a] | 0.44164 |
| | *p* value | 0.0005 | 0.0057 | 0.0038 |
| VMT | Correlation | 0.42423 | 0.22092 | 0.29506 |
| | *p* value | 0.0057 | 0.1651 | 0.0611 |

[a]Pearson correlations between CCT and DST, VOT, FRT-2 were analyzed, respectively given the normally distributed variables while other correlations were spearman correlations due to ordinal variables or non-normal distributions of continuous variables.

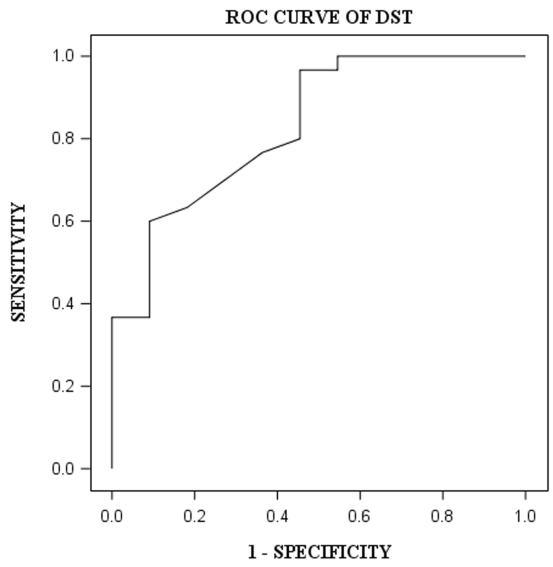


FIGURE 1
ROC curve of DST for visuospatial disorder.

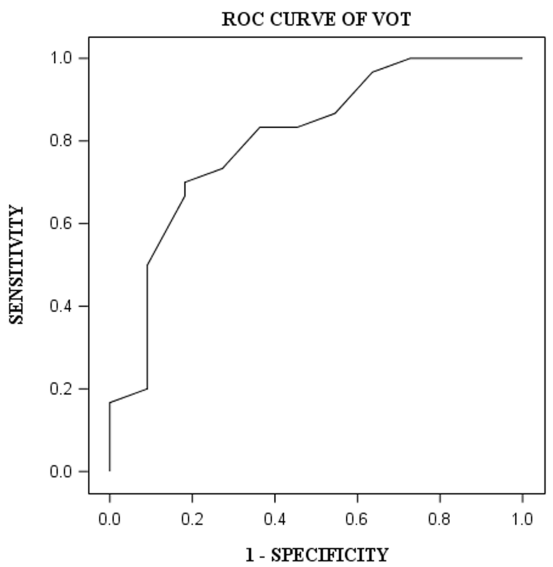


FIGURE 2
ROC curve of VOT for visuospatial disorder.

Test can be selected for visuospatial tests (Garcia-Diaz et al., 2018). As classic assessment tools, CDT and CCT are widely used in the assessment of PD patients because they are easy to understand and requiring less time (Scarpina et al., 2020; Mori et al., 2021; Srivastava et al., 2022). However, their limitations and shortcomings are also obvious. CDT and CCT require complex and delicate movements, which means more difficult to perform for PD patients. It is perplexing to judge whether their test results imply the decline of visuospatial ability or the difficulty of execution caused by motor dysfunction. Some clinicians even think that the visuospatial disorder may not exist if the factors of motor ability decline are removed (Brown and Marsden, 1986). Therefore, the selection of assessment tools with high sensitivity and good specificity can get more objective results and improve the efficiency of clinical work. The APP assessments used in our study could be applied in mobile devices such as mobile phones or tablets, which can facilitate the assessment by clinicians, requiring a smaller range of limb movement and a lower level of delicate movement. For patients, the difficulty of completing tests is significantly reduced by using their fingers to click on the screen compared with drawing clock and copying cube. In the meantime, it has excellent sensitivity and specificity for visuospatial function test.

Cognitive function is affected by many factors. The present study had shown that general cognitive function, executive function, memory, and information processing speed in PD patients were related to educational level, while no significant association was showed between educational level and visuospatial function, language in PD patients (Gu and Xu, 2022). At present, most of the commonly used neuropsychological assessment tools were developed in Anglosphere cultures.

Statucka and Cohn (2019) studied the cognitive function differences between Canadian immigrants and aborigines after diagnosis of PD, and found that the immigrant group showed lower scores and greater rates of deficits on all visuospatial and some executive function tasks, but not on attention or memory measures. These biases could not be explained by demographic and clinical variables as groups were comparable. Because the differences between groups were strongly mediated by the Historical Index of Human Development of the participant's country of birth, which reflects economic, health, and educational potential of a country at the time of birth. The assessment APP used in our study took full account of the cultural differences between China and the West, with less internal correlation on factors such as educational level, cultural differences, ethnic habits, and economic status. The requirements of the APP were simple and feasible for the PD patient to complete, and allowed clinicians to achieve more objective and consistent neurophysiological assessments. Because the evaluation criteria of APP were invariable, which was different from MMSE, CDT and CCT, there had no interference from subjective factors of clinicians.

At present, the treatment of visuospatial disorders is mainly cognitive rehabilitation training, which can be combined with transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) (Veldema et al., 2020), as well as pharmacological treatment and DBS surgery. Pharmacotherapy include dopaminergic and cholinergic treatment. Related studies have shown that there are different outcomes in the effect of dopaminergic treatment, and no unified conclusion can be obtained from those studies. Cholinergic treatment may be the best option for future research (van der Kemp et al., 2017). Because the existing literature is very sparse and studies have various methodological limitations, it is currently not possible to either support or reject the effects of DBS surgery on cognitive function. Some studies reported that DBS might lead to the decline in visual constructive and visuospatial skills, while other articles shown no significant change pre and post-operatively (BarbozaE Barbosa and Fichman, 2019).

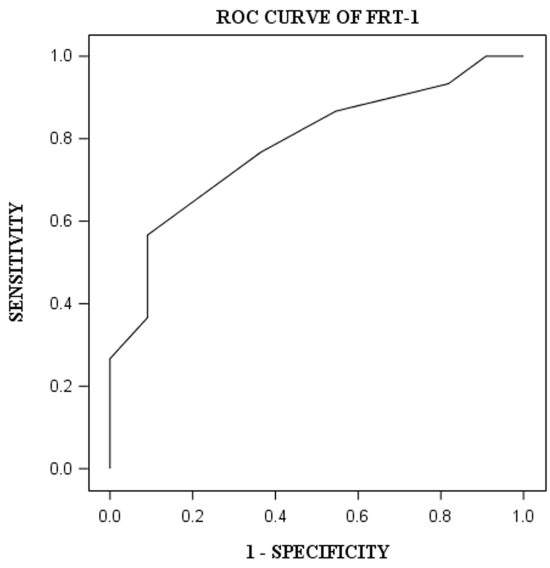


FIGURE 3
ROC curve of FRT-1 for visuospatial disorder.

## 4.1. Limitations

Three limitations of this study must be considered. First, during the COVID-19 pandemic, the government recommended reducing unnecessary social activities, and people preferred reduce the frequency of visits to the hospital. As a result, it was difficult to recruit participants, and the sample size was small. PD patients in this study were from one medical center and the findings may not reflect the state of PD general population. Second, the details of the pharmacotherapy history of participants were not recorded and the possible intrinsic correlation between the timing of the test and the patient's medication schedule was imperceptible. Third, the incidence of visuospatial disorders might vary according to the different neuropsychological assessments used, and the most suitable tool for estimating visuospatial disorders in PD is still a matter of controversy. CDT, CCT and MMSE were chosen as traditional assessment tools, but they may still have some limitations and may lead to bias in the results. In the future, we need more research about consistency of assessment tools in particular and longitudinal studies about possible risk factor associated with visuospatial dysfunction.

# 5. Conclusion

The results of this study showed that the incidence of visuospatial disorders in PD patients was high, and there was still a lack of rapid, effective and unified cognitive assessment battery. Assessments in APP had higher sensitivity and better specificity, which could help clinicians to diagnose PD patients with visuospatial disorders simply and quickly, and we also explored threshold score for diagnosing visuospatial disorders through this APP. These findings could also improve early rehabilitation guidance and pharmacological interventions.

TABLE 3 Sensitivity, specificity, and predictions of DST, VOT, and FRT-1 for visuospatial disorder.

| Variable | Estimate (95% confidence interval*) | | | |
|---|---|---|---|---|
| | Sensitivity | Specificity | Positive prediction | Negative prediction |
| DST ≤26 | 0.9667 (0.8278, 0.9992) | 0.5455 (0.2338, 0.8325) | 0.8529 (0.6894, 0.9505) | 0.8571 (0.4213, 0.9964) |
| VOT ≤17 | 0.7000 (0.5060, 0.8527) | 0.8182 (0.4822, 0.9772) | 0.9130 (0.7196, 0.9893) | 0.5000 (0.2602, 0.7398) |
| FRT-1 ≤ 19 | 0.5667 (0.3743, 0.7454) | 0.9091 (0.5872, 0.9977) | 0.9444 (0.7271, 0.9986) | 0.4348 (0.2319, 0.6551) |

*Exact binomial confidence limits.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of China-Japan friendship Hospital (2020-129-K82). The patients/participants provided their written informed consent to participate in this study.

## Author contributions

XS, HT, and KW contributed to conception and design of the study. YZ and XZ contributed to the data extraction and organized the database. XS and FD contributed to methodology and performed the statistical analysis. HT and YY contributed to supervision, funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1108166/full#supplementary-material

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# Role of the subthalamic nucleus in perceiving and estimating the passage of time

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Sense of time (temporal sense) is believed to be processed by various brain regions in a complex manner, among which the basal ganglia, including the striatum and subthalamic nucleus (STN), play central roles. However, the precise mechanism for processing sense of time has not been clarified. To examine the role of the STN in temporal processing of the sense of time by directly manipulating STN function by switching a deep brain stimulation (DBS) device On/Off in 28 patients with Parkinson's disease undergoing STN-DBS therapy. The test session was performed approximately 20min after switching the DBS device from On to Off or from Off to On. Temporal sense processing was assessed in three different tasks (time reproduction, time production, and bisection). In the three temporal cognitive tasks, switching STN-DBS to Off caused shorter durations to be produced compared with the switching to the On condition in the time production task. In contrast, no effect of STN-DBS was observed in the time bisection or time reproduction tasks. These findings suggest that the STN is involved in the representation process of time duration and that the role of the STN in the sense of time may be limited to the exteriorization of memories formed by experience.

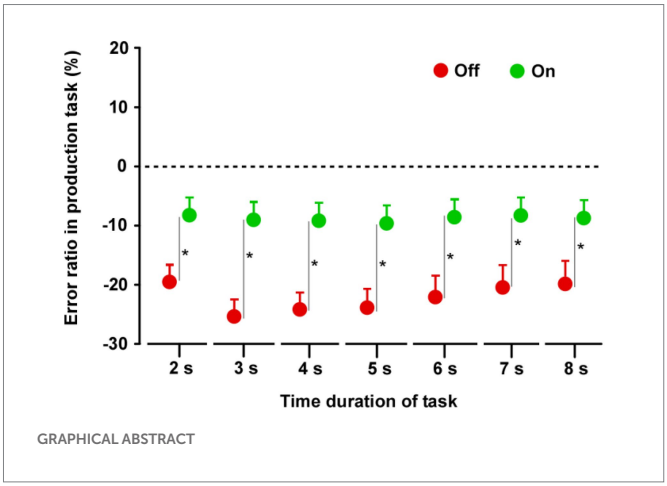
KEYWORDS

**Parkinson's disease, subthalamic nucleus, deep brain stimulation, temporal sense, representation**

## Introduction

Subjective sense of time (temporal sense) is essential for perceiving and estimating the passage of time in daily life. Although the temporal sense is influenced by many factors, including circadian rhythms, emotion, and disease (Hancock et al., 1992; Honma et al., 2016; Mella et al., 2019), we previously showed that temporal sense is robust in each individual and consolidated at a stable value under certain conditions (Honma et al., 2021). This also holds for patients with Parkinson's disease (PD) in whom this is also robust and resistant to change; in the presence of dopamine deficiency, accurate time duration learned by feedback training quickly returns to inherent underestimated levels, and even after applying repetitive transcranial magnetic stimulation over the prefrontal cortex for inducing cortical plasticity and manipulating/consolidating time sense (Honma et al., 2022). This shortened temporal sense or memory representation may be likened to the shuffling gait in PD patients, with steps gradually asymptoting to a smaller level in the absence of an external cue.

Details of temporal processing for the sense of time or its neural correlate, however, have not been clarified. Unlike light and sound, time does not have a dedicated sensory organ. Temporal




GRAPHICAL ABSTRACT

processing may be mediated not by a single or a few brain areas but by a complex network involving multiple brain regions (Buhusi and Meck, 2005; Shi et al., 2013), including the prefrontal cortex, striatum, and subthalamic nucleus (STN) (Nani et al., 2019), but the precise role of individual brain regions remains unresolved. While the brain regions involved in temporal processing largely overlap with those for motor control and sensory perceptual processing, regions serving memory, in this case, the temporal representation of time, may be also involved, consistent with the view that time and memory are closely interlinked (Teki et al., 2017).

Psychologically, the scalar expectancy theory (SET) postulates that temporal sensory processing comprises different processes, including clock (pacemaker, switch, and accumulator), memory (short-term and reference memory), and decision stages (Gibbon, 1977; Gibbon et al., 1984). It also assumes that different mechanisms may serve different temporal processes, e.g., mechanisms of representation and perception of time. The time production task reflects a function to read out time duration in reference to the representation of time duration (reference memory); the representation of time refers to the sense of time or a kind of "time scale" acquired through what one has experienced and stored in long-term memory as a scale for reference (for example, the time scale for subjective 10-s duration is established by repeated experience of the physical 10-s duration) (Baudouin et al., 2006; Jozefowiez and Machado, 2013). In contrast, the time bisection task reflects perceptual function, a process of perceiving and recognizing the current time with respect to the subjective sense of time learned shortly in advance (Atakan et al., 2012; Ogden et al., 2018). Meanwhile, time perception involves inputting physical time duration into the short-term memory to recognize its duration. In SET, temporal information processing is considered a cognitive process coordinating time perception and memory across a wide range of memory processes both utilizing the internal clock, and the abnormality in temporal production and perception has been explained by the abnormal pace of the internal clock. This may be addressed by evaluating time processing and perception functions at the same time, but few studies have studied both simultaneously in the same study.

PD patients, in whom basal ganglia dysfunction with dopamine deficiency likely causes bradykinesia (slowness of movements), have pronounced deficits in temporal processing compared with normal participants (Smith et al., 2007; Honma et al., 2017, 2018). Slowness may also involve the mind's temporal processing. The basal ganglia have been postulated to set the pace of the "internal clock." If we postulate that the mind uses an internal clock ticking at a regular rate to perceive the passage of time, it would tick more slowly in dopamine deficiency. Earlier studies have provided evidence consistent with the slowed clock hypothesis, which states that dopamine deficiency slows down the pace of the internal clock, which is corrected by dopaminergic medication improving the estimation of duration in the time production task in PD patients (Pastor et al., 1992; Lange et al., 1995; Smith et al., 2007; Koch et al., 2008; Wild-Wall et al., 2008). However, later studies have not necessarily supported this view. For example, in the time production task, PD patients evaluate (produce a specified time duration) the subjective time duration as shorter than normal participants (Honma et al., 2016). When PD patients estimate the duration of the period that a figure is visible on a screen as shorter or longer relative to two standard durations, PD patients are more likely to judge the duration as longer compared with healthy participants (Zhang et al., 2016). The pace of the internal clock can also be studied by the synchronized tapping task, which requires participants to press a button or tap a keyboard in synchrony with repetitive tones presented at fixed intervals (synchronization task, S) and to continue tapping at the same pace after the tones have been removed (continuation task, C), have found inconsistent results, reporting the pace of the internal clock to be faster (Ivry and Keele, 1989; O'Boyle et al., 1996; Harrington et al., 1998; Jones et al., 2011), slower (Pastor et al., 1992), or unchanged (Duchek et al., 1994; Spencer and Ivry, 2005; Wojtecki et al., 2011; Joundi et al., 2012) relative to normal participants. While temporal processing deficits in parkinsonism remain to be characterized, dopamine deficiency may not be the sole mechanism leading to the various temporal processing deficits in PD patients. Finally, in terms of the sense of time, some PD patients exhibit short production of duration compared to actual time, indicating a "faster" flow of time (Honma et al., 2018). Many findings are thus difficult to explain simply by the slowed clock hypothesis (Terao et al., 2021), and revision of the SET view should be considered. Additionally, because dopaminergic medication operates on various brain regions, it is difficult to verify the role of each region(s) alone play a critical role in temporal sensory processing and how (the cause-and-effect relationship) (Pastor et al., 1992; Nani et al., 2019).

Recently, deep brain stimulation (DBS) of the subthalamic nucleus (STN), playing a physiologically pivotal role in the pathomechanism of PD (Nambu, 2004; Wichmann and Soares, 2006), has come to be used widely for reducing PD patients' motor symptoms (Sasaki et al., 2021; Tai, 2022). The inconsistent findings regarding dopamine deficiency and the pace of the internal clock can be addressed by manipulating the function of the STN, providing novel insights into temporal processing in terms of the internal clock and temporal sensory processing (temporal sense). STN-DBS also affects cognitive functions (Oyama et al., 2011; Tokushige et al., 2018) by altering the function of the basal ganglia-thalamo-cortical loop (Santaniello et al., 2012). A study of the effect of STN-DBS on temporal sense in PD showed that STN-DBS had no significant effect on perceptual timing in the hundreds of milliseconds range, unlike its effect on motor symptoms (Cope et al., 2014). STN-DBS also has a significant effect on the time reproduction task to measure the ability of short-term memory unrelated to internal clock (Koch et al., 2004). However, it is unclear whether STN-DBS affects temporal sense in the few seconds range, in which memory and other factors are likely to interact.

Animals and humans can process different ranges of timescales, ranging from microseconds, milliseconds, seconds to minutes, and a day (circadian rhythms), and it has been suggested that the neural structures responsible for temporal processing differs for these different time rages (Merchant and de Lafuente, 2014). Although these systems for different timescales may all contribute to the formation of the sense of time, in

this study, we focused on the time scale of seconds to minutes range, which is considered to be closely associated with and processed within the motor system such as the basal ganglia and the cerebellum. We investigated the role of the STN in temporal sense processing by looking at what happens when the DBS device is switched on/off in PD patients receiving STN-DBS.

Three temporal processing tasks have been widely used to address distinct aspects of time perception. In the production task, subjects produce the duration of time instructed verbally, according to time scale formed by experienced and stored in memory, but does not require the ability to discriminate different time durations; in the reproduction task subjects are asked to reproduce the presented duration, for which it is neither required that the time scale stored in reference memory or that the ability to discriminate different duration is normal. In the bisection task to ask subjects whether the immediate duration of time presented is longer/shorter compared to the immediately preceding one (discrimination between different durations), whereas it does not depend on whether or not the reference duration formed by experience and stored in memory is normal.

By comparing performance of temporal cognitive tasks, we investigated whether STN DBS affects the ability referring to time duration formed by experience and stored in memory, the ability to discriminate different durations, of the ability to reproduce different durations, or any combination thereof. We predicted that performance is improved in time production task if DBS-STN affects the ability of reference duration formed by experience. Alternatively, if DBS-STN affects the ability to discriminate differences of duration, performance should be improved in the time bisection task. Finally, if DBS-STN affects the ability to reproduce duration, performance is improved in time reproduction task.

# Materials and methods

## Participants

This study was approved by the ethics committee of Juntendo University School of Medicine and conducted according to the principles of the Declaration of Helsinki (identifier: 18-215). This study was registered in the University hospital Medical Information Network (UMIN)-CTR (ID: UMIN000033776, 20/08/2018). All patients provided written informed consent before the experiments. G*Power (Version 3.1.9) specified that a sample size of 27 would be needed to obtain 70% power to detect a medium effect with an alpha of 0.05. Effect size (0.50) was determined by previous researches using temporal task (Honma et al., 2016, 2017, 2018, 2021, 2022; Terao et al., 2021).

There were 28 PD patients with an implanted DBS device (4 women and 24 men; mean age: 62.7 years, range: 51–74 years). The average duration of illness was 14.5 ± 3.7 years. All patients were right-hand dominant. PD severity was measured using the Unified Parkinson's Disease Rating Scale-part III (Martinez-Martin et al., 1994) (average: 19.1 ± 7.8). We also examined general cognitive functions using the Mini-Mental Status Examination (Folstein et al., 1975) (28.6 ± 1.3) and Montreal Cognitive Assessment (Nasreddine et al., 2005) (26.8 ± 2.7). The neurologist diagnosed that none of the participants had dementia. All patients were tested for dopamine transporter (DaT) activity using DaT imaging (Kagi et al., 2010). The radioactive agent bound to DaT was expressed using a specific binding ratio, which is the ratio of the radiation in the striatum to those in the whole brain, calculated by the Bolt method (Tossici-Bolt et al., 2006). The average value of DaT was 1.78 in total (range: 0.11–4.64), and 1.83 in the right (0–4.83, SD = 1.8), and 1.72 in the left (0.22–4.44, SD = 1.7). Parkinson's disease-related medications were discontinued at least 12 h before the tests were performed. Some subjects also had comorbid symptoms or diseases other than PD (Supplementary Figures).

All patients underwent surgery for bilateral implantation of stimulation electrodes (Model 3,389, Medtronic, Minneapolis, MN, United States) in the STN (13 patients, Vercise Gevia, Boston Scientific, Boston, MA, United States; 3 patients, Vercise PC, Boston Scientific, Boston, MA, United States; 7 patients, Vercise Genus RC, Boston Scientific, Boston, MA, United States; 1 patient, Activa RC, Medtronic, Minneapolis, MN, United States; 2 patients, Activa SC, Medtronic, Minneapolis, MN, United States; 2 patients, Percept PC, Medtronic, Minneapolis, MN, United States) (Supplementary Table S1). The average months since implantation was 15.86 ± 16.8 months. During the study, the parameters were optimized for anti-Parkinson therapy. Stimulation amperes were [right: 1.5–3.5 (2.6 ± 0.5) mA; left: 1.4–3.2 (2.5 ± 0.5) mA] and Hertz [right: 130–200 (135.9 ± 17.8) Hz; left: 130–200 (135.6 ± 16.8) Hz]. Twenty patients showed symptoms predominantly on the right side.

## Study design

In this study, a prospective, single-blinded and within-subject repeated measures design was used to investigate and compare the effects of DBS. The participants were divided into two groups (groups A and B), in a randomized manner, to assess the effects of order and repetition on the same tasks. In group A, the test was conducted thrice in the order of On-1 (first test of On condition of STN-DBS), Off, and On-2 (second test of On condition of STN-DBS). In group B, the test was conducted two times in the order of Off and On (On and Off conditions performed once each, Figure 1). The next test session was performed approximately 20 min after switching the DBS device from On to Off or from Off to On. Each time, the neurologist confirmed whether the effects of DBS were clearly lost when DBS was switched Off or emerged when it was switched On based on the patients' symptoms, including tremor at rest, muscle rigidity, akinesia, and postural maintenance. The same five tasks were conducted in each session. Temporal sense processing was assessed in three different tasks (time reproduction, time production, and bisection). Additionally, length production and simple reaction tasks were conducted.

## Procedures

In the time production task, the duration of the interval to be produced was presented on the monitor screen as a number of seconds for 3 s at the beginning of each trial. Patients were not informed the duration of the cue. After the number presentation disappeared from the screen, patients produced the instructed time duration by pressing the button twice at the start and end of the duration, such that the time interval between the first two and last two button presses corresponded to the required duration (Supplementary Figure S1A). The durations to be produced were 2, 3, 4, 5, 6, 7, and 8 s. Patients were not provided with feedback on their produced duration. Each duration was repeated thrice (total: 21 repetitions), with the trial order randomized and counterbalanced among participants. Data for the time production task

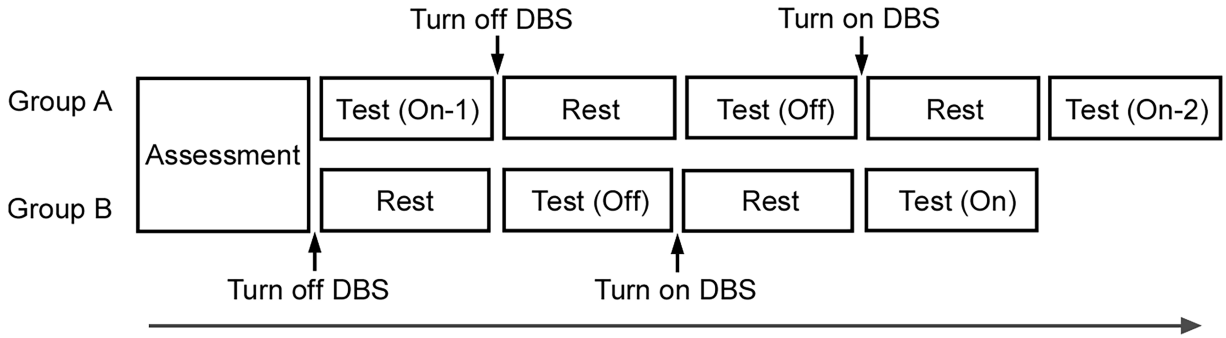



FIGURE 1
Experimental design. After conducting the assessment, in group A, the test was conducted thrice in the order of On-1, Off, and On-2. In group B, the test was conducted twice in the order of Off and On. After approximately 20min of turning the DBS device On or Off, the next test was performed. The same five tasks were conducted in a test.

were calculated by the duration produced compared to the specified number of seconds, expressed as a percentage.

The time bisection task comprised two phases: learning and test (Supplementary Figure S1B). In the learning phase, circles appeared on the screen for a long (8 s) or short duration (2 s). These were considered the "standard durations." During the learning phase, each standard (long and short) was shown on the screen 10 times, for a total of 20 repetitions. In the test phase, the circles were shown for durations of 2, 3, 4, 5, 6, 7, or 8 s. In each trial, subjects were asked to indicate whether the duration shown was "closer to the short standard" or "closer to the long standard." Each duration was repeated five times in the test phase (35 repetitions), and the trial order was randomized. Results for the bisection task were calculated as the proportion of "long" responses shown as a percentage.

The time reproduction task was conducted to examine the role of short-term memory in the sense of time duration. A circle was shown on screen for a specified duration at the beginning of each trial. After the sample disappeared from the screen, patients reproduced the circle presentation duration by pressing the button twice, one for start and another for end, so that the time interval between the two button presses corresponded to the patient's estimate of the duration (Supplementary Figure S1C). The durations of 2, 3, 4, 5, 6, 7, and 8 s were presented in each trial. The patients had no way of knowing the actually presented duration of the circle. Each duration was repeated thrice (total: 21 repetitions), and the trial order was randomized. Results on the time reproduction task were calculated by the duration estimated by the patients compared to the actual duration, expressed as a percentage.

To examine basic motor function, a simple reaction task was conducted. Patients were instructed to press a response button using their dominant hand as soon as a figure (circle) appeared on the computer screen. The same trial was repeated thrice per session. To assess whether DBS affects spatial sense processing, we conducted the length production task. The patients were asked to move a circular figure on the computer screen 10 cm to the right in the absence of any distance measuring cue. The same trial was also repeated thrice per session. Results for the length production task were calculated as the patient's estimate of 10 cm compared to an actual distance of 10 cm, expressed as a percentage.

The order of the three temporal tasks was randomized among patients. The simple reaction and length production tasks were done after the temporal tasks. No feedback was provided to patients in all tasks.

## Statistical analyses

To examine an effect of repetition and trial order of the same tasks, a paired *t*-test was performed to analyze differences between the On-1 and On-2 conditions in each index in group A. Using an unpaired *t*-test, we analyzed the difference between the On-2 condition in group A and that in group B, and the difference between the Off conditions of the two groups. Next, after the On condition (On-2 condition in group A and On condition in group B) and Off condition (Off condition in group A and group B) data were averaged separately, we analyzed differences between the On and Off conditions using the paired *t*-test. All tests were two-tailed. Results are shown as mean ± standard error of the mean (SEM). Statistical significance was set at adjusted $p < 0.05$. SPSS version 26 for Windows (IBM, Inc., Chicago, IL, United States) was used for the analyses.

# Results

## Time production task

In both groups, the produced time durations in the Off condition were shorter than those in the On condition (Supplementary Figure S2). In group A ($n = 14$), there was no difference between the On-1 and On-2 conditions. Furthermore, there was no difference between the On-2 condition in group A and the On condition in group B, nor was there a difference in the Off condition between the two groups. When the On condition (On-2 condition in group A and On condition in group B) and Off condition (Off condition in the groups A and B) data were averaged separately, there were significant differences between the On and Off conditions. The durations in the Off condition were significantly shorter than those in the On condition for all task durations (2, 3, 4, 5, 6, 7, and 8 s) (2 s: $t_{27} = 3.997$, $p < 0.0001$; 3 s: $t_{27} = 7.092$, $p < 0.0001$; 4 s: $t_{27} = 7.649$, $p < 0.0001$; 5 s: $t_{27} = 8.902$, $p < 0.0001$; 6 s: $t_{27} = 8.756$, $p < 0.0001$; 7 s: $t_{27} = 8.729$, $p < 0.0001$; 8 s: $t_{27} = 9.955$, $p < 0.0001$) (Figure 2).

## Time bisection task

In both groups, the proportion of "long" response for the 2 s duration was 0%, and the proportion for 6, 7, and 8 s were 100%, in both

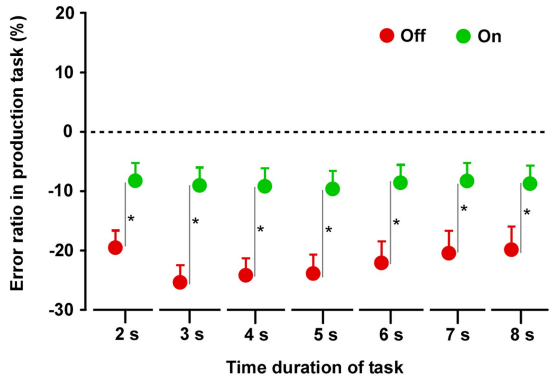



FIGURE 2
Results of the time production task. Paired *t*-tests were performed across the two groups. The duration in the Off condition was shorter than that in the On condition for all task durations. Error bars show standard error mean (SEM). Asterisks indicate significant differences ($p<0.0001$).

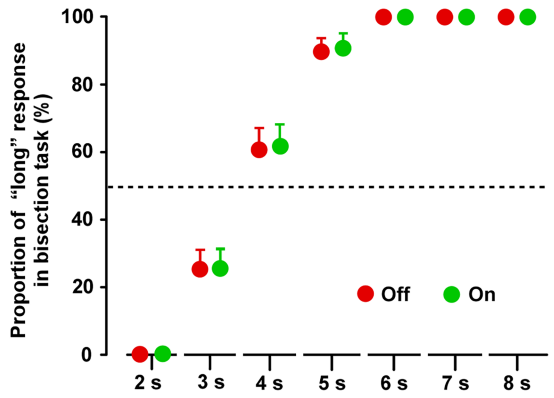



FIGURE 3
Results of the time bisection task. Across the two groups, repeated measures analysis of variance (RM-ANOVA) showed that there were no main effects of STN-DBS for all durations (2, 3, 4, 5, 6, 7, and 8s). Error bars show SEM.

the Off and On conditions (Supplementary Figure S3). In group A, there were no significant differences between the On-1 and On-2 conditions, between the On-2 condition in group A and the On condition in group B, or between the Off condition in the two groups. After the On condition and Off condition data were averaged separately, the paired *t*-test revealed no significant difference between the On and Off conditions for all task durations (2–8 s) (Figure 3).

## Time reproduction task

In both groups, there was no difference in reproduced durations between Off and On conditions (Supplementary Figure S4) on all tasks. There was no significant difference between On-1 and On-2 conditions in group A, between the On-2 condition in group A and the On condition in group B, or between the Off conditions in the two groups. Finally, there was no difference between the On and Off conditions for all task durations (2–8 s) (Figure 4).

## Length production task

In both groups, there was no difference in the estimated lengths between Off and On conditions (Supplementary Figure S5). In group A, there was no significant difference between On-1 and On-2 conditions, between the On-2 condition in group A and the On condition in group B, or between the Off condition in the two groups. Overall, there was no difference between the On and Off conditions (Figure 5A).

## Simple reaction task

There was no difference in reaction time for the simple reaction task between the Off and On conditions (Supplementary Figure S6). In group A, there was no significant difference between On-1 and On-2 conditions, between the On-2 condition in the group A and the On condition in group B, or between the Off condition in the two groups. There was no significant difference in reaction time between the On and Off conditions (Figure 5B).

# Discussion

The current study investigated the role of STN in temporal sensory processing especially in the sense of time, by manipulating STN function in PD patients off medication. Only the time production task showed a change with STN-DBS, with the estimate of time duration becoming shorter when STN-DBS was turned Off. In contrast, in the time bisection and time reproduction tasks, there was no difference between the Off and On conditions. These indicate that the functional manipulation of STN mainly affected the time production task, or the exteriorization (read out) of reference memory formed by experience, but not the temporal processing for time bisection and reproduction.

The bisection task requires subjects to remember long/short durations during the learning session and then judge the present time duration in reference to the learned duration during the test session, after the clock stage processing has been completed, and only engages reference memory to a small extent. Lack of effect of STN-DBS on the bisection task implies that the internal clock speed, or the "scale" used to perceive and estimate the present time duration, is not affected by STN-DBS. Rather, the difference in results among different temporal tasks can be ascribed to STN-DBS affecting the time representation in the memory stage, which may be classified into two types: reference and short-term memory. The production task would mainly require referral to and retrieval from reference memory but does not engage short-term memory (read out). In contrast, in the reproduction task, the time reproduced will be close to the veridical time duration as long as it is encoded and reproduced using the same clock; short-term memory but not reference memory in long-term memory is mainly used to perform the task. The effect of STN-DBS on the production task suggests that STN-DBS affects the reference memory processing in the memory stage (long-term memory). In contrast, lack of STN-DBS effect in the bisection task suggests that STN does not affect short-term memory processing. The effect of STN-DBS was even less evident in the reproduction task, suggesting that STN-DBS does not affect short-term memory processing. Our results thus suggest that reference and short-term memory may be affected differently during temporal processing in PD.

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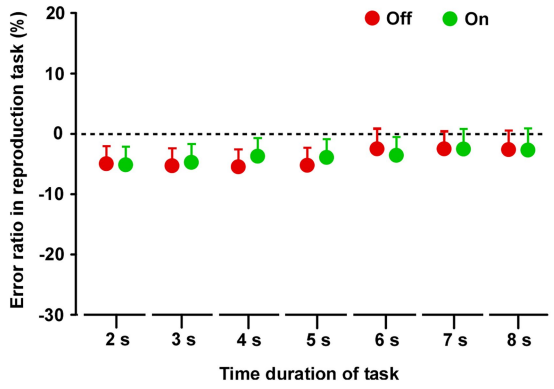


FIGURE 4
Results of the time reproduction task. Across the two groups, repeated measures analysis of variance (RM-ANOVA) showed that there were no main effects of STN-DBS for all task durations (2, 3, 4, 5, 6, 7, and 8s). Error bars show SEM.

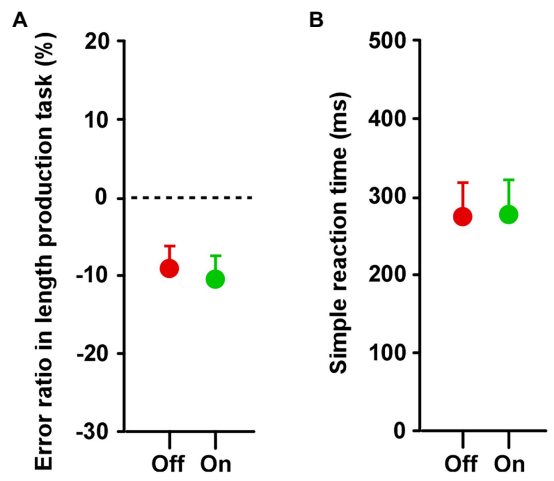


FIGURE 5
Results of **(A)** length production and **(B)** simple reaction tasks. Across the two groups, paired *t*-tests showed that there was no difference in the duration between Off and On conditions in both the length production and simple reaction tasks. Error bars show SEM.

Meanwhile, the lack of STN-DBS effect on the length production and simple reaction tasks suggest that STN-DBS does not affect spatial representation and basic motor function. Since all the temporal tasks in this study involved button presses and were dependent on motor function, we have to differentiate whether the effect of DBS was specific to temporal processing or was rather due to its effect on basic motor function. We assumed that if PD affected basic motor function and that the main reason for the altered task performance in PD patients was the result of this or the effect of DBS on motor function, it would affect motor function to a similar degree in all temporal tasks (production, reproduction, and bisection), with similar results in all three tasks, but this was not the case. On the other hand, the fact that there was no effect of DBS On/Off in the simple reaction task suggests that, at least, the influence of DBS on basic motor function as assessed by the speed of button responses was minimal.

The basal ganglia-thalamocortical circuit comprises the direct, indirect, and hyper-direct pathways. In the direct and indirect pathways, the striatum is the input stage, whereas internal segments of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) constitute the output stages of the basal ganglia. The indirect pathway leads from the striatum to the external segment of the globus pallidus (GPe) and STN, which in turn project to the GPi and SNr. Here the STN is positioned as an important relay nucleus (Albin et al., 1989; DeLong, 1990), receiving excitatory input from the cerebral cortex and inhibitory input from the GPe, and sends excitatory projections to the GPe, GPi, and SNr. Furthermore, *via* the hyper-direct pathway, STN receives inputs directly from the frontal areas involved in inhibition and executive control (Jahanshahi, 2013).

In PD, dopamine depletion in the striatum leads to decreased activity of neurons in the direct pathway and increased activity of neurons in the indirect pathway, resulting in increased activity of the GPi, decreased activity of the GPe, and increased activity of the STN (Miller and DeLong, 1988; Bergman et al., 1990). STN-DBS inhibits the overactivity of the STN (Breit et al., 2004). This inhibition may affect the pathways that project to the GPi and SNr and affect higher order functions, mainly the prefrontal cortex in the basal ganglia-thalamo-cortical circuit (Alexander and Crutcher, 1990; Oswal et al., 2013; Terao et al., 2021). Our findings suggest that the role of the STN in temporal sense processing may be reference to the time representation in long-term memory. Conversely, STN-DBS may have little effect on the function of perceiving the present time duration and may also have little effect on maintaining temporal short-term memory for performing the time reproduction task. Consistent with these views, a study showed in primates that L-DOPA treatment ameliorated PD signs, particularly akinesia/bradykinesia, and normalized cortically evoked responses in both the GPi and GPe, whereas STN blockade by muscimol injection ameliorated motor deficits and unmasked cortically evoked inhibition in the GPi (Chiken et al., 2021).

Based on this neural network, we may speculate how the coding of time representation in the short-and long-term is differently implemented by the basal ganglia thalamo-cortical circuit, leading to the differential effect of STN-DBS on different temporal tasks. Two memory-related brain structures are involved in temporal processing: the prefrontal cortex and the hippocampus. Honma et al. previously showed that the time scale representation for time sense, possibly in the long-term temporal memory, is abnormally shortened in PD patients (Honma et al., 2021), and this false learning of time scale learning may be consolidated by quadripulse transcranial magnetic stimulation over the prefrontal cortex (Honma et al., 2022). This may be stored in the prefrontal cortex as a form of temporal long-term memory, and may be directly affected by the functional manipulation induced by STN-DBS. Another pathway projecting from the striatum through the hippocampus to SNr bypasses STN, but projects to the thalamus and is relayed through the cortex. The time representation in short-term memory using this pathway may be less affected by PD or by the functional manipulation of STN-DBS.

This study has several limitations. The primary effect of STN DBS is on motor symptoms of PD patients (Hariz and Blomstedt, 2022). In the present study, we assessed basic motor function by using the simple reaction task and found no effect of DBS-STN on it. However, this task represents only one aspect of the overall motor symptoms. In the future, it may be necessary to use a more extensive experimental measure to assess motor symptoms, such as the UPDRS part-III for On/Off conditions in each subject. Second, it is

possible that DBS-STN influenced patients' emotional/psychological state. For example, the temporal processing may have been highly affected by the presence of unpleasant symptoms (Mella et al., 2019). Experiments with designs controlling for the DBS-STN effects on emotional/psychological state would need to be conducted. Third, all of the temporal tasks in this study were related to the visual domain. Temporal processing has been shown to have a more strong effect in the auditory domain (Wehrman and Sowman, 2021). Using a task that estimates the duration of sounds instead of visual signal might produce more robust results. Fourth, the sex of the patients in this experiment was biased for males. Since gender differences are known in time perception (Geer et al., 1964; Hancock et al., 1992), it would be important for future studies to conduct an experiment with subjects of both genders of equal sample size. Fifth, only medications related to Parkinson's disease were discontinued in the current study. Half-lives of some medicines may be so long as to be confounding variables, and non-Parkinson's disease-related medications could easily impact time perception. Some subjects also had comorbid symptoms or diseases other than PD, which may have also affected their time perception. Finally, the present study was necessarily performed in PD patients treated with DBS because the DBS-STN is not performed in healthy subjects. PD thus becomes the only human model, but since time can be distorted by a degenerative neurologic conditions, any conclusions are very tentative. Studies using primate models of Parkinsonism have provided insights into the mechanism of action of DBS in PD, in which STN DBS has been suggested to functionally interfere the function of the overactive STN-GPi/SNr pathway, which works to normalize the function of basal-ganglia thalamocortical pathway (Albin et al., 1989; Bergman et al., 1990; Calabresi et al., 2014; Pappas et al., 2014). However, DBS has not been studied in the context of time perception, partly since temporal sense in humans and primates may not be equated, and the size differences between rodent and primate anatomy makes it difficult to translate the findings from rodents to humans (Hardman et al., 2002). In the future, temporal sense experiments using DBS-STN in animals will be necessary.

## Conclusion

This study examined the role of STN in the temporal sense processing in the few seconds range in PD patients using three temporal processing tasks. Unlike the hundreds of milliseconds range addressed in previous study (Cope et al., 2014), STN-DBS affects temporal sense in the seconds range but only for the performance of the temporal production task (Rao et al., 2001; Meck et al., 2008). STN-DBS may enhance the function of the prefrontal cortex through the basal ganglia-thalamo-cortical circuit, and improve access to memory representation (time scale) for reading out time duration using reference memory.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

## Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of Juntendo University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

MH and YT designed the study. FS, HKa, MN, HKu, AU, HI, YS, GO, and NH recruited patients and conducted the investigation. MH, FS, HK, and TA analyzed the data. MH and YT wrote the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1090052/full#supplementary-material

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# Uric acid regulates α-synuclein transmission in Parkinsonian models

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Ample evidence demonstrates that α-synuclein (α-syn) has a critical role in the pathogenesis of Parkinson's disease (PD) with evidence indicating that its propagation from one area of the brain to others may be the primary mechanism for disease progression. Uric acid (UA), a natural antioxidant, has been proposed as a potential disease modifying candidate in PD. In the present study, we investigated whether UA treatment modulates cell-to-cell transmission of extracellular α-syn and protects dopaminergic neurons in the α-syn-enriched model. In a cellular model, UA treatment decreased internalized cytosolic α-syn levels and neuron-to-neuron transmission of α-syn in donor-acceptor cell models by modulating dynamin-mediated and clathrin-mediated endocytosis. Moreover, UA elevation in α-syn-inoculated mice inhibited propagation of extracellular α-syn which decreased expression of phosphorylated α-syn in the dopaminergic neurons of the substantia nigra leading to their increased survival. UA treatment did not lead to change in markers related with autophagolysosomal and microglial activity under the same experimental conditions. These findings suggest UA may control the pathological conditions of PD via additive mechanisms which modulate the propagation of α-syn.

KEYWORDS

**uric acid, α-Syn, transmission, endocytosis, Parkinson's disease**

## Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder pathologically characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of Lewy bodies and Lewy neurites, which mainly consist of aggregates of α-synuclein (α-syn). Intracellular proteinaceous aggregates of α-syn, which can be monomeric, oligomeric intermediate, or fibrillar, have a critical role in the pathogenesis of PD (Eschbach and Danzer, 2014). Recent studies suggest that α-syn can propagate from one area of the brain to others via cell-to-cell transmission, which might be the underlying mechanism for pathological propagation of Lewy bodies and disease progression of motor and non-motor symptoms (Lee et al., 2005; Desplats et al., 2009; Hansen et al., 2011; Gaugler et al., 2012). α-Syn may be transmitted via endocytosis to neighboring neurons, which is known to play a key role in spreading of α-syn (Lee et al., 2008; Cheng et al., 2011; Oh et al., 2016). Several studies also provided evidence of cell surface receptor-mediated endocytosis of α-syn such as FcγRIIB or lymphocyte activation gene-3 (Mao et al., 2016; Choi et al., 2018).

Moreover, we have previously demonstrated that α-syn may be internalized and propagated via interaction with *N*-methyl-D-aspartate receptors (Lee et al., 2021). Thus, the modulation of α-syn propagation would be an important strategy to delay the progression of PD as one of disease modifying therapeutics.

Uric acid (UA) is an important natural antioxidant in brain tissue and blood that is produced as a byproduct of purine metabolism. Preclinical studies have demonstrated that the neuroprotective effects of UA by scavenging oxygen radicals and reactive nitrogen and chelating metal ions in animal models (Davies et al., 1986; Yu et al., 1998; Schlesinger and Schlesinger, 2008; Huang et al., 2017; Bi et al., 2018). Clinical studies have demonstrated that serum UA levels are much lower in patients with PD compared to those without. Moreover, several epidemiological studies have demonstrated that higher UA levels are linked to reduced risk of PD and slow PD progression after the diagnosis of PD (Davis et al., 1996; de Lau et al., 2005; Weisskopf et al., 2007; Schwarzschild et al., 2008; Ascherio et al., 2009; Gao et al., 2016). These findings suggest that UA may have neuroprotective properties against PD-associated microenvironment, possibly acting as a disease modifier in PD. In the present study, we hypothesized that UA may exert neuroprotective effects via regulation of α-syn transmission in Parkinsonian models. To prove this, we triggered α-syn pathology by intrastriatal injection of preformed α-syn fibrils (PFF) in mice, where the injection of the α-syn has been shown to spread to other anatomically interconnected brain regions, including the dorsal motor nucleus of the vegal nerve, locus coeruleus, and substantia nigra pars compacta. Previous animal studies have reported progressive loss of dopaminergic neurons in substantia nigra and behavioral deficits between 30 and 180 days after intrastriatal injection of PFFs (Luk et al., 2012; Tarutani et al., 2018). We investigated whether treatment of UA modulates the cell-to-cell transmission of extracellular α-syn and protects dopaminergic neurons in α-syn enriched cellular and animal PD models.

# Materials and methods

## SH-SY5Y culture

The human neuroblastoma cell line SH-SY5Y was obtained from the Korean Cell Line Bank (Seoul National University, Seoul, Republic of Korea). SH-SY5Y cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, HyClone) supplemented with 10% fetal bovine serum (JC Bio) and a mixture of penicillin and streptomycin (1%, HyClone). When the cells reached 70–80% confluence, they were trypsinized and subcultured, and maintained at a temperature of 37°C in a humidified atmosphere containing 5% CO2/air. For differentiation, SH-SY5Y cells were plated at a density of. The next day, the cells were incubated in fresh DMEM with 10 μM of retinoic acid (Sigma). The medium was replaced on alternate days and the cells were allowed to differentiate for 5 days. The differentiated cells were then pretreated with UA (Sigma, 400 μM) for 24 h prior to treatment with α-syn (1 μM). Lysosomal inhibitor, bafilomycin A1 (Sigma, 50 nM) was added to the SH-SY5Y cells to exclude the function of lysosomal degradation.

## Preformed fibrillary α-syn preparation

Recombinant human α-syn [5 mg/ml in phosphate buffered saline (PBS) containing 50 mM Tris and 100 mM NaCl] was agitated at 37°C (1000 rpm) for 5 days. After then, fragmentation of α-syn fibrils was carried out by sonication using a Qsonica 4423 Q55 sonicator with 5/64-inch probe tip and sonicated at 20% amplitude, for a total time of 2 min (10 s pulse on/off). The α-syn fibrils were then visualized using electron microscopy. The confirmed preformed fibrillar protein was briefly sonicated to allow it to readily internalize into cells. The α-syn fibrils were visualized after agitation using electron microscopy.

## Cell viability analysis

SH-SY5Y cells were plated in 24-well polystyrene plates (SPL Life Sciences) at a density of and incubated at 37°C for 24 h to allow cells to stabilize. SH-SY5Y cells were then treated with various concentration of UA. Simultaneously, the same volume of fresh DMEM was added to the control groups. Plates were incubated at 37°C for a 24. Cell viability was measured using a cell proliferation assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, [Promega]) in accordance with the manufacturer's protocol. Mixture of a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES) was then added to a final concentration of 0.5 mg/ml. After incubation for 1 h, the medium was transferred to 96-well plates (SPL Life Sciences) in triplication and absorbance was measured by using the ELISA microplate reader (VersaMax, Molecular Devices, USA) at 490 nm.

## Bimolecular fluorescence complementation (BiFC) system

SH-SY5Y cells were transfected with Venus1-αSyn (V1S) or αSyn–Venus2 (SV2) plasmid using electroporation. Stable cell lines were maintained with 200 μg/ml G418 (Invitrogen) (Bae et al., 2014). These two stable cell lines express αSyn fused to either the amino (N) terminus (V1S) or carboxy (C) terminus (SV2) fragment of Venus, a variant of yellow fluorescence protein. When the two cell lines were co-cultured, fluorescence resulting from dimerization or oligomerization of the V1S and SV2 fusion proteins (Outeiro et al., 2008; Goncalves et al., 2010) during cell-to-cell transfer of αSyn was visualized using BiFC system. V1S and SV2 cell lines were plated in separate 60-mm dish (SPL Life Sciences) at a density of 6 cm × $10^{6}$/cm and incubated at 37°C for 24 h. Simultaneously, UA-treated co-culture group was incubated with UA (400 μM) during 24 h stabilization. After stabilization, V1S and SV2 cell lines were subcultured for co-culture. V1S and SV2 stable cells were mixed in a coverslip at a density of 6 cm × $10^{6}$/cm and cultured for 48 h before visualization.

## Immunocytochemistry

SH-SY5Y cells were washed three times using PBS and incubated 0.1% Triton X-100 for 10 min. They were fixed with 4% paraformaldehyde for 20 min at room temperature. After then, cells were washed three times and they were blocked with 0.5% bovine serum albumin for 1 h at room temperature. After blocking, they were incubated overnight at 4°C with specific primary antibodies. The primary antibodies that were used for staining are as follows: rabbit anti-α-syn (Abcam, ab138501), mouse anti-clathrin (Abcam, ab2731), rabbit anti-Rab5 (Abcam, ab18211). Immunofluorescence labeling was carried out by incubating cells for 2 h in donkey anti-mouse IgG (Alexa 488, green) and goat anti-rabbit IgG (Alexa 647, red) secondary antibodies. Cell nuclei were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI; Invitrogen, D1306). The immunostained cells were visualized using a Zeiss LSM 700 confocal imaging system. Quantification of the fluorescence intensities of α-syn, clathrin, and Rab5 was performed through Zen software program (Zeiss).

## Animal study

All procedures were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Experiments provided by the Institutional Animal Care and Use Committee (IACUC) at the Yonsei University Health System. Male C57BL/6 mice (Orient Bio) were acclimated in a climate-controlled room with a constant 12 h light/dark cycle for 1 week prior to drug administration. The mice were divided into the following three groups ($n$ = 10 per group): (1) control group; (2) only α-syn-treated group; and (3) UA-elevated group in α-syn-treated mice. To elevate UA serum levels, the mice were administered a daily intraperitoneal (IP) injection of potassium oxonate (KOx; 500 mg/kg; Sigma) and guanine monophosphate (GMP; 500 mg/kg; Sigma) for 2 weeks, whereas the control group mice received normal saline. To construct Parkinsonian model, Preformed fibrillary α-syn (1 μl, total 5 μg/hemisphere) was slowly injected into the striatum bilaterally (0.2 mm posterior to the bregma, ± 2.0 mm lateral to the midline, and −2.6 mm ventral to the brain surface) using a stereotaxic apparatus. After α-syn inoculation, the UA-elevated group received a daily IP injection for additional 4 weeks (total 6 weeks). All mice were sacrificed 1 month after α-syn inoculation (**Figure 3A**).

## Preparation of serum and brain tissue

All mice were deeply anesthetized by isoflurane inhalation at the end of the experimental period and their blood and brains for western blotting analysis were collected. To measure serum UA levels, blood samples from the abdominal aorta were collected in serum separator tube (BD Diagnostic Systems, Sparks, MD, USA). Serum and blood cells were separated by centrifugation (16,000 rcf, 20 min), then immediately frozen and stored at −20°C until analysis. For immunohistochemistry, mice were perfused with 4% paraformaldehyde. Brains were carefully harvested from the skulls, post-fixed overnight in 4% paraformaldehyde, and stored in 30% sucrose in PBS for 1–2 days at 4°C until they sank. Coronal brain tissue sections (25 μm thickness) were cut using a cryostat and stored in sterile tissue storage solution (30% glycerol, 30% ethylene glycol, 30% distilled water, 10% 0.2 M PB) at 4°C until analysis.

## Rotarod test

To assess motor function and coordination, along with balance, mice were tested on the rotarod apparatus (MED-Associates, USA). On the day prior to initiating the training session, mice were habituated to the apparatus for 15 min. In training trials, mice were trained to run on the rotarod (20 rpm) for 10 min without falling, twice a day for three consecutive days prior to α-synuclein administration. In test trials, mice were placed on the rotarod at 30 rpm (cut-off time of 700 s maximum). The latency time to fall was recorded.

## Measurements of serum UA level

Each serum samples obtained from mice were used for measuring serum UA level. Enzyme colorimetric assay was performed by Seoul Clinical Laboratories (SCL). Uricase converts UA into allantoin and hydrogen peroxide. In the presence of hydrogen peroxide, 4-aminophenazone is oxidized by hydrogen peroxide and become quinone-diimine. The color intensity of generated quinone-diimine is directly proportional to the concentration of UA. Absorbance was measured at a 552 nm wavelength using a Cobas 8000 c502 (Roche Diagnostics [HITACHI], Japan).

## Western blotting analysis

Cells harvested from the cell culture plates for western blotting analysis were dissolved in ice-cold Radio-Immunoprecipitation Assay (RIPA) buffer (50 mM Tris–HCl, pH 7.5, with 150 mM sodium chloride, 1% triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 2 mM EDTA sterile solution; Lugen Sci Co.) plus protease inhibitors and phosphatase inhibitors (Xpert Duo inhibitor cocktail solution; GenDEPOT). Mice were anesthetized with isoflurane and their brains were carefully collected for western blotting analysis as mentioned in "Preparation of serum and brain tissue" section. Brain tissue was homogenized and also dissolved in RIPA buffer for protein extraction. Lysates were centrifuged (13,000 rpm at 4°C) for 20 min (13,000 rpm), and supernatants were transferred to sterile tubes. Briefly, 10 or 20 μg of protein were separated by SDS-gel electrophoresis and transferred to hydrophobic polyvinylidene difluoride (PVDF) membranes (GE Healthcare) which were blocked in 5% skim milk in PBST (0.1% Tween 20). Membranes were probed with the following primary antibodies: mouse anti-actin (Santa Cruz, sc 47778), mouse anti-α-tubulin (Santa Cruz, sc-32293), rabbit anti-α-syn (Abcam, ab138501), rabbit anti-phospho S129 α-syn (Abcam, ab59264), anti-aggregated α-synuclein antibody (Millipore, MABN389), rabbit anti-dynamin (Santa Cruz, sc-11362), mouse anti-clathrin

(Abcam, ab2731), rabbit anti-Rab5 (Abcam, ab18211), rabbit anti-EEA1 (Abcam, ab2900), rabbit anti-Iba-1 (Abcam, ab178847), mouse anti-SQSTM1/p62 (Abcam, ab56416), rabbit anti-LC3B (Sigma, L7543), rabbit anti-LAMP2 (BioVision, 3900-100). After overnight incubation with primary antibodies at 4°C, membranes were incubated with a secondary antibody conjugated with horse radish peroxidase for 2 h at room temperature. Antigen-antibody complexes were visualized with ECL solution (GenDEPOT), For quantitative analysis, immunoblotting band densities were measured by Image J.

## Immunohistochemistry

Brain sections were washed twice in 0.01% Triton X-100 (Sigma) and incubated in 0.5% Triton X-100 for 15 min at room temperature for permeabilization. Sections were blocked with 0.5% bovine serum albumin (BSA, Sigma) for 1 h, then washed twice in 0.01% Triton X-100 and incubated overnight 4°C with primary antibodies. The primary antibodies were: rabbit anti-α-syn (Abcam, ab138501), mouse anti-NeuN (Abcam, ab104224), rabbit anti-phosphorylated α-syn (Abcam, ab59264), and mouse anti-Tyrosine hydroxylase (TH) (Sigma, T2928). Immunofluorescence labeling was carried out by incubating tissue slides for 2 h in donkey anti-mouse IgG and goat anti-rabbit IgG (both Alexa Fluor-488, green and Alexa Fluor-647, red) secondary antibodies (1:200, Invitrogen). Cell nuclei were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI; Invitrogen, D1306). For the TH staining of midbrain, the brain tissue was incubated in biotinylated secondary antibodies in blocking solution (1:400) for 2 h at room temperature. The TH antibodies were visualized using 0.05% diaminobenzidine (DAB, Dako, Carpinteria, CA, USA). The immunostained tissue samples were visualized using bright-field microscopy and immunofluorescence images were viewed with a Zeiss LSM 700 confocal imaging system (Jena, Germany). To analyze the localization of antigens in double stained tissues, immunofluorescence images were created from the same tissue sections and merged using Zeiss ZEN software. The fluorescence intensity was quantified using ZEN software program.

## Measurement of α-syn and phosphorylated α-syn

The mass of α-syn was measured using the sandwich ELISA kit (AnaSpec, USA) and the mass of phosphorylated α-syn was measured using the sandwich ELISA kit (MybioSource, USA). Cell culture medium collected from each experimental group (control, α-syn, and α-syn/UA) was used for ELISA analysis. Brain tissue was homogenized and also dissolved in RIPA buffer for protein extraction. Brain lysates were centrifuged (13,000 rpm at 4°C) for 20 min (13,000 rpm), and supernatants were transferred to sterile tubes. The supernatants were used for ELISA analysis. Each diluted sample and standard included in the kit were applied to microtiter strip plates precoated with antibody that specifically binds to α-syn or phosphorylated α-syn in cell culture medium and brain lysates. Diluted detection antibodies that were indirectly linked to an enzyme were applied to each sample diluent and standard. Following overnight incubation at 4°C, washing solution was added to each well and then aspirated six times. To ensure accurate optical reading, the plates were inverted and then patted until no moisture remained. 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution was added to each well and incubated for 10 min until the blue gradient was clearly observed across the wells. Stop solution was added to each well until the color completely changed from blue to yellow. An automatic ELISA microplate reader (BioTek) was used with the wavelength setting at 450 nm. The Bio-Rad software was used to generate standard curves and to calculate the target antigen concentration of the samples.

## TH-positive cell counts

Tyrosine hydroxylase -immunostained neurons in the left and right SNpc of every fourth section were counted throughout the entire extent of the SNpc. Each stained tissue sample was visualized at low power and the number of TH-immunostained cells was counted at high power. To accurately count the number of TH-positive cells, TH-positive cells were counted only when the nucleus was optimally visualized, which occurred in only one focal plane. The average cell number of five mice in each experimental group was shown in graph.

## Statistical analysis

Mean differences among experimental groups were determined by one-way analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test. Differences were considered statistically significant at $P < 0.05$. Statistical analysis was performed using the commercially available software SPSS Statistics 26 (IBM Corp: IBM SPSS Statistics for Windows, Armonk, NY, USA).

# Results

## UA modulates cell-to-cell transmission of extracellular α-syn in SH-SY5Y cells

Based on the cell viability test that UA treatment at various concentrations (0–400 μM) for 24 h did not affect the cell viability (**Figure 1A**), UA concentration was fixed as 400 μM. We utilized a BiFC system to directly demonstrate the effect of UA on transmission of α-syn between neuronal cells. BiFC intensity was markedly decreased in the UA treatment group compared to the V1S and SV2 co-culture groups. However, neither V1S-expressing cells nor SV2-expressing cells were fluorescent in the individual cultures (**Figure 1B**). To examine the modulatory effect of UA on internalization of α-syn fibrils, we treated UA to differentiated SH-SY5Y cells prior to α-syn incubation. Immunocytochemistry showed that treatment with UA significantly attenuated the immunoreactivity of α-syn compared to treatment with α-syn alone (**Figure 1C**). Western blotting also demonstrated that UA treatment decreased the expression of α-syn compared to α-syn alone (**Figure 1E**). To measure the amount of extracellular

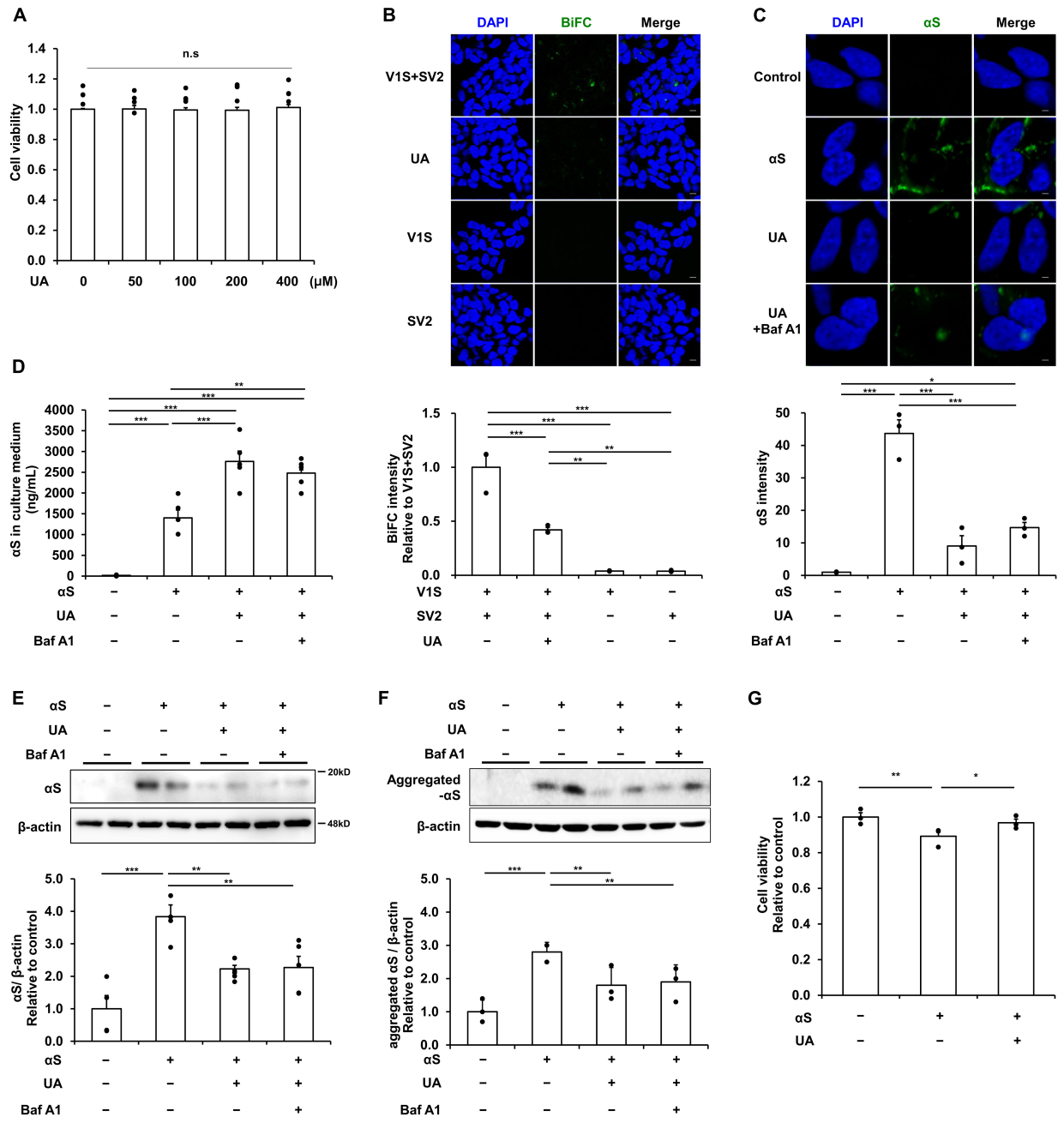


FIGURE 1
Uric acid (UA) modulates cell-to-cell transmission of extracellular α-syn in neuronal cells. **(A)** MTS analysis in UA-treated differentiated SH-SY5Y cells with a concentration of 0, 50, 100, 200, and 400 μM. UA treatment did not induce cytotoxicity within the set concentration range ($n$ = 4 per group). **(B)** A donor-acceptor co-culture method for transmission of αS showing that the intensity of BiFC signals (green) were markedly decreased in the UA treatment group compared to the V1S and SV2 co-culture groups. Scale bar, 50 μm. **(C)** Immunostaining for internalization of αS fibrils (αS, green) and quantification of αS fluorescence intensity in SH-SY5Y cells among control, αS, and αS/UA-treated cells. Scale bar, 2 μm. **(D)** Quantification using ELISA analysis of extracellular αS in the culture medium of the control, αS, αS/UA, and αS/UA/bafilomycin-treated group ($n$ = 5 per group). Western blot for αS **(E)** and aggregated αS **(F)** with control ($n$ = 4), αS ($n$ = 5), αS/UA ($n$ = 5), and αS/UA/bafilomycin-treated ($n$ = 5) groups. **(G)** MTS analysis in the control, αS, and αS/UA-treated group after incubation of αS fibrils for 24 h ($n$ = 3 per group). Differences among conditions were evaluated by ANOVA with Bonferroni's correction for multiple comparisons. All data are presented as mean ± SE. *$P$ < 0.05, **$P$ < 0.01, and ***$P$ < 0.001.

α-syn not internalized into cells, we performed ELISA analysis in culture medium harvested from each experimental group. The amount of extracellular α-syn in the UA treatment group was markedly higher than the α-syn alone group, presumably resulting from the inhibition of α-syn internalization (Figure 1D). Bafilomycin A1 was added to exclude lysosomal degradation of α-syn in neurons and resulted in no influence on α-syn internalization or extracellular α-syn levels. In addition, we characterized conformation-specific antibodies that preferentially recognize aggregated forms of α-syn fibril. As a results, the internalization of aggregated form of α-syn was also reduced by UA and bafilomycin A1 did not affect the levels of α-syn (Figure 1F

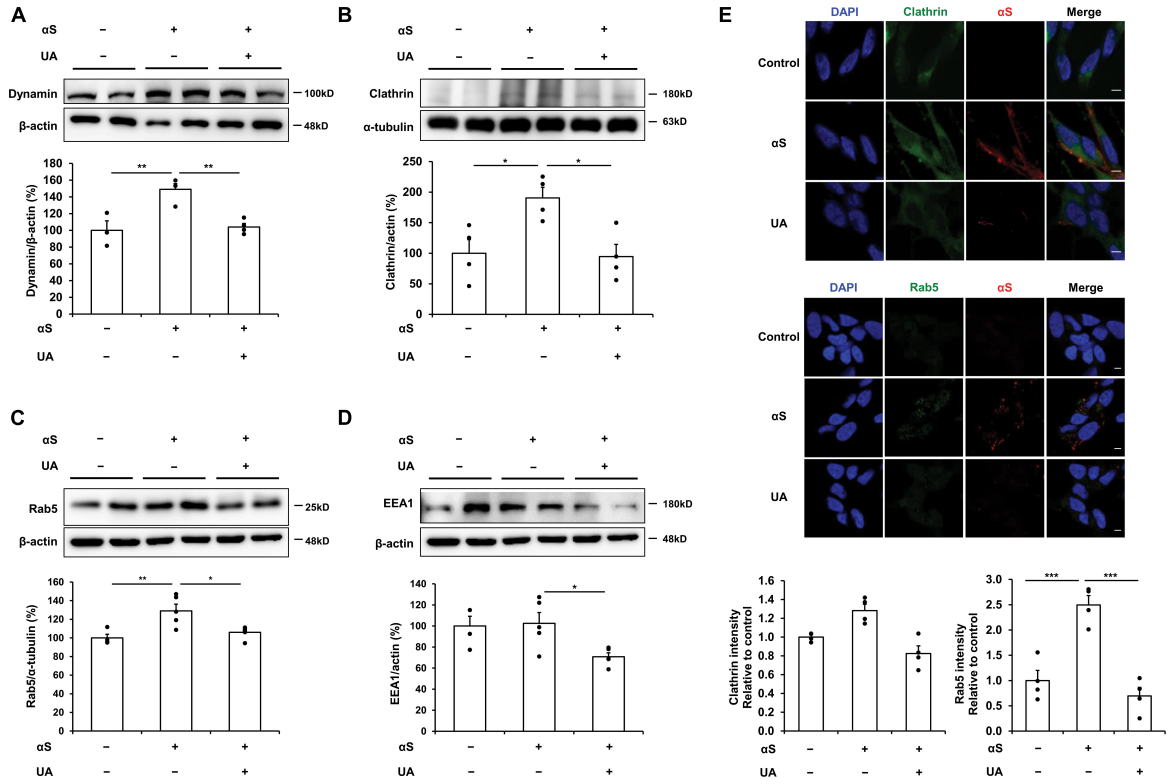


FIGURE 2
Uric acid (UA) inhibits cell-to-cell transmission of α-syn via blocking endocytic pathway. **(A)** Western blot for dynamin in control ($n$ = 4), αS ($n$ = 5), and αS/UA-treated ($n$ = 5), neuronal cells and quantification graph. **(B)** Western blot for clathrin and quantification graph ($n$ = 4 per group). **(C)** Western blot for Rab5, early endosome marker, and quantification graph ($n$ = 4 for control, $n$ = 5 for αS, and αS/UA-treated group). **(D)** Western blot for EEA1, a Rab5 effector protein, and quantification graph ($n$ = 4 for control, $n$ = 5 for αS-treated, and αS/UA-treated group). **(E)** Immunostaining of clathrin or Rab5 and αS with quantification of clathrin or Rab5 fluorescence intensity. Scale bar, 5 μm. Differences among conditions were evaluated by ANOVA with Bonferroni's correction for multiple comparisons. All data are presented as mean ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

and Supplementary Figure 1). The incubation of α-syn fibrils for 1 h decreased SH-SY5Y cell viability, but treatment of UA decreased α-syn-induced cell death compared to the α-syn alone (Figure 1G).

## UA inhibits cell-to-cell transmission of α-syn via blocking endocytic pathway

We evaluated the expression of endocytic pathway markers to verify how UA blocks the internalization of α-syn fibrils in differentiated neuronal cells. Western blotting showed that the expression of dynamin was significantly increased in α-syn treated neuronal cells relative to control cells, whereas treatment with UA significantly decreased dynamin expression compared to cells treated with α-syn alone (Figure 2A). Similarly, α-syn-treated neuronal cells exhibited increased clathrin expression compared to control cells, whereas treatment with UA in α-syn-treated neuronal cells significantly attenuated the expression of clathrin. This indicates that UA suppresses clathrin-mediated endocytosis (CME) of α-syn (Figure 2B). The suppression of dynamin-mediated and clathrin-mediated endocytosis caused the expression of Rab5 localized with early endosomes to markedly decrease in UA-treated cells compared to cells treated with α-syn alone (Figure 2C). In addition, the expression of early endosome antigen, EEA1 (also known as "Rab5 effector") which is involved in endosomal trafficking, decreased in UA-treated neuronal cells compared to α-syn alone-treated cells (Figure 2D). Moreover, immunocytochemistry showed that α-syn-treated neuronal cells increased the immunoreactivity of clathrin or Rab5 co-localized with α-syn, compared to control cells, whereas treatment with UA markedly decreased the immunoreactivity of clathrin or Rab5 compared to cells treated with α-syn alone (Figure 2E).

## UA elevation modulates α-syn transmission in the striatum of α-syn-inoculated mice

To examine the effect of UA elevation on α-syn transmission in α-syn-inoculated mice, mice were intraperitoneally co-injected with guanine 5′-monophosphate (GMP) and uricase inhibitor, potassium oxonate (KOx) to elevate their UA serum levels. Recombinant human α-syn fibrils were slowly inoculated bilaterally into the striatum. The detailed *in vivo* study design is illustrated in Figure 3A. Two weeks' treatment with GMP and KOx led to a significant increase in the serum levels of UA (3.4 mg/dl) relative to the control mice (2.2 mg/dl) or only α-syn-inoculated mice (2.1 mg/dl) (Figure 3B). We then evaluated whether UA elevation

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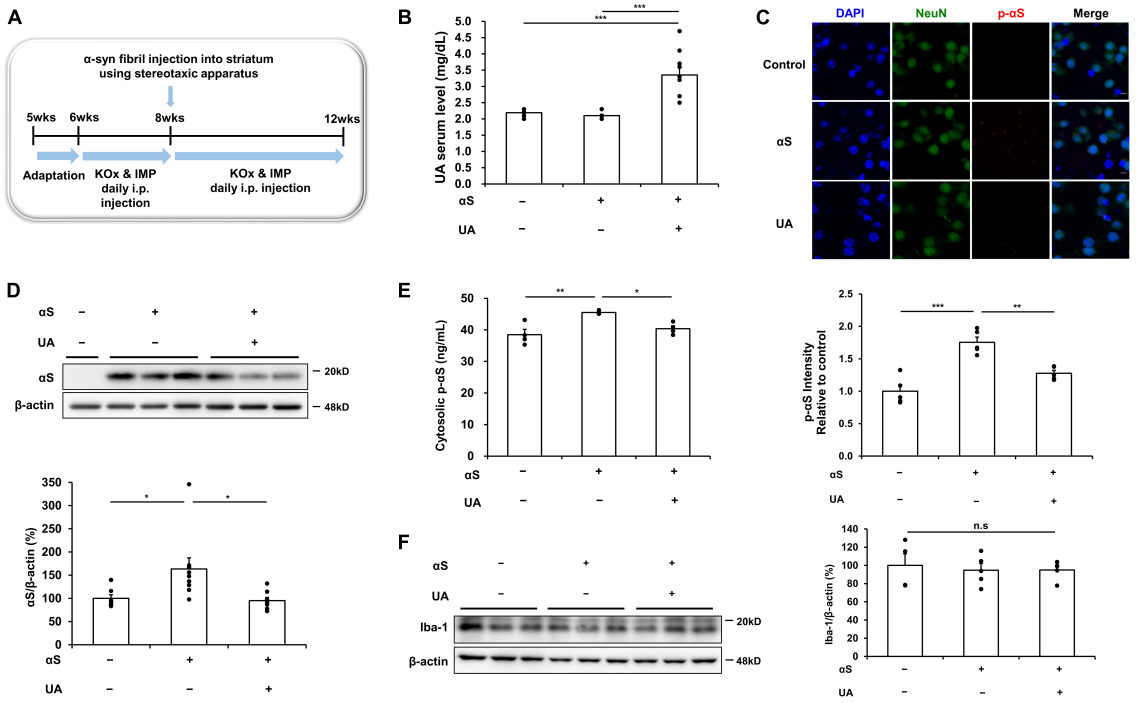


FIGURE 3
Uric acid (UA) elevation modulates α-syn transmission in the striatum of α-syn-inoculated mice. **(A)** Animal experiment schedule. **(B)** Serum UA levels were significantly higher in mice that were co-injected with GMP and KOx compared to control and αS-inoculated mice. **(C)** Immunohistochemical analysis showed that the immunoreactivity of p-αS in the striatal region was markedly increased in αS-inoculated mice, whereas the immunoreactivity of p-αS was significantly decreased in UA-elevated mice. Scale bar, 5 μm. **(D)** Western blotting showing that UA-elevated mice significantly attenuated the expression of cytosolic αS in the striatal region ($n$ = 4 for control, $n$ = 9 for αS-inoculated, and $n$ = 8 for UA-elevated mice). **(E)** ELISA analysis showing that UA-elevated mice markedly decreased the amount of cytosolic p-αS in the striatal region compared to control and only αS-inoculated mice ($n$ = 4 per group). **(F)** Western blotting showed that the expression of Iba-1 in the striatal region was comparable among control ($n$ = 4), αS-inoculated ($n$ = 5), and UA-elevated mice ($n$ = 5). Differences among conditions were evaluated by ANOVA with Bonferroni's correction for multiple comparisons. All data are presented as mean ± SE. *$P$ < 0.05, **$P$ < 0.01, and ***$P$ < 0.001.

modulated transmission of α-syn 30 days after inoculation in the striatum. Immunohistochemical analysis showed that the immunoreactivity of phosphorylated α-syn in NeuN-positive cells of the striatum was markedly increased, whereas UA elevation in α-syn-inoculated mice significantly attenuated the immunoreactivity of phosphorylated α-syn in striatal neurons (Figure 3C). In addition, western blotting showed that UA elevation in α-syn-inoculated mice significantly decreased the expression of α-syn in the striatal region compared to only α-syn-inoculated mice (Figure 3D). ELISA analysis demonstrated that UA elevation in α-syn-inoculated mice significantly decreased the amount of phosphorylated α-syn in the striatum compared to α-syn-inoculated mice (Figure 3E). Furthermore, we assessed whether UA elevation led to microglia activation in the striatum of α-syn-inoculated mice. The expression of Iba-1 in the striatum was comparable among control mice, α-syn-inoculated mice, and UA-elevated mice (Figure 3F).

## UA elevation modulates α-syn propagation in the midbrain of α-syn-inoculated mice

Thirty days after inoculation of α-syn into the striatum, α-syn expression in the midbrain was significantly increased, as was the immunoreactivity of α-syn in dopaminergic neurons of the SN. However, UA elevation in α-syn-inoculated mice significantly attenuated the expression and immunoreactivity of α-syn in the midbrain compared to α-syn-inoculated mice (Figure 4A). Phosphorylated α-syn expression was examined using an ELISA analysis to evaluate whether exogenous α-syn induces pathogenic α-syn. Results indicated that α-syn inoculation markedly increased the levels of phosphorylated α-syn in the midbrain compared to control mice. However, UA elevation in α-syn-inoculated mice significantly attenuated phosphorylated α-syn levels compared to only α-syn-inoculated mice (Figure 4B). Phosphorylated α-syn was prominently immunostained with dopaminergic neurons of the SN in α-syn-inoculated mice, whereas the immunoreactivity of phosphorylated α-syn co-merged with dopaminergic neurons was markedly decreased in UA-elevated mice (Figure 4C). Consequently, α-syn inoculation led to a significant decrease in the number of TH-positive neurons in the SN compared to controls. However, UA elevation resulted in a significant increase in the number of TH-positive neurons (Figure 4D). Furthermore, the expression of Iba-1 in the midbrain was comparable among control, α-syn-inoculated, and UA-elevated mice (Figure 4E). Behavioral analysis showed that α-syn inoculation led to progressive patterns of the latency to fall on the rotarod test compared to the control group. However, UA treatment in α-syn-inoculated mice tended

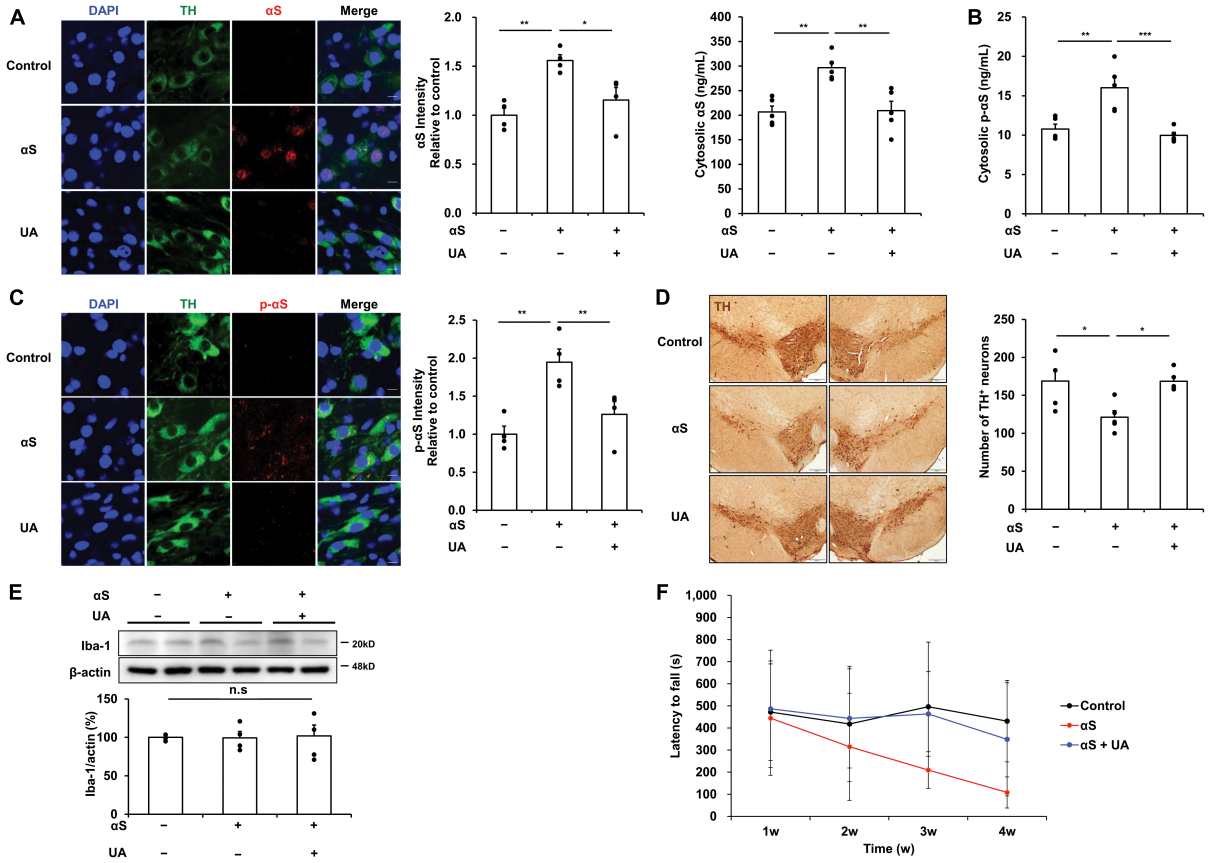


FIGURE 4
Uric acid (UA) elevation modulates α-syn propagation in the midbrain of α-syn-inoculated mice. **(A)** Immunostaining of αS co-localized with TH-positive neurons and quantification of fluorescence intensity ratio in the SNpc of midbrain. Immunoreactivity of αS was significantly decreased in UA-elevated mice compared to control and only αS-inoculated mice. Scale bar, 10 μm. **(B)** ELISA analysis showing that UA-treated mice had markedly decreased cytosolic p-αS in the midbrain compared to control and only αS-inoculated mice ($n$ = 5 per group). **(C)** Immunostaining of phosphorylated αS co-localized with TH-positive neurons with quantification of fluorescence intensity ratio in the SNpc of midbrain. UA-elevated mice markedly attenuated the immunoreactivity of p-αS compared to control and only αS-inoculated mice with significant increase in TH-positive neurons. Scale bar, 5 μm. **(D)** Photomicrographs of SNpc of TH-immunostained sections. Graph shows the number of TH-positive cells in SNpc counted by stereology. Scale bar, 200 μm ($n$ = 5 per group). **(E)** Western blotting showed that expression of Iba-1 in the midbrain was comparable among control, αS-inoculated, and UA-elevated mice ($n$ = 4 per group). **(F)** UA treatment in α-syn-inoculated mice tended to improve motor performance on the rotarod test ($n$ = 6, each group). Differences among conditions were evaluated by ANOVA with Bonferroni's correction for multiple comparisons. All data are presented as mean ± SE. *$P$ < 0.05, **$P$ < 0.01, and ***$P$ < 0.001.

to improve motor coordination and balance on the rotarod test (Figure 4F).

## UA elevation modulates transmission of α-syn via blocking endocytic pathway in α-syn-inoculated mice

Next, we examined whether the elevation of UA levels modulated transmission of extracellular α-syn by blocking dynamin-mediated and clathrin-mediated endocytosis. In α-syn-inoculated mice, the expression of dynamin and clathrin in the midbrain markedly increased compared to control mice, whereas UA-elevated mice showed significantly decreased expression of dynamin and clathrin compared to only α-syn-inoculated mice (Figures 5A, B). In addition, UA elevation in α-syn-inoculated mice led to marked decrease in the expression of Rab5 localized to early endosome compared to only α-syn-inoculated mice (Figure 5C). Moreover, the expression of EEA1 significantly decreased in UA-elevated mice compared to only α-syn-inoculated mice (Figure 5D).

## α-syn modulation by UA elevation may not be associated with induction of autophagolysosome

Finally, we examined whether UA elevation induced the autophagic pathways to exclude the modulatory effect of UA on α-syn via autophagic clearance under the same experimental conditions. In a cellular model, α-syn incubation for 6 h did not induce any autophagolysosomal markers of p62, LC3B, or LAMP-2 compared to control neuronal cells. In addition, the expression of autophagy markers did not differ between α-syn-treated cells and UA-treated cells (Figures 6A–C). Similarly, UA elevation in α-syn-inoculated mice did not change the expression of p62, LC3B, or

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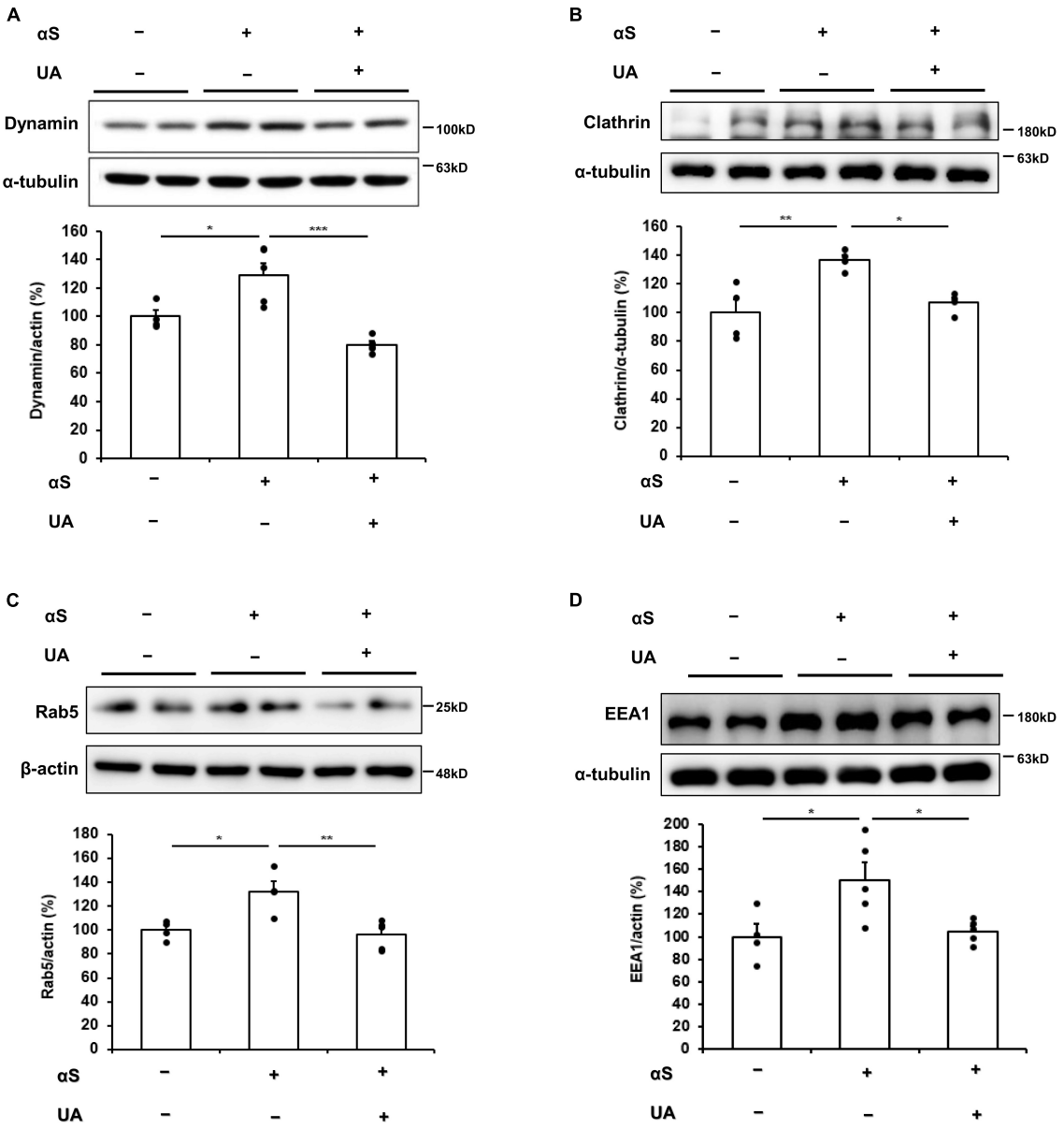


FIGURE 5
Uric acid (UA) elevation modulates transmission of α-syn via blocking endocytic pathway in α-syn–inoculated mice. **(A)** Western blot for dynamin in control ($n$ = 4), αS ($n$ = 5), αS/UA-treated ($n$ = 5) neuronal cells, and quantification graph. **(B)** Western blot for clathrin and quantification graph ($n$ = 4 per group). **(C)** Western blot for Rab5 and quantification graph ($n$ = 4 for control, $n$ = 5 for αS-inoculated and UA-elevated mice). **(D)** Western blot for EEA1 and quantification graph ($n$ = 4 for control, $n$ = 5 for αS-inoculated and UA-elevated mice). Differences among conditions were evaluated by ANOVA with Bonferroni's correction for multiple comparisons. All data are presented as mean ± SE. *$P$ < 0.05, **$P$ < 0.01, and ***$P$ < 0.001.

LAMP-2 in the midbrain compared to only α-syn-inoculated mice (Figures 6D–F). These data indicate that the effect of UA elevation on α-syn modulation may be associated with direct inhibition of α-syn propagation, but not due to autophagy-mediated α-syn clearance.

## Discussion

The present study investigated whether elevation of serum UA levels modulated the transmission of α-syn, resulting in a neuroprotective effect on dopaminergic neurons in Parkinsonian models. The major findings were: (1) UA inhibited cell-to-cell transmission of extracellular α-syn by modulating endocytosis; and (2) inhibition of α-syn propagation by the elevation of serum UA levels led to a pro-survival effect on nigral dopaminergic neurons in α-syn-inoculated mice. Our data suggest that UA may modulate the propagation of α-syn as an additive mechanism which controls the pathological conditions of PD.

There is ample evidence that neurons have an ability to internalize extracellular aggregates by endocytosis. Internalized α-syn aggregates can be transmitted from neuron-to-neuron via the extracellular milieu and can be propagated by a seeding mechanism (Guo and Lee, 2014; Lee et al., 2014; Tyson et al., 2017). Also,

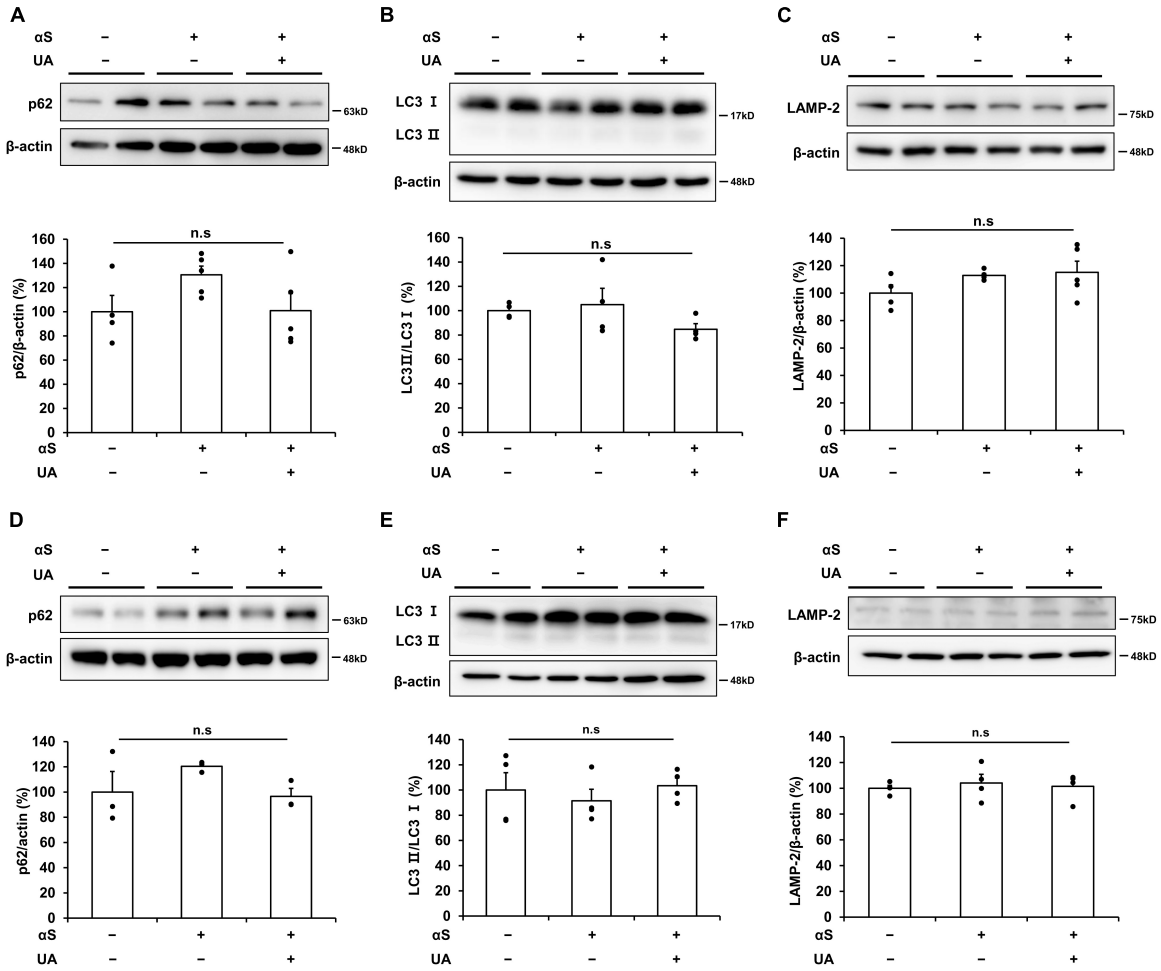


FIGURE 6
α-Syn modulation by UA elevation may not be associated with induction of autophagolysosome. **(A–C)** Western blots showing that expression of p62, LC3B, and LAMP-2 was comparable among the control ($n$ = 4), αS ($n$ = 5), and αS/UA-treated ($n$ = 5) cells. **(D–F)** Western blots showing that expression of p62, LC3B, and LAMP-2 was comparable among control, αS-inoculated, and UA-elevated mice in the midbrain ($n$ = 4 per group). Differences among conditions were evaluated by ANOVA with Bonferroni's correction for multiple comparisons. All data are presented as mean ± SE.

propagation of α-syn aggregates can induce neurotoxicity and neuronal loss in α-synucleinopathies, accompanied by impairment of motor and cognitive function (Conway et al., 1998; Forloni et al., 2000; Da Cunha et al., 2002; Volpicelli-Daley et al., 2011; Luk et al., 2012). Thus, the inhibition of α-syn propagation may be clinically relevant as a key pharmacological target for disease-modifying treatment for α-synucleinopathies. On this basis, our data suggested that the neuroprotective property of UA elevation in regulating the transmission of α-syn may be applicable to the development of future disease modifying strategies for patients with α-synucleinopathies.

The present study demonstrated that UA inhibits α-syn endocytosis and leads to the inhibition of α-syn transmission. In cellular models, UA inhibited the internalization of extracellular α-syn in an α-syn-enriched cellular environment and blocked neuron-to-neuron transmission of α-syn in donor-acceptor cell models. Previous report has demonstrated that the internalization of α-syn may be mediated by endocytosis and have shown that endocytosis inhibitors have an ability to decrease the internalization of α-syn both *in vitro* and *in vivo* (Hansen et al., 2011). Furthermore, in cultured cell lines, the overexpression of a dominant-negative dynamin efficiently reduces the extent of internalized α-syn aggregates (Lee et al., 2008, 2010) and suppression of dynamin GTPase decreases α-syn uptake by neuronal cells (Konno et al., 2012). In addition, clathrin-mediated endocytosis is another pathway of extracellular α-syn endocytosis. Studies have shown that α-syn interacts and co-localizes with components of the clathrin-coated pit (Erb and Moore, 2020; Schechter et al., 2020; Lee et al., 2021; Teixeira et al., 2021), suggesting that the endosomal pathway of α-syn may start with clathrin-mediated endocytosis. Our previous data also showed that suppression of clathrin-mediated endocytosis may lead to a reduction of internalized extracellular α-syn (Oh et al., 2016; Lee et al., 2021). In the present study, we found that α-syn fibrils were internalized into neuronal cells through dynamin-mediated and clathrin-mediated endocytosis, with up-regulated expression of dynamin and clathrin in α-syn-treated cells. However, UA-treated cells had significantly decreased expression of dynamin and clathrin as well as expression of Rab5. Small GTPases Rab5, a marker of early endosomes and regulator of endocytosis, is critical for the

endocytosis of exogenous α-syn into neuronal cells (de Hoop et al., 1994; Stenmark et al., 1995; Neve et al., 1998; Sung et al., 2001). As a result, the expression of EEA1, a Rab5 effector protein, markedly decreased in UA-treated cells compared to cells treated with α-syn alone. Similarly, we found that UA treatment in α-syn-inoculated mice significantly decreased the expression of clathrin and dynamin in the midbrain, which subsequently decreased the expression of Rab5 and EEA1. Accordingly, the present study provides evidence that UA modulates extracellular α-syn propagation by inhibiting both dynamin-mediated and clathrin-mediated endocytosis.

We found that UA significantly decreased the levels of internalized cytosolic α-syn and attenuated α-syn-induced cell death in α-syn-enriched models. In cellular models, UA treatment decreased the expression of intracellular α-syn with an increase in the amount of extracellular α-syn. In addition, it has been reported that extracellular α-syn can act as seed that initiate the aggregation of endogenous α-syn, in both cellular and animal models (Mahul-Mellier et al., 2020; Awa et al., 2022). In our study, it was also observed that aggregated α-syn increased by extracellular α-syn was reduced by UA. This was possibly caused by inhibition of α-syn internalization, which led to a pro-survival effect in neuronal cells. In α-syn-inoculated mice, elevation of serum UA levels significantly decreased transmission of α-syn in the striatum as well as the midbrain region which is distant from the inoculation site. Consistent with the results of our *in vitro* study, the expression of several markers related to extracellular α-syn entry significantly decreased in UA-elevated mice inoculated with α-syn compared to mice that were inoculated with α-syn-alone. Specifically, 1 month after α-syn was inoculated into the dorsal striatum, UA elevation decreased the immunoreactivity and expression of the phosphorylated α-syn in the striatum and SN of midbrain. Thus, UA elevation in α-syn-inoculated mice resulted in increased survival of dopaminergic neuron in the SN. In terms of other mechanisms of UA on α-syn modulation, it is possible that reduction in the amount of α-syn could be secondary to modulation of autophagy or microglial activation because UA can enhance autophagy activity (Sheng et al., 2017) and inhibit microglia activation in PD model (Bao et al., 2018). Moreover, by blocking intracellular transmission, the subsequent increase in the burden of extracellular α-syn could lead to the induction of neuroinflammation (Gelders et al., 2018; Domingues et al., 2022). However, in the current study, we found that UA treatment did not lead to change in markers related with autophagolysosomal or microglial activity under the same experimental conditions. Accordingly, these data indicate that the beneficial effect of UA on α-syn modulation may be associated with direct modulation of α-syn propagation.

Although there are many studies on the beneficial effect of UA in neurodegenerative disease, serum uric acid concentrations above the saturation threshold promote the deposition monosodium urate crystals, leading to chronic inflammatory response such as goat, renal and cardiovascular diseases (Sautin and Johnson, 2008; So and Thorens, 2010; Vassalle et al., 2016). In the present study, when administered KOx 500 mg/kg and GMP 500 mg/kg for increasing UA, the serum uric acid level of UA-treated group was about 3.4 mg/dL, which was within the normal uric acid range of mice (serum urate concentration 0.1 to 760 μM (Watanabe et al., 2014). Given that UA can switch between having protective antioxidant capacity and having detrimental pro-oxidizing effects, depending on its concentration and the surrounding microenvironment, further laboratory and clinical studies are needed to uncover the optimum UA concentration for disease-modifying effect in PD.

## Conclusion

The present study demonstrated that UA has neuroprotective effects on dopaminergic neurons via the inhibition of extracellular α-syn transmission. Along with the pleiotropic effects of UA, repositioning use of UA focusing on α-syn propagation should be further investigated in patients with α-synucleinopathies.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

## Ethics statement

All procedures were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Experiments provided by the Institutional Animal Care and Use Committee (IACUC) at the Yonsei University Health System. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

YS: conception and design, collection and/or assembly of data, manuscript writing, and final approval of manuscript. JEL, YSK, JWL, and HK: technical assistance. YJK and JS: conception and design, collection and/or assembly of data, and discussion. PL: supervision of study, data analysis and interpretation, financial support, and final approval of manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

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# Research status and hotspots in the surgical treatment of tremor in Parkinson's disease from 2002 to 2022: a bibliometric and visualization analysis

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**Objective:** This study aims to investigate the research status and hotspots of surgical treatment for tremor in Parkinson's disease (PD) from 2002 to 2022, utilizing bibliometric and visual analysis. Additionally, it aims to offer insights into future research trends in this field.

**Methods:** This study collected publications on the surgical treatment of tremor in PD from 2002 to 2022 using the Web of Science (WOS) database. CiteSpace, VOSviewer, and Scimago Graphica were employed to quantify the number of publications and analyze the bibliographic information networks, including the contributions of countries/cities, authors, keywords, and co-cited references.

**Results:** A total of 2,815 publications were included in the study, revealing that 541 scientific institutions experienced an increase in publications from 2002 to 2022. Michael Okun emerged as the most productive author, and the United States emerged as the leading hub for research. The study identified 772 keywords. Noteworthy citation bursts and long-term activity were observed in pallidotomy, bilateral stimulation, and focused ultrasound thalamotomy. The top 10 highly co-cited references comprised eight deep brain stimulation (DBS) studies (including two follow-up studies and six randomized controlled trials), one randomized controlled trial on focused ultrasound, and one consensus on tremor.

**Conclusion:** This study uses an in-depth and systematic bibliometric and visualization analysis to visualize the evolution of research and identify emerging hotspots. The identified hotspots are as follows: Firstly, DBS has received significant attention and widespread recognition as a surgical treatment for tremor in PD. Secondly, there are various key aspects to consider in DBS, such as operative indications, operative targets, and surgical protocols. Lastly, magnetic resonance-guided focused ultrasound (MRgFUS) has emerged as a promising treatment option in the surgical management

of tremor in Parkinson's disease. This research also provides insights into the phenomenon of these hotspots, offering valuable prompts and reminders for further research.

KEYWORDS

tremor, Parkinson's disease, surgical treatment, visualization, research hotspots

# 1. Introduction

Parkinson's disease (PD) is a chronic and progressive complex neurodegenerative disorder characterized by the core motor symptom of tremor. Although drug therapy is the primary treatment for PD (Hayes, 2019), its efficacy diminishes due to the disease's gradual progression and unpredictable adverse effects, making surgical intervention a consideration. Current surgical modalities for PD tremor can be primarily classified into neuromodulation and neurodestructive procedures. Neuromodulation comprises spinal cord stimulation and deep brain stimulation (DBS) (Merola et al., 2021). DBS, approved by the Food and Drug Administration (FDA) in 2002 for PD treatment, involves the stimulation of specific deep brain nuclei using different chronic microcurrents. The procedure comprises two parts: implantation of the DBS device and post-operative program control. Its mechanism of action is complex, involving hypotheses like the interruption, inhibition, and excitation hypotheses, which are interrelated and vary in importance based on treatment conditions and stimulation target.

However, neurodestructive procedures mainly encompass magnetic resonance-guided focused ultrasound (MRgFUS), magnetic resonance-guided laser interstitial thermotherapy, and radiofrequency ablation (Harris et al., 2019; Garcia-Gomar et al., 2020; Yamamoto et al., 2021). Due to the post-operative complications associated with traditional radiofrequency ablation and advancements in imaging and ultrasound technology, novel neuron-destruction procedures, like magnetic resonance-guided laser interstitial thermotherapy and MRgFUS, have emerged. MRgFUS received FDA approval in 2019 for PD treatment. Surgical modalities for PD also encompass gene therapy and cell transplantation, alongside spinal cord electrical stimulation, currently under clinical research. However, there have been no studies reporting significant efficacy for PD tremor as of yet. Therefore, it is urgent to investigate the research status and hotspots of surgical treatment for tremor in Parkinson's disease (PD) from 2002 to 2022.

# 2. Materials and methods

## 2.1. Search strategy and data collection

We conducted a search in the WOS database on August 31, 2022, to identify relevant studies evaluating surgical interventions for tremor in PD published from 2002 to 2022. The search terms were based on medical subject headings (MESH) and synonyms of relevant text terms, including (Parkinson*) and (*tremor* or pill-rolling), and (*surgery* or surgical* or *stimulation or Radiofrequency ablation or MRI-guided laser interstitial thermal or MRI-guided focused ultrasound or stereotactic radiotherapy or Gamma knife thalamotomy or cell transplantation therapy or gene therapy). Literature-type language was not restricted, and the search period was set from January 1, 2002, to December 31, 2022. We used CiteSpace to remove duplicate publications, resulting in 2,815 unique articles. The search process is illustrated in **Supplementary Figure 1**.

## 2.2. Bibliometric and visual analysis

A Java-based visualization program called CiteSpace software, designed by Professor Chen Chaomei from Drexel University in the United States, utilizes the theory of co-citation (Chen, 2004; Chen and Song, 2019). In this study, we employed CiteSpace software (v6.1.R2, v6.1.R3), VOSviewer (v1.6.18), and Scimago Graphica (v1.0.25) to conduct a scientometric analysis. The dataset was managed using Microsoft Excel (2018), with duplicates and rejects removed before applying CiteSpace for data plotting, keyword co-occurrence analysis, and co-citation analysis. Additionally, VOSviewer (v1.6.18) was utilized to map cooperation networks (authors, institutions, and countries), while Scimago Graphica (v1.0.25) was employed for country/region analysis and to visualize the number of publications in different regions and countries.

CiteSpace parameters were set as follows: time slice: 1 year; period: January 2002 to December 2022; term source: all items, one node type at a time; other settings were set to default values.

## 2.3. Interpretation of the visualization map

The knowledge maps contain nodes and links. The round areas, termed "citation nodes," represent the citation history of a paper. The node size indicates the frequency of varied items (author, Country/region, Institution and so on) and is positively correlated with the number of references. The links between nodes signify the strength of cooperation, co-occurring keywords, and co-citation relationships. Bursts mean that the value of a variable has changed significantly in a short period of time, which also indicates that the variable is an important turning point in the field of study, which is worth paying attention to. The Burst analysis not only identifies papers attracting more attention from peer researchers, but also

helps discover articles with significant influence on future research promptly. The centrality is a key indicator to the importance of keywords. If the centrality is more than 0.1, it means that the node is a central node, which is more important and influential in the study. The strength value reflects the citation frequency. The blue blocks represent the years 2002 to 2022, while the red blocks indicate the beginning, end, and duration of citation bursts. Clustering#: Each cluster consists of closely related keywords (each tag can be divided into several categories, e.g., #0, #1, #2. . . . . ). The smaller the serial number, the more keywords its cluster contains—timeline: Each cluster is displayed as a horizontal timeline in a left-to-right order. The size of the circles on the map represents the number of citations. The timeline graph also reflects the time interval between 2002 and 2022 when the citation was noticed (Chen, 2017; Chen and Song, 2019).

# 3. Results

## 3.1. Annual publication volume

A total of 2,815 publications were identified in the WOS database for the period from 2002 to 2022 (**Supplementary Figure 2**).

## 3.2. Authors

The study identified 322 authors. Among them, Michael Okun ($n$ = 83), Andres Lozano ($n$ = 50), and Kelly Foote ($n$ = 31) were the top three authors in terms of the number of articles (**Supplementary Figure 3**).

## 3.3. Country/region

The study involved researchers from 43 countries/regions. **Table 1** presents the contributions from each country, with the United States leading with the highest number of publications ($n$ = 1,204; 42.8%), followed by Germany ($n$ = 400; 14.2%), the United Kingdom ($n$ = 279; 10.0%), Canada ($n$ = 221; 7.9%), and China ($n$ = 204; 7.2%) (**Table 1** and **Supplementary Figure 4**).

## 3.4. Institution

Out of 541 scientific institutions, seven published more than 50 papers each, namely, the University of Toronto, University of Florida, University of Oxford, Mayo Clinic, Emory University, University of California San Francisco, and Baylor College of Medicine (**Table 2** and **Supplementary Figure 5**).

## 3.5. Co-occurring keywords

The study involved 772 keywords, with the top 10 most frequent ones being essential tremor ($n$ = 671), movement disorder ($n$ = 424), tremor ($n$ = 375), basal ganglia ($n$ = 370), globus pallidus ($n$ = 289), subthalamic nucleus stimulation ($n$ = 280), and thalamic stimulation ($n$ = 254) (**Supplementary Figure 6**).

Clustering analysis revealed 10 co-occurring keyword clusters: #0 basal ganglia, #1 deep brain stimulation, #2 quality of life, #3 zona incerta, #4 high frequency stimulation, #5 orthostatic tremor, #6 blood-brain barrier, #7 double-blind, #8 machine learning, #9 tremor suppression, and #10 transcranial magnetic stimulation (**Supplementary Figure 7**).

A timeline map was constructed for the co-citation graph based on the time nodes. **Supplementary Figure 8** displays the top three keywords with the highest level of activity and increasing attention since 2002: #0 basal ganglia, #1 deep brain stimulation, and #2 quality of life (**Supplementary Figure 8**).

The top 10 keywords with the most powerful citation bursts were: pallidotomy (12), thalamotomy (9.59), bilateral stimulation (13.43), multicenter (10.25), focused ultrasound thalamotomy (9.57), DB (9.53), Parkinson (10.04), consensus statement (8.78), s disease (11.16), and functional neurosurgery (9.57) (**Supplementary Figure 9**). Since our research theme was

TABLE 1 Top 10 countries/regions by publications.

| Rank | Countries/ Regions | Publications | Percentage (total of 2,815; %) |
|---|---|---|---|
| 1 | United States | 1204 | 42.8 |
| 2 | Germany | 400 | 14.2 |
| 3 | United Kingdom | 279 | 10.0 |
| 4 | Canada | 221 | 7.9 |
| 5 | China | 204 | 7.2 |
| 6 | Italy | 178 | 6.3 |
| 7 | France | 139 | 4.9 |
| 8 | Japan | 129 | 4.6 |
| 9 | Netherlands | 116 | 4.1 |
| 10 | Spain | 109 | 3.9 |

TABLE 2 Top 10 institutions according to publications.

| Rank | Publications | Institutions | Original country |
|---|---|---|---|
| 1 | 128 | University of Toronto | Canada |
| 2 | 111 | University of Florida | United States |
| 3 | 78 | University of Oxford | England |
| 4 | 59 | Mayo Clinic | United States |
| 5 | 57 | Emory University | United States |
| 6 | 54 | University of California San Francisco | United States |
| 7 | 50 | Baylor College of Medicine | United States |
| 8 | 49 | University of Kiel | Germany |
| 9 | 48 | University College London | United States |
| 10 | 48 | Duke University | United States |

surgical treatment of PD we excluded Parkinson (10.04), consensus statement (8.78). We also excluded keywords with poor spelling, including DB (9.53), s disease (11.16).

### 3.6. Co-citations reference

**Supplementary Figure 10** displays 1,467 nodes, and **Table 3** lists the top 10 references with high co-citations. The table provides a summary of essential information, including the number of citations, journal, study type, sample size, patients, interventions, outcomes, and highlights for each reference (Schuurman et al., 2000; Deep-Brain Stimulation for Parkinson's Disease Study Group et al., 2001; Krack et al., 2003; Deuschl et al., 2006; Follett et al., 2010; Little et al., 2013; Schuepbach et al., 2013; Elias et al., 2016; Cury et al., 2017; Bhatia et al., 2018).

## 4. Analysis of results

### 4.1. Analysis of annual publications, countries/regions, institutions, authors, and co-cited authors

This study focused on publications related to the surgical treatment of tremor in PD between 2002 and 2022. A bibliometric analysis was performed using the WoS database to gain insights into research dynamics and facilitate future investigations. A total of 2,815 articles were included in the analysis. Over the specified period, there was a consistent increase in annual publications, indicating a growing interest in and efforts toward the surgical treatment of tremor in PD. The United States emerged as the leading contributor, with the highest number of publications and citations. In the country/region co-authorship analysis network, the United States ranked first, underscoring its significant influence and prominent role in research on the surgical treatment of PD tremor. Remarkably, seven out of the top 10 institutions conducting research in this field were based in the United States. These results suggest that the United States may have substantial influence and play a leading role in the direction of research on the surgical treatment of PD tremor. Through a comprehensive examination of prolific authors and their articles, current research hotspots in the surgical treatment of PD tremor were identified. Notably, Michael Okun ($n$ = 83) from the University of Florida was the most prolific author, followed by Andres M Lozano ($n$ = 50) from the University of Toronto, and Kelly D Foote ($n$ = 31) from the University of Florida. Highly productive authors could reflect the current research frontiers in the field. The research areas of the highly productive authors also verify the reliability of citespace's hotspot results. Therefore, we have summarized the research directions of Michael Okun, Andres M Lozano and Kelly D Foote. Specifically, Michael S. Okun and Kelly D Foote, both affiliated with the University of Florida, co-founded and co-direct the renowned Norman Fixel Institute for Neurological Diseases at UF health. Their focus on DBS and neuromodulation has demonstrated that DBS is a practical surgical approach for treating tremor. Dr. Andres M Lozano focuses on functional neurosurgery and the development of new therapies for the treatment of movement disorders and psychiatric disorders. His team conducts research on brain mapping, DBS, and focused ultrasound (FUS) in both patients and animal models of the disease.

### 4.2. Analysis of co-occurring keywords

Keywords play a crucial role in reflecting the themes and frontiers of research articles. Through the co-occurring keywords clustering analysis, we observed a strong focus on tremor in the context of the surgical treatment of PD. Notably, the subthalamic nucleus and DBS emerged as prominent research hotspots. The clustering of keyword modules, as indicated by Modularity Q ($>0.3$) and Mean Silhouette ($>0.7$), is highly convincing. In the co-occurring keyword time zone map, #0 basal ganglia and #1 DBS were found to be actively studied, and they garnered sustained attention over an extended period.

Citation burst identifies keywords with high frequencies at specific times, indicating the evolutionary trends in a particular study. Notably, pallidotomy (burst = 12; 2002–2010), bilateral stimulation (burst = 13.43; 2004–2010), and focused ultrasound thalamotomy (burst = 9.57; 2017–2022) exhibited prolonged periods of continuous research focus. Furthermore, tracing back, we observed a renewed interest in pallidotomy in 1992 after a dormant period of PD tremor surgery, followed by a remarkable surge in pallidotomy-related publications (Laitinen et al., 1992; de Bie et al., 1999; Hariz, 2003; Vitek et al., 2003).

During this period, DBS surgery emerged, and related research rapidly developed. During the early 20th century, numerous studies confirmed the efficacy of bilateral DBS (Limousin et al., 1995; Esselink et al., 2004; Smeding et al., 2005; Gross, 2008). In 2011, the World Movement Disorders Society guidelines reviewed reports on motor symptoms in PD from 2004 to 2010, ranking bilateral subthalamic nucleus DBS, bilateral pallidum DBS, and unilateral pallidotomy as the highest level of evidence (Fox et al., 2011). Concurrently, advancements in technology enabled the use of phased-array transducers to deliver precise, incisionless, transcranial acoustic energy, leading to the development of focused ultrasound (FUS) for lesion destruction. Consequently, several studies explored the application of FUS in various brain diseases, and in 2017, a randomized controlled trial (RCT) by JAMA Neurol provided further evidence that ultrasound-focused thalamotomy effectively relieved tremor in drug-resistant PD, a significant milestone (Martin et al., 2009; Elias et al., 2016; Bond et al., 2017). Technological advancements continued, and in 2019, the FDA approved MRgFUS for the treatment of tremor in PD and primary tremor, marking a significant advancement in the field.

The co-occurring keyword analysis reveals essential themes in the research on the surgical treatment of tremor in PD. Notably, neuron destruction procedures, including pallidotomy, thalamotomy, and focused ultrasound thalamotomy, along with neuromodulation techniques like DBS, subthalamic nucleus stimulation, and thalamic stimulation, emerge as significant areas of interest. Moreover, studies of the brain regions

TABLE 3 Top 10 highly cited references details.

| References | Citation counts | Journal | Design or type of study | Sample size | Participants | Intervention | Outcomes | Highlights |
|---|---|---|---|---|---|---|---|---|
| Elias et al., 2016 | 109 | *NEJM* | Single-blind, randomized, controlled trial | 76 | Patients diagnosed with essential tremor, with moderate to severe postural or intentional tremor of the hand, who have failed at least two trials of drug therapy and whose current drug dose is stable for primary tremor. | MRgFUS vs. sham procedure. | The Clinical Rating Scale for tremor and the Quality of Life in Essential tremor Questionnaire | 1. Demonstrated the efficacy of FUS in drug-refractory primary tremor.<br>2. Long-lasting efficacy (12 months) |
| Bhatia et al., 2018 | 97 | *Movement Disorders* | Consensus | \ | \ | \ | \ | 1. Classification of tremor based on two axes.<br>2. To provide a framework for clinical diagnosis of new tremor syndromes for further etiological studies. |
| Krack et al., 2003 | 79 | *NEJM* | Follow-up study | 49 | Patients aged less than 70 years with advanced PD after bilateral stimulation of the subthalamic nucleus | Bilateral STN-DBS | UPDRS, Mattis Dementia Rating Scale, an assessment of frontal-lobe dysfunction, Beck depression inventory | 1. Demonstrated that STN-DBS significantly improved all post-operative PD motor symptoms.<br>2. No stimulus tolerance was found (5-year follow-up).<br>3. It is proposed that the procedure is more effective in patients with PD dyskinesia who respond well to medication and are relatively young. |
| Little et al., 2013 | 78 | *Annals of Neurology* | Randomized controlled study | 8 | Patients with advanced idiopathic PD with motor fluctuations and/or dyskinesias | aDBS vs. no stimulation vs. cDBS. | UPDRS | 1. Optimize the DBS stimulation protocol.<br>2. Design a BCI-controlled adaptive DBS (aDBS). |
| Schuepbach et al., 2013 | 77 | *NEJM* | Randomized, multicenter, parallel-group | 251 | PD patients with early motor complications | DBS plus medical therapy or medical therapy alone | UPDRS, the time with good mobility and no dyskinesia. | Neurostimulation was indicated for earlier, younger patients (mean duration of disease of 7.5 years; mean age of 52 years; fluctuations and motor deficits for 1.7 and 1.5 years, respectively). |

*(Continued)*





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TABLE 3 (Continued)

| References | Citation counts | Journal | Design or type of study | Sample size | Participants | Intervention | Outcomes | Highlights |
|---|---|---|---|---|---|---|---|---|
| Deuschl et al., 2006 | 71 | *NEJM* | Non-blind, randomized, paired trial | 156 | Patients younger than 75 years with impaired motor symptoms of PD affecting the ability to perform daily living with optimal drug therapy. | DBS group vs. drug group | PDQ-39, UPDRS | 1. Used quality of life as the primary outcome indicator<br>2. The risks of surgery need to be weighed against the benefits of surgery. |
| Schuurman et al., 2000 | 70 | *NEJM* | Randomized controlled trial | 68 | Severe unilateral or bilateral arm tremor due to PD, primary tremor, or multiple sclerosis for at least one year in the case of medication. | Thalamotomy or thalamic stimulation | Frenchay Activities Index, UPDRS, Essential tremor Rating Scale, Modified Tremor Scale. | 1. DBS is more effective and safer than thalamotomy.<br>2. STN is a superior target than thalamus. |
| Deep-Brain Stimulation for Parkinson's Disease Study Group et al., 2001 | 69 | *NEJM* | Prospective, double-blind, crossover study | 134 | Patients aged 30–75 years with at least two major features of PD (tremor, rigidity, and bradykinesia) that are not controlled by medication and respond well to levodopa | Randomly turn on/off the DBS to STN or globus pallidus (GPi) | UPDRS, a dyskinesia-rating scale, a home diary | 1. Demonstrated the effectiveness and safety of two DBS targets.<br>2. STN may be superior to the pallidum as a target. |
| Cury et al., 2017 | 65 | *Neurology* | Follow-up study | 98 | PD, ET, and dystonia due to refractory tremor | VIM-DBS | UPDRS; Fahn, Tolosa, Marin tremor Rating Scale | 1. Long follow-up period (over 10 years)<br>2. VIM is effective for PD tremor in the long term but does not delay PD progression.<br>3. VIM is the preferred target for tremor. |
| Follett et al., 2010 | 65 | *NEJM* | Multi-center, randomized, blinded trial | 299 | PD patients over 21 years of age with Hoehn Yahr score greater than 2 who are stable on medication and have poor efficacy | Pallidal DBS or STN-DBS | Hoehn and Yahr scale, Schwab and England scale of activities of daily living, stand–walk–sit test; UPDRS, PDQ-39, Beck depression inventory-II. | 1. Demonstrated the efficacy of DBS in the two target areas and the reduction of dopaminergic medication.<br>2. Improvements in pulse generators can reduce the impact of amplitude in DBS surgery. |





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involved in Parkinson's disease tremor, such as the subthalamic nucleus, basal ganglia, and globus pallidus, also hold crucial importance in this field.

### 4.3. Analysis of top 10 highly co-cited references

Highly co-cited references are regarded as seminal contributions in the field and represent articles with significant influence. The frequency of citations indicates the literature's impact. Among these references, seven were clinical trials, two were follow-up studies, and one was a consensus statement. The top 10 most frequently cited references provide insights into the key topics that have garnered researchers' attention in the surgical treatment of PD tremor and reveal essential research areas in this field. Among these references, Elias et al. (2016) stands out as the most frequently co-cited, with a centrality value of 0.03. This paper focuses on MRI-guided focused ultrasound technology (MRgFUS), which has emerged as a prominent area of research in recent years, presenting new hotspots and trends that warrant attention. Notably, the remaining nine references are DBS-related, highlighting the current significant interest in DBS among researchers. By analyzing these eight papers, we identified three primary areas related to DBS research: (1) operative indications, encompassing timing, purpose, post-operative adverse effects, and prognosis of the surgery; (2) exploration of new targets; and (3) advancements in DBS devices.

## 5. Discussion

### 5.1. Bibliographic characteristic

This discussion centers on the research status and hotspots concerning the surgical treatment of tremor in PD from 2002 to 2022. We collected a total of 2,815 relevant publications from the WOS database and primarily analyzed them using CiteSpace software.

The analysis of relevant publications revealed insightful findings from diverse perspectives. Firstly, a notable upward trend was observed in the number of publications, indicating an increasing focus on the surgical treatment of tremor in PD. Secondly, the geographic distribution of publications showed early research initiatives from European countries (Germany, the United Kingdom), Canada, and China, while the United States emerged as the leading contributor, with eight out of the top 10 institutions that produced the most publications situated in the country. These outcomes strongly suggest the prominent role of the United States in this field. We should put more attention to the research from the United States.

The top three authors in terms of the number of articles and co-cited authors were Michael Okun, Andres Lozano, and Kelly Foote, showcasing their significant contributions to the field of surgical treatment of tremor in PD. Particularly, Andres Lozano's article on a randomized trial of focused ultrasound thalamotomy, published in the New England Journal of Medicine, held the first position among co-cited references, underscoring the pivotal role of MRgFUS in the surgical treatment of tremor in PD. Researchers could start from the works of these prolific authors in order to know the hotspots in the field of surgical treatment of PD tremor, and could also grasp the frontier directions in the field of surgical treatment of PD tremor by studying the research directions of these prolific authors.

Keywords co-occurrence and co-occurring keywords clustering analysis have enabled us to rapidly and directly identify the general themes and hotspots in the field of surgical treatment of tremor in PD. In our analysis, the subthalamic nucleus and DBS garnered significant attention from researchers. Meanwhile, the basal ganglia and DBS remained active subjects of longstanding interest. This observation indicates that DBS and operative targets represent prominent hotspots in the field of surgical treatment of tremor in PD. Initially, early researchers primarily focused on pallidotomy, but as time progressed, there was a gradual shift toward a safer and more effective alternative—DBS, which has maintained sustained attention over the years. Moreover, recent advancements in non-invasive nerve destruction techniques have brought focused ultrasound thalamotomy to the forefront as a new and promising hotspot in the field of surgical treatment of tremor in PD.

In our investigation of co-cited references, we identified the top 10 highly co-cited articles, recognized as significant landmarks in the field of surgical treatment of tremor in PD. We then conducted a detailed examination of these 10 publications. Notably, eight out of the 10 references were dedicated to DBS, highlighting its pivotal role in the field of surgical treatment of tremor in PD. These references not only underscore the importance of DBS but also shed light on several crucial research areas and key aspects related to DBS. Accordingly, we meticulously analyzed these eight references to gain insights into the overall landscape of DBS in the context of surgical treatment for tremor in PD.

The eight references acknowledged the efficacy of DBS and primarily addressed three crucial aspects within the realm of surgical treatment for tremor in PD: operative indication (encompassing timing, purpose, post-operative adverse effects, and surgery prognosis), operative target, and operative stimulation projection.

### 5.2. Operative indication

Among the eight references focused on DBS, two discussed the timing of DBS, highlighting its significance as a research hotspot. The findings suggest that DBS surgery could be considered for PD patients with a disease duration of $\geq$5 years (or $\geq$3 years if severe tremor persist after standardized drug treatment), those experiencing the "on-off phenomenon" and Hoehn Yahr stage in the range of 2.5–4, or individuals aged below 75 years. However, flexibility in these restrictions is recommended based on individual patient health (FNG Chinese Society of Neurosurgery et al., 2020). Numerous studies have demonstrated that DBS surgery is beneficial for PD patients with motor complications in the middle to late stages of the disease. In contrast, Schuepbach et al.

(2013) WMM's study included PD patients with relatively early disease stages (disease duration <5 years and young age) for DBS treatment, yielding positive outcomes (Schuepbach et al., 2013). Krack et al. (2003) proposed that the procedure is more effective in patients with PD dyskinesia who respond well to medication and are relatively young. Hacker ML et al. included PD patients who had received pharmacological treatment for an average of 7.5 years and had recently developed motor complications (Hacker et al., 2015). These patients underwent DBS surgery with favorable results, suggesting that the timing of undergoing DBS surgery is advanced from patients with a long disease course to patients who present early with motor complications. After a thorough evaluation by a team of specialized physicians, patients with early stage PD and recently developed motor complications can benefit from DBS surgery.

Few studies have been conducted on older (>75 years) PD populations, and none of the highly co-cited references in this study specifically examined older patients with PD tremor. This indicates a gap in DBS research concerning aged PD patients. As a neurodegenerative disease, PD primarily affects individuals in middle and old age. However, most studies included in the current analysis and consensus recommendations for surgery focused on patients aged less than 75 years. There is a need for in-depth research on surgical interventions for PD patients older than 75 years. DeLong et al. collected and analyzed data from 1,750 patients treated with DBS between 2000 and 2009, and found that the overall complication rate did not increase with aging (DeLong et al., 2014). Some argue that performing DBS early may lead to very short disease duration, making it difficult to achieve a precise diagnosis and potentially resulting in misdiagnosis (Charles et al., 2014). On the other hand, others suggest that performing DBS very late may exacerbate primary conditions, increase post-operative complications and mortality, and impact the long-term efficacy of STN-DBS. The timing of surgery remains a subject of heated debate. Whether the constraints on disease duration and age restrictions could be relaxed, allowing DBS surgery to be performed on younger or older patients, has been a topic of significant discussion in recent years but remains uncertain.

Four out of the eight references examined in this study focused on operative targets. Schuurman et al. (2000) demonstrated the superiority of the Subthalamic Nucleus (STN) over the thalamus as a target. Deep-Brain Stimulation for Parkinson's Disease Study Group et al. (2001) acknowledged the effectiveness and safety of two DBS targets, namely, the GPi and STN. Cury et al. (2017) highlighted the long-term effectiveness of the ventral intermediate nucleus of the thalamus (Vim) target for PD tremor without delaying PD progression. Krack et al. (2003) showed that STN-DBS significantly improved all post-operative PD motor symptoms.

## 5.3. Surgical targets

The efficacy of DBS surgery in treating PD tremor varies based on the chosen target. Among the top 10 highly co-cited references, four discussed STN, GPi, and Vim as operative targets. These three targets, STN, GPi, and Vim, have been extensively studied for PD tremor. In our investigation, STN surgery constituted over half of the clinical studies, which aligns with the prevailing trend among research teams.

Schuurman et al. (2000) and Deep-Brain Stimulation for Parkinson's Disease Study Group et al. (2001) conducted discussions on the effects of different targets for DBS. There should be an authoritative recommendation on target for surgical treatment of tremor in Parkinson's disease. The primary targets for PD surgical treatment are STN and GPi, which demonstrate similar improvements in motor symptoms, including tremor, in PD patients (Ramirez-Zamora and Ostrem, 2018; Wong et al., 2019). STN-DBS offers significant benefits in terms of post-operative reduction in anti-PD drug dosage (Odekerken et al., 2015). Research by Volkmann et al. (1998) reported a remarkable 65% decrease in drug use following STN stimulation, with less electrical stimulation required, leading to a more cost-effective treatment. Additionally, some animal experiments suggest that STN stimulation may also have neuroprotective effects (Wallace et al., 2007). STN-DBS is recommended for patients experiencing dopaminergic drug-induced behavioral disorders (Fasano et al., 2010; Weaver et al., 2012; Odekerken et al., 2015). However, it is crucial to consider that STN-DBS carries slightly higher surgical risks concerning cognitive function, particularly for patients with preoperative language and cognitive deficits associated with PD. In such cases, GPi-DBS is a more suitable choice. GPi provides a more cost-effective approach to alleviate and treat motor deficits, and its long-term stability and cognitive benefits offer distinct advantages (St George et al., 2014; Toft and Dietrichs, 2014).

Subthalamic Nucleus-DBS requires more post-operative modulation and has a higher incidence of adverse effects associated with levodopa withdrawal compared to GPi-DBS. On the other hand, the Vim target has proven effective and long-lasting for PD patients with significant tremor but shows limited effectiveness for other symptoms such as rigidity, bradykinesia, and drug-induced allodynia (Deuschl et al., 2006; Schuepbach et al., 2013). Additionally, a study at the University of Kansas Medical Center indicated that Vim-DBS had no significant improvement in post-operative affective cognition (Troster et al., 1998). However, Woods et al. (2001) conducted a 1-year follow-up study of six PD patients treated with Vim-DBS and found that PD patients could benefit from Vim-DBS in terms of both neurocognitive safety and quality of life. Nonetheless, further studies are necessary to confirm the role of Vim in affective cognition. Patients with relatively good motor function but disabling tremor may experience better outcomes from Vim-DBS. **Table 4** provides a comparison of the advantages and disadvantages of STN, GPi, and Vim targets.

## 5.4. DBS protocols

Two references address the improvement of DBS devices. Little et al. (2013) demonstrated the design of a Brain-Computer Interface (BCI)-controlled adaptive DBS (aDBS) and optimized

TABLE 4 Comparison of the advantages and disadvantages of STN, GPi, and Vim targets.

| Target | Advantages | Disadvantages |
|---|---|---|
| STN | 1. Reduced levodopa dose.<br>2. More comprehensive PD symptom control.<br>3. Lower energy consumption and higher cost performance.<br>4. Possible neuroprotective function | Complications of surgery include cognitive decline, psychological problems (anxiety and depression), speech, balance, and postural gait disorders, which require more medications. |
| GPi | 1. Control tremor in PD.<br>2. Significantly improves motor retardation and rigidity.<br>3. No significant speech, psychological, or neurological damage | Inability to reduce drug dose |
| Vim | Highly effective for controlling tremor.<br>Long duration of action. | 1. It works only for tremor and not for other symptoms, such as rigidity and bradykinesia.<br>2. Bilateral stimulation can produce cognitive problems, worsening dysphonia, sensory abnormalities, gait disturbances, pain, and other adverse effects.<br>3. Controversy about affective cognition. |

the stimulation protocol. Follett et al. (2010) highlighted that advancements in pulse generators can mitigate the impact of amplitude in DBS surgery. Beyond electrodes, hardware innovations in recent years have focused on pulse generators, moving toward energy-efficient and miniaturized technology. Some pulse generators can generate novel waveforms with an independent current control system (Weiss and Pal, 2018). These advancements have provided novel stimulation waveforms and modalities with temporal changes that activate corresponding neurons. By varying waveforms and pulse intervals, different stimulation modalities can be achieved. During long-term post-operative follow-up, an increasing number of PD patients experience adverse reactions or poor symptom control, leading researchers to develop innovative settings for stimulation voltage, current, polarity, pulse width, waveform, and frequency to obtain new program control strategies. Additionally, improvements in Directional Leads and pulse generators have enhanced the diversification of post-operative program control modes, aiming to achieve maximum symptom control with minimal stimulation (Aubignat et al., 2020). To explore optimal program control strategies, multiple program control technology software or algorithms can be utilized. However, no single software or algorithm can address all symptoms, necessitating further research in breakthroughs related to electrode and cell design, stimulation paradigms, closed-loop, on-demand stimulation, and sensing technologies to enhance the effectiveness and tolerability of DBS. These potential directions hold promise for future research.

## 5.5. MRgFUS

In addition, the result that the article about MRgFUS ranked the first of the top 10 most frequently co-cited references, combined with the result of the co-occurring keywords burst, indicated that, with significant research potential, MRgFUS might be a new hotspot in the field of surgical treatment of tremor in PD.

This novel non-invasive nerve destruction technique utilizes the combination of MRI and high-intensity focused ultrasound. By employing MRI for precise real-time target localization and intraoperative temperature monitoring, focused ultrasound ablation generates thermal effects on the target area *in vivo*. The technique is irreversible (Xiong and Yq, 2020).

The paper compares the treatment effects of DBS and MRgFUS for tremor in PD. DBS currently serves as the mainstream surgical approach, while MRgFUS offers advantages as a new type of nerve destruction technique. MRgFUS has demonstrated efficacy in managing significant tremor and is particularly effective in drug-refractory PD tremor (Magara et al., 2014; Schlesinger et al., 2015). It is generally considered suitable for patients with more severe unilateral symptoms of PD tremor (Martinez-Fernandez et al., 2018). Moreover, MRgFUS serves as a viable surgical option for PD patients who are not candidates for craniotomy, have contraindications to DBS, or have limited access to regular device reprogramming resources. **Table 5** provides a comparison of the principles, advantages, and disadvantages of DBS and MRgFUS.

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Although MRgFUS has garnered increasing attention, it remains an emerging surgical treatment. Prospective multicenter large-scale randomized controlled trials and cohort studies are necessary to further assess its merits. In summary, MRgFUS, as a novel nerve destruction technique, can serve as a complementary surgical modality to neuromodulation and is currently a prominent area of research in the surgical treatment of tremor in PD.

## 5.6. Research trends in the surgical treatment of tremor in PD

Surgery is an indispensable treatment for tremor in PD. Our study spanning from 2002 to 2022 reveals a shift in the research hotspot within the field of surgical treatment for PD tremor. Attention and interest have transitioned from pallidotomy to DBS. Although the treatment efficacy of DBS for PD tremor is widely acknowledged, numerous and hotly debated issues and

TABLE 5 Principles, advantages, and disadvantages of DBS and MRgFUS.

| Surgery | Principle | Advantages | Disadvantages |
|---|---|---|---|
| DBS | Neuromodulatory mechanism | 1. Adjustable range to increase precision. Unlimited treatment targets | 1. Requires craniotomy. |
| | | 2. Multiple programmable modalities for repeated stimulation | 2. Higher risk of intracranial hemorrhage and infection. |
| | | 3. Reversibility. | 3. Need for long-term implantation of artificial materials. |
| | | | 4. General anesthesia. |
| | | | 5. Multiple post-operative procedures and device maintenance are required. |
| | | | 6. DBS device implantation does not allow ultrasound or MRI. |
| MRgFUS | Neural destruction mechanisms | Non-invasive. No anesthesia required. No ionizing radiation. Mild adverse reactions and rare severe adverse reactions (Fishman et al., 2018). | Skin preparation required. Claustrophobia. Irregular destruction of target tissues by ultrasound ablation, with possible over- or under-destruction and risk of recurrence (Zong et al., 2020). Irreversible (Elias et al., 2013). May burn the scalp. |

controversies persist regarding operative indications, targets, and DBS devices. Recently, MRgFUS has garnered increasing attention. Our study also provides a comprehensive comparison of the advantages and disadvantages between DBS and MRgFUS.

This study presents several notable advantages. Firstly, this study represents a pioneering effort as the first bibliometric and visualization analysis conducted in the field of surgical treatment of tremor in PD, demonstrating significant innovation. Secondly, our study utilizes the WOS database, a prominent tool in bibliometric analysis, for objective and systematic retrieval. Thirdly, we primarily employ a novel bibliometric software, Citespace, which provides a fresh approach to elucidating the underlying mechanisms of various changes and patterns in scientific networks. By identifying critical nodes based on objective data, we can discern the research hotspots in surgical treatment for PD tremor, thus offering valuable directions for future clinical practices and research in this field. Finally, our data analysis is comprehensive and rigorous, encompassing various aspects such as annual publication volume, authors, institutions, countries/regions, and co-cited literature. This in-depth analysis of surgical treatment for PD tremor enables readers to gain a comprehensive understanding of peer education, training, and scientific research guidance.

This study also exhibits some limitations. Firstly, it solely searched the English literature in the WoS database, limiting the scope to the years 2002 to 2022. Consequently, the selected keywords might not fully represent all relevant studies in the field, potentially introducing bias. Additionally, considering that surgery for PD tremor encompasses a vast area, a more comprehensive understanding of specific surgical aspects may be necessary for this study.

## 6. Conclusion

This research employs a comprehensive and systematic bibliometric and visualization analysis to visualize the evolution of research and emerging hotspots. The identified hotspots are as follows: Firstly, DBS has garnered considerable attention and widespread recognition in the surgical treatment of PD tremor. Secondly, there are numerous critical issues in operative indications, operative targets, and surgical protocols related to DBS. Thirdly, MRgFUS has emerged as a promising treatment in the field of surgical treatment for PD tremor. This study also elucidates these hotspots, providing valuable insights and guidance for future research.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

JCZ and HC: conceptualization, writing-original draft, and formal analysis. YQL and XX: methodology, software, and validation. LML: visualization and investigation. JJL: conceptualization and supervision. GAL: data curation. LSL and LHL: writing—review and editing. NGX and SXW: project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1157443/full#supplementary-material

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# Enhanced brain functional connectivity and activation after 12-week Tai Chi-based action observation training in patients with Parkinson's disease

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**Introduction:** Motor-cognitive interactive interventions, such as action observation training (AOT), have shown great potential in restoring cognitive function and motor behaviors. It is expected that an advanced AOT incorporating specific Tai Chi movements with continuous and spiral characteristics can facilitate the shift from automatic to intentional actions and thus enhance motor control ability for early-stage PD. Nonetheless, the underlying neural mechanisms remain unclear. The study aimed to investigate changes in brain functional connectivity (FC) and clinical improvement after 12 weeks of Tai Chi-based action observation training (TC-AOT) compared to traditional physical therapy (TPT).

**Methods:** Thirty early-stage PD patients were recruited and randomly assigned to the TC-AOT group ($N = 15$) or TPT group ($N = 15$). All participants underwent resting-state functional magnetic resonance imaging (rs-fMRI) scans before and after 12 weeks of training and clinical assessments. The FCs were evaluated by seed-based correlation analysis based on the default mode network (DMN). The rehabilitation effects of the two training methods were compared while the correlations between significant FC changes and clinical improvement were investigated.

**Results:** The results showed that the TC-AOT group exhibited significantly increased FCs between the dorsal medial prefrontal cortex and cerebellum crus I, between the posterior inferior parietal lobe and supramarginal gyrus, and between the temporal parietal junction and clusters of middle occipital gyrus and superior temporal. Moreover, these FC changes had a positive relationship with patients' improved motor and cognitive performance.

**Discussion:** The finding supported that the TC-AOT promotes early-stage PD rehabilitation outcomes by promoting brain neuroplasticity where the FCs involved in the integration of sensorimotor processing and motor learning were strengthened.

KEYWORDS

**Parkinson's disease, motor-cognitive intervention, default mode network, restingstate functional MRI, rehabilitation**

# 1. Introduction

Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, resulting in a wide spectrum of motor symptoms, such as bradykinesia, rigidity, gait disorders, and instability (Bloem et al., 2021). The alterations in the cortico-basal ganglia networks play a pivotal role in the coordination of cognitive and motor functions, while cognitive impairments significantly influence movement automaticity and motor control ability (DeLong and Wichmann, 2007; Aarsland et al., 2017; Florio, 2018). Motor-cognitive interactive training is crucial for early-stage PD rehabilitation to slow disease progress.

Motor-cognitive interactive rehabilitation has proven to have an impact on neural pathways related to cognitive and motor functions. Attention and executive functions are a set of top-down processes that modulate goal-based movements (Calleo et al., 2012; Bloem et al., 2015). The integration of cognitive training and motor exercise would enhance PD patients' ability to plan and execute movements while activating brain regions related to memory, attention, and problem-solving (Maidan et al., 2017; King et al., 2020; Johansson et al., 2022). Current studies have reported various motor-cognitive interactive training frameworks that combine motor training with virtual reality, secondary cognitive tasks, or motor imagery (Ferrazzoli et al., 2018; Herold et al., 2018; Ferrazzoli et al., 2020).

Action observation training (AOT) has drawn more and more attention as an effective motor-cognitive interactive training framework that requires participants to observe and imitate specific movements (Caligiore et al., 2017, 2019). Agosta et al. (2017) showed that 4 weeks of AOT with mobility training could reduce the severity of gait freezing, where gait improvement was associated with increased brain activation of the mirror neuron system. The recruitment of cognitive processing within the AOT would lead to the functional reorganization of brain regions in motor control and movement execution (Sarasso et al., 2021). The incorporation of complex inter-limb coordination with AOT may facilitate cognitive-motor interplay (Li et al., 2012; Ferrazzoli et al., 2020). The AOT, incorporating Tai Chi-based continuous and spiral movements, could serve as a novel, effective motor-cognitive training. However, their rehabilitation effectiveness on early-stage PD patients and the underlying mechanisms are unclear.

Resting-state functional MRI (rs-fMRI), as a non-invasive neuroimaging technique, could provide deep insights into the mechanisms of cognitive-motor interplay where functional connectivity (FC) changes can be assessed (Snyder and Raichle, 2012; Soares et al., 2016). The default mode network (DMN) was commonly used in the fMRI analysis and was found to be related to internal mental-state processes (Buckner et al., 2008). The reduced FCs based on the DMN are correlated with executive dysfunction and progressive cognitive decline in PD (Baggio et al., 2015; Thibes et al., 2017). Cognitive training improved functional integration within the DMN in healthy older adults (Cao et al., 2016). Brain changes in the DMN have revealed that aerobic training could modulate brain metabolism in patients with mild cognitive impairment (Porto et al., 2018). Only a few studies have revealed the effect of the AOT on activating the cortical–subcortical region (Agosta et al., 2017; Sarasso et al., 2021).

This study aimed to investigate the rehabilitation effects and the underlying motor-cognitive mechanisms of Tai Chi-based AOT (TC-AOT) training compared to conventional physical therapy. We explored brain FC changes following the 12-week AOT using rs-fMRI. We hypothesized that the TC-AOT, which integrated action observation, motor imagery, and imitation, would be more effective in improving motor and cognitive functions that can be related to network connectivity reorganization of functional brain regions.

# 2. Materials and methods

## 2.1. Participants and clinical measurement

Thirty idiopathic, early-stage PD patients were recruited at the Tianjin General Hospital according to the following inclusion criteria: (1) aged 50–75 years; (2) Hoehn & Yahr (H & Y) stage ranging from 1 to 2.5 while in "on state" (Hoehn and Yahr, 2001); (3) on an anti-parkinsonian medical treatment with a stable daily dose for at least 4 weeks; (4) a Mini-Mental Status Examination score (MMSE) $\geq$ 24 with more than 12 years of education as the education level needs to be considered to be the strongest noncognitive factor that can affect the performance of MMSE (Folstein et al., 1975; Chen et al., 2016; Opdebeeck et al., 2016); (5) volunteered to participate in the rehabilitative exercise. Exclusion criteria were as follows: (1) other neurological disorders, such as stroke and post-traumatic brain injury; (2) cognitive impairment or dementia; (3) orthopedic problems that affect mobility; and (4) significant head tremor or any contraindications to MRI examination.

Demographic information and clinical assessment scores, including age, gender, disease duration, levodopa equivalent daily dose (LEDD), H & Y stage, and the MMSE score, were collected at enrollment. Thirty participants with PD were finally enrolled and randomly assigned to the Tai Chi-based AOT group (TC-AOT, $n = 15$) or the traditional physical therapy group (TPT, $n = 15$). General motor and cognitive function, balance and posture control capacity, quality of life, and rs-fMRI scan were evaluated at baseline (W0): General motor function was assessed using the Unified Parkinson's Disease Rating Scale-Part III (UPDRS-III) (Goetz, 2003); Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) assessed global cognitive function; Berg Balance Scale (BBS) (Franchignoni and Velozo, 2005); and the Mini Balance Evaluation Systems Test (Mini-BESTest) (Bloem et al., 2016) were used to objectively determine the participant's ability to safely balance and maintain posture. All aforementioned clinical measurements were repeated after 12 weeks of training (W12).

All participants provided written informed content, and the study was approved by the Ethics Committee of Tianjin General Hospital. Our trial was registered at www.chictr.org.cn at ChiCTR22000062596.

## 2.2. Intervention procedure

The study was a randomized, assessor-blind exercise trial. Participants were randomized in a 1:1 ratio to the TC-AOT or TPT groups with a block size of 15. To facilitate unbiased group assignment, a concealed allocation procedure was implemented. The allocations were recorded within sealed and opaque envelopes containing computer-generated random numbers, prepared by a research assistant who was intentionally kept blinded to the contents. The

researchers who performed the assessments at the time points of W0 and W12 remained blinded to group assignment. Both TC-AOT and TPT groups were conducted in a controlled clinical setting in Tianjin General Hospital under the supervision of a licensed physiotherapist. Participants' movement execution and safety were closely monitored to ensure consistency and adherence to the prescribed protocols. No participants had engaged in any exercise or physiotherapy before the study. They were instructed not to initiate any new exercise or physiotherapy during the training period.

Both groups received a 1-h training intervention twice a week for 12 weeks. The TC-AOT intervention was administered in a group setting, with each group comprising 2–6 participants. Participants in the TC-AOT group were informed that a training trial consisted of three phases: (1) watched a 60-s video clip containing one specific Tai Chi sequence movement accompanied by voice instruction and concentrated on learning the performance of the actions; (2) imagined themselves performing these actions for 3 min; (3) imitated the Tai Chi actions as precisely as possible. Each training trial took about 5 min. One participant accomplished eight trials in a training session in total, with necessary breaks. Tai Chi movements were selected from the most popular Yang style (Lan et al., 2013). The physiotherapist would systematically progress the intervention based on the participant's abilities. On the other side, the TPT was administered on a one-on-one basis. The TPT group took a stretching and flexibility exercise consisting of proprioceptive neuromuscular facilitation and gait/balance training with cueing strategies. The therapists followed the same intervention protocol for each participant, but the intervention level was adjusted based on the individual's conditions. All training programs began with a short warm-up session and ended with a cool-down session.

## 2.3. fMRI analysis

### 2.3.1. MRI acquisition

Imaging data were captured using a 3.0 Tesla MRI system (GE 3.0 T DISCOVERY MR 750). Foam padding and earplugs were used to minimize head motion and reduce scanner noise. All participants were instructed to hold still and keep their eyes closed without thinking about anything in particular, but not to fall asleep during the period. T1-weighted images were acquired using a magnetization-prepared rapid acquisition gradient echo sequence with the following imaging parameters: repetition time (TR) = 8.2 ms, echo time (TE) = 3.2 ms, flip angle (FA) = 12°, field of view (FOV) = 256 × 256 $mm^2$, matrix = 256 × 256, slice thickness = 1.0 mm, no gap, and 188 slices in total. Resting-state fMRI data were acquired using a gradient-echo single-shot echo-planar imaging sequence with the following imaging parameters: TR/TE = 2,000/30 ms, FOV = 220 × 220 $mm^2$, matrix = 64 × 64, flip angle = 90°, slice thickness = 3 mm, 36 interleaved transverse slices, and 180 volumes.

### 2.3.2. Preprocessing of fMRI data

All resting-state functional and structural data were analyzed using SPM12 software (Wellcome Trust Center for Neuroimaging, London, United Kingdom) and CONN toolbox (version 21.a) (Whitfield-Gabrieli and Nieto-Castanon, 2012).[1] Data preprocessing was conducted using the following procedures: (1) functional images were realigned and unwrapped for head motion correction where data were excluded if the head movements exceeded 1.5 mm/1.5° translation/rotation on any axis (one subjects were excluded due to the exceeded head motion), slice-timing corrected, and ART-based identification of outlier scans for scrubbing; (2) functional and anatomical data were normalized into standard Montreal Neurological Institute (MNI) space using a direct normalization procedure and segmented into grey matter, white matter, and cerebrospinal fluid (CSF) tissues (Ashburner and Friston, 2005; Ashburner, 2007); (3) functional data were smoothed using spatial convolution with a Gaussian kernel of 8 mm full width half maximum (FWHM) and resampled to 2 $mm^3$ × 2 $mm^3$ × 2 $mm^3$; and (4) finally, potential confounding effects including noise components from white matter, CSF, scrubbing regressors, subject-motion realignment parameters, and session effects were included as regressors, and a band-pass filter (0.008–0.09 Hz) was used to reduce low-frequency drift and high-frequency physiological noise in each voxel.

### 2.3.3. Functional connectivity analysis

FC was processed using a seed-based correlation approach. According to currently available literature (Andrews-Hanna et al., 2010; Hou et al., 2016), the default modes network with 18 priori seeds was selected, including the anterior, dorsal, and ventral medial prefrontal cortices (amPFC, dmPFC, and vmPFC), superior frontal gyrus (SFG), inferior frontal gyrus (IFG), posterior inferior parietal lobule (pIPL), precuneus, posterior cingulate cortex (PCC), anterior temporal lobe (ATL), superior temporal sulcus (STS), temporal parietal junction (TPJ), and hippocampal formation (HF). The MNI coordinates of the 18 seeds are listed in Supplementary Table S1. All seeds were defined as spheres of 6 mm radius with a resolution of 2 $mm^3$. Seed-based correlation functional analyses were performed by computing the temporal correlation between each priori seed and the rest of the brain. Fisher's transformed bivariate correlation coefficients between a seed BOLD time series and each individual voxel BOLD time series were calculated while Fisher's r-to-z transformed correlation maps were generated. Therefore, an entire brain z-value map was created for each subject.

## 2.4. Statistical analysis

Statistical analysis was performed using SPSS version 26 (SPSS Inc., Chicago, IL). Means and standard deviations were calculated for all demographic and clinical assessment information. Shapiro–Wilk normality test was used to assess the normality of variables. Differences between groups in terms of demographic and clinical data were evaluated by a two-sample *t*-test, Mann–Whitney U test, or chi-square test, as appropriate. Longitudinal changes within groups and the time × group interactions were assessed by using an analysis of variance (ANOVA). The significance threshold was set at $p < 0.05$ (two-tailed).

A group-level analysis of FC was evaluated using a general linear model (GLM). A 2 × 2 mixed analysis of variance (ANOVA) was performed to investigate the main effect of the intervention and the time×group interaction effect. Cluster-level inferences were based on parametric statistics from Gaussian Random Field theory (Worsley et al., 1996) with a threshold setup consisting of a voxel-level ($p < 0.001$) and a family-wise corrected cluster level (p-FWE < 0.05) (Chumbley

1 https://www.fil.ion.ucl.ac.uk/spm/

et al., 2010). For explorational purposes, an uncorrected cluster level $p < 0.05$ was also considered, as the present study aims to investigate any possible effects of intervention (Bender and Lange, 2001).

To further investigate the motor-cognitive interaction mechanism, two-tailed Pearson's correlation was applied to calculate the correlation between the FC values at the time points W0 and W12, as well as their changes over time within the measured significant clusters and clinical score changes. The significance threshold was set at $p < 0.05$.

# 3. Results

## 3.1. Baseline characteristics and clinical outcomes

Thirty patients were randomly assigned to the TC-AOT group and the TPT group. All patients were in the early stages of PD. Two training groups had no significant difference in all demographic and clinical characteristics, including age, gender, disease duration, LEDD, H & Y stage, and UPDRS-III, as well as in MoCA, MMSE, BBS, Mini-BESTest, and PDQ-39 at baseline. None of the participants received additional physical therapy during the training period. The demographic and clinical data are detailed in Table 1.

After 12 weeks of training (W12), both the TC-AOT and TPT groups showed improved mobility with reduced UPDRS-III scores ($p$ TC-AOT = 0.008, $p$ TPT = 0.022), and enhanced balance performance with greater BBS ($p$ TC-AOT = 0.002, $p$ TPT = 0.019) and Mini-BESTest scores ($p$ TC-AOT = 0.006, $p$ TPT = 0.044), as well as a significant improvement in PDQ-39 scores ($p$ TC-AOT < 0.001, $p$ TPT = 0.030) compared to W0 (Figure 1). There was no significant time × group interaction in clinical assessments between the two groups, except that the TC-AOT group had a greater MoCA score after the intervention while the TPT group exhibited a decreased trend ($p = 0.04$, Table 1).

## 3.2. Seed-based correlation analysis

### 3.2.1. TC-AOT group vs. TPT group at baseline

There were no significant FC differences were observed at the time point of baseline between the TC-AOT and TPT groups.

TABLE 1 Clinical variables of the two PD training groups at baseline.

| Variables | TC-AOT | TPT | $p$ |
|---|---|---|---|
| n | 15 | 15 | – |
| Age (years) | 65.60 ± 4.53 | 64.93 ± 6.76 | 0.75 |
| Gender (F/M) | 9/6 | 7/8 | 0.54 |
| LEDD (mg) | 386.00 ± 162.31 | 311.05 ± 158.50 | 0.69 |
| HY (stage) | 1.50 ± 0.97 | 1.60 ± 0.57 | 0.61 |
| UPDRS-III | 15.40 ± 10.21 | 16.73 ± 13.40 | 0.34 |
| MoCA | 24.27 ± 2.99 | 24.47 ± 3.48 | 0.59 |
| MMSE | 26.93 ± 2.05 | 28.07 ± 1.91 | 0.08 |
| BBS | 48.47 ± 2.53 | 48.20 ± 2.51 | 0.78 |
| Mini-BESTest | 22.87 ± 1.96 | 23.33 ± 2.50 | 0.57 |
| PDQ-39 | 25.00 ± 11.96 | 21.64 ± 9.15 | 0.41 |
| After-intervention difference (Δ, W12 – W0) | | | |
| Δ UPDRS-III | −5.00 ± 5.77** | −3.00 ± 3.30* | 0.35 |
| Δ MoCA | 2.10 ± 2.60 | −0.20 ± 2.10 | 0.04* |
| ΔMMSE | 1.40 ± 2.95 | −0.40 ± 1.43 | 0.10 |
| Δ BBS | 2.90 ± 2.18** | 1.50 ± 1.84* | 0.14 |
| Δ Mini-BESTest | 1.70 ± 1.34** | 1.30 ± 1.77* | 0.58 |
| Δ PDQ-39 | −18.10 ± 10.78*** | −9.50 ± 12.28* | 0.11 |

Values are mean ± standard deviations. F, female; M, male; LEDD, levodopa equivalent daily dose; UPDRS-III, Unified Parkinson's Disease Scale; MoCA, Montreal Cognitive Assessment; MMSE, Mini-Mental Status Examination score; BBS, Berg Balance Scale; Mini-BESTest, Mini Balance Evaluation Systems Test; PDQ-39, Parkinson's Disease Questionnaire-39. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

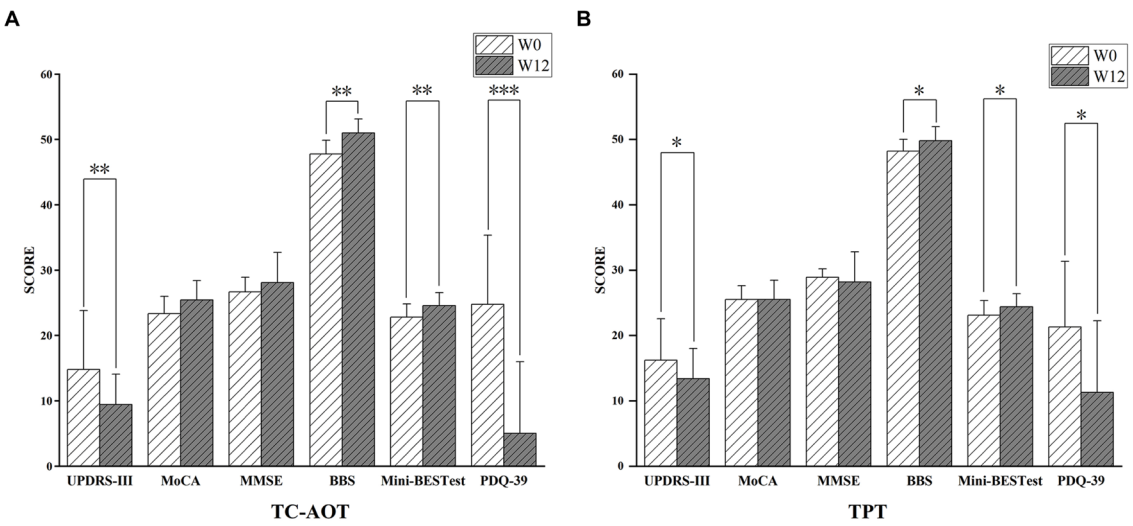


FIGURE 1
Clinical outcomes after the 12 weeks of TC-AOT and TPT training. **(A)**, Participants in the TC-AOT group showed significantly reduced UPDRS-III scores, improved BBS, Mini-BESTest, and PDQ-39 scores; **(B)**, the TPT group demonstrated decreased scores in UPDRS-III scores, and improvements in BBS, Mini-BESTest, and PDQ-39 scores. UPDRS-III, Unified Parkinson's Disease Scale; MoCA, Montreal Cognitive Assessment; MMSE, Mini-Mental Status Examination score; BBS, Berg Balance Scale; Mini-BESTest, Mini Balance Evaluation Systems Test; PDQ-39, Parkinson's Disease Questionnaire-39. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 2 Significant functional connectivity differences within groups (W12 > W0).

| Seed area | Cluster size | Cluster location | MNI coordinate | | | *p* | *T* |
|---|---|---|---|---|---|---|---|
| *TC-AOT group, W12 > W0* | | | | | | | |
| Right IFG | | | | | | | |
| Cluster 1 | 177 | Precuneus | −2 | −74 | 30 | <0.001 | 11.35 |
| Left pIPL | | | | | | | |
| Cluster 1 | 216 | Right insula | 38 | 16 | −2 | <0.001 | 14.07 |
| Cluster 2 | 195 | Right middle frontal | 26 | 40 | 14 | <0.001 | 7.84 |
| | | BA 10 | | | | | |
| Cluster 3 | 149 | Right superior frontal | 26 | 6 | 48 | 0.002 | 14.07 |
| | | BA 6 | | | | | |
| PCC | | | | | | | |
| Cluster 1 | 138 | Right vlPFC | 46 | 40 | −8 | 0.003 | 10.13 |
| Left ATL | | | | | | | |
| Cluster 1 | 190 | Right supramarginal | 50 | −38 | 32 | <0.001 | 9.58 |
| Left TPJ | | | | | | | |
| Cluster 1 | 440 | Left middle occipital | −30 | −94 | 0 | <0.001 | 16.51 |
| | | | | | | | |
| *TPT group, W12 > W0* | | | | | | | |
| dmPFC | | | | | | | |
| Cluster 1 | 214 | Right frontal operculum | 42 | 20 | 2 | <0.001 | 16.63 |
| Left STS | | | | | | | |
| Cluster 1 | 185 | Right lingual | 0 | −76 | −6 | <0.001 | −11.01 |

*p* values are corrected for multiple comparisons by FWE correction. MNI, Montreal Neurological Institute; IFG, inferior frontal gyrus; pIPL, posterior inferior parietal lobule; BA, Brodmann area; PCC, posterior cingulate cortex; ATL, anterior temporal lobe; TPJ, temporal parietal junction; dmPFC, dorsal medial prefrontal cortex; STS, superior temporal sulcus; FWE, family-wise error.

### 3.2.2. Changes within groups after training (W0 vs. W12)

Table 2 shows significant FC changes within the TC-AOT and TPT groups after 12 weeks of intervention. The TC-AOT group exhibited more significant changes in specific brain regions, as shown in Figure 2. The coupling of the right IFG to the left precuneus cortex was strengthened ($p < 0.001$, FWE-corrected) (Figure 2A). Figure 2B shows increased connectivity patterns within the areas including the right insular cortex ($p < 0.001$, FWE-corrected), right middle frontal gyrus (MFG), and Brodmann Area (BA) 10 ($p < 0.001$, FWE-corrected), as well as the right SFG and BA 6 ($p = 0.002$, FWE-corrected), when selecting the left pIPL as a seed ($p < 0.001$, FWE-corrected). Significant active patterns were observed at clusters located at the PCC and right MFG ($p = 0.003$, FWE-corrected) (Figure 2C), as well as the left ATL and the right supramarginal gyrus ($p < 0.001$, FWE-corrected) (Figure 2D). The left TPJ and the left middle occipital gyrus (MOG) also showed an increased connectivity pattern ($p < 0.001$, FWE-corrected) (Figure 2E).

On the other side, the TPT group demonstrated an increased FC between the dmPFC and right frontal operculum cortex ($p < 0.001$, FWE-corrected) and a significantly reduced FC between the left STS and right lingual gyrus ($p < 0.001$, FWE-corrected), as shown in Figures 2F,G.

### 3.2.3. Changes between groups after training

We observed significant time × group interactions between specific DMN seeds and related brain areas, as detailed in Table 3. Compared to the TPT group, the TC-AOT group exhibited increased FC after 12 weeks of training based on different seeds, including the dmPFC to the cerebellum crus 1, the right pIPL, and right supramarginal gyrus as well as between the left TPJ and area of the left middle occipital and superior temporal gyrus ($p < 0.05$, cluster level uncorrected) (Figure 3). There were no other significant time × group interactions when comparing the TPT group to the TC-AOT group.

## 3.3. Behavioral and neural correlation

Table 4 demonstrates significant correlations between clinical assessment scores and FC changes from seed-based fMRI analysis. In the TC-AOT group, the increased FC between the left TPJ and left MOG after 12 weeks of training was significantly related to the reduction of the UPDRS-III score ($r = -0.698$, $p = 0.025$), while the FC level of the left pIPL and right insula showed a positive correlation with the improvement of the BBS score ($r = 0.644$, $p = 0.044$). The change in FC between the left ATL and right supramarginal was positively correlated with the change in MMSE score ($r = 0.670$, $p = 0.034$). In the TPR group, the FC between the dmPFC and right frontal operculum showed a positive relationship with the enhancement in Mini-BESTest score ($r = 0.666$, $p = 0.035$), and that between the left STS and right lingual gyrus had a negative relationship with the improvement in MoCA score ($r = -0.669$, $p = 0.035$).

# 4. Discussion

This study investigated the effect of the TC-AOT intervention on brain FC changes in early-stage PD patients compared to a physical rehabilitation method. The resting-state DMN was used, and the

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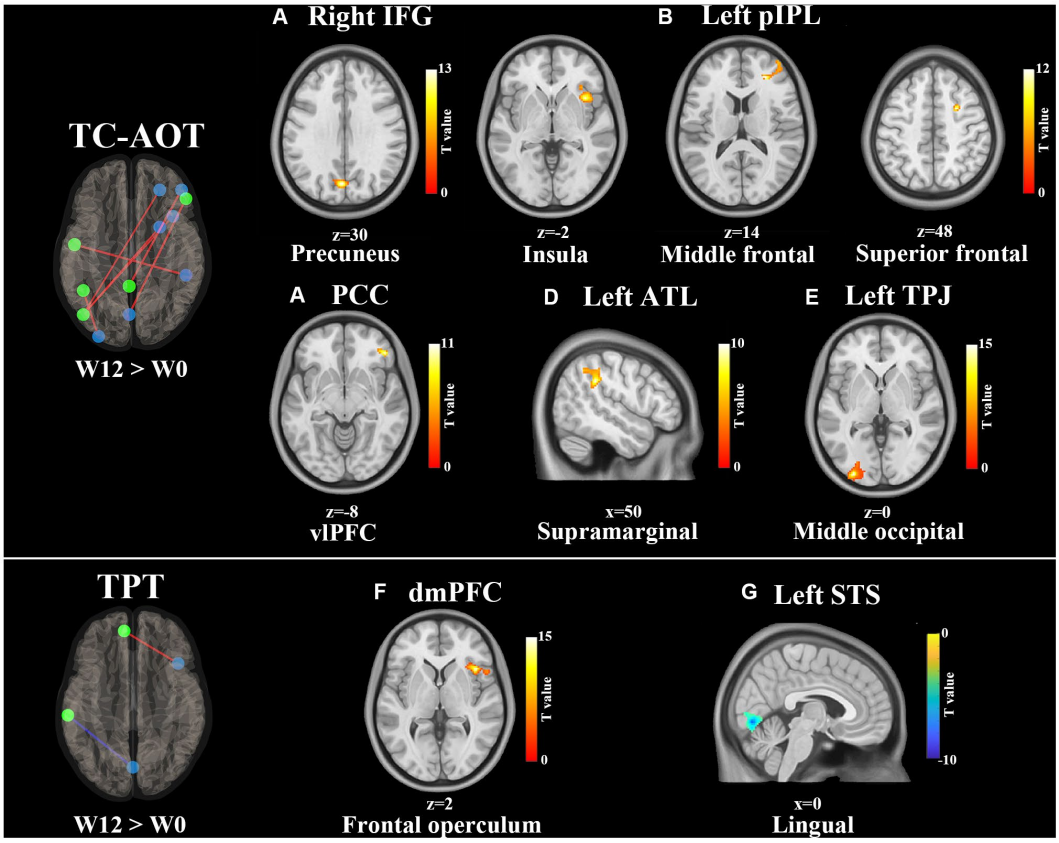


FIGURE 2
Training effects on FC of the DMN after 12 weeks of intervention (W12 > W0). All FC connections between the DMN seeds and significantly correlated clusters are shown on the left side. Red lines represent the increased FCs, and blue lines refer to the decreased FCs. The results of clusters are shown on axial and sagittal sections of the Montreal Neurological Institute standard brain, where the color bar denotes T values. Increased FC clusters are shown in the hot color and decreased in the cold color. The significance is set at $p < 0.01$ with FWE-corrected. Significant FC changes within the TC-AOT group: **(A)** seed area: right IFG, activated clusters: precuneus; **(B)** seed area: left pIPL, activated clusters: right insula, middle, and superior frontal gyrus; **(C)** seed area: PCC, activated clusters: vlPFC; **(D)** seed area: left ATL, activated clusters: right supramarginal gyrus; **(E)** seed area: left TPJ, activated clusters: middle occipital gyrus. Significant FC changes within the TPT group; **(F)** seed area: dmPFC, activated clusters: right frontal operculum; **(G)** seed area: left STS, activated clusters: right lingual gyrus. Abbreviations: IFG, inferior frontal gyrus; pIPL, posterior inferior parietal lobe; PCC, posterior cingulate cortex; vlPFC, ventrolateral prefrontal cortex; ATL, anterior temporal lobe; TPJ, temporal parietal junction, dmPFC, dorsal medial prefrontal cortex; STS, superior temporal sulcus.

TABLE 3 Significant functional connectivity differences between groups (W12 > W0) using time × group model.

| Seed area | Cluster size | Cluster location | MNI coordinate | | p | T | |
|---|---|---|---|---|---|---|---|
| *TC-AOT > TPT, W12 > W0* | | | | | | | |
| dmPFC | | | | | | | |
| Cluster 1 | 88 | Left cerebellum crus1 | −26 | −84 | −22 | 0.009 | 6.21 |
| Right pIPL | | | | | | | |
| Cluster 1 | 77 | Right supramarginal | 56 | −46 | 34 | 0.017 | 5.69 |
| Left TPJ | | | | | | | |
| Cluster 1 | 117 | Left middle occipital | −34 | −96 | 0 | 0.003 | 6.79 |
| Cluster 2 | 59 | Right superior temporal | −44 | −32 | 8 | 0.026 | 5.75 |

*p* values are cluster-level uncorrected. MNI, Montreal neurological; dmPFC, dorsal medial prefrontal cortex; pIPL, posterior inferior parietal lobule; TPJ, temporal parietal junction.

correlations between changes in FCs and clinical scores were analyzed. The FCs between the brain regions that are involved in executive attention and motor learning, including the TPJ, SMG, IFG, and insula, were significantly increased after 12 weeks of TC-AOT training. The increased FCs were correlated to the improvements in UPDRS-III and BBS scores. Our results suggested that the TC-AOT intervention, which incorporates cognitive neural pathways in motor learning, has great potential benefits for early-stage PD.

A novel AOT intervention was proposed in the study, where continuous Tai Chi movements were first incorporated with AOT training. Agosta et al. have proved that the AOT combined with mobility exercise can increase brain activity in frontoparietal areas

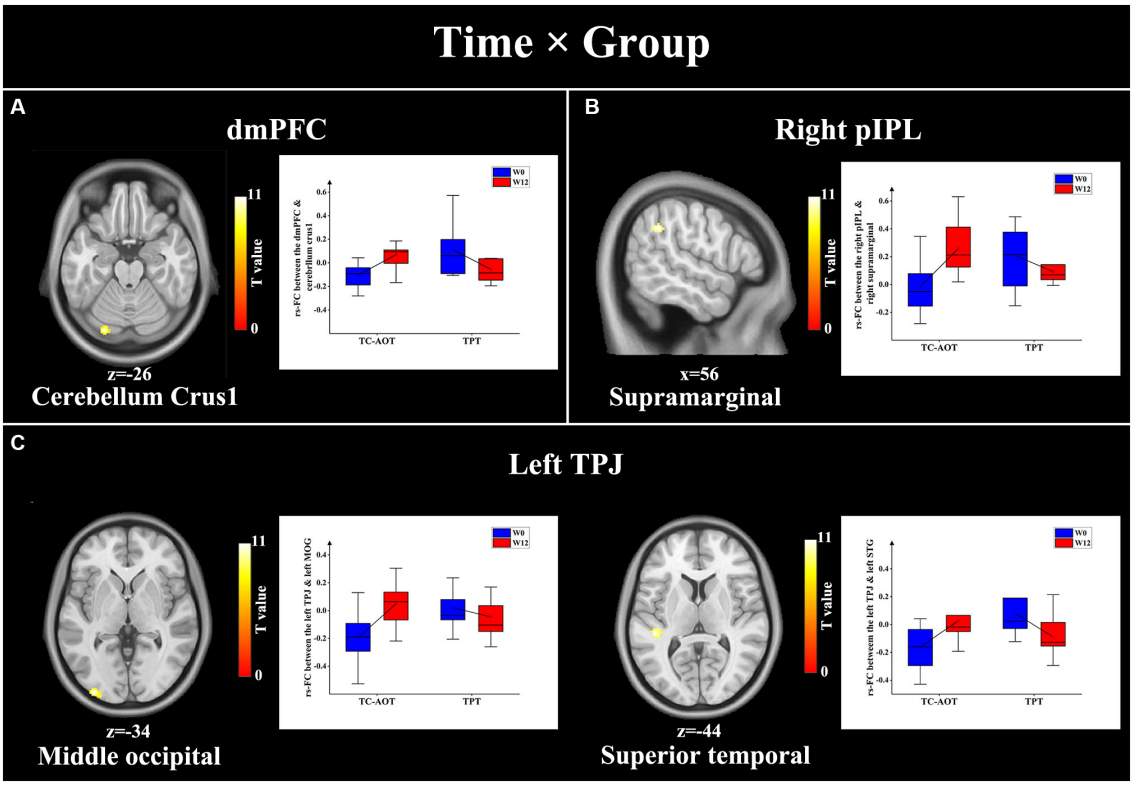


FIGURE 3
Results of significant time × group interactions between the DMN seeds and specific brain regions. The TC-AOT group exhibited a significant increase in FC values between several DMN seeds and brain regions, whereas the TPT group had decreased FCs. The significance is set at $p < 0.05$ cluster level uncorrected. **(A)** seed area: dmPFC, activated clusters: cerebellum crus1; **(B)** seed area: right pIPL, activated clusters: right supramarginal gyrus; **(C)** seed area: left TPJ, activated clusters: middle occipital gyrus and left superior temporal gyrus. dmPFC, dorsal medial prefrontal cortex; pIPL, posterior inferior parietal lobe; TPJ, temporal parietal junction.

TABLE 4 Significant correlations between the fcs and clinical assessment variables using a pearson correlation analysis.

| Group | Time | FC variables | Clinical assessment | *r*(*p*) |
|---|---|---|---|---|
| TC-AOT | W12 | Left TPJ and left MOG | Δ UPDRS-III | −0.698 (0.025) |
| | W12 | Left pIPL and right insula | Δ BBS | 0.644 (0.044) |
| | W12 | Δ Left ATL and right SMG | Δ MMSE | 0.670 (0.034) |
| TPT | W12 | dmPFC and right FO | Δ Mini-BESTest | 0.666 (0.035) |
| | W12 | Left STS and right lingual | Δ MoCA | −0.669 (0.035) |

Δ means change before and after rehabilitation (W12 – W0). TPJ, temporal parietal junction; MOG, middle occipital gyrus; pIPL, posterior inferior parietal lobe; ATL, anterior temporal lobe; SMG, supramarginal gyrus; dmPFC, dorsal medial prefrontal cortex; FO, frontal operculum; UPDRS-III, Unified Parkinson's Disease Scale; MMSE, Mini-Mental Status Examination score; MoCA, Montreal Cognitive Assessment; BBS, Berg Balance Scale; Mini-BESTest, Mini Balance Evaluation Systems Test.

during fMRI tasks (Agosta et al., 2017). The TC-AOT protocol requires participants to observe, imagine, and imitate sequential movements, which involves more interplay between cognition and motor control. Recent studies have shown that modulation of the TPJ and SMG can influence motor attention performance for complex skilled actions (Saxe et al., 2004; Davis et al., 2018; Farina et al., 2020). Moreover, the IPL and IFG regions are critical for motor learning (Johnson-Frey et al., 2003; Buccino et al., 2004; Grafton et al., 2007). The insula plays a crucial role in the integration of sensory information for motor planning (Christopher et al., 2014; Huang et al., 2020). Consistent with these studies, we also observed increased FCs in the abovementioned brain areas after 12 weeks of rehabilitation. As shown in Table 4, the increased FC between left TPJ and left MOG was significantly related to the decrease in UPDRS-III scores, while the increased FC between left ATL and right SMG was associated with the improvement of cognitive function. The results demonstrated that the cognitive-motor interaction in the TC-AOT could promote brain plasticity and enhance motor control ability in PD patients (Herold et al., 2018).

The TC-AOT and TPT have different underlying mechanisms for PD rehabilitation. Significant time × group interactions were observed in FC changes in specific brain areas. The TC-AOT group exhibited increased FCs between the dmPFC and cerebellum crus1, right pIPL, and supramarginal cortex, as well as the TPJ to the region of the occipital and temporal gyrus, while the opposite trends were found in the TPT group (Figure 3). The temporoparietal junction, as well as the right pIPL and supramarginal cortex, are essential for visuospatial

recognition, which may be related to the involvement of motion observation in the TC-AOT (Aracil-Bolaños et al., 2019). On the other hand, the PFC plays an important role in the regulation of behavior and cognition within the network (Friedman and Robbins, 2022). The strengthened cerebellar-prefrontal pathway could facilitate the transfer of sensorimotor information into ongoing cortical processing during goal-directed behaviors (Watson et al., 2014). It can be concluded that the TC-AOT has a unique advantage in improving cognitive functioning in PD associated with efficient motor learning.

The TPT performs less contribution to modulation in FCs of brain regions for early-stage PD patients. Only an increased FC between the dmPFC and the area of the right frontal operculum was observed (Figure 2F). Li et al. (2022) demonstrated that physical exercise could modify the plasticity of the frontal cortex. The increased FC was significantly correlated with improved balance ability. The intervention has also been regarded as a complementary option for PD management (Frazzitta et al., 2011; Bloem et al., 2015). However, in contrast with previous studies that revealed that physical exercise has a positive effect on cognitive function by influencing activation and metabolism of the frontal lobe circuit, there were no significant changes in the motor-cognitive pathway in the TPT group. Notably, we observed a decreased FC between the left STS and lingual gyrus, which was correlated with a declining trend in cognitive function, especially in visual perceptual integration (Pagonabarraga et al., 2013). Although both the TC-AOT and TPT groups exhibited improvements in motor and balance performance as well as quality of life, the TPT may not have a preventative effect on PD progression.

There are some limitations to be mentioned. First, the sample size was relatively small because it was a pilot study of a novel PD rehabilitation intervention. However, the calculation result of the *post-hoc* power analysis was 0.79, demonstrating that the statistical analysis and related conclusions were still effective in our study. Second, there were no significant differences in clinical assessments between the TC-AOT and TPT groups, which might be because the pen-and-paper tests may not be sensitive enough to detect subtle changes in PD symptoms. Quantitative measurement, especially for assessing motor automaticity ability, such as dual-task tests, should be considered in future studies.

Overall, the study was the first attempt to investigate the underlying mechanisms of a TC-AOT intervention for early-stage PD rehabilitation by analyzing resting-state fMRI based on the DMN compared to physical therapy. Both groups showed significant improvement in motor functions, enhancement in balance ability, and quality of life. However, increased FCs among the DMN nodes and several brain regions, including the frontal, insula, supramarginal, and occipital lobes, were observed and found to be significantly associated with cognitive and motor function improvement in the TC-AOT group. The proposed TC-AOT intervention demonstrated great potential in preventing cognitive decline and motor dysfunctions by promoting the interplay between cognition and motor control in early-stage PD patients.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving humans were approved by The Ethics Committee of Tianjin General Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

LM, XZ, and DM: conception and design of the study. DW and YS: rehabilitation training and data collection. ZL and JZ: patient recruitment and clinical assessment. LM and DW: data interpretation and manuscript drafting. HL: critical manuscript revision for important intellectual content. XZ: clinical administration. DM: project administration. All authors approved the final version to be submitted.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1252610/full#supplementary-material

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# Different impulse control disorder evolution patterns and white matter microstructural damage in the progression of Parkinson's disease

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**Background:** The course of impulse control disorders (ICD) varies in the early stage of Parkinson's disease (PD).

**Aim:** We aimed to delineate the association between the evolution pattern of ICD and the progression of PD.

**Methods:** A total of 321 *de novo* PD patients from the Parkinson's Progression Markers Initiative database were included. Patients were followed up for a mean of 6.8 years and were classified into different groups according to the evolution patterns of ICD. Disease progression was compared among groups using survival analysis, in which the endpoint was defined as progression to Hoehn and Yahr stage 3 or higher for motor progression and progression to mild cognitive impairment for cognitive decline. In the fourth year of follow-up, four types of ICD evolution patterns were identified: (1) non-ICD-stable (68.2%), a patient who is consistently free of ICD; (2) late-ICD (14.6%), ICD developed during the follow-up of patients; (3) ICD-stable (11.5%), patients showed persistent ICD; and (4) ICD-reversion (5.6%), baseline ICD disappeared during the follow-up of patients with ICD.

**Results:** The ICD-reversion type shows daily life non-motor symptoms [Movement Disorder Society-Unified Parkinson Disease Rating Scale (MDS-UPDRS) part I], daily life motor symptoms (MDS-UPDRS part II), rapid eye movement sleep behavior disorder, and anxiety symptoms has a greater impact. PD patients with different ICD evolution patterns had different changes in white matter microstructure at the onset of the disease. Those relevant brain regions are involved in ICD and non-motor functions.

**Conclusion:** Four early ICD evolution patterns are identified in *de novo* PD, with different prognoses and brain white matter microstructural damage patterns, and they may predict motor progression and cognitive decline in PD patients.

KEYWORDS

**Parkinson's disease, impulse control disorders, evolution patterns, white matter microstructural, non-motor symptoms**

## Introduction

Impulse control disorder (ICD) is a non-motor symptom (NMS) in patients with Parkinson's disease (PD). ICD was described as excessive or harmful urges and behaviors that cause substantial impairment in social and occupational functioning, such as gambling (pathological gambling), hypersexuality (increased pursuit of sex), overeating, shopping too much, dysregulation of dopamine (a tendency to overuse PD medications), and punding (behavior that is repetitive and purposeless) (Joutsa et al., 2012). Between 13 and 37% of Parkinson's patients have ICD. Jaakkola et al. have found that newly diagnosed PD patients are more likely to develop ICD during follow-up (Jaakkola et al., 2014). PD patients without ICD symptoms at onset could develop ICD over time (Corvol et al., 2018). Studies have shown that PD-ICD is closely related to the use of Da and has a dose-effect relationship (Corvol et al., 2018), suggesting that PD-ICD may represent a different phenotype.

Diffusion tensor imaging (DTI) is a non-invasive form of magnetic resonance imaging (MRI) that clarifies the integrity of the microscopic structure of brain tissue, assesses neuronal connections and the change in microscopic structure, provides a unique quantitative measurement window, and determines the integrity of the brain (Surkont et al., 2021). A previous DTI study, found that the cerebellum, basal ganglia, cortex, and spinal cord projected fiber link interruption are risk factors for ICD (Smith et al., 2016). Yoo et al. found that the PD-ICD group and the PD-non-ICD group had differences in the callous anterior to the brain, left thalamic radiations, partial right thalamus radiations, the right dorsal and posterior cingula, the right internal capsule (genu and posterior limbs), the right superior temporo-occipital lobe, and the right thalamus (Yoo et al., 2015). Research on PD-related ICD is largely based on brain imaging studies, but the evidence is inconclusive. Neuroimaging studies of PD combined with ICD are still mainly involved in abnormal changes in the midbrain-cortex-limbic system-striatal loops. The in-depth study of PD-ICD-related imaging is expected to provide new ideas for the intervention of PD-ICD. White matter microstructural alterations have also been related to neurodegeneration in PD (Atkinson-Clement et al., 2017; Sarasso et al., 2021). Imperiale et al. found extensive disruption of the white matter tracts in patients with Parkinson's disease-ICD, with increased radial and axial dispersion of the corpus callosum genu and pontine bundle (Imperiale et al., 2018). There may be an important correlation between the changes in white matter microstructure and PD-ICD. In PD patients, the heterogeneity of symptoms may be related to the stage and brain areas affected. Hence, this evolution pattern may have prognostic value.

The present study aimed to identify the different evolutionary types of ICD in PD, analyze the effects of different ICD evolution types on motor and NMSs of PD, and analyze the potential changes of white matter microstructure.

## Results

### Patterns of ICD evolution in PD patients

A total of 321 patients with *de novo* PD were included in this study, with an average follow-up of 6.8 years. As shown in Figure 1, 266 patients had no ICD symptoms at baseline, accounting for 82.8% of patients; 55 patients had ICD at baseline, accounting for 17.1% of patients. In the initial 4 years of follow-up, 37 patients had stable ICD symptoms, accounting for 11.5% of patients, which was the ICD stable group; ICD disappeared in 18 patients, accounting for 5.6% of patients, which was the ICD reversal group, and the average phenotypic transition time was 35.3 months. In addition, 47 PD patients without ICD at baseline developed ICD symptoms, accounting for 14.6% of patients, which was the late ICD group, and the average phenotypic transition time was 38.5 months. However, 219 PD patients still did not have ICD, which was the non-ICD stable group, accounting for 68.2% of patients.

### Clinical characteristics and demographics at baseline of patients from four groups of ICD evolution patterns

A summary of the clinical characteristics and demographics at baseline of patients can be found in Table 1. There were no significant differences between the four groups regarding age, sex, years of schooling, or length of follow-up. Similarly, there was no significant difference in baseline motor function, cognitive function, or index t-score. Patients in the ICD-stable and ICD-reversion groups showed higher MDS-UPDRS part I and II distributions at baseline than patients with non-ICD-stable and late-ICD ($p = 0.000 < 0.05$ and $p = 0.000 < 0.05$, respectively). At baseline, seven patients with H&Y stage ≥3, 37 with mild cognitive impairment (MCI), and 80 with MoCA <26 were identified. The ESS scaled score, Rapid-eye-movement Sleep Behavior Disorder Screening Questionnaire (RBDSQ) scaled

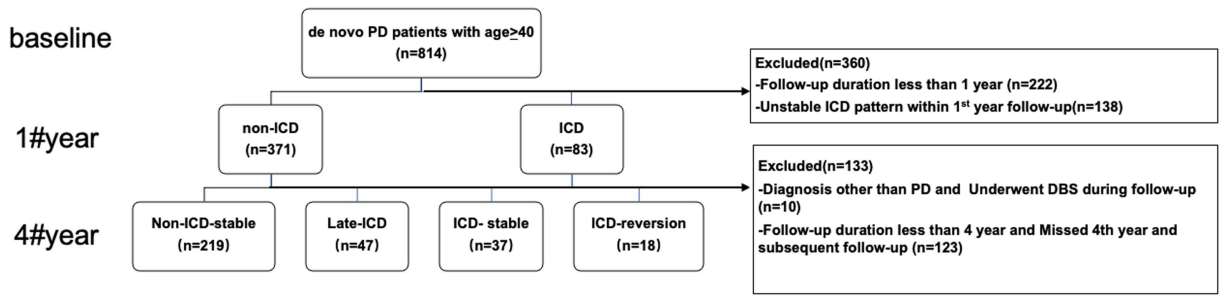


FIGURE 1
Participants and study flow chart. DBS, deep brain stimulation; PD, Parkinson's disease; ICD, impulse control disorders.

TABLE 1 Baseline and demographic characteristics of the study population.

| | Non-ICD-stable | Late-ICD | ICD-reversion | ICD-stable | *p*-value |
|---|---|---|---|---|---|
| | *n* = 219 | *n* = 47 | *n* = 18 | *n* = 37 | |
| **Demographics** | | | | | |
| Age, y | 62.58 ± 8.81 | 62.03 ± 8.08 | 58.41 ± 7.67 | 62.76 ± 9.09 | 0.265 |
| Male, *n* (%) | 133 (60.7%) | 32 (68.1%) | 10 (55.6%) | 24 (64.9%) | 0.721 |
| Years of education, y | 15.51 ± 3.43 | 14.64 ± 4.47 | 14.28 ± 4.56 | 15.35 ± 4.81 | 0.633 |
| Follow-up duration, y | 6.68 ± 3.19 | 5.79 ± 3.8 | 5.56 ± 2.99 | 5.86 ± 2.95 | 0.058 |
| Time to ICD status change, y | | 3.21 ± 0.85 | 2.94 ± 0.85 | | |
| **Motor function** | | | | | |
| MDS-UPDRS part I | 0.9 ± 1.26 | 1.32 ± 1.64 | 3.67 ± 3.27 | 2.49 ± 2.41 | **0.000** |
| MDS-UPDRS part II | 5.54 ± 4.62 | 5.98 ± 4.37 | 10.17 ± 6.91 | 7.97 ± 4.71 | **0.000** |
| MDS-UPDRS part III | 20.03 ± 9.16 | 22.32 ± 9.33 | 27.44 ± 15.18 | 20.05 ± 8.93 | 0.116 |
| H&Y stage (stage 1/stage 2, *n*) | 94/120 | 18/29 | 4/13 | 15/20 | 0.394 |
| H&Y stage ≥ 3 at baseline, *n* (%) | 4 (1.8%) | 0 | 1 (5.6%) | 2 (5.4%) | 0.268 |
| **Non-motor function** | | | | | |
| ESS scaled score | 5.47 ± 3.56 | 6.66 ± 3.25 | 8.17 ± 5.96 | 7.78 ± 4.44 | **0.002** |
| RBDSQ scaled score | 3.81 ± 2.66 | 4.3 ± 2.61 | 5.89 ± 3.38 | 4.7 ± 2.92 | **0.007** |
| GDS scaled score | 4.36 ± 1.5 | 4.45 ± 1.87 | 5.39 ± 1.85 | 4.76 ± 1.69 | 0.130 |
| S-AI scaled score | 31.79 ± 9.53 | 33.89 ± 10.35 | 42 ± 10.94 | 34.92 ± 10.64 | **0.000** |
| T-AI scaled score | 32.91 ± 8.42 | 34.45 ± 8.98 | 42.56 ± 9 | 37 ± 7.96 | **0.000** |
| **Cognitive function** | | | | | |
| MoCA | 26.86 ± 2.44 | 27.3 ± 2.36 | 25.67 ± 4.03 | 25.84 ± 5.07 | 0.277 |
| MoCA < 26 at baseline | 53 (24.2%) | 7 (14.9%) | 6 (33.3%) | 14 (37.8%) | 0.087 |
| HVLT-R total recall *t*-test | 46.29 ± 10.46 | 45.49 ± 10.28 | 43.33 ± 14.56 | 44.11 ± 11.25 | 0.505 |
| HVLT-R delay recall | 45.63 ± 10.55 | 44.62 ± 10.14 | 43.39 ± 14.21 | 45.76 ± 10.64 | 0.796 |
| HVLT-R retention | 47.64 ± 10.86 | 47.04 ± 10.28 | 45.94 ± 14.58 | 49.51 ± 12.06 | 0.662 |
| HVLT-R recognition Dis | 45.75 ± 10.93 | 44.19 ± 10.51 | 42.67 ± 17.23 | 48.03 ± 9.95 | 0.315 |
| **Index *t*-score** | | | | | |
| JLO scaled score | 25.47 ± 4.6 | 25.87 ± 4.12 | 24.71 ± 4.95 | 24.81 ± 4.63 | 0.667 |
| LNS scaled score | 10.44 ± 2.63 | 10.11 ± 2.32 | 10.67 ± 3.14 | 10.38 ± 2.82 | 0.846 |
| SFT t-score | 51.58 ± 10.12 | 50.89 ± 9.55 | 46.72 ± 16.32 | 51.11 ± 10.66 | 0.313 |
| SDMT t-score | 44.66 ± 9 | 44.24 ± 8.78 | 44.69 ± 12.02 | 47.58 ± 10.7 | 0.332 |
| MCI at baseline, *n* (%) | 24 (11%) | 6 (12.8%) | 4 (22.2%) | 3 (8.1%) | 0.460 |

Significant *p*-values are shown in bold ($p < 0.05$). Pairwise comparisons were made using Bonferroni correction. ESS, Epworth Sleepiness Scale; GDS, Geriatric Depression Scale; HVLT-R, Hopkins verbal learning test revised; H&Y, Hoehn–Yahr; JLO, judgment of line orientation; LNS, Letter-Number Sequencing; MCI, mild cognitive impairment; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; S-AI, State Anxiety Inventory; SFT, Semantic Fluency Test; SDMT, Symbol Digit Modalities Test; T-AI, Trait Anxiety Inventory.

score, State Anxiety Inventory (S-AI) scaled score, and Trait Anxiety Inventory (T-AI) scaled score of the non-ICD-stable group were lower than those of the other three groups ($p = 0.002 < 0.05$, $p = 0.007 < 0.05$, $p = 0.000 < 0.05$, and $p = 0.000 < 0.05$, respectively).

## The relationship between ICD evolution patterns and non-motor function progression In PD patients

The Kaplan–Meier analysis was used to determine whether different patterns of ICD evolution are associated with the progression of non-motor function in PD. For the increase of 0 points in the MDS-UPDRS Part I score, the PFS time in the ICD-reversion group was shorter than that in the non-ICD-stable group and the late-ICD group (Figure 2A): 71.4 vs. 99 months ($p = 0.000 < 0.05$) and 71.4 vs. 98.5 months ($p = 0.000 < 0.05$). The PFS time in the ICD-stable group was also shorter than that in the non-ICD-stable and late-ICD groups: 82.1 vs. 99 months ($p = 0.000 < 0.05$) and 82.1 vs. 98.5 months ($p = 0.000 < 0.05$).

For the 15-point increase in MDS-UPDRS II score, the progression-free survival (PFS) of the non-ICD-stable group was longer than that in the late-ICD group, ICD-reversion group, and ICD-stable group (Figure 2B): 123 vs. 114 months ($p = 0.0004 < 0.05$), 123 vs. 112 months ($p = 0.000 < 0.05$), 123 vs. 113 months ($p = 0.0005 < 0.05$). There is a

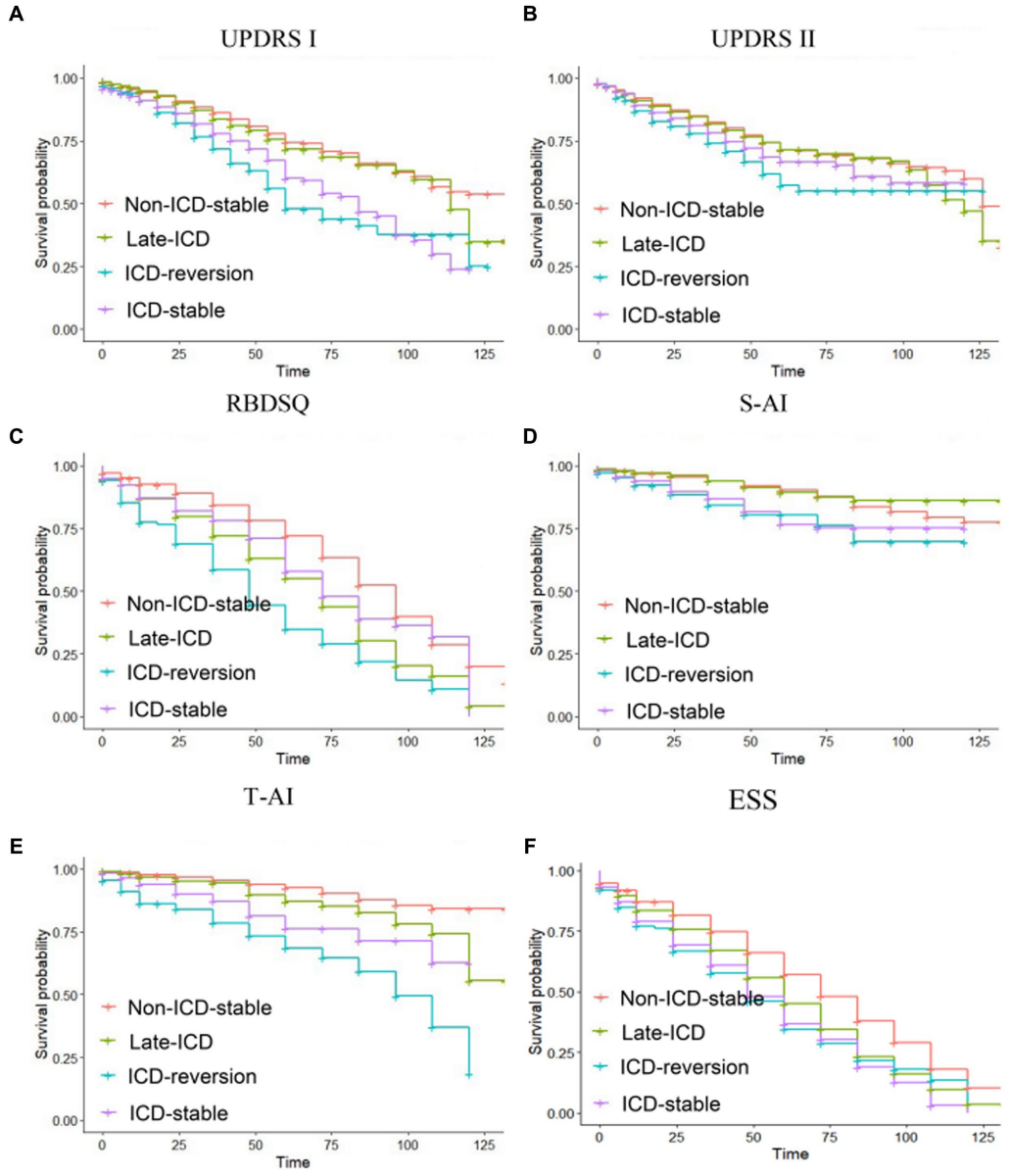


FIGURE 2
Kaplan–Meier progression-free survival curves depending on ICD evolution patterns. Time from baseline to **(A)** a zero-point increase in MDS-UPDRS part I, **(B)** a 15-point increase in MDS-UPDRS part II, **(C)** a RBDSQ <5, **(D)** a S-AI scaled score (40–49 years old: male <51, female <53; more than 50 years old: male <55, female <58), **(E)** a T-AI scaled score (40–49 years old: male <55, female <58; more than 50 years old: male <52, female <47), and **(F)** an ESS < 7. Ticks indicate censoring events.

tendency for the ICD-reversion group to have a shorter survival period for progression. There were no statistical significance comparisons with other groups.

For RBDSQ <5, the progression-free survival of the non-ICD-stable group was longer than that in the late-ICD, ICD-reversion, and ICD-stable groups (Figure 2C): 86 vs. 67.7 months ($p = 0.000 < 0.05$), 86 vs. 53.9 months ($p = 0.000 < 0.05$), 86 vs. 74.9 months ($p = 0.0015 < 0.05$). The PFS time in the ICD-reversion group was shorter than in the late-ICD and ICD-stable groups: 53.9 vs. 67.7 months ($p = 0.016 < 0.05$) and 53.9 vs. 74.9 months ($p = 0.000 < 0.05$).

For the S-AI scaled score, the PFS time in the ICD-reversion group was shorter than that in the non-ICD-stable group and the late-ICD group (Figure 2D): 105 vs. 117 months ($p = 0.002 < 0.05$), 105 vs. 120 months ($p = 0.0039 < 0.05$). The PFS time in the ICD-stable group was shorter than in the non-ICD-stable and late-ICD groups: 108 vs. 117 months ($p = 0.0002 < 0.05$) and 108 vs. 120 months ($p = 0.0023 < 0.05$).

For the T-AI scaled score, the PFS time comparison of the four groups was significant: the ICD-reversion group < the ICD-stable group < the late-ICD group < the non-ICD-stable group (85.5 months <104.4 months <112.5 months <120.8 months, and the $p < 0.05$; Figure 2E). For the ESS score, compared with the ICD reversal group, the ICD stabilization group, and the ICD late-onset group, the PFS time in the non-ICD group was longer (72.4 vs. 54.2 months, 72.4 vs. 53.9 months, and 72.4 vs. 60.7 months; all $p < 0.05$; Figure 2F).

TABLE 2 Kaplan–Meier estimates for HR in different ICD evolution patterns of non-motor progression.

| Cox | Late-ICD | *p*-value | ICD-reversion | *p*-value | ICD-stable | *p*-value |
|---|---|---|---|---|---|---|
| | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | |
| MDS-UPDRS I | 1.117 (0.928–1.305) | 0.252 | **2.135 (1.892–2.379)** | **0.000** | **1.811 (1.625–1.997)** | **0.000** |
| MDS-UPDRS II | 1.092 (0.91–1.274) | 0.344 | **1.499 (1.236–1.762)** | **0.003** | **1.294 (1.093–1.496)** | **0.012** |
| RBDSQ | **1.732 (1.57–1.894)** | **0.000** | **2.774 (2.535–3.013)** | **0.000** | **1.349 (1.132–1.566)** | **0.007** |
| S-AI | 1.054 (0.664–1.444) | 0.790 | **2.104 (1.628–2.58)** | **0.002** | **2.693 (2.35–3.035)** | **0.000** |
| T-AI | **1.812 (1.454–2.17)** | **0.001** | **4.997 (4.605–5.388)** | **0.000** | **3.567 (3.217–3.916)** | **0.000** |
| ESS | **1.397 (1.253–1.54)** | **8E-16** | **1.745 (1.511–1.978)** | **8E-16** | **1.693 (1.53–1.857)** | **8E-16** |

Cox regression, compared with the different ICD evolution patterns. Significant *p*-values are shown in bold ($p < 0.05/3 = 0.017$). ESS, Epworth Sleepiness Scale; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; S-AI, State Anxiety Inventory; T-AI, Trait Anxiety Inventory.

## Kaplan–Meier estimates for HR in different ICD evolution patterns on non-motor progression

As shown in Table 2, we found that the ICD reversion group was associated with the progression of NMS, including an increase of 0 points in the MDS-UPDRS Part I score and 15 points in MDS-UPDRS II, REM sleep disorders, S-AI, T-AI, and ESS (HR = 2.135, 95% CI 1.892–2.379, $p = 0.000$; HR = 1.499, 95% CI 1.236–1.762, $p = 0.003$; HR = 1.499, 95% CI 1.236–1.762, $p = 0.003$ 2.774, 95% CI 2.535–3.013, $p = 0.000$; HR = 2.104, 95% CI 1.628–2.58, $p = 0.002$; HR = 4.997, 95% Cl 4.605–5.388, $p = 0.000$; and HR = 1.745, 95% Cl 1.551–1.978, $p < 0.001$). The ICD stable group was also associated with the progression of NMS, including an increase of 0 points in MDS-UPDRS Part I score and 15 points in MDS-UPDRS II, REM sleep disorders, S-AI, T-AI, and ESS (HR = 1.811, 95% CI 1.625–1.997, $p = 0.000$; HR = 1.294, 95% CI 1.093–1.496, $p = 0.012$; HR = 1.349, 95% CI 1.132–1.566, $p = 0.007$; HR = 2.693, 95% CI 1.35–3.035, $p = 0.000$; HR = 3.567, 95% CI 3.217–3.916, $p = 0.000$; and HR = 1.693, 95% CI 1.53–1.857, $p < 0.001$). The late ICD group may affect REM sleep disorders, T-AI, and ESS (HR = 1.732, 95% CI 1.57–1.894, $p = 0.000$; HR = 1.821, 95% CI 1.454–2.17, $p = 0.001$; and HR = 1.397, 95% CI 1.253–1.54, $p < 0.001$).

## Patterns of white matter microstructure change with different ICD evolution patterns at baseline in PD patients

We hypothesized that in PD patients with different patterns of ICD evolution, brain regions associated with non-motion had significant lesions at baseline. Therefore, 49 patients with non-ICD-stable, 16 with late-ICD, 6 with ICD-stable, and 4 with ICD-reversion were analyzed through whole-brain diffusion tensor imaging (DTI). Table 1 shows no significant differences between the four groups regarding age, sex, years of schooling, or length. Additionally, there were no statistically significant differences in mean Levodopa Equivalent Daily Dose during follow-up between the four groups (Supplementary Table S1).

PD patients with different ICD evolution patterns have fractional anisotropy (FA) evolution patterns, mainly located on the right uncinate. The FA values of the right uncinate decreased in the late-ICD group compared with the non-ICD-stable group, the ICD-reversion group, and the ICD-stable group (FA values were $0.383 \pm 0.018$, $0.409 \pm 0.01$, $0.419 \pm 0.028$, and $0.415 \pm 0.039$, respectively; $p = 0.037 < 0.05$; Figure 3A). The FA values were positively correlated with axonal integrity; this shows that the shaft breakout of the late-ICD group was the most serious.

PD patients with different patterns of ICD evolution also have different patterns of mean diffusion (MD) change, mainly located in the right cingulum bundle and the left superior longitudinal fasciculi (SLF). In the right cingulum bundle, the ICD-reversion group had higher MD values than the non-ICD-stable, late-ICD, and ICD-stable groups (MD values were $0.787 \pm 0.073$, $0.726 \pm 0.01$, $0.743 \pm 0.029$, and $0.750 \pm 0.04$, respectively; $p = 0.024 < 0.05$; Figure 3B). In the left SLF, the ICD-reversion group had higher MD values than the non-ICD-stable, late-ICD, and ICD-stable groups (MD values were $0.748 \pm 0.075$, $0.685 \pm 0.011$, $0.699 \pm 0.022$, and $0.700 \pm 0.027$, respectively; $p = 0.024 < 0.05$; Figure 3C), which shows that the cellular edema in the ICD-reversion group was the most severe.

# Discussion

A longitudinal study of ICD in *de novo* PD patients was conducted. First, we found that ICD in PD fluctuated over time and could be divided into non-ICD-stable, late-ICD, ICD-stable, and ICD-reversion patterns. Second, we showed that ICD evolution patterns are associated with non-motor impairment in PD patients. ICD-reversion patients showed the fastest non-motor disease progression, whereas non-ICD-stable patients showed relatively mild disease progression. Third, we found that PD patients with different ICD evolution patterns had different changes in white matter microstructure at the onset of the disease. Interestingly, those relevant brain regions are involved in ICD and non-motor functions. Based on these results, ICD evolution patterns in PD may have prognostic value.

Previous longitudinal studies have found that the overall prevalence of ICD changes as the course of PD progresses. An identical incidence of 18% has been found in the normal population and patients with newly diagnosed drug-naive PD (Yoo et al., 2015). Follow-up studies have found that patients with idiopathic PD are more likely to have ICD than healthy controls and patients with newly diagnosed untreated PD (Antonini et al., 2011), with an incidence of up to 25% (Bostwick et al., 2009), suggesting that patients with PD who do not have an ICD at the time of onset may develop an ICD. Our study found that in PD patients, the incidence of ICD was 26.16% as of the fourth year of follow-up; longitudinal data show that ICD symptoms in PD patients do not always remain stable. In our study, as of year 4 of

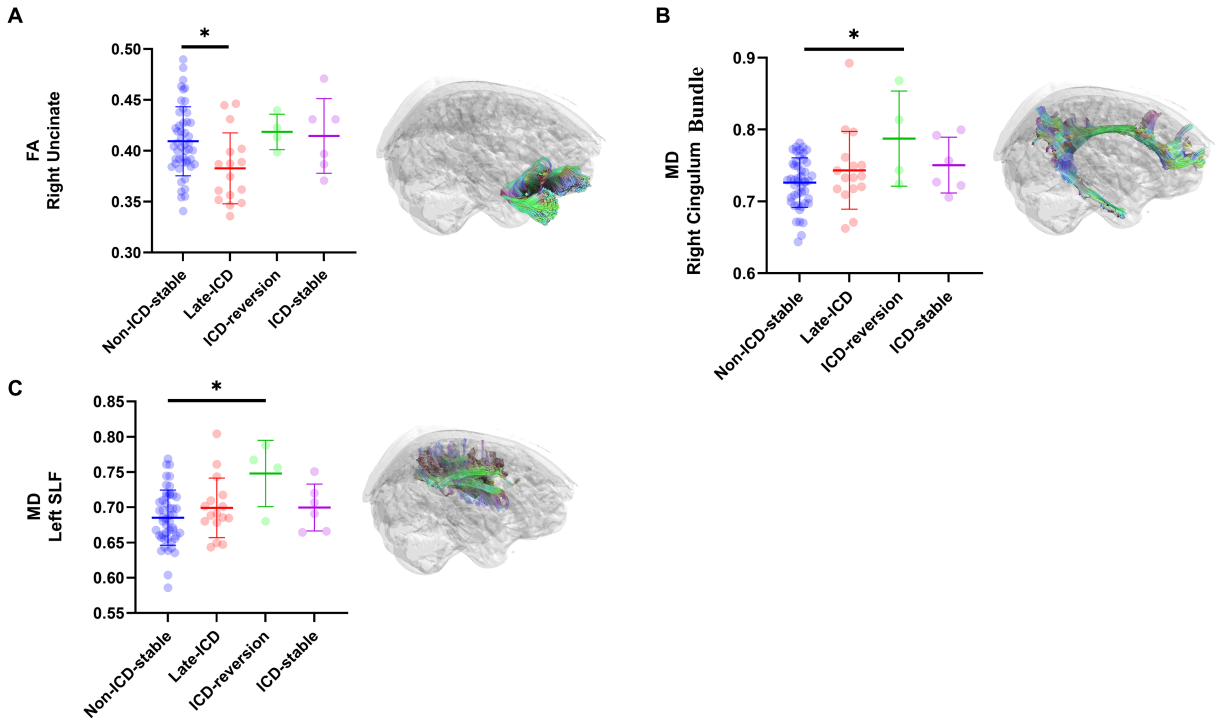


FIGURE 3
DTI differences among ICD evolution patterns. Post hoc ROI analysis in **(A)** the right uncinate for FA ($p < 0.05$), **(B)** the right Cingulum Bundle for MD ($p < 0.05$), **(C)** the left SLF for MD ($p < 0.05$), Bonferroni correction, error bars represent SD. *$p < 0.05$.

follow-up, 14.6% of PD patients without ICD at baseline developed ICD symptoms, while 5.6% of ICD patients no longer had ICD symptoms. The number of patients with PD-ICD has increased. In a recent 4-year prospective cohort study of DA therapy, the incidence of ICD was 39% (Bastiaens et al., 2013).

The incidence of ICD in PD patients treated with DA has been shown in previous studies, fluctuating between 15 and 40% (Garcia-Ruiz et al., 2014; Weintraub et al., 2015). We reported the disappearance of ICD symptoms in 5.6% of patients with ICD, and the reversibility of ICD and related behaviors after dopamine agonist discontinuation has been fully demonstrated in a few previous studies (Bostwick et al., 2009). According to this study, we observed that ICDs vary with the course of PD and that ICDs may persist or reverse their disappearance. This difference may reflect the heterogeneity of PD, with different subtypes. Therefore, the evolution model of ICD may have predictive value.

Second, we determined the progress of PD patients with ICD evolution patterns and NMSs. Specifically, non-ICD-stable patients showed relatively mild disease progression, whereas ICD-reversion patients showed the fastest NMS progression. This study focuses on the link between the ICD change course and PD progress. A previous study showed that compared with non-ICD in PD patients, patients with ICD have more than NMS, consistent with the results of our study. Further, we analyzed the effects of each group on NMS of daily life (MDS-UPDRS Part I), the severity was as follows: the ICD-reversion group > the ICD-stable group > the late-ICD group≈ the non-ICD-stable group. Here, we also found that the evolution patterns of ICD correlated with different rates of progression of NMS, generally manifested as the following trends: the ICD-reversion group > the ICD-stable group > the late-ICD group > the non-ICD-stable group.

Previous reports have shown that patients with ICD have more frequent rapid eye movement sleep behavior disorders (RBDs) than those without ICD (Fantini et al., 2015). Those results are consistent with our study. The effects of each group on rapid eye movement behavior disorder (RBDSQ-5) were further analyzed, and the severity was as follows: the ICD-reversion group > the ICD-stable group > the late-ICD group > the non-ICD-stable group.

Consistent with our results, patients with ICD have more obvious anxiety symptoms than those without ICD (Voon et al., 2011). We further analyzed different groups to determine the T-AI score and S-AI score. Among them, the S-AI score affects the severity of the sequence: the ICD-reversion group ≈ the ICD-stable group > the late-ICD group ≈, and the non-ICD-stable group. The T-AI-score affects the severity of the sequence: the ICD-reversion group > the ICD-stable group > the late-ICD group > the non-ICD-stable group.

Siri's study has shown that compared with patients with ICD and those without ICD, there are no differences in cognitive function in PD patients, consistent with our results (Siri et al., 2010). However, there is a paucity of research on the role of PD patients with and without ICD in motor symptoms had no effect. However, our study also found that the ICD-stable group had a better experience of exercise in daily life compared to the non-ICD-stable group. Moreover, the effect of severity on everyday life movement symptoms (MDS-UPDRS part II) in different groups was as follows: the ICD-reversion group > the ICD-stable group ≈ the late-ICD group > the non-ICD-stable group.

Previous studies on PFS using the Epworth Sleepiness Scale (ESS) scores of patients with PD-ICD are rare. For the ESS score, our study

found that compared with the ICD-reversion group, the ICD-stable group, and the late-ICD, the PFS time in the non-ICD-stable was longer (72.4 vs. 54.2 months, 72.4 vs. 53.9 months, and 72.4 vs. 60.7 months; all $p < 0.05$). Above all, the two types with the worst prognosis (the ICD-reversion and ICD-stable groups) constitute a baseline ICD group. Therefore, ICD evolution patterns and associations between PD progress. Furthermore, we found that the ICD-reversion group was more relevant to the progress of RBD, anxiety symptoms, and motor symptoms.

Third, we demonstrated that the alteration patterns of white matter microstructure differed among PD patients whose ICD evolution patterns differed. Interestingly, both ICD and non-motor functions are associated with relevant brain regions. These findings suggest that the ICD evolution pattern is potentially prognostic in PD.

Multiple imaging studies have found that changes in brain structure, function, and metabolism prior to drug therapy in PD patients increase the risk of developing an ICD (Ray and Strafella, 2013; Aracil-Bolanos and Strafella, 2016; Mojtahed Zadeh et al., 2018; Santangelo et al., 2019; Baagil et al., 2023; Hernadi et al., 2023). Yoo et al. (2015), using DTI technology, found that in the anterior corpus callosum, right internal capsule posterior limbs, right posterior cingulum, and right thalamic radiations, FA levels in the PD-ICD were significantly higher than in PD-non-ICD patients (corrected $p < 0.05$). In our study, the PD-ICD group had a higher FA trend than the non-ICD group in the right uncinate. The late-ICD group had the lowest FA ($p < 0.05$).

Previous research has suggested that the uncinate fasciculus plays a hypothetical role in several psychiatric disorders, including episodic memory, language, and social–emotional processing (Von Der Heide et al., 2013). Therefore, for PD patients, whether the change in the right uncinate FA value is related to the development of ICD and whether it is a predictor of future ICD needs to be further studied.

Based on previous studies, cognitive dysfunction in specific areas was related to damage to certain tract profiles, including the posteromedial component of the right cingulum bundle, the posterior portion of the left SLF, the bilateral anterior thalamic radiation, and the occipital lobe portion of the callosum forceps major (Huang et al., 2020). Increased MD is often associated with a loss of microstructural integrity (Aslan and McCarty, 2013). In our study, in the right cingulum bundle and left SLF, MD values were higher in the ICD-reversion group than in the other three groups ($p < 0.05$). According to the results, the ICD-reversion group had the most serious microstructural damage in the right cingulum bundle and left SLF, which may explain the factors that progressed most rapidly to non-motor function in the ICD-reversion group. This can explain the clinical features of different ICD evolutionary patterns.

## Limitations

First, the ICD diagnosis is based on an impulse and obsessive-compulsive disorder PD screening questionnaire (QUIP) (Weintraub et al., 2009). The QUIP was designed and validated as a screening tool, not a diagnostic or rating tool (Weintraub et al., 2009). In combination with clinical manifestations in patients with final confirmation, clinical doctors must improve clinical diagnosis accuracy. In assessing ICD and other compulsive behaviors during PD, we used an "anytime" time frame to avoid recall bias (Weintraub et al., 2009). However, in recent clinical studies, questionnaire-based evaluation is still widely used because it is relatively time-saving and practical (Marin-Lahoz et al., 2022). The QUIP, a self-administered questionnaire for recognizing ICD in Parkinson's patients, is a "gold standard" diagnosis using formal diagnostic criteria with good discriminant validity (Weintraub et al., 2009).

Second, the ICD reverse group sample size is relatively limited (18 patients). Especially MRI data, ICD-reversion group only included four patients. The underlying mechanisms can be better examined with larger cohorts to validate our preliminary findings. Large longitudinal studies are needed to validate the prognostic value of ICD evolutionary models, an important issue that can significantly impact the outcome. It could be improved by discussing the clinical implications of the observed ICD evolution patterns, particularly regarding patient management and treatment strategies. Finally, because we focused exclusively on *de novo* PD patients, medication and dosage variations may interfere with ICD and PD symptoms.

In conclusion, based on our study, four early ICD evolutions are identified in *de novo* PD, with different prognoses and brain white matter microstructural damage patterns, and may predict motor progression and cognitive decline in PD patients to develop precise intervention strategies as early as possible.

# Methods

## Study design and participants

Our data is from the Parkinson's Progression Markers Initiative (PPMI) database; PPMI is a multicenter study of early-stage PD subjects in longitudinal studies, and detailed objectives and methods for this study have previously been published (Evans et al., 2019).

The PPMI database was downloaded in March 2022. PD patients meeting both criteria were included in this study: (1) a baseline age of 40 years for men or women, and (2) clinical assessment data were available. After enrollment, participants were followed up every 3 months for the first year and followed every 6 months after that. Patients were excluded if they have: (1) follow-up for less than 4 years; (2) DBS surgery during follow-up; and (3) during the follow-up period, they were diagnosed with multiple system atrophy, essential tremor, and dementia with Lewy bodies.

## Ethical approval

The PPMI study has been registered with ClinicalTrials.gov under registration number NCT01141023. Each participating site of the study was approved by the Human Experimentation Ethics Standards Committee, and participants provided informed consent to participate.

## Assessment and classification of ICD transformation

### The diagnosis of ICD was assessed using the QUIP-rating scale

To standardize the clinical diagnosis and research of PD-ICD, the International Association of Movement Disorders recommends using QUIP and the QUIP-RS for the screening,

classification, and evaluation of the occurrence of PD-ICD after systematically evaluating the 50 reported ICD evaluation and grading scales (Von Der Heide et al., 2013). The QUIP scale is a quick and easy PD-ICD rating scale designed by scholars such as Weintraub of the University of Pennsylvania School of Medicine in 2009. Based on the previous ICD screening scale, Professor Weintraub followed the diagnostic criteria and clinical features of the revised fifth edition of the American Diagnostic and Statistical Manual of Psychiatry and divided the QUIP scale into three parts: (1) five questions for the four most common ICDs of PD; (2) five questions for compulsive impulse behaviors in PD; and (3) five questions for dopamine dysregulation syndrome (DDS). Under the premise of ensuring more than 80% sensitivity and specificity, the positive answer to two questions ≥ pathological gambling is positive on the QUIP scale; impulse shopping ≥ positive answer to one question; compulsive eating ≥ positive answer to two questions; and compulsive sexual behavior ≥ positive answer to one question. The QUIP scale requires clinicians to make final confirmation based on the patient's clinical manifestations to improve the accuracy of clinical diagnosis. The sensitivity and specificity of the QUIP scale have been verified in many countries and regions. QUIP-RS is a scale developed by QUIP to measure the severity of PD-ICD, involving 28 questions on pathological gambling, impulsive shopping, compulsive eating, compulsive sexual behavior, stereotypic movements, special hobbies, and the occurrence of DDS within 1 month. Each item is evaluated on a five-point scale, and its score ranges from 0 (never) to 4 points (very), with a total score of 0–112 points. The higher the score, the heavier the severity of the disease, under the premise of ensuring more than 90% sensitivity and specificity. On the QUIP-RS scale, pathological gambling ≥6 points is higher. Impulse shopping ≥8 is considered more severe. Compulsive eating ≥ a score of 7 is considered more severe. Compulsive sexual behavior ≥ a score of 8 is considered more severe (Evans et al., 2019).

During the fourth year of follow-up, the pattern of ICD evolution was gaged according to the ICD status during the baseline year. PD patients were defined as (1) ICD with a positive QUIP-RS score at baseline and subsequent visits or (2) non-ICD with a negative QUIP-RS score at baseline and subsequent visits.

Patients were excluded in the first year of follow-up if they did not achieve a stable ICD status. Patients were divided into four groups based on symptom fluctuations in the fourth year of follow-up. (1) Non-ICD stable: patients were ICD-free at baseline and throughout the 4-year monitoring period. (2) Late ICD: patients were ICD-free at baseline but developed an ICD during the 4-year follow-up period. (3) Stable ICD: patients with ICD symptoms during the 4-year follow-up period. (4) ICD recovery: the ICD symptoms were present at baseline but disappeared during the 4-year follow-up period.

## Clinical assessments

During follow-up, the investigators assessed the patient for multiple motor and NMSs to understand disease progression, including the Movement Disorders Association in conjunction with the Parkinson's Disease Rating Scale (MDS-UPDRS) Parts I (non-motor experiences of daily living), II (motor experiences of daily living), III (motor examination), and Hoehn-Yahr (H&Y). NMSs were evaluated, including the ESS scaled score, RBDSQ scaled score, GDS scaled score, S-AI scaled score, and T-AI scaled score.

The global and domain-specific cognitive status of the participants was assessed with neuropsychological tests, including the Hopkins Verbal Learning Test-Revised (HVLT-R) for verbal memory, the Montreal Cognitive Assessment (MoCA) for global cognition; the Semantic Fluency Test animal category for verbal fluency; the Judgment of Line Orientation for visuospatial ability; the Letter-Number Sequencing for working memory; and the Symbol Digit Modalities Test for executive function. As previously described, the performance on these assessments was converted to t-scores or scaled scores (Wyman-Chick et al., 2018).

## Outcomes

Criteria for the progression of NMSs are as follows:

(1) Criteria for sleep progression: RBDSQ <5.
(2) The standard for MDS-UPDRS Part I is MDS-UPDRS Part I < 0.
(3) MDS-UPDRS Part II < 15.

The State–Trait Anxiety Scale (STAI) is applied to determine whether a client has a transient anxiety attack and state or more stable personality traits with chronic anxiety levels. It is a self-report scale (10.1037/h0020743). In males aged 40–49, a cutoff of 51 or greater is used to define State-Anxiety. In females aged 40–49, a cutoff of 53 or greater is used to define State-Anxiety. In males aged 40–49, a 55 or greater cutoff is used to define Trait-Anxiety. In females aged 40–49, a cutoff of 58 or greater is used to define Trait-Anxiety. In males aged 50 or more, a cutoff of 55 or greater is used to define State-Anxiety. In females aged 50 or more, a cutoff of 58 or greater defines State-Anxiety. In males aged 50 or more, a cutoff of 52 or greater is used to define Trait-Anxiety. In females aged 50 or more, a cutoff of 47 or greater is used to define Trait-Anxiety.

## Neuroimaging and DTI analysis

During the 6 months of follow-up, some patients underwent high-resolution three-dimensional T1-weighted MRI scans, which ruled out significant abnormalities due to excessive head movement artifacts. A total of 75 patients met the requirements.

In addition to the above details, the PPMI MRI operation manual contains additional information. DTI analysis was performed following the previous publication (Sun et al., 2015). Briefly, we used FSL 6.0.5 (FMRIB Software Library, FMRIB, Oxford, United Kingdom). An automated fiber quantification (AFQ) v0.1 program is available at https://github.com/jyeatman/AFQ (Yeatman et al., 2012). The diffusion images were preprocessed using FSL. The preprocessing steps, including B0, were registered with a DWI image, head motion correction, and an exclusion of non-brain tissue. After preprocessing, we used the AFQ to perform fiber tracking and tract segmentation. A detailed description of the method of neuroimaging processing is shown in Supplementary File S1.

A structured array with tensor-based measures was returned for each group of 20 tracts. We focused specifically on FA, MD, axial diffusivity (AD), and radial diffusivity (RD) using 100 nodes per tract delineated: left and right thalamic radiations, minor of the corpus callosum and forceps major, left and right inferior frontal-occipital, superior longitudinal, inferior longitudinal, corticospinal tract, arcuate and uncinate fasciculi, and cingulum.

### Statistical analysis

Analysis of statistical results using SPSS 25 (IBM Corp., Armonk, NY). Continuous variables are expressed as mean ± standard deviation and categorical variables are expressed as quantities (percentages). Chi-square tests are used to find associations between categorical variables. One-way analysis of variance (ANOVA) is used for continuous variable calculations.

Kruskal–Wallis with Bonferroni correction was used for multiple comparisons. The chi-square or Fisher's exact test was used with categorical data to represent and compare frequencies and percentages. PFS was calculated using the Kaplan–Meier method, and a log-rank test compared survival rates. A Cox proportional hazard model was used to estimate the risk ratio (HR).

A $p$-value <0.05 was a considered statistically significant. For DTI analysis, we extracted the average FA, MD, AD, and RD values of different fiber celluloses. Our study generated 1,000 bootstrap samples and applied ANOVA to each bootstrap sample. After testing for the homogeneity of variances, Bonferroni was used to correct t-values for multiple comparisons; otherwise, Dunnett's $t$-test was used. In this instance, statistical significance was defined as a $p$-value of less than 0.001.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

## Ethics statement

The studies involving humans were approved by The PPMI study has been registered with ClinicalTrials.gov under registration number NCT01141023. Each participating site of the study was approved by the Human Experimentation Ethics Standards Committee, and participants provided informed consent to participate. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

LH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. CL: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. FL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing, LW: Investigation, Supervision, Validation, Visualization, Writing – original draft. ZL: Investigation, Supervision, Validation, Visualization, Writing – review & editing. ZC: Investigation, Supervision, Validation, Visualization, Writing – review & editing. XL: Investigation, Supervision, Validation, Visualization, Writing – review & editing. QY: Conceptualization, Data curation, Project administration, Supervision, Validation, Writing – original draft. YW: Conceptualization, Data curation, Project administration, Supervision, Validation, Writing – review & editing. GC: Conceptualization, Data curation, Project administration, Supervision, Validation, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1260630/full#supplementary-material

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# Research progress on the cannabinoid type-2 receptor and Parkinson's disease

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Parkinson's disease (PD) is featured by movement impairments, including tremors, bradykinesia, muscle stiffness, and imbalance. PD is also associated with many non-motor symptoms, such as cognitive impairments, dementia, and mental disorders. Previous studies identify the associations between PD progression and factors such as α-synuclein aggregation, mitochondrial dysfunction, inflammation, and cell death. The cannabinoid type-2 receptor ($CB_2$ receptor) is a transmembrane G-protein-coupled receptor and has been extensively studied as part of the endocannabinoid system. $CB_2$ receptor is recently emerged as a promising target for anti-inflammatory treatment for neurodegenerative diseases. It is reported to modulate mitochondrial function, oxidative stress, iron transport, and neuroinflammation that contribute to neuronal cell death. Additionally, $CB_2$ receptor possesses the potential to provide feedback on electrophysiological processes, offering new possibilities for PD treatment. This review summarized the mechanisms underlying PD pathogenesis. We also discussed the potential regulatory role played by $CB_2$ receptor in PD.

KEYWORDS

**Parkinson's disease, $CB_2$ receptor, mitochondrial function, neuroinflammation, iron transport**

## Introduction

Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases (de Lau and Breteler, 2006; Subramaniam and Chesselet, 2013). Patients with PD are commonly suffering from movement disorders, such as tremors, involuntary movements, rigidities, and imbalance. Many patients also demonstrate non-movement disorders, including cognitive impairments, sleep disorder, chronic pain, olfactory dysfunction, anxiety, and depressive disorder (Garcia-Ruiz et al., 2014; Tolosa et al., 2021). Many patients diagnosed with PD eventually develop dementia during the advanced stage (Szeto et al., 2020). The main pathological feature of PD includes gradual loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) located at the midbrain, and the accumulation of Lewy bodies (LBs) containing mainly α-synuclein (α-syn) intracellular inclusions all over the brain (Warren et al., 2017). Multiple mechanisms, including α-syn aggregation (Roy, 2017), mitochondrial dysfunction (Subramaniam and Chesselet, 2013), oxidative stress, abnormal iron accumulation (Hare and Double, 2016), and neuroinflammation (Gelders et al., 2018), have been implicated in the neurodegenerative process of PD (Figure 1). However, the exact cause of PD is still not clear. Consequently, clinical therapies for PD treatment, including medicines and surgeries, are mostly symptomatic. No treatment can stop or reverse the development of PD. The endocannabinoid system (ECS) comprises a network of endocannabinoids (eCBs) and their

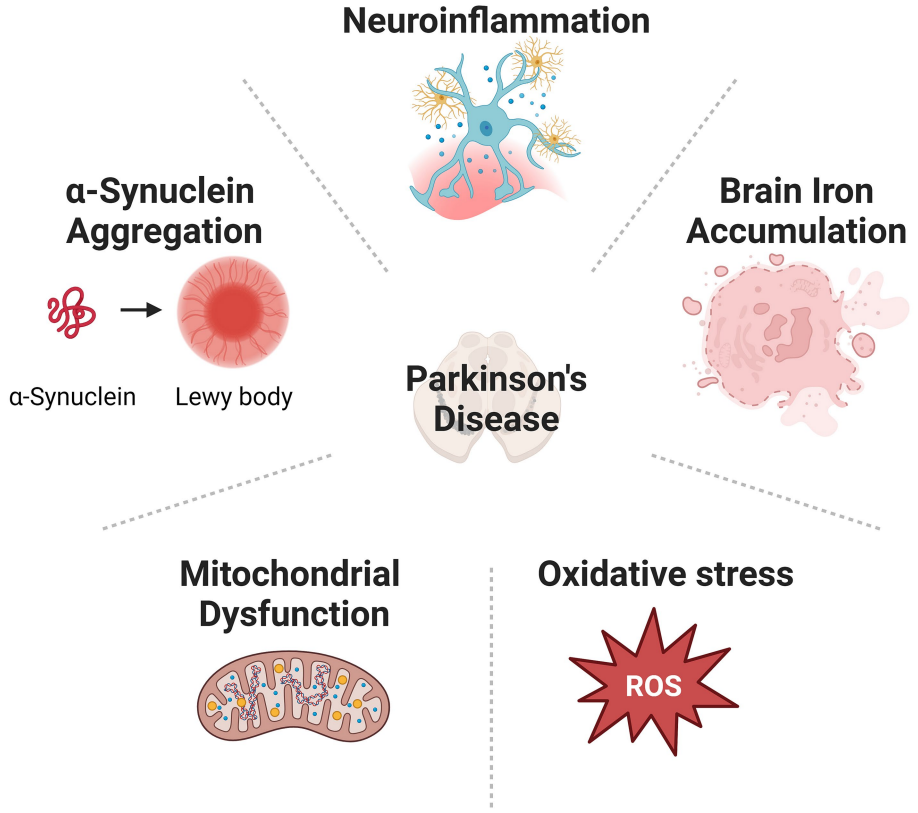


FIGURE 1
Cause of PD is various, including α-synuclein aggregation, mitochondrial dysfunction, oxidative stress, abnormal iron accumulation, and neuroinflammation.

receptors that are widespread throughout the central nervous system (CNS) and immune system. This tightly regulated system modulates the transmission of chemical signals via an immediate feedback mechanism. Dysregulation of eCB signalling has been suggested in the development of neuropsychiatric disorders and neurodegenerative disease (Yin et al., 2019; Cooray et al., 2020). eCBs are recognized by cannabinoid receptors (CB receptors): the cannabinoid type-1 receptor ($CB_2$ receptor) and the cannabinoid type-2 receptor ($CB_2$ receptor). Among them, the $CB_2$ receptor is mainly located in immune cells. Its activation is reported to exert protective effects in neurological disorders and thus receives extensive attention as a new treatment target. Here, we summarized the current research progress of how the $CB_2$ receptor is involved in the pathogenesis and progression of PD and discussed the potential of targeting the $CB_2$ receptor for the treatment of this disease.

## The endocannabinoid system (ECS)

Cannabinoids, as an emerging therapeutic agent, have attracted wide attention for their great potential in the treatment of various diseases. They are best understood for their inhibitory effects on the release of γ-aminobutyric acid (GABA) and glutamate through $CB_1$ and $CB_2$ receptors (Urits et al., 2020). The ECS consists of two major branches: the $CB_1$ receptor is highly enriched in the brain and its surrounding nerves (Herkenham et al., 1991), meanwhile, the CB2 receptor is mainly found in the immune system (Facci et al., 1995). Cannabinoids are generally classified into three types based on their source: phytocannabinoids (found in cannabis plants, for example, $\Delta^9$-tetrahydrocannabinol, THC), synthetic cannabinoids (chemically synthesized), and endocannabinoids (eCBs, i.e., naturally occurring in the human body). Cannabinoids bind to CB receptors located on the cell membrane, exerting corresponding psychotropic effects (Howlett et al., 1990). The eCBs, CB receptors, and enzymes catalyze the synthesis and degradation collectively form the ECS. The activation of the ECS is related to decreased dopaminergic activity and can regulate various neural functions related to emotions, cognitions, motor controls, feeding behaviors, and pain (Castillo et al., 2012; Pacher and Kunos, 2013).

### The eCBs

N-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which share highly similar structures with $\Delta^9$-THC, are the two major and most well-understood eCBs. Generally, they are released from the postsynaptic terminal after neuronal activation, modulate presynaptic neurotransmissions, and produce physiological feedback mechanisms dedicated in preventing excessive excitation of neurons (Lovinger, 2008; Zou and Kumar, 2018). This retrograde feedback initiates depolarization-induced suppression of inhibition (DSI) at GABAergic synapses and depolarization-induced suppression of excitation (DSE) at glutamatergic synapses (Makara et al., 2005). AEA and 2-AG, unlike other neurotransmitters and

neuropeptides that are stored in the intracellular compartments, are produced on demand from the cleavage of their precursors, N-arachidonyl-phosphatidyl ethanolamine (NAPE) and diacylglycerol (DAG), respectively (Maccarrone and Finazzi-Agro, 2003).

In the CNS, the eCBs are synthesized by both neuronal cells and glial cells such as microglia (Kelly et al., 2020). *In vitro* study reveals the production of both AEA and 2-AG by microglia (Walter et al., 2003; Carrier et al., 2004). Adenosine triphosphate (ATP) stimulation of microglia increases the production of 2-AG through the activation of P2X purinoceptor 7 (P2X7) ionotropic receptor (Witting et al., 2004). Microglia is suggested as the one of the main source of eCBs under neuroinflammation (Stella, 2009). Upregulated eCB levels are implicated in anti-inflammatory effects, and therefore are believed to exert neuroprotective effects in various diseases. 2-AG is reported to limit acute neuroinflammation induced by the Theiler's murine encephalomyelitis virus (TMEV) by modulating microglial activation and promoting the activation of brain-derived suppressor cells, indicating a potent regulatory function of 2-AG on peripheral and central immunity (Mecha et al., 2018). AEA treatment is found to attenuate the lipopolysaccharide (LPS)-induce microglia activation via the $CB_2$ receptor (Malek et al., 2015). Clinical study recently reveals that deficiency of diacylglycerol lipase β (*DAGLB*), the synthase of 2-AG, is associated with early onset of PD. Knockdown of *Daglb* impairs locomotor skill learning in mice (Liu et al., 2022). In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model, increased level of 2-AG is reported in the ventral midbrain after MPTP treatment (Mounsey et al., 2015). Exogenous addition of 2-AG or monoacylglycerol lipase (MAGL, enzyme for 2-AG hydrolysis) inhibitors demonstrate potent protective effect against MPTP-induced cell death (Mounsey et al., 2015). Collectively, those studies suggest the potential neuroprotective effects of eCBs in PD via regulation of microglia and neuroinflammation.

## The CB receptors

Biological effects of the eCBs and other synthetic cannabinoids (such as WIN55,212–2 and HU210) are mainly mediated by the G-protein-coupled CB receptors: the $CB_1$ and $CB_2$ receptors (Munro et al., 1993). The activation of $CB_1$ receptors involves the coupling of pertussis toxin (PTX)-sensitive G proteins (Gαi/o), leading to the inhibition of adenylate cyclase (AC) and cyclic adenosine monophosphate (cAMP) formation. Activation of $CB_1$ receptor also activates the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathways, which both participate in the regulation of cell proliferation, cell cycle, and cell death (Pertwee, 2006; Howlett et al., 2010; Turu and Hunyady, 2010; Blázquez et al., 2015). $CB_1$ receptors can also exert their effects through G protein-dependent or other ligand-dependent mechanisms (Demuth and Molleman, 2006). In addition to the typical G protein-dependent signaling, $CB_1$ receptors also transmit signals through interaction with other molecules (such as β-arrestin) in a G protein-independent manner (Howlett et al., 2010). Moreover, $CB_1$ receptors also regulate several types of ion channels (Turu and Hunyady, 2010). Upon activation of $CB_1$ receptors, the inhibition of $G\alpha_{i/o}$-mediated cAMP reduction regulates inwardly rectifying potassium channels (GIRKs) and inhibits N-type and P/Q-type voltage-gated calcium channels (Howlett et al., 2002; Fisyunov et al., 2006), thereby suppressing presynaptic neurotransmitter release. Research has shown that $CB_2$ receptors regulate the activity of N-type $Ca^{2+}$ channels located at the presynaptic membrane, thereby modulating calcium influx to inhibit GABA release in mouse hippocampal slices (Szabo et al., 2014).

Like the $CB_1$ receptor, the $CB_2$ receptor is coupled to $G\alpha_{i/o}$ proteins. However, unlike the $CB_1$ receptor, the $CB_2$ receptor does not appear to be coupled to potassium channels (McAllister et al., 1999). The $CB_2$ receptor is initially thought to be predominantly expressed in the peripheral immune system. However, recent studies have found $CB_2$ receptor expression in the CNS (Mackie, 2008). The $CB_2$ receptor is expressed by microglia, astrocytes and certain subpopulations of neurons (Fernández-Ruiz et al., 2008). Upregulation of $CB_2$ receptor has been implicated in neurodegenerative diseases. Activation of this receptor in animal models demonstrate disease-modifying effects against the process of neurodegeneration, suggesting $CB_2$ receptor is a promising therapeutic target for the treatments of such disease. Also, compared to $CB_1$ receptor, the activation of $CB_2$ receptor has been shown to have fewer psychoactive and other side effects (Pacher et al., 2006; Liu et al., 2021), making selective $CB_2$ receptor targeting a better option for this approach. In the following parts, we summarized the current understanding of how $CB_2$ receptor participates in the progression of PD, and its potential as a treatment target in the treatment of this disease.

# $CB_2$ receptor in PD

## The role of $CB_2$ receptor in PD

Both clinical and animal studies reveal the alternation of $CB_2$ receptor in PD. Postmortem studies reveal the increased level of $CB_2$ receptor in microglial cells at substantia nigra (SN) of PD patients, indicating the recruitment and activation of microglia at the site of lesion (Gómez-Gálvez et al., 2016). This finding is supported by the observation in animal models of PD. $CB_2$ receptor level is significantly increased in both LPS- and 6-hydroxydopamine (6-OHDA)-induced PD model, and this elevation is associated with the activation of microglia (Concannon et al., 2015). Those findings suggest the upregulation of $CB_2$ receptor in microglia. However, downregulation of $CB_2$ receptor is also reported in neurons and other brain regions. Reduced level of $CB_2$ receptor is reported in the tyrosine hydroxylase (TH)-containing in the SN of PD patients, indicating increased DA neuronal cell death (García et al., 2015). Reduced transcription of $CB_2$ receptor is observed in the cerebellum and hippocampus of PD patients, as compared to healthy controls (Grünblatt et al., 2007). Similarly, in the MPTP-induced PD mouse model, a downregulation of $CB_2$ receptor is observed 3 weeks after MPTP injection (Shi et al., 2017; Xin et al., 2020). Further research demonstrates neuroprotective potentials of $CB_2$ receptor in PD. Specifically, $CB_2$ receptor-deficient mice demonstrate more severe loss of tyrosine TH-containing neurons in the SN, indicating the protective role of $CB_2$ receptor in PD (Gómez-Gálvez et al., 2016). In an *in vitro* PD model established by $MPP^+$ treatment, JWH133 (a potent $CB_2$ receptor agonist) is shown to promote cell survival (Aymerich et al., 2016). *In vivo* study also demonstrates that the administration of nonselective CB receptor agonist WIN55,212–2 and selective $CB_2$ receptor agonist JWH015 alleviate the MPTP-induced neuron death and microglial activation in SN (Price et al., 2009). GW842166x (a selective $CB_2$ receptor agonist) exerts protective effects against the 6-OHDA-induced loss of dopamine neurons (Yu et al., 2021). Another selective $CB_2$ receptor

agonist AM1241 is reported to alleviate the MPTP-induced PD-like symptoms and promote the regeneration of DA neurons in mice (Shi et al., 2017). Moreover, administration of β-caryophyllene (BCP, a $CB_2$ receptor agonist) is reported to exert neuroprotective effects in both rotenone (ROT)-induced and MPTP-induced PD animal models (Javed et al., 2016; Viveros-Paredes et al., 2017). Those research findings collectively suggest the potential protective effects of $CB_2$ receptor agonist in PD, rising the discussion of targeting $CB_2$ receptor as a potential treatment approach for PD. Therefore, we further discuss the potential roles of $CB_2$ receptor in PD from different perspectives and possible mechanisms in the following sections.

## Role of $CB_2$ receptor in α-syn pathology

α-syn is one of the major components involved in the formation of LBs. α-syn oligomers exert strong cytotoxic effects to neuron (Ghosh et al., 2017; Calabresi et al., 2023). The formation of α-syn oligomers is influenced by multiple factors. Clinical studies have shown significantly elevated level of α-syn oligomers in the plasma, serum, and red blood cells of PD patients, as compared to healthy controls (Zhao et al., 2022). Interestingly, it has been reported that the peripheral autonomic nervous system may be a key pathway for the spread of α-syn pathology from the periphery to the CNS (Chen et al., 2020). Numerous research and clinical findings reemphasize the central role of α-syn, and α-syn-induced neurotoxicity and neuroinflammation in PD (Fayyad et al., 2019; Wang et al., 2019). However, the interaction between $CB_2$ receptor and α-syn has been largely over-looked. Recently, Feng et al. demonstrate that the fibrillar α-syn treatment causes significantly promoted neuroinflammation and phagocytosis, as revealed by higher level of cluster of differentiation 68 (CD68) and interleukin-1β (IL-1β), reduced level of brain-derived neurotrophic factor (BDNF) in mice with $CB_2$ receptor knockout, as compared to wild-type (WT) mice (Feng et al., 2023). Indeed, they also find that $CB_2$ receptor knockout promotes the activation of microglia and pruning of cholinergic synapses induced by α-syn treatment (Feng et al., 2023), suggesting the important role played by $CB_2$ receptor in α-syn pathology.

## The inhibitory effect of $CB_2$ receptor in neuroinflammation

Extensive post-mortem examinations, brain imaging studies, epidemiological data, and animal studies have demonstrated the contribution of innate and adaptive immunities in neurodegeneration (McGeer et al., 1988; Gerhard et al., 2006; Theodore et al., 2008). It is widely believed that the degeneration and death of neurons in neurodegenerative diseases are primarily influenced by the release of inflammatory factors and neurotoxic mediators, such as IL-1β, tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-33 (IL-33), chemokine ligand 2 (CCL2), chemokine ligand 5 (CCL5), prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), and increased ROS (Skaper et al., 2014; Kempuraj et al., 2015). These mediators bind to corresponding receptors on neurons or glia cells, directly or indirectly induce neurodegeneration and affect neuronal survival through interactions with neuroglial cells. Meanwhile, the activation of glial cells, including microglial cells and astrocytes, promote the expression of pro-inflammatory mediators in neurodegenerative diseases (Kim and Lee, 2014), causing aggravated neurodegeneration, which further exacerbates the progression of the disease course. In PD, the contribution of neuroinflammation has been intensively studied and suggested as a promising target for effective treatment (Tansey et al., 2022).

$CB_2$ receptor has been identified as a potential anti-inflammatory component in various inflammation-related diseases. Its activation disrupts the self-sustained neuroinflammation status that contributes to the disease progression of neurodegeneration. Activation of $CB_2$ receptor reduces the release of pro-inflammatory cytokine and thereby prevents neuronal cell death in neurodegeneration diseases. LPS injection in mice leads to an increase in TNF-α levels and oxidative stress in the brain, resulting in disease-like behavior. Acute injection of the $CB_2$ receptor agonist 1-phenylisatin (PI) significantly rescues the behavioral changes induced by LPS administration in mice (Sahu et al., 2019). Moreover, PI inhibits the transcription of TNF-α and oxidative stress in the brain, demonstrating that both acute and long-term activation of $CB_2$ receptor may exert protective effect against the development of various disease related to neuroinflammation and oxidative stress (Sahu et al., 2019). Activation of $CB_2$ receptor is reported to inhibit the activation of NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome, a potent contributor of neuroinflammation and neurodegenerative diseases (Ke et al., 2016; Yu et al., 2019). In human microglial cells derived from the temporal lobe, JWH015 exerts neuroprotective effects by reducing the release of TNF-α and IL-1β (Klegeris et al., 2003).

Non-selective $CB_2$ receptor agonist WIN55,212–2 and selective $CB_2$ receptor agonist JWH015 have been shown to reduce MPTP-induced microglial infiltration. This effect can be reversed by the $CB_2$ receptor antagonist JTE907, confirming the $CB_2$ receptor-mediated inhibitory effect via the modulation of microglia (Price et al., 2009). $CB_2$ receptor activation by JWH133 is reported to reduce the level of pro-inflammatory cytokines and promote the M2 polarization of microglia via the activation of the PI3K/Akt signalling pathway (Wang et al., 2023). In the MPTP-induced PD mouse model, $CB_2$ receptor knockout exhibits aggrieved microglial activation, along with neuropathology and functional deficits (Komorowska-Muller and Schmole, 2020). In an environmental and viral inflammation-induced PD model established by unilateral intrastriatal injection of ROT or polyinosinic:polycytidylic acid (Poly I:C) in male rats, a significant increase of $CB_2$ receptor expression is observed, which is strongly correlated with activated microglia in the model (Concannon et al., 2016). Similarly, in ROT-induced and MPTP-induced PD animal models, $CB_2$ receptor agonizing using BCP demonstrates disease-alleviating effect via the suppression of neuroinflammation (Javed et al., 2016). ROT injection leads to microglial activation and subsequent inflammation. Further research has showed that the activation of $CB_2$ receptor by BCP can inhibit ROT-induced microglial activation, improve the release and expression of inflammatory mediators in CNS, and attenuate the expression of inflammatory factors such as NF-κB, COX-2, and Inducible nitric oxide synthase (iNOS) (Javed et al., 2016).

Excessive inflammation not only involves the activation of microglial cells but also the activation and proliferation of astrocytes, which play a crucial regulatory role in the inflammatory response. Currently, there is limited research on the effects of $CB_2$ receptor on astrocytes. It has been demonstrated that rat astrocytes express both $CB_1$ receptor and $CB_2$ receptor (Stella, 2004; Sheng et al., 2005). Recent studies also report the colocalization of $CB_2$ receptor with astrocytes by immunohistochemical localization. Increased immunoreactivity of $CB_2$ receptor in astrocytes is reported in PD patients (Navarrete et al.,

2018). This suggests that the expression changes of $CB_2$ receptor in astrocytes have potential regulatory roles in PD, and warrant further investigation. In primary cultured astrocytes, the nonspecific CB receptor agonist WIN 55,212–2 has been shown to regulate cell viability, inflammatory mediators, and oxidative stress. Specifically, the amyloid-β (Aβ) 1–42, the aberrant protein aggregation contributes to the pathogenesis of Alzheimer's disease (AD), reduces astrocyte viability while increasing the expression of TNF-α, IL-1β, COX-2, and iNOS. Meanwhile, pre-treatment with WIN 55,212–2 significantly rescues the inflammatory and astrocyte vulnerability to Aβ1-42 treatment (Aguirre-Rueda et al., 2015). Furthermore, JWH133 is reported to exert neuroprotective effects by inhibiting blood–brain barrier (BBB) damage, astrocytic targeting myeloperoxidase (MPO) expression, peripheral immune cell infiltration, and the production of inflammatory and chemotactic factors by activated microglial cells (Chung et al., 2016). Collectively, those results indicate $CB_2$ receptor as a promising disease-modifying treatment target for PD via its regulation of neuroinflammation.

## The inhibitory effect of $CB_2$ receptor on oxidative stress

The motor dysfunction in PD is caused by the loss of DA neurons in the SNpc. Increasing evidence suggests that oxidative stress is a key driving factor in the complex degenerative cascade of dopaminergic neurodegeneration in all forms of PD (Dias et al., 2013; Blesa et al., 2015). Markers of oxidative stress in the CNS increase with aging and the occurrence of neurodegenerative diseases (Boveris and Navarro, 2008). Oxidative stress arises from a disruption in cellular redox homeostasis, where the production of reactive oxygen species (ROS) exceeds the clearance rate by endogenous antioxidant enzymes and molecular chaperones. Uncontrolled oxidative reactions within cells cause destructive damage to normal cellular structures, leading to cellular degeneration and death (Wiseman and Halliwell, 1996; Rego and Oliveira, 2003). Accumulation of ROS induces oxidative damage to lipids, proteins, DNA, and RNA, impairing neuronal function and structural integrity (Schieber and Chandel, 2014). Due to the increased chances of spontaneous mutations resulting from oxidative stress, it may trigger mutations that make cells more susceptible to functional impairments, and the vulnerability of the SN to oxidative stress contributes to selective neuronal degeneration (Floor and Wetzel, 1998). The damaging effects of oxidative stress are well recognized, and research focusing on inhibiting neuronal oxidative stress has become a mainstream direction in PD treatment.

Previous research demonstrates that activation of $CB_2$ receptor can protect DA neurons against degeneration in a ROT-induced PD model (Javed et al., 2016). ROT injection causes extensive loss of DA neurons in the SNpc and striatal fibers, leading to oxidative damage characterized by reduction of anti-oxidant enzymes and upregulated nitrite level (Thakur and Nehru, 2013). Treatment with the $CB_2$ receptor agonist BCP prevents glutathione depletion, enhances antioxidant enzyme activity in the midbrain, and inhibits the elevation of nitrite levels. It has been found that the GW405833 (a $CB_2$ receptor-specific agonist) administration inhibits inflammatory response by suppressing the levels of cytokine and oxidative stress (Parlar et al., 2018). Other research results report that the $CB_2$ receptor agonist HU308 reduces the production of ROS-generating enzymes NOX4, NOX2, and NOX1, as well as subsequent renal oxidative stress in mice (Zhang et al., 2009). An *in vitro* study demonstrates that $CB_2$ receptor is involved in the antioxidant stress process in RAW264.7 macrophages, blocking cell death (Giacoppo et al., 2017). These results indicate that activation of $CB_2$ receptor can inhibit oxidative stress and protect neuronal cells.

## $CB_2$ receptor and iron transport

Excessive accumulation of iron in the brain is a major characteristic of brain degeneration in patients with PD, known as brain iron accumulation. Non-physiological accumulation of iron in specific brain regions is associated with various diseases. This phenomenon is referred to as neurodegeneration with brain iron accumulation (NBIA) (Schneider et al., 2012). It has been reported that iron levels in the SN of PD patients increase significantly. This change is accompanied by upregulation of divalent metal transporter 1 (DMT1), a protein involved in iron transport (Jia et al., 2015). Iron accumulation may exert its pathogenic activity by increasing ROS and causing widespread damage to intracellular proteins. However, there is also evidence suggesting that it leads to neuronal death through interactions with pathological protein aggregates found in these diseases by promoting the process of cellular apoptosis (Ward et al., 2014).

Maintaining iron homeostasis in the brain has long been considered a potential target for drug treatment related to aging-related diseases. Iron is involved in various cellular functions, such as the synthesis of myelin phospholipid, mitochondrial respiration, and the biosynthesis and metabolism of neurotransmitters. Therefore, the regulation of iron transport through DMT1 plays a significant role in maintaining normal brain physiological function. It has been reported that $\Delta^9$-THC, CP 55940, WIN 55,212–2, and AEA inhibit the uptake of 55Fe and 54Mn in HEK293T cells expressing DMT1 by stabilizing the expression of the transporter protein and inhibiting DMT1 expression. Small-molecule tests have shown that Δ9-THC inhibits DMT1 activity (Wetli et al., 2006). Furthermore, gene knockout of the $CB_2$ receptor eliminates its regulatory effects, indicating that the inhibitory effect of Δ9-THC is mediated by the $CB_2$ receptor. Moreover, activation of $CB_2$ receptor negatively regulates signaling cascades related to serine/threonine kinases. Immunoprecipitation experiments have shown that phosphorylation of serine 43 of DMT1 promotes its transport activity, thereby facilitating iron absorption. $\Delta^9$-THC blocks serine phosphorylation of DMT1, and $CB_2$ receptor knockout abolishes the blockade of iron transport by $\Delta^9$-THC (Seo et al., 2016).

## The regulatory effect of $CB_2$ receptor on mitochondrial function

Mitochondria play a pivotal role in the vitality of eukaryotic cells as they are involved in bioenergetics, metabolism, and signaling, and are associated with many diseases (Pfanner et al., 2021). The involvement of mitochondrial dysfunction in the pathogenesis of PD is discovered when individuals who consumed illegally contaminated drugs containing MPTP developed PD-like symptoms (Langston et al., 1983). It has been demonstrated that mitochondrial dysfunction can induce degeneration and death of DA neurons (More and Choi, 2015), promoting the occurrence of neurodegenerative in PD (Bose and Beal, 2016).

Previous research has shown that cannabinoids such as $\Delta^9$-THC and synthetic cannabinoid HU210 impair mitochondrial respiratory function via the suppression of oxygen consumption and mitochondrial membrane potential ($\Delta\Psi m$) (Athanasiou et al., 2007). $\Delta\Psi m$ manifests the functional status of mitochondria. Additionally,

both AEA and 2-AG suppress the transcription of genes associated with mitochondrial biogenesis, and decrease mitochondrial DNA content and oxygen consumption in white adipocytes of mouse (Tedesco et al., 2010). Further studies have found that activation of $CB_2$ receptor using JWH133 conveys an anti-apoptotic effect in animal model of myocardial ischemia (Li et al., 2013), which aligns with the protective outcome of JWH133 against ischemia-induced $\Delta\Psi m$ loss and cytochrome c release from mitochondria to the cytoplasm. Moreover, $CB_2$ receptor is involved in AEA-stimulated mitochondrial cation transport (Zoratti et al., 2003). Collectively, $CB_2$ receptor is believed to play a regulatory role in modulating mitochondrial respiratory activity. How this regulatory effect of $CB_2$ receptor related to PD is therefore worth further investigation.

### $CB_2$ receptor and autophagy

Autophagy is a lysosome-dependent self-degradation and recycling process. It is an essential metabolic process that targets protein and dysfunctional cellular components (Kim and Lee, 2014; Saha et al., 2018). Autophagy is a conserved cellular process that maintains cellular homeostasis. Autophagy impairments are closely related to the pathogenesis of PD (Cheng et al., 2020; Lu et al., 2020). Further studies reveal the association between autophagy and $CB_2$ receptor. It has been demonstrated that autophagy is related to the protective functions of $CB_2$ receptor in several diseases (Shao et al., 2014; Denaës et al., 2016). Ke et al. (2016) found that activation of $CB_2$ receptor alleviates the effects of NLRP3 inflammasome activation by inducing autophagy in rat macrophages, thereby reducing inflammation in a mouse model of inflammatory bowel disease (IBD). Additionally, there is a similar association between $CB_2$ receptor and autophagy in a mouse model of multiple sclerosis. It has been shown in mice that activation of $CB_2$ receptor can induce autophagy to prevent diabetic cardiomyopathy (Wu et al., 2018). These studies suggest that inducing autophagy through the activation of $CB_2$ receptor has potential therapeutic value in the progression of PD.

### The electrophysiological regulatory effects of $CB_2$ receptor

There is electrophysiological evidence suggesting that activation of $CB_2$ receptors can regulate neuronal activity and excitability. $CB_2$ receptors have been found to be expressed in ventral tegmental area (VTA) DA neurons (Foster et al., 2016), and systemic and local administration of JWH133 has been shown to enhance M-type potassium currents, leading to neuronal inhibition and hyperpolarization, significantly reducing the firing frequency of VTA DA neurons both *in vivo* and *in vitro* (Zhang et al., 2014, 2017). Specifically, in whole-cell perforated and cell-attached membrane patch clamp recordings from individual neurons or brain slices in wild-type mice, JWH133 dose-dependently suppressed the firing of VTA DA neurons, and this effect is blocked by AM630 and observed in $CB_2$ receptor knockout mice. Similar effects have also been observed in rats, indicating that activation of $CB_2$ receptor in the brain can regulate the firing of VTA DA neurons, exerting electrophysiological regulatory effects and providing new avenues for the treatment of PD.

### The neuroprotective effects of $CB_2$ receptor on DA neuron

Given that the main characteristic of PD is the loss of DA neurons in SN and a significant reduction in striatal dopamine, the current mainstay of PD clinical treatment involves the use of levodopa (L-DOPA). However, long-term use of L-DOPA often leads to fluctuations and motor complications that offset its beneficial effects (Utsumi et al., 2013). Therefore, many studies are focused on developing novel non-dopaminergic drugs that can prevent or even reverse the degeneration of DA neurons. $CB_2$ receptors are detected in central nervous system regions including the striatum, hippocampus, basal ganglia, frontal cortex, amygdala as well as the VTA (Morris et al., 2021), and their activation is involved in various diseases associated with DA neuron injuries. Mice overexpressing $CB_2$ receptor show significantly reduced damage to DA neurons induced by 6-OHDA, reduced motor impairment, and decreased activation of glial cells in the affected area (Ternianov et al., 2012). Activation of $CB_2$ receptor using the $CB_2$ receptor agonist AM1241 can protect against MPTP-induced PD mouse models, leading to an increase in the number of TH-positive cells in the SN, indicating the regeneration of DA neurons in PD mice and suggesting AM1241 as a potential candidate for PD treatment (Shi et al., 2017). Research data obtained from DA neuron-specific $CB_2$ receptor knockout mice indicates that the absence of $CB_2$ receptor in DA neurons modulate psychomotor and reward behavior (Liu et al., 2017). This further confirms the protective functions of $CB_2$ receptor on DA neurons and establishes a new target for PD treatment.

### $CB_2$ receptor prevents motor dysfunction

Motor dysfunction is a prominent feature in the progression of PD and poses significant inconvenience and harm to patients (Bologna et al., 2020). In PD models established by unilateral lesion of DA neurons, induced by 6-OHDA or LPS injection in male Sprague Dawley rats, behavioral tests for motor dysfunction and $CB_2$ receptor detection are conducted on days 7, 14, and 28. The animal exhibits motor dysfunction, and the expression of $CB_2$ receptor is significantly upregulated in the PD models (Concannon et al., 2015). Previous studies have found that activation of $CB_2$ receptor using agonists improve certain aspects of motor dysfunction, providing a solution to alleviate the motor deficits caused by PD. In C57BL mice, treatment with the $CB_2$ receptor agonist JWH015 alleviates anxiety-like behavior during chronic mild stress, while AM630 enhances anxiety-like behavior (Ishiguro et al., 2018). An increase in $CB_1$ and $CB_2$ receptor expression in the striatum has been reported in chronic L-DOPA treatment for motor dysfunction, and a correlation between motor dysfunction, striatal activation, and microglial cell activation in the PD model after L-DOPA treatment (Navarro et al., 2018). In a mouse model of PD induced by MPTP treatment, treatment with AM1241 can mitigate weight loss, attenuate MPTP-induced motor impairment, and reduce climbing time in mice (Shi et al., 2017). This indicates the critical role of $CB_2$ receptor in preventing MPTP toxicity and highlights the significant therapeutic value of the $CB_2$ receptor agonist AM1241 in PD, including the potential regeneration of dopaminergic neurons following neurotoxicity induced by MPTP.

## Therapeutic potential of $CB_2$ receptor agonists

Currently, no selective $CB_2$ receptor drug has been approved for the treatment of PD. However, several studies have proposed the use of cannabinoids in the treatment of PD (Stampanoni Bassi et al., 2017;

Buhmann et al., 2019). In preclinical studies, different phytocannabinoids has demonstrated potent neuroprotective effect in animal models of PD and other neurodegenerative diseases. Phytocannabinoid $\Delta^9$-tetrahydrocannabivarin ($\Delta^9$-THCV), a potent agonist of $CB_2$ receptor and antagonist of $CB_1$ receptor, is reported to attenuate the loss of TH-containing neurons in the SN caused by 6-OHDA administration (García et al., 2011). Similar effect of $\Delta^9$-THCV is also reported in the PD animal model induce by L-DOPA (Espadas et al., 2020). However, its low BBB-permeability largely limits its application in clinic (Deiana et al., 2012). BCP, a phytocannabinoid and $CB_2$ receptors agonist, is demonstrated to attenuates oxidative stress, neuroinflammation and apoptosis, and produces neuroprotective effects in PD animal models (Javed et al., 2016; al-Taee et al., 2019). Moreover, $\Delta^9$-THC has been shown to reduce agitation in the late stages of AD (Walther et al., 2006). In 2003, the FDA granted a patent for cannabinoids as antioxidants and neuroprotectants, but their clinical application in PD is yet to be determined (Krishnan et al., 2009).

Studies using synthetic cannabinoids recently have brought new exciting news in this research area. Nabilone, a synthetic form of $\Delta^9$-THC, mimicking the structure and pharmacological activities of $\Delta^9$-THC via both $CB_1$ and CB2 receptors. This drug is approved by the U.S. Food and Drug Administration (FDA) for treatment of nausea and vomiting caused by chemotherapy. Recently, 2 clinical trials using Nabilone for the treatment of the non-motor symptoms of PD patients have completed (NCT03769896; NCT03773796). The obtained results indicate that Nabilone is able to produce beneficial effects on sleep disorders associated with PD (Peball et al., 2019, 2020, 2022).

## Conclusion and perspective

As a progressive neurodegenerative disorder, the prevalence of PD significantly increases in the past decades. Meanwhile, the incidence of PD is also demonstrating a trend of early onset at younger ages. Intensive studies unravel multiple theories that contributes to the pathogenesis of PD. However, the fundamental mechanisms are not fully understood. Consequently, the current treatment for PD is most symptomatic. For this reason, identifying effective therapeutic targets for PD is critically important. The discovery of $CB_2$ receptor by Munro in 1993 (Munro et al., 1993) and subsequent evidence of $CB_2$ receptor expression in the brain and neurons of rodents and primates (Zhang et al., 2014; Stempel et al., 2016), as well as alterations in $CB_2$ receptor expression in PD, have led to investigations in this area. $CB_2$ receptor, as an important component of ECS, plays a protective role in various neurodegenerative diseases (Jordan and Xi, 2019). Selective activation of $CB_2$ receptor regulates mitochondrial function, inhibits oxidative stress, suppresses the release of inflammatory factors, and involves in various regulations such as iron transport, electrophysiology, and autophagy. $CB_2$ receptor agonists have emerged as promising neuroprotective drugs with considerable therapeutic potential (Spinelli et al., 2017). However, many questions about $CB_2$ receptor and its function in PD still remain open, which potentially limits the development and application of the $CB_2$ receptor-targeting therapy. First, most of the current research on the neuroprotective effects of $CB_2$ receptor has focused on its anti-inflammatory properties in microglia and astrocytes. As neurons also express $CB_2$ receptor, its function in neuron and association with neurodegeneration warrant further studies. Second, clinical researches intensively focus on the use of phytocannabinoids such as $\Delta^9$-THC in the treatment of neurodegenerative disease such as AD. As those compounds are potent agonist of both $CB_1$ and $CB_2$ receptors, further in-depth clinical research of selective $CB_2$ receptor agonists is necessary to fully understand the therapeutic potential of $CB_2$ receptor in PD. Finally, the function of $CB_2$ receptor in PD is generally believed as neuroprotective and anti-inflammatory, and results little or no adverse CNS effects. However, giving its abundance in the immune system, further investigation of the potential adverse effects of $CB_2$ receptor agonizing is critically important for the clinical application of selective $CB_2$ receptor agonists.

## Author contributions

XY: Writing – original draft. YJ: Writing – original draft. YD: Funding acquisition, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Effects of STN-DBS on cognition and mood in young-onset Parkinson's disease: a two-year follow-up

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**Background:** The effects of subthalamic nucleus deep brain stimulation (STN-DBS) on the cognition and mood of patients with PD are still not uniformly concluded, and young-onset Parkinson's disease (YOPD) is even less explored.

**Objective:** To observe the effectiveness of STN-DBS on the cognition and mood of YOPD patients.

**Methods:** A total of 27 subjects, with a mean age at onset of 39.48 ± 6.24 and age at surgery for STN-DBS of 48.44 ± 4.85, were followed up preoperatively and for 2 years postoperatively. Using the Unified Parkinson disease rating scale (UPDRS), H&Y(Hoehn and Yahr stage), 39-Item Parkinson's Disease Questionnaire (PDQ-39), Mini-mental state examination (MMSE), Montreal Cognitive Assessment (MoCA), Hamilton depression scale (HAMD), Hamilton anxiety scale (HAMA) to assess motor, cognition, and mood.

**Results:** At the 2-year follow-up after STN-DBS, YOPD patients showed significant improvements in motor and quality of life (UPDRS III: $p < 0.001$, PDQ-39: $p < 0.001$); overall cognition was not significantly different from preoperative (MMSE: $p = 0.275$, MoCA: $p = 0.913$), although language function was significantly impaired compared to preoperative (MMSE: $p = 0.004$, MoCA: $p = 0.009$); depression and anxiety symptoms also improved significantly (HAMD: $p < 0.001$, HAMA: $p < 0.001$) and the depression score correlated significantly with motor (preoperative: $r = 0.493$, $p = 0.009$), disease duration (preoperative: $r = 0.519$, $p = 0.006$; postoperative: $r = 0.406$, $p = 0.036$) and H&Y (preoperative: $r = 0.430$, $p = 0.025$; postoperative: $r = 0.387$, $p = 0.046$); total anxiety scores were also significantly correlated with motor (preoperative: $r = 0.553$, $p = 0.003$; postoperative: $r = 0.444$, $p = 0.020$), disease duration (preoperative: $r = 0.417$, $p = 0.031$), PDQ-39 (preoperative: $r = 0.464$, $p = 0.015$) and H&Y (preoperative: $r = 0.440$, $p = 0.022$; postoperative: $r = 0.526$, $p = 0.005$).

**Conclusion:** STN-DBS is a safe and effective treatment for YOPD. The mood improved significantly, and overall cognition was not impaired, were only verbal fluency decreased but did not affect the improvement in quality of life.

KEYWORDS

**Parkinson's disease, deep brain stimulation, cognition, verbal fluency, mood**

## Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects the central nervous system. Its pathogenesis is associated with a variety of pathologies including neuroinflammation due to misfolding of α-synuclein, mitochondrial dysfunction and neurotransmitter-driven alterations in the neural network of the brain (Titova et al., 2017). It is characterized by typical motor symptoms such as tremors, rigidity, bradykinesia, postural gait disturbances, and a range of non-motor symptoms (NMS; Duncan et al., 2014). With the current aging of the population, it is estimated that by 2030, China will account for about 50% of the world's PD patients (Dorsey et al., 2007). Age of onset is highly significant in various neurodegenerative diseases, as it correlates with the disease's clinical phenotype and progression. Parkinson's disease can be divided into two subtypes according to the time of disease onset: young-onset Parkinson's disease (YOPD) and late-onset Parkinson's disease (LOPD). The criteria for classifying patients with YOPD and LOPD are not uniform and are mainly based on age of onset. The maximum age reported for YOPD ranges from 40 to 55 years (Butterfield et al., 1993; Schrag et al., 2003; Mehanna and Jankovic, 2019; Do et al., 2023). Some scholars use 40 years of age as the dividing point, while others refer to patients with onset before 50 years of age as YOPD and those with onset after 50 years of age as LOPD (Mahale et al., 2014; Liu et al., 2015; Mehanna and Jankovic, 2019; Kaiyrzhanov et al., 2021). The mechanisms and manifestations of the two types of Parkinson's disease are not the same. It was found that most patients with PINK1 gene mutations had an earlier age of onset, mostly between 32 and 48 years old (Huang et al., 2023). In addition, YOPD patients taking levodopa had a lower risk of dementia and gait disorders compared with LOPD patients, and there are also differences in the incidence and severity of various NMS (Mehanna and Jankovic, 2019).

As PD continues to be explored, levodopa and other dopaminergic drugs have been widely used. However, as the disease progresses in the mid to late stages, the effectiveness of drug therapy decreases, and long-term high-dose application eventually leads to motor complications and the more effective deep brain stimulation (DBS) has emerged due to this treatment bottleneck (Mohr et al., 2011; Warren Olanow et al., 2013; Asahi et al., 2014). The ventral intermediate nucleus (VIM), the globus pallidus internal (GPI), and the subthalamic nucleus (STN) are the most commonly used clinical targets. STN is the most chosen target in the current DBS treatment of PD because of its ability to control motor symptoms relatively comprehensively (Kleiner-Fisman et al., 2006). The research suggests that patients with STN-DBS have improved motor symptoms in the short and long term (Witt et al., 2008; Fasano et al., 2010; Wu et al., 2014). However, the physiological basis of DBS surgery for Parkinson's disease is not well understood, the degree of improvement in DBS varies between patients, and minimal data are focusing on the role of age at the onset of PD on the outcome of patients treated for STN-DBS. More significant improvements in the motor have been reported in YOPD compared to LOPD with STN-DBS, which may be related to the fact that patients with YOPD have slower disease progression (Kempster et al., 2007; Otaka et al., 2010; Tsai et al., 2013). Based on these results, STN-DBS may be more effective if surgery is performed early in the onset of PD (Merola et al., 2012; Schuepbach et al., 2013).

Although DBS can dramatically improve motor symptoms, its effect on NMS in PD patients has long been overlooked. In addition to motor symptoms, PD patients also suffer from various NMS, such as hyposmia, depression/anxiety, cognitive dysfunction, sleep disturbances, and constipation. Braak proposed that NMS frequently occurs in all stages of PD and is a pre-motor symptom (Braak et al., 2003). NMS has become a severe condition that plagues patients after motor symptoms have been controlled.

Cognitive impairment and altered mood symptoms in NMS are more prevalent in PD, where patients develop cognitive decline in areas such as memory, executive ability, and language, as well as anxiety and depressive symptoms as the disease progresses. There has been increasing interest in the effects of DBS on cognition and mood in PD patients, but the findings remain controversial. Some scholars believe that PD patients have reduced memory and verbal fluency after DBS (Weaver et al., 2009); while another study suggests that patients' memory improves after surgery, with only verbal fluency and executive function declining (Halpern et al., 2009); even as 32% of patients in the STN-DBS surgery group were observed to transform into dementia after 2 years of follow-up in one study (Williams et al., 2011). There are also conflicting studies on mood state, with some reporting that bilateral STN stimulation significantly improves anxiety and that this improvement is more often seen in patients with the more excellent recovery of motor function in response to stimulation (Fabbri et al., 2017). Nevertheless, randomized studies with unilateral STN or GPi-DBS have found that patients' anxiety symptoms were worse at 2, 4, 6, and 12 months postoperatively than at baseline (Okun et al., 2014); other studies have shown no significant Impairment post-DBS (Wang et al., 2016; Sarno et al., 2019). Meanwhile, in various studies on the effect of depression in PD patients, the same differing results of improvement or no influence or even worsening were presented (Deuschl et al., 2006; Mehta and Sethi, 2009; Pariwatcharakul et al., 2013; Chandran et al., 2014).

In conclusion, the results of the studies on NMS, such as the cognition and mood of PD patients with STN-DBS, are still diverse, and most of the subjects in these reports are LOPD, while there are few kinds of research on YOPD. In contrast to LOPD patients with similar disease duration and severity, YOPD patients have greater social and family responsibilities or stresses and can be more concerned about their physical status. There is also growing evidence that increasing the age of onset of PD is associated with low cognition (Levy, 2007; Wickremaratchi et al., 2009). The relationship between the age of onset in depression and anxiety states has been inconsistently shown in various studies (Pagano et al., 2016; Hu et al., 2018; Park et al., 2018). Therefore, using YOPD patients as study subjects is even more crucial. To this end, we conducted this clinical study to observe the postoperative cognitive function, mood, and motor symptoms of YOPD patients who received bilateral STN-DBS to investigate their impact and help doctors make the best clinical decisions to optimize the neuromodulation treatment of PD.

## Materials and methods

### Participants

This study included patients with YOPD who underwent bilateral STN-DBS at the General Hospital of the Chinese People's

Liberation Army from May 2019 to September 2021. The 46 patients with YOPD who entered the initial screening were evaluated with separate scale tests, and 12 were excluded because they did not meet the inclusion criteria or refused to participate in this study. The remaining 34 patients with YOPD underwent STN-DBS, of which 27 completed the 2-year follow-up and were included in the study (Figure 1).There were 9 female cases, overall age of onset of 39.48 ± 6.24 years, duration of disease of 8.96 ± 2.78 years, and 11.70 ± 3.94 years of education (Table 1). The inclusion criteria for having STN-DBS surgery were as follows: fulfilling the diagnostic criteria for Parkinson's disease (Postuma et al., 2015); age < 50 years at onset of PD (YOPD); favorable response to levodopa on the Unified Parkinson's Disease Rating motor assessment (UPDRS III; >30% improvement); no structural lesions on brain magnetic resonance imaging (MRI); and no contraindications to neurosurgery. The local ethics committee approved the study protocol, and eligible patients signed an informed consent form before entering the study.

## Surgical procedure

YOPD Patients were placed in a stereotactic head frame (Leksell Model F head frame) under local anesthesia. Stereotactic magnetic resonance imaging (MRI) was performed with a 3 T scanner (Siemens Espree), scanning to obtain a plane containing the anterior commissure (AC) and posterior commissure (PC). Coronal and sagittal images were taken orthogonal to the axial image. The anatomical target coordinates of the STN were located 5 mm inferior to the midpoint of the AC-PC line, 2 mm posterior, and 12 mm laterally. Targets were adjusted to the center of the STN due to individual differences in STN morphology. The entry point was determined based on MRI images with target coordinates, and intraoperative microelectrode recording was used to place the DBS electrode (Medtronic 3,389 s, Medtronic, or PINS L301, PINS Medical Co.). Awareness of the patients and external stimulators (Programmer 8,840) for testing verbal feedback and physical activity. This was followed by intraoperative MRI (iMRI) to verify the accuracy of electrode placement. If the iMRI showed accurate electrode placement, the implantable pulse generator (IPG; Medtronic Activa RC, Medtronic, or PINS G102RZ, PINS Medical Co.) was placed on the chest; else, the coordinates were adjusted to ensure electrode position. Postoperatively, the MRI and CT scans were performed to revalidate the accuracy of the targets and to exclude the risk of intracranial hematoma (Figure 2). Postoperative stimulation was performed 1–2 weeks after surgery to select the optimal stimulation contacts and parameters to achieve satisfactory patient improvement.

## Assessment methods

The Patients with STN-DBS were assessed for H&Y, quality of life, motor symptoms, cognition, depression, anxiety, daily levodopa equivalent dose (LEDD), and other relevant clinical symptoms 1 week before and 2 years after surgery (Tomlinson et al., 2010). All motor symptoms were evaluated in the medication-on (Med-on) and medication-off (Med-off) status; the preoperative Med-on was the period of optimal symptom control after taking PD medication, and the preoperative Med-off was when PD medication had been stopped for at least 12 h. The postoperative Med-off means that the pulser was working optimally and the medication had been stopped for 12 h or more, while the postoperative Med-on refers to the best working condition of the pulser and the peak performance after medication. A clinical professional assessed the cognitive and mood symptoms during the "on" state of medication.

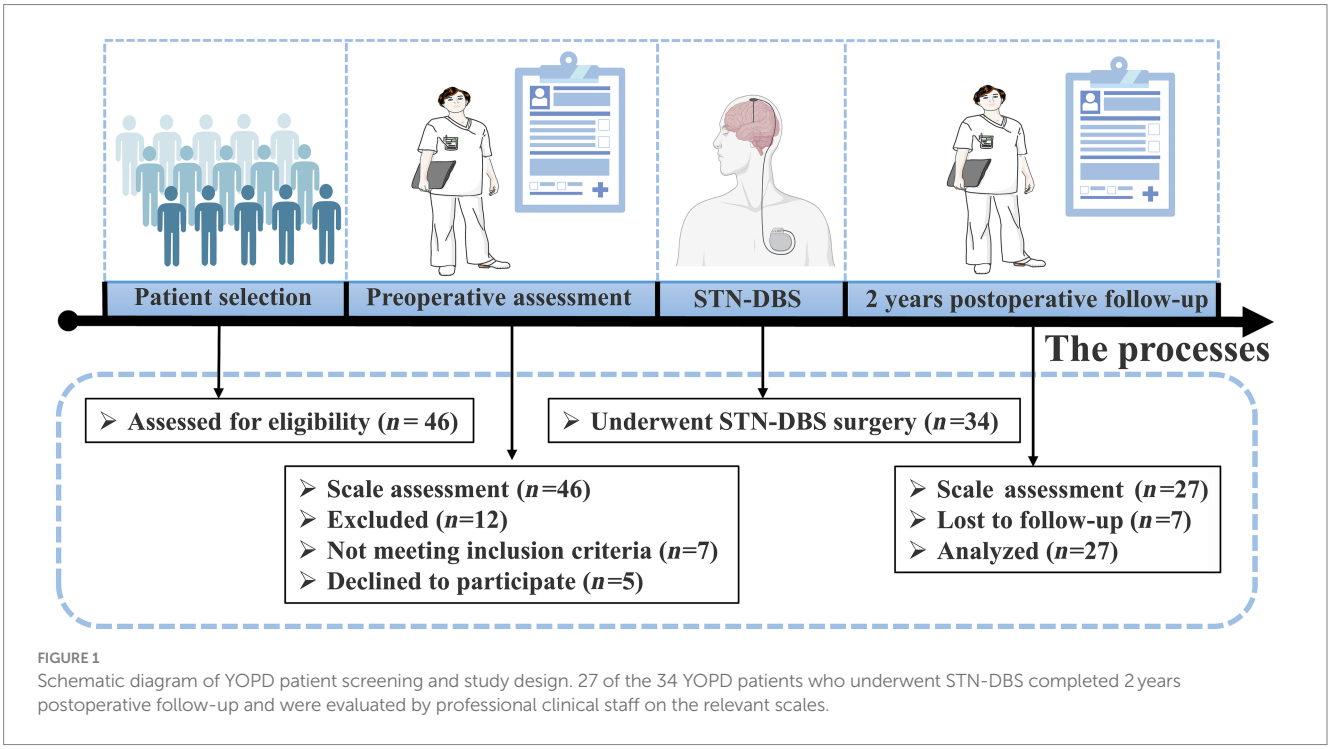


FIGURE 1
Schematic diagram of YOPD patient screening and study design. 27 of the 34 YOPD patients who underwent STN-DBS completed 2 years postoperative follow-up and were evaluated by professional clinical staff on the relevant scales.

Motor function: The Unified Parkinson's Disease Rating Scale (UPDRS) evaluated patients' mental, behavioral, emotional, activities of daily living, motor, and complications, and consists of four subscales: UPDRS I, UPDRS II, UPDRS III, and UPDRS VI. Each item was scored on a five-point scale of 0, 1, 2, 3, and 4, with the higher the score, the more severe the PD symptoms (Martínez-Martín et al., 1994). The UPDRS III primarily evaluated the motor abilities of YOPD patients, and the UPDRS III subscales consisted of bradykinesia, tremor, rigidity, and axial symptoms. The axial subscore was calculated as the sum of speech, gait, postural stability, neck rigidity, posture, and arising from a chair, with the first three items focused mainly on in the study. The H&Y was used to assess the severity of the disease and the scores were also inversely related to the function.

Quality of life: The 39-Item Parkinson's Disease Questionnaire (PDQ-39) was used to assess patients' quality of life in terms of their ability to perform activities of daily living and motor function. Thirty-nine questions were asked on eight dimensions, including mobility, activities of daily living (ADL), emotional well-being, stigma, social support, cognition, communication, and bodily discomfort (Hamilton, 1960; Peto et al., 1995). The scores were inversely related to the quality of life.

Cognition: The patient's cognitive function was initially assessed using the Mini-mental state examination (MMSE), which has five components: directionality, memory, attention and calculation, delayed recall, and language, for a total of 30 points, with the advantage of high specificity (Folstein et al., 1975); the cognitive state of the YOPD patient was further evaluated using the Montreal Cognitive Assessment (MoCA). The MoCA has seven components: Visual space and execution, picture recognition, attention and calculation, language, abstraction, delayed recall, and directionality, for a total of 30 points, and has the strength of sensitivity (Nasreddine et al., 2005). The scores were proportional to symptoms.

Mood state: Hamilton depression scale (HAMD) and Hamilton anxiety scale (HAMA) assess the depression and anxiety states of YOPD patients (Hamilton, 1959). HAMD consists of 24 questions with seven dimensions, and the criteria for determining the results are: <8 scores: no depression; 8–20 scores: possible depression; >20 scores: mild to moderate depression; >35 scores: severe depression. HAMA is mainly divided into two domains of somatic anxiety and mental anxiety, with 14 items, and the criteria are: <7 scores: no anxiety; >7 scores: possible anxiety; >14 scores: definitely anxiety; >21 scores: apparent anxiety; >29 scores: severe anxiety. The scores were inversely related to the quality of life.

TABLE 1 Clinical characteristics of YOPD patients ($n = 27$).

| Demographic and clinical variable | Baseline, Mean ± SD |
|---|---|
| Sex (male/female) | 18/9 |
| Age (years) | 48.44 ± 4.85 |
| Age at PD onset (years) | 39.48 ± 6.24 |
| Duration of the disease (years) | 8.96 ± 2.78 |
| Education (years) | 11.70 ± 3.94 |
| The total score of UPDRS | |
| UPDRS I | 12.33 ± 5.42 |
| UPDRS II | 15.85 ± 5.98 |
| UPDRS III(Med-off) | 52.67 ± 12.17 |
| UPDRS III(Med-on) | 22.89 ± 6.14 |
| UPDRS VI | 8.04 ± 4.00 |
| LEDD (mg/day) | |
| Baseline | 818.19 ± 240.51 |
| Follow-up | 369.48 ± 109.46 |

## Statistical analyses

Statistical analysis was performed using SPSS13.0 and origin2021 for statistical processing and descriptive analysis. Changes in each follow-up indicator before and after STN-DBS surgery were expressed in the form of improvement rate as a percentage, improvement rate (%) = absolute value of (postoperative score − preoperative score) × 100% / preoperative score. Measures that conformed to a normal distribution were analyzed using the paired t-test. In contrast, measures that did not conform to a normal distribution were analyzed using the Wilcoxon non-parametric rank sum test. Correlations were analyzed using Pearson correlation, with $p < 0.05$ indicating a statistically significant difference.

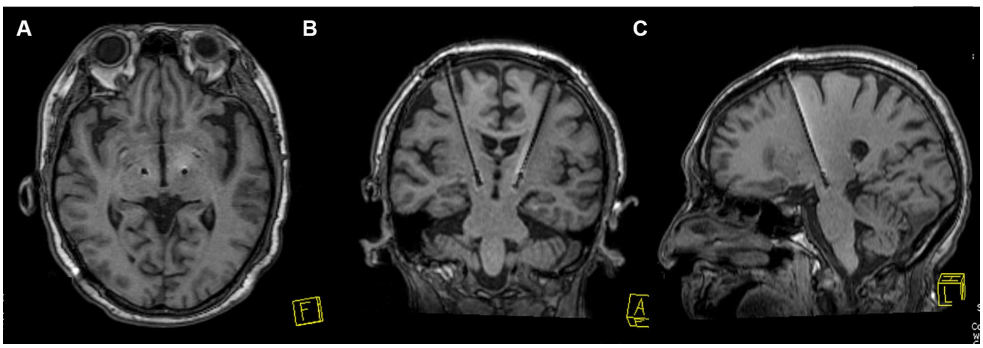


FIGURE 2
STN-DBS electrode position verification image. **(A–C)** An MRI example of electrode placement in the STN is shown to verify the accuracy of electrode placement.

# Results

A total of 27 patients with PD were included in the study, of whom 18 were male, overall mean age of 48.44 ± 4.85 years. As shown in Table 1, the mean preoperative duration of the disease was 8.96 ± 2.78 years, the mean age of onset was 39.48 ± 6.24, and the UPDRS I, UPDRS II, and UPDRS IV scores were 12.33 ± 5.42, 15.85 ± 5.98, and 8.04 ± 4.00. The motor subscale UPDRS III Med-on and off scores were 52.67 ± 12.17 and 22.89 ± 6.14. all patients underwent bilateral STN-DBS surgery, received preoperative levodopa medication, and had >30% effectiveness. Two years after surgery, LEDD decreased from 818.19 ± 240.51 mg preoperatively to 369.48 ± 109.46 mg, a 54.84% reduction ($p < 0.001$).

All data are expressed as means ± standard deviations; YOPD, young onset Parkinson's disease; UPDRS I, Unified Parkinson's Disease Rating Scale part I (non-motor) score; UPDRS II, Unified Parkinson's Disease Rating Scale part II (activities of daily living) score; UPDRS III, Unified Parkinson's Disease Rating Scale part III (motor) score; UPDRS VI, Unified Parkinson's Disease Rating Scale part VI (complications) score; Med-on, evaluation performed under the pharmacological effect of dopaminergic therapies; Med-off, evaluation performed at least 12 h after the last levodopa dose; LEDD, levodopa equivalent daily dose.

## Motor outcome

The 27 YOPD patients with STN-DBS in this study showed significant improvement in H&Y and motor function compared to the corresponding period before surgery, both in the Med-on and Med-off state ($p < 0.001$). Table 2 shows, in the Med-off state, the total UPDRS III scores were 52.67 ± 12.17 after surgery with only STN-DBS treatment, which was reduced significantly compared to the total preoperative scores of 25.11 ± 3.95 ($p < 0.001$), with an improvement rate of 52.32%. Among the subscales, the improvement rates of bradykinesia, tremor, and rigidity were 58.75% ($p < 0.001$), 58.52% ($p < 0.001$), and 44.79% ($p < 0.001$), respectively. The improvement rate of axial was 37.16% ($p < 0.001$), but speech function was significantly impaired ($p = 0.034$). In the Med-on state, the total UPDRS III scores at follow-up were 22.89 ± 6.14 compared to the total preoperative scores of 16.19 ± 3.25 ($p < 0.001$), with the combination of STN-DBS treatment and medication. Rigidity, tremor and bradykinesia all improved more ($p < 0.001$). However, no significantly improved axial symptoms were observed compared to the preoperative Med-on period ($p = 0.061$). In addition, H&Y was greatly reduced in the postoperative Med-off and Med-on states compared to the corresponding preoperative period (2.26 ± 0.32 vs. 3.24 ± 0.76, and 1.83 ± 0.24 vs. 2.28 ± 0.42, $p < 0.001$). The total score revealed that YOPD patients had the best results in the Med-on state 2 years after surgery, suggesting that the combination of DBS and medication is more productive than DBS alone. That postoperative anti-Parkinsonian medication is still needed to achieve the greatest results.

Normal data are expressed as means ± standard deviations; Nonnormal data are expressed as median (lower quartile, upper quartile); YOPD, young onset Parkinson's disease; UPDRS III, Unified Parkinson's Disease Rating Scale part III (motor) score; H&Y, Hoehn and Yahr stage; Med-on, evaluation performed under the pharmacological effect of dopaminergic therapies; Med-off, evaluation performed at least 12 h after the last levodopa dose; A probability value of $p < 0.05$ was considered significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

## Quality of life

The quality of daily life for patients with YOPD was evaluated by the PDQ-39 (Table 3). This study revealed that the total PDQ-39 scores at baseline and 2 years postoperatively were 45.07 ± 14.41 and 27.52 ± 5.86, representing an improvement of 38.94% ($t = 9.128$, $p < 0.001$). The enhancement in the motor ability and ADL components of the PDQ-39 scores directly resulted from the patients' improved motor symptoms postoperatively. The PDQ-39 subscales demonstrated the greatest increase in motor activity ($z = -4.482$, $p < 0.001$), and in the other subscales, ADL ($z = -4.380$, $p < 0.001$), emotional well-being ($z = -4.566$, $p < 0.001$), stigma ($t = 3.770$, $p = 0.001$) and bodily discomfort ($z = -3.501$, $p < 0.001$) scores also all showed significant improvements compared to the preoperative

TABLE 2 YOPD patients' motor scores in Med-off and Med-on conditions before and after bilateral STN stimulation ($n = 27$).

| Motor symptoms | Range | Med-off | | Med-on | | *p* value | |
|---|---|---|---|---|---|---|---|
| | | Baseline | Follow-up | Baseline | Follow-up | Med-off | Med-on |
| UPDRS III | | | | | | | |
| Total | 0–132 | 52.67 ± 12.17 | 25.11 ± 3.95 | 22.89 ± 6.14 | 16.19 ± 3.25 | **<0.001***** | **<0.001***** |
| Tremor | 0–40 | 9.74 ± 4.47 | 4.04 ± 1.51 | 3 (2, 4) | 2 (1, 3) | **<0.001***** | **0.001**** |
| Rigidity | 0–20 | 10.07 ± 2.50 | 5.56 ± 1.76 | 6.30 ± 3.23 | 3.44 ± 1.28 | **<0.001***** | **<0.001***** |
| Bradykinesia | 0–48 | 23.78 ± 7.67 | 9.81 ± 1.84 | 8.93 ± 2.13 | 6.96 ± 1.56 | **<0.001***** | **<0.001***** |
| Total axial | 0–24 | 9.07 ± 3.27 | 5.70 ± 1.86 | 4.52 ± 2.21 | 3.93 ± 1.41 | **<0.001***** | 0.061 |
| Speech | 0–4 | 1 (1, 1) | 1 (1, 2) | 1 (1, 1) | 1 (1, 1) | 0.034* | 0.180 |
| Postural stability | 0–4 | 1 (1, 2) | 1 (0, 2) | 0 (0, 1) | 0 (0, 1) | **<0.001***** | 0.046* |
| Gait | 0–8 | 2(2, 2) | 1 (1, 2) | 1 (0, 2) | 1 (0, 1) | **<0.001***** | **0.033*** |
| **H&Y** | 0–5 | 3.24 ± 0.76 | 2.26 ± 0.32 | 2.28 ± 0.42 | 1.83 ± 0.24 | **<0.001***** | **<0.001***** |

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 3 Quality of life, cognitive and mood scores of YOPD patients before and after bilateral STN stimulation ($n = 27$).

| | Range | Baseline | Follow-up | t/z value | *p* value |
|---|---|---|---|---|---|
| PDQ-39 | | | | | |
| Total score | 0–156 | 45.07 ± 14.41 | 27.52 ± 5.86 | 9.128 | **<0.001***** |
| Mobility | 0–40 | 9 (5, 15) | 4 (3, 6) | −4.482 | **<0.001***** |
| Activities of daily living | 0–24 | 8 (5, 9) | 4 (3, 6) | −4.380 | **<0.001***** |
| Emotional well-being | 0–24 | 6 (5, 9) | 3 (2, 5) | −4.566 | **<0.001***** |
| Stigma | 0–16 | 6.67 ± 2.40 | 4.81 ± 1.59 | 3.770 | **0.001**** |
| Social support | 0–12 | 1 (1, 2) | 1 (1, 2) | −1.732 | 0.083 |
| Cognition | 0–16 | 3.96 ± 1.26 | 3.52 ± 0.98 | 1.894 | 0.069 |
| Communication | 0–12 | 2.78 ± 1.05 | 2.63 ± 0.88 | 0.811 | 0.425 |
| Bodily discomfort | 0–12 | 3(2, 5) | 2 (2, 3) | −3.501 | **<0.001***** |
| MMSE | | | | | |
| Total score | 0–30 | 28.11 ± 1.78 | 27.81 ± 1.55 | 1.114 | 0.275 |
| Directionality | 0–10 | 10.00 ± 0.00 | 9.93 ± 0.27 | 1.442 | 0.161 |
| Memory | 0–3 | 2.85 ± 0.36 | 2.93 ± 0.27 | −1.442 | 0.161 |
| Attention and calculation | 0–5 | 4.19 ± 1.00 | 4.30 ± 0.82 | −0.769 | 0.449 |
| Delayed recall | 0–3 | 2.56 ± 0.64 | 2.63 ± 0.56 | −0.527 | 0.602 |
| Language | 0–9 | 8.52 ± 0.64 | 8.04 ± 0.71 | 3.118 | **0.004**** |
| MOCA | | | | | |
| Total score | 0–30 | 25.59 ± 2.66 | 25.55 ± 1.97 | 0.110 | 0.913 |
| Visual space and execution | 0–5 | 4.19 ± 0.79 | 4.26 ± 0.71 | −1.000 | 0.327 |
| Picture recognition | 0–3 | 2.78 ± 0.51 | 2.70 ± 0.61 | 1.442 | 0.161 |
| Attention | 0–6 | 5.37 ± 0.79 | 5.33 ± 0.73 | 0.254 | 0.802 |
| Language | 0–3 | 2.19 ± 0.56 | 1.89 ± 0.58 | 2.842 | **0.009**** |
| Abstraction | 0–2 | 1.85 ± 0.36 | 1.81 ± 0.40 | 0.570 | 0.574 |
| Delayed recall | 0–5 | 3.37 ± 0.84 | 3.63 ± 0.84 | −1.763 | 0.090 |
| Directionality | 0–6 | 5.85 ± 0.36 | 5.93 ± 0.27 | −0.811 | 0.425 |
| HAMD | | | | | |
| Total score | 0–96 | 15.07 ± 3.57 | 9.41 ± 1.31 | 8.909 | **<0.001***** |
| Anxiety/somatization | 0–24 | 3 (3, 5) | 2 (2, 4) | −2.535 | **0.011*** |
| Weight | 0–4 | 0 (0, 1) | 0 (0, 0) | −1.069 | 0.285 |
| Cognitive disturbance | 0–24 | 1 (0, 2) | 0 (0, 1) | −3.562 | **<0.001***** |
| Diurnal variation | 0–4 | 1 (1, 1) | 0 (0, 1) | −2.714 | **0.007**** |
| Retardation | 0–16 | 3 (2, 4) | 2 (1, 2) | −4.409 | **<0.001***** |
| Sleep disturbance | 0–12 | 3 (2, 4) | 2 (2, 3) | −3.158 | **0.002**** |
| Hopelessness | 0–12 | 3 (2, 4) | 2 (1, 3) | −3.934 | **<0.001***** |
| HAMA | | | | | |
| Total score | 0–56 | 10.89 ± 2.49 | 6.74 ± 2.23 | 6.056 | **<0.001***** |
| Somatic anxiety | 0–28 | 4.19 ± 1.27 | 2.59 ± 0.84 | 8.522 | **<0.001***** |
| Psychic anxiety | 0–28 | 7 (4, 9) | 4 (3, 5) | −3.926 | **0.001**** |

Normal data are expressed as means ± standard deviations; Nonnormal data are expressed as median (lower quartile, upper quartile); YOPD, young onset Parkinson's disease; PDQ-39, 39-Item Parkinson's Disease Questionnaire; MMSE, Mini-mental state examination; MoCA, Montreal Cognitive Assessment; HAMD, Hamilton depression scale; HAMA, Hamilton anxiety scale; A probability value of $p < 0.05$ was considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

period. There was, however, a downward trend in social support ($z = -1.732$, $p = 0.083$), cognition ($t = 1.894$, $p = 0.069$), and communication ($t = 0.811$, $p = 0.425$) at the 2-year postoperative follow-up, but it was not statistically significant.

## Cognitive function

Using the MoCA and MMSE scales to assess the cognitive state of YOPD patients before and after surgery (Table 3), the total MMSE

scores were 28.11 ± 1.78 and 27.81 ± 1.55 at baseline and follow-up ($t = 1.114$, $p = 0.275$). In the MMSE scores for each cognitive dimension, YOPD patients' directionality ($t = 1.442$, $p = 0.161$), memory ($t = -1.442$, $p = 0.161$), attention and calculation ($t = -0.769$, $p = 0.449$) and delayed recall ($t = -0.527$, $p = 0.602$) were not compared to preoperative statistically significant, with only language showed a significant decrease ($t = 3.118$, $p = 0.004$). 12 of the 27 patients in this group (44%) received an impact on language, mainly in language fluency. Again a significant decline in all cognitive domains of MoCa was found only in language function in comparison to preoperative ($t = 2.842$, $p = 0.009$), with 9 patients (33%) having lower scores postoperatively. Outcomes for overall cognition were consistent with the MMSE, with total MoCA scores of 25.59 ± 2.66 and 25.55 ± 1.97 pre- and postoperatively, with no significant differences found ($t = 0.110$, $p = 0.913$). Also Visual space and execution ability ($t = -1.000$, $p = 0.327$), Picture recognition ($t = 1.442$, $p = 0.161$), attention ($t = 0.254$, $p = 0.802$), abstraction ($t = 0.570$, $p = 0.574$), delayed recall ($t = -1.763$, $p = 0.090$), Directionality ($t = -0.811$, $p = 0.425$) scores also all did not change meaningfully.

## Mood state

As shown in Table 3, the outcomes of the HAMD scores for most dimensions before and after surgery were dramatically reduced for anxiety/somatization ($z = -2.535$, $p = 0.011$), cognitive disturbance ($z = -3.562$, $p < 0.001$), diurnal variation ($z = -2.714$, $p = 0.007$), retardation ($z = -4.409$, $p < 0.001$), Sleep disturbance ($z = -3.158$, $p = 0.002$), and Hopelessness ($z = -3.934$, $p < 0.001$). The total HAMD score was 15.07 ± 3.57 vs. 9.41 ± 1.31 ($t = 8.909$, $p < 0.001$) and an improved rate of 37.56% overall. 3 of the 27 surgical patients had a mild to moderate depressive state (score > 20), and the remainder were possibly depressed (score: 8–20). Postoperatively, most patients showed a significant reduction in symptoms. The HAMA results reveal an effective postoperative anxiety improvement of 38.11% in total ($t = 6.056$, $p < 0.001$), from 10.89 ± 2.49 preoperatively to 6.74 ± 2.23. Both subscales of somatic anxiety ($t = 8.522$, $p < 0.001$) and Psychic anxiety ($z = -3.926$, $p = 0.001$) were also significantly decreased. At baseline, 2 of 27 patients was determined to have anxiety symptoms (score > 14), and 25 were possible anxiety (score > 7). The number of patients with postoperative anxiety was reduced to 13, and the other 14 had no anxiety symptoms (score < 7). Compared with the baseline, the HAMA and HAMD scores of the YOPD patients in this group decreased significantly during the follow-up, and the mood disorders were relieved.

## Overall outcome and correlation analysis

YOPD patients showed significant increases in motor or mood ($p < 0.001$) and further improvements in quality of life after STN-DBS, except for no significant changes in overall cognition, which confirmed the effectiveness and safety of STN-DBS for YOPD patients (Figure 3A). Pearson correlation analysis further explored the correlation between cognitive ability or mood and various other variables at different time points (Figures 3B,C; Supplementary Table 1). Preoperative depressive symptoms were significantly correlated with the disease duration ($r = 0.519$, $p = 0.006$), motor ability ($r = 0.493$, $p = 0.009$) and H&Y ($r = 0.430$, $p = 0.025$), and postoperatively also correlated with disease duration ($r = 0.406$, $p = 0.036$) and H&Y ($r = 0.387$, $p = 0.046$). Preoperative anxiety symptoms were significantly correlated with the disease duration ($r = 0.417$, $p = 0.031$), motor ability ($r = 0.553$, $p = 0.003$), H&Y ($r = 0.440$, $p = 0.022$) and PDQ-39 ($r = 0.464$, $p = 0.015$), and improvement in postoperative anxiety was significantly correlated with improved motility ($r = 0.444$, $p = 0.020$) and H&Y ($r = 0.526$, $p = 0.005$). Nevertheless, no correlation was seen between postoperative total cognition and other variables ($p > 0.05$).

# Discussion

DBS is rapidly evolving; among them, bilateral STN-DBS has become the primary choice for PD. However, several studies have not reached consistent conclusions regarding the postoperative effects of NMS. Many patients, especially YOPD, are concerned about the adverse impact on other NMS while improving motor symptoms. Thus, this study investigated the effects of DBS on NMS with a high prevalence of YOPD patients, such as cognition, anxiety, and depression.

This study is consistent with previous studies regarding improving motor and quality of life (Fasano et al., 2010; Lahtinen et al., 2020; Bove et al., 2021; Bezdicek et al., 2022; Golfrè Andreasi et al., 2022; Zeng et al., 2023). It is shown that postoperative Med-off treatment with only STN-DBS improved PD motor compared with preoperative Med-off status. All motor scores improved 2 years after surgery (tremor 58.52%, rigidity 44.79%, bradykinesia 58.75%, and axial symptoms 37.16%), demonstrating that DBS alone can significantly enhance various motor symptoms in patients with YOPD, and the effect is sustained. However, STN-DBS has a limited effect on the axial of YOPD patients. A further 5-year long-term follow-up study showed worse scores for axial than preoperatively, which may also be related to progressive disease progression (Fasano et al., 2010). The rate of improved postoperative Med-on status in YOPD patients was significantly higher than at baseline Med-off, suggesting that the combination of DBS and drugs maximized efficacy and significantly reduced postoperative medication doses. Significant progress in overall PDQ-39 scores, consistently with other studies (Lee et al., 2006; Büttner et al., 2019; Schuepbach et al., 2019; Chen et al., 2023). Changes in PDQ-39 were significantly associated with improvements in motor function, further substantiating the effectiveness of STN-DBS and laying the foundation for slowing NMS in YOPD patients.

Regarding cognition, some studies have argued that long-term DBS treatment may cause abnormal cognitive function (Aybek et al., 2007; Williams et al., 2011; David et al., 2020; Pal et al., 2023). Nevertheless, studies with up to 2 years of follow-up did not find any cognitive changes in patients (Georgiev et al., 2021). Several studies have shown that cognition, especially verbal fluency, is degraded in PD patients after DBS, which may be related to the different criteria for the inclusion of patients in each center, the different cognitive rating scales used, and the progression of PD itself (Hogg et al., 2017; Gratwicke et al., 2018; Kawaguchi et al., 2020). This study indicates that 2 years after STN-DBS in YOPD patients, both MoCA and MMSE total scores were not statistically significant compared to preoperative, there were no significant changes in each cognitive subscale, and only language function was impaired, again mainly in the form of a

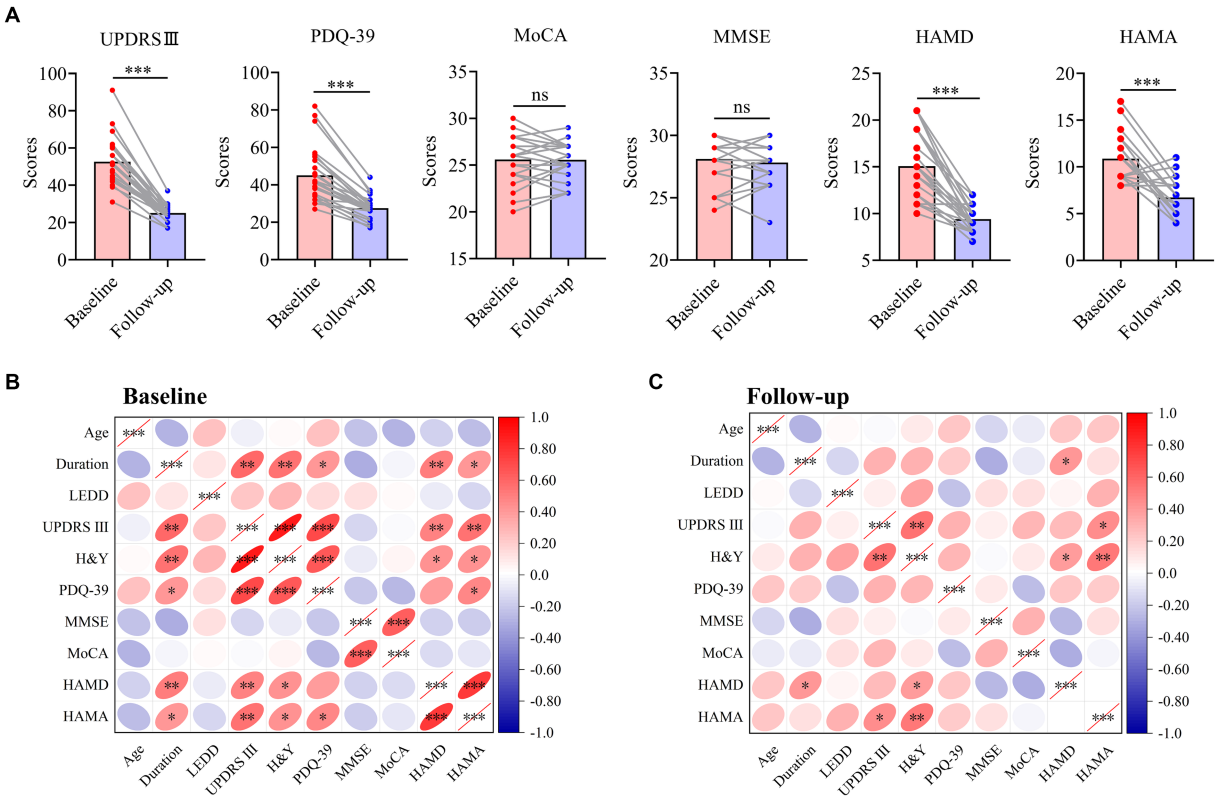


FIGURE 3
Overall outcome and correlation analysis of patients with YOPD. **(A)** The change of clinical outcomes in patients with YOPD at baseline and 2-year follow-up. The pink indicates each clinical symptom score for YOPD patients at baseline; The blue indicates each clinical symptom score for YOPD patients at 2-year follow-up. **(B,C)** Heat map of the correlation matrix between clinical variables at baseline and 2-year follow-up. YOPD, young onset Parkinson's disease; UPDRS III, Unified Parkinson's Disease Rating Scale part III (motor) score; PDQ-39, 39-Item Parkinson's Disease Questionnaire; H&Y, Hoehn and Yahr stage; LEDD, levodopa equivalent daily dose; MMSE, Mini-mental state examination; MoCA, Montreal Cognitive Assessment; HAMD, Hamilton depression scale; HAMA, Hamilton anxiety scale; A probability value of $p < 0.05$ was considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: not significant.

significant lowering of verbal fluency, consistent with the relevant papers (Duncan et al., 2014b; Demeter et al., 2017; Tröster et al., 2017; Hyder et al., 2021; John et al., 2021). However, no improvements in Visual space function and attention reported in other studies were found. In contrast, the present study found no significant reduction in total cognitive performance in patients before and after DBS. The reasons could be: (1) this study is a two-year follow-up study and lacks longer-term follow-up data; (2) Differences in the type of anesthesia taken during surgery in different patients between the studies may have an impact on the postoperative outcome, leading to inconsistent results (Brodsky et al., 2017; Blasberg et al., 2018; Jiang et al., 2021). (3) Patients with YOPD are younger, and it has been reported that intracranial tau protein levels are lower in YOPD patients than in LOPD. The increase in tau protein is associated with disrupted neural network connections in the brain and progressive degeneration of the substantial nigra (Gomperts et al., 2016). (4) And there exists a significantly lower level of Aβ42 in the cerebrospinal fluid of PD patients compared with the average population, which leads to the aggravation of intracranial amyloid plaque deposition and becomes a strong predictor for the assessment of cognitive impairment in PD patients (Alwardat et al., 2019). It can be speculated that the level of Aβ42 is further decreased in LOPD patients compared with YOPD patients. This may be one of the reasons for the absence of significant overall cognitive impairment in YOPD after surgery in this study. (5) The intervention of DBS may affect the corticobasal ganglia loop and alter the output from the basal ganglia to the frontal lobe. This mechanism is mainly associated with impaired verbal fluency(Manes et al., 2014). Our study showed that some patients had language fluency Impact after surgery. However, it did not affect the total cognitive level, so STN-DBS is generally safe for YOPD, and that mild language fluency decline is not a contraindication to STN-DBS surgery.

Depression and anxiety are common in patients with PD, and the factors associated with their occurrence are equally controversial. Several studies have identified neurotransmitter abnormalities associated with developing anxiety and depression in PD, such as decreased dopamine levels and abnormal secretion of neurotransmitters, including adrenaline and 5-hydroxytryptamine (Remy et al., 2005; Prediger et al., 2012). Neurofunctional imaging studies also suggest that PD and anxiety and depression may share the same impaired chemical pathways, so the role of DBS in the brain's neural network cannot be ignored (Black et al., 2005; Wen et al., 2016; Carey et al., 2021). The effect of STN-DBS on mood in our study may also be related to the neural network system. However, the results of the studies on the impact of STN-DBS on the mood of PD patients are

diverse, with some studies reporting improvement in motor function as well as anxiety and depression in PD patients after surgery (Lieberman, 2006; Couto et al., 2014; Fabbri et al., 2017; Chuquilín-Arista et al., 2020; Santos-García et al., 2020; Cartmill et al., 2021). Another part of the study showed no change or a significant decrease in the mood at different times after surgery (Soulas et al., 2008; Chang et al., 2012; Seritan et al., 2021). The results of this study showed that 2 years after STN-DBS, the improvement rates of anxiety and depression in YOPD patients were 38.11 and 37.56%. The dimensions of anxiety/somatization, cognitive disturbance, diurnal variation, retardation, sleep disturbance, and hopelessness in HAMD were statistically distinct, and postoperative HAMA indicated that both somatic and psychic anxiety significantly progressed more than those before surgery. Analysis of the reasons: (1) It could be related to bilateral STN-DBS stimulation that alters brain structure and affects patients' moods. The electrode contacts of STN-DBS can directly inhibit the limbic subregion of STN and indirectly affect the corticobasal ganglia limbic loop, and pulse stimulation of this loop can mediate mood responses and thus control the mood behavior of PD patients (Soulas et al., 2008). (2) STN-DBS may also have the effect of reducing mood disorder by improving the metabolism of neuronal cells and regulating the relevant transmitters that trigger depression and anxiety or regulating mood in PD patients by affecting other monoaminergic neural pathways, such as the serotonin-containing nucleus of the middle suture and the norepinephrine-containing nucleus of the blue spot (Gallagher and Schrag, 2012; Etiévant et al., 2015). (3) The correlation analysis implies that there was a correlation between the relief of depression and anxiety with the improved ability in patients' daily life and motor, indicating that the enhancement of motor function and quality of life had an impact on the mood of YOPD. STN-DBS influences movement disorders by inhibiting the frontal lobe and further strengthens mood and neurological function (Castrioto et al., 2014; Combs et al., 2015).

## Limitations

There are some limitations in this study: (1) the follow-up period is short, only observed the changes of cognitive function, depression and anxiety after 2 years of bilateral STN-DBS treatment, and the patient population is YOPD, younger age, the potential impact produced may be reduced due to the younger age. Therefore, follow-up should be continued to observe the long-term effects of bilateral STN-DBS treatment on each clinical symptom in patients with YOPD. (2) The sample size of this study was limited, which may limit the accuracy of the statistical analysis. Further expansion of the sample size is needed to reduce the bias caused by insufficient sample size. (3) Scale tests are highly subjective, and more detailed cognitive neuropsychological tests should be used in addition to the MMSE and MoCA, and it is also recommended to incorporate objective assessment criteria, such as MR imaging, to help assess changes in brain structure and function before and after STN-DBS surgery. (4) More randomized controlled studies and further research to confirm the association between stimulation sites and neuropsychiatric disorders are needed in the future to help clinicians choose the best stimulation targets to guide better clinical decisions.

## Conclusion

In conclusion, STN-DBS for YOPD is a safe, minimally invasive, and effective treatment. This study revealed that YOPD patients had significantly lower postoperative levels of depression and anxiety, and that this improvement was in part associated with better motor and quality of life after STN-DBS. Regarding cognition, our results showed that STN-DBS causes cognitive decline in verbal fluency for YOPD patients. However, the MMSE and MoCA total scores indicated no significant impairment in overall cognitive level and did not affect improvement in quality of life. STN-DBS is a promising treatment modality for YOPD, and studying the effects of STN-DBS on the cognition and mood of YOPD will require larger sample sizes and longer-term randomized controlled trials at a later stage to select more accurate stimulation targets for DBS.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving humans were approved by Ethics Committee of the General Hospital of the Chinese People's Liberation Army. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

## Author contributions

JH and ZM contributed to conceptualization. JH and DL contributed to data collection and verification. HX contributed to the methodology. JH, HX, YC, TW, and ZM contributed to the data analysis. JH contributed to writing the original draft. ZM and KX contributed to writing, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1177889/full#supplementary-material

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# Fish oil supplementation, physical activity and risk of incident Parkinson's disease: results of longitudinal analysis from the UK Biobank

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**Objective:** Evidence on the individual and combined relationship of physical activity (PA) and fish oil supplement use on the incidence of Parkinson's disease (PD) risk remains lacking.

**Materials and methods:** This UK population-based prospective cohort study, involving 385,275 UK Biobank participants, collected PA and fish oil supplement data via touchscreen questionnaires. Using Cox proportional hazards models and restricted cubic splines to examined the associations between use of fish oil supplements, PA and PD risk.

**Results:** During a median 12.52-year follow-up, 2,131 participants incident PD. Analysis showed that fish oil supplement users had a lower PD risk [hazard ratio (HR), 0.89; 95% confidence interval (CI), 0.82–0.98]. The adjusted HRs for the PD incidence were 0.96 (95% CI, 0.95–0.98) for total PA; 0.93 (95% CI, 0.90–0.96) for moderate PA; 0.95 (95% CI, 0.91–0.99) for vigorous PA and 0.93 (95% CI, 0.89–0.98) for walking activity. Significant interactions were found between fish oil supplement use and total PA (*P* for interaction = 0.011), moderate PA (*P* for interaction = 0.015), and walking activity (*P* for interaction = 0.029) in relation to PD incidence.

**Conclusion:** Both fish oil supplement use and PA were associated with a reduced risk of PD, and the effect of PA in reducing the risk of PD was more pronounced when fish oil supplement was used.

KEYWORDS

**physical activity, fish oil, Parkinson's disease, genetic predisposition, UK Biobank**

# 1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder in worldwide (Dorsey et al., 2007). It can cause a wide range of motor and non-motor symptoms, including resting tremors, muscle rigidity, slow movement, postural instability, autonomic dysfunction, cognitive impairment, and mood disorders (Cerri et al., 2019; Tolosa et al., 2021). Since 1990, PD has been the fastest-growing neurological disease in terms of prevalence, disability, and mortality worldwide (GBD 2016 Parkinson's Disease Collaborators, 2018). Considering the disease burden of PD, identifying its modifiable environmental risk factors and developing preventive interventions are important public health issues (Tysnes and Storstein, 2017; Aaseth et al., 2018; Reichmann et al., 2022).

Fish oil mainly contains the long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Liu et al., 2022). These nutrients have a wide range of biological activities related to cardiovascular health, including lowering blood pressure and triglycerides and improving endothelial vasodilator function. Several prospective observational studies have shown an association between diets rich in n-3 PUFAs and a lower risk of PD (Avallone et al., 2019). Omega-3 fatty acids are also found to be beneficial in alleviating PD symptoms in a randomized clinical trial (RCT) (Taghizadeh et al., 2017). However, in a case–control study based on Japanese population showed that consumption of n-3 PUFAs were not significantly associated with PD risk (Miyake et al., 2010). To provide further evidence, this study explored the association between fish oil supplements and PD risk based on UK Biobank.

Physical activity (PA) has been suggested as a potentially effective means to reduce PD morbidity (Ascherio and Schwarzschild, 2016; LaHue et al., 2016; Reichmann et al., 2022). Epidemiological evidence suggested that higher levels of moderate to vigorous activity associated with a lower risk of PD (Fang et al., 2018). Besides, aerobic exercise has been found to reduce motor and non-motor symptoms in PD patients in some RCTs (van der Kolk et al., 2019; Johansson et al., 2022).

Although a large number of studies have shown that PA is recommended as a robust preventive factor for the development of PD. However, evidence for the protective effect of low-intensity PA and the amount of PA at different intensities is still limited. Considering that vigorous PA may not be feasible for some specific groups, such as morbidly obese or elderly people, and prolonged exercise may not be scientific. It is important to provide information on associations between the amount of PA and the risk of PD for different types of PA. Therefore, the present study explored the association between amount of sum PA and the risk of PD and further explored the association between amount of PA and PD risk for different types of PA (vigorous, moderate, and walking).

Previous studies have not examined the effect of the interaction between fish oil supplementation use and PA on the risk of PD. Dietary intake of fish oil has multiple potential biological mechanisms associated with increased muscle protein synthesis and improved muscle mass, which is strongly associated with loss of physical function (Smith et al., 2011; Rodacki et al., 2012). Therefore, we hypothesized that the effect of PA on the onset of PD would be modified by the intake of fish oil supplementation. Specifically, combining PA with fish oil supplementation may enhance the effect on biological pathways and thus have a greater impact on physical function. In addition, considering potential sex-specific biological responses to PA (Yang et al., 2015; Da Boit et al., 2017), it would be of interest to explore sex differences in the combined effects of fish oil supplementation and PA on the risk of PD. Moreover, as evidence suggested genetic risk may modify the effect environmental factors on PD (Jacobs et al., 2020; Bloem et al., 2021), there is a need to explore whether the relationship between fish oil, PA and PD risk is altered by differential genetic risk.

The aim of this study was to investigate the independent as well as combined effects of fish oil supplementation use and PA on the risk of PD. In addition, we further explored whether there were differences in the associations of fish oil supplement use and PA with PD risk in different sex.

# 2 Methods

## 2.1 Study population

UK Biobank is a large prospective study with 502,387 participants designed to collect detailed information on a wide range of phenotypes through questionnaires, physical measurements, sample testing, accelerometry, multimodal imaging, and long-term follow-up of a range of health-related outcomes. After excluding those with baseline PD ($n = 900$), 501,487 participants remained. We further excluded individuals with missing data for age, sex, race, education, and Townsend deprivation index (TDI), smoking, drinking, and body mass index (BMI), PA, and fish oil supplements use. Ultimately, 385,275 participants were included in this study. The detailed screening process is presented in Supplementary Figure S1.

The North West Multi-Centre Research Ethics Committee approved the UK Biobank study, and all participants provided written informed consent to participate. The study protocol is publicly available on the UK Biobank website.[1]

## 2.2 Parkinson's disease diagnosis

As recommended by the UK Biobank [Algorithmically-defined outcomes (ADOs) Version 2.0, 2022], algorithmically-defined outcomes were used to identify PD onset in the cohort's participants. PD definition is shown in detail in Supplementary Table S1. The disease information was obtained from inpatient electronic health records and death registers, which are linked to the Hospital Episode Statistics for England, Scottish Morbidity Record, or Patient Episode Database for Wales. The censoring dates for these databases were 31 October 2022, 31 July 2021, and 28 February 2018, respectively. The follow-up time was calculated from baseline to the time of PD

Abbreviations: PA, Physical activity; PD, Parkinson's disease; BMI, Body mass index; TDI, Townsend deprivation index; MET, Metabolic equivalent task; CVD, Cardiovascular diseases; PRS, Polygenic risk score.

1 http://www.ukbiobank.ac.uk/

diagnosis, death, loss to follow-up, or censorship, whichever occurred first.

## 2.3 Assessment of fish oil supplement use

Information about fish oil supplement use was collected through a touch screen questionnaire in which participants were asked, "Do you regularly take any of the following supplements?" Various supplements, including fish oil, were listed in this question for the participants to mark the relevant ones. Data on fish oil supplement use collected at baseline were used for analysis.

## 2.4 Assessment of physical activity

Data on the type (walking, moderate, or vigorous), frequency and duration of PA were obtained from the completed touch screen questionnaires. Furthermore, the metabolic equivalent task (MET) scores were calculated based on the International Physical Activity Questionnaire guidelines. The MET score has been described in detail elsewhere [Guidelines for data processing and analysis of the international physical activity questionnaire (IPAQ)—short and long forms, 2005]. In this study, we assessed the PA types (moderate, vigorous, and walking) using MET minutes per week (MET-minutes/week). The sum of the MET-minutes/week for all three PAs was also calculated (total PA).

## 2.5 Other measurements

Other assessed variables included age (continuous), sex (male or female), ethnicity (White or non-White), TDI, BMI, smoking status (on most or all days, only occasionally, or never), drinking status (current, previous, or never), disease status, and dietary composition. The TDI was used to identify the socio-economic status. It measures regional deprivation derived from national census data on unemployment, car ownership, household overcrowding, and owner's occupation. Higher scores indicate higher levels of deprivation (Townsend, 1987). BMI was calculated by dividing the weight (kg) by the height squared ($m^2$). History of diabetes was defined based on self-reported diabetes at baseline, having been diagnosed with diabetes by a physician, or taking medications to treat diabetes. Cardiovascular diseases (CVD) were defined based on self-reported hypertension, heart problems, cerebrovascular disease, peripheral vascular disease, or other cardiovascular-related diseases. Dietary data were obtained from the touch screen food frequency questionnaire. As in previous studies (Wang et al., 2022), we coded the frequency of various food intakes into scores: never = 0, less than once a week = 0.5, once a week = 1, 2–4 times a week = 3, 5–6 times a week = 5.5, and once or more daily = 7. We then calculated the frequency scores for vegetables, fruit, fish, unprocessed red meat, and processed meat by grouping and summarizing the scores obtained from the above coding rules for each food item.

## 2.6 Polygenic risk score

The polygenic risk score (PRS) shows the correlation between genotype and risk of Parkinson's disease through a score format. In the present study, we applied the standard PRS for Parkinson's disease released from the UK Biobank (Field ID: 26260), the calculation of which has been specifically described in the study by Thompson et al. (2022). The study by Thompson et al. calculated polygenic risk scores for 28 diseases and 25 quantitative traits. Standard PRS were generated from an external GWAS meta-analysis dataset and the RPS algorithm was built from trait-specific meta-analyses using Bayesian approach. The PRS value for each individual was calculated as the genome-wide sum of the per-variant posterior effect size multiplied by allele dosage. For the generated raw PRS, centering and standardization steps were followed to generate a corrected PRS for subsequent analysis.

## 2.7 Statistical analysis

Descriptive statistics are expressed as means [standard deviations (SDs)] for continuous variables and numbers (percentages) for categorical variables. Baseline characteristics of participants with and without PD onset during follow-up were compared by analysis of variance (ANOVA) for normally distributed continuous variables, Mann–Whitney U test for non-normally distributed continuous variables, and chi-squared tests for categorical variables.

First, we used the Cox proportional hazards regression model to assess the relationship between PA and PD incidence. Specifically, we analyzed the association between individual PA types (moderate, vigorous, walking, and total) and PD incidence. When the PA types were analyzed as continuous variables, the results are expressed as the association between change per 1,000 MET-minutes/week and PD incidence. We further divided the PAs into four subgroups based on the quartiles to analyze them as categorical variables, using the lowest quartile subgroup as a reference. Second, we used the Cox proportional hazards regression model to analyze the association between fish oil supplement use and PD incidence. Finally, in search of an interaction between fish oil supplement use and PA in affecting PD incidence, we stratified the participants according to their fish oil supplement use and explored the effect of the various PA levels on PD progression. We used likelihood ratio tests to assess for interaction when PA was considered a categorical variable. When PA was considered a continuous variable, we included a cross-product term for PA and fish oil supplement use in the model to test the interaction. Besides, we used restricted cubic spline to further observe the dose-dependent relationship between PA and PD incidence in participants with different fish oil supplement use status. In this study, model 1 was adjusted for age and sex; model 2 was further adjusted for race, TDI, BMI, smoking status, drinking status, CVD, diabetes, and fish, vegetable, fruit, unprocessed red meat, and processed meat intake frequency scores. Model 2 was further adjusted for fish oil supplement use when analyzing the association between PA and PD progression. Model 2 was further adjusted for total PA when analyzing the association between fish oil supplement use and PD progression. The results are expressed as hazard ratios (HRs) and their 95% confidence intervals (CIs).

In addition, we conducted a series of additional analyses. First, considering the possible sex variability of the above associations, we further explored the associations of fish oil supplement use and PA with PD risk in different sex populations. Second, to test whether the effect of fish oil or physical activity on the occurrence of PD differed across population with different PD genetic risk, we examining the

interaction effect of genetic risk and fish oil supplement use or physical activity on the risk of PD separately. Populations with high PD genetic risk, Intermediate PD genetic risk and low PD genetic risk were classified by the PD-PRS tertiles. Finally, we performed a sensitivity analysis by excluding participants who developed Parkinson's disease in the 2 years prior to study follow-up in order to exclude participants whose occurrence of PD might not be related to PA and fish oil supplements use.

Statistical analysis was performed with R software, Version 4.2.1. Two-sided $p < 0.05$ was considered statistically significant.

# 3 Results

## 3.1 Baseline characteristics

The cohort baseline characteristics were presented in Supplementary Table S2. Participants incident PD during the follow up were more likely to be older, male, white, with higher BMI, and a history of CVD and diabetes ($p < 0.001$), compared with participants no incident PD.

## 3.2 Association between fish oil supplement use or physical activity and Parkinson's disease development

During a median follow-up of 12.52 years, 2,131 participants developed PD. The association between PA or fish oil supplement use and PD incidence was presented in Tables 1, 2. We found that after adjusting for a range of variables, participants using fish oil supplements had a lower risk of developing PD than non-users (HR, 0.89; 95% CI, 0.82–0.98). For each 1,000 MET-minute/week increase, the adjusted HRs for PD occurrence were 0.96 (95% CI, 0.95–0.98) for total PA, 0.93 (95% CI, 0.90–0.96) for moderate PA, 0.95 (95% CI, 0.91–0.99) for vigorous PA, and 0.93 (95% CI, 0.89–0.98) for walking. When the amounts of PA were analyzed as categorical variables, participants with highest PA level (Q4) showed a significantly lower risk of PD occurrence than those with lowest PA level (Q1). The trend analysis showed that the associations between the activity level for all the three types of PA, as well as total PA level and the risk of developing PD showed significant negative linear trends ($P$ for trend $< 0.001$).

## 3.3 Joint effect of fish oil supplement use and physical activity on Parkinson's disease incidence

The association between PA and the occurrence of PD after stratification by fish oil supplement use was shown in Table 3. When analyzing the PA levels as categorical variables, a significant interaction was noted between total or moderate PA levels and fish oil supplement use ($P$ for interaction <0.05). The HR of the highest total PA level (Q4), when compared to the lowest PA level (Q1), was 0.62 (95% CI, 0.50–0.76) in the fish oil supplement-using population and 0.84 (95% CI, 0.72–0.97) in the non-users. The HR of the highest moderate PA level (Q4), when compared to the lowest PA level (Q1), was 0.59 (95% CI, 0.48–0.73) for the fish oil supplement-using population and 0.86 (95% CI, 0.74–0.99) for non-users. When PA levels was analyzed as a continuous variable, we found a significant interaction between fish oil supplement use and total PA ($P$ for interaction = 0.011), moderate PA ($P$ for interaction = 0.015), and walking activity ($P$ for interaction = 0.029) in their effect on PD incidence. This finding was reflected in the more significant reduction in PD risk in fish oil supplement users than in non-users with increasing of total, moderate, and walking PA levels.

Restricted cubic splines (Figure 1) examined the dose–response associations between the PA levels and PD occurrence in populations with different fish oil supplement use statuses. We found no significant association between levels of various types of PA and PD risk in the group not using fish oil supplements ($P$ for overall > 0.05). In those using fish oil supplements, for total PA ($P$ for overall < 0.001), and walking ($P$ for overall = 0.004), the risk of PD continued to decrease with increasing PA levels. For vigorous PA, we found a significant negative association between lower vigorous PA levels and PD occurrence in fish oil supplement users. However, this association reversed to a positive correlation and lost statistical significance above a certain PA level ($P$ for overall = 0.007, $P$ for nonlinear = 0.041). In the population using fish oil supplements, moderate PA levels were found to be "U-shaped" associated with the risk of PD ($P$ for overall < 0.001, $P$ for nonlinear = 0.002).

## 3.4 Sex differences in the joint effect of fish oil supplement use and physical activity on Parkinson's disease

Baseline characteristics for males and females were detailed in Supplementary Tables S3, S4. Supplementary Table S5 shows the association between PA and PD occurrence after stratification by fish oil supplement use in males. We found a significant interaction between the total, walking, and moderate PA levels and fish oil supplement use when PA level was analysed as categorical variables ($P$ for interaction < 0.05). The HR for the highest total PA level (Q4), when compared to the lowest level (Q1), was 0.55 (95% CI, 0.42–0.71) in fish oil supplement users and 0.84 (95% CI, 0.69–1.01) in non-users. The HR for the highest moderate PA level (Q4), when compared to the lowest level (Q1), was 0.51 (95% CI, 0.39–0.66) for fish oil supplement users and 0.82 (95% CI, 0.68–0.99) for non-users. The HR for the highest walking PA level (Q4), when compared to the lowest level (Q1), was 0.56 (95% CI, 0.42–0.75) for fish oil supplement users and 0.92 (95% CI, 0.75–1.13) for non-users. When the PA level was analyzed as a continuous variable, we also found a significant interaction between fish oil supplement use and total PA ($P$ for interaction = 0.0039), moderate PA (for interaction $p = 0.0028$), and walking activity (for interaction $p = 0.0342$) in their effect on the incidence of PD.

Supplementary Table S6 shows the association between PA and PD occurrence after stratification by fish oil supplement use in females. No interaction was found between categorical total, moderate, vigorous, and walking PA levels and fish oil supplement use ($P$ for interaction >0.05).

Restricted cubic splines examined the dose–response relationship between PA levels and PD incidence in males and females with various fish oil supplement use statuses (Supplementary Figure S2). The dose–response relationship between the PA level and PD incidence in males

TABLE 1 Associations of physical activity with incident PD ($n$ = 385,275).

| | Number | Model 1 | | Model 2 | |
|---|---|---|---|---|---|
| | | HR (95%CI) | $p$-value | HR (95%CI) | $p$-value |
| Physical activity (sum) | | | | | |
| Continues[a] | | 0.96(0.94,0.98) | **<0.001** | 0.96(0.95,0.98) | **<0.001** |
| Q1 (0,810) | 96,534 | Ref | | Ref | |
| Q2 (810,1773) | 96,449 | 0.89(0.80,1.00) | 0.056 | 0.91(0.81,1.02) | 0.116 |
| Q3 (1773,3,546) | 96,094 | 0.77(0.68,0.87) | **<0.001** | 0.79(0.70,0.90) | **<0.001** |
| Q4 (3,546,19,278) | 96,198 | 0.73(0.65,0.82) | **<0.001** | 0.75(0.67,0.85) | **<0.001** |
| P-trend | | | **<0.001** | | **<0.001** |
| Physical activity (moderate) | | | | | |
| Continues[a] | | 0.92(0.89,0.95) | **<0.001** | 0.93(0.90,0.96) | **<0.001** |
| Q1 (0,120) | 102,703 | Ref | | Ref | |
| Q2 (120,480) | 106,907 | 0.85(0.76,0.95) | **0.005** | 0.87(0.78,0.98) | **0.018** |
| Q3 (480,1,200) | 84,736 | 0.74(0.66,0.84) | **<0.001** | 0.76(0.67,0.87) | **<0.001** |
| Q4 (1,200,5,040) | 90,929 | 0.73(0.65,0.82) | **<0.001** | 0.75(0.67,0.85) | **<0.001** |
| P-trend | | | **<0.001** | | **<0.001** |
| Physical activity (vigorous) | | | | | |
| Continues[a] | | 0.94(0.91,0.98) | **0.003** | 0.95(0.91,0.99) | **0.01** |
| Q1 (0,0) | 155,914 | Ref | | Ref | |
| Q2 (0,240) | 53,537 | 0.94(0.83,1.07) | 0.383 | 0.98(0.86,1.12) | 0.793 |
| Q3 (240,960) | 96,116 | 0.93(0.83,1.03) | 0.167 | 0.96(0.86,1.08) | 0.513 |
| Q4 (960,10,080) | 79,708 | 0.77(0.68,0.87) | **<0.001** | 0.80(0.71,0.91) | **<0.001** |
| P-trend | | | **<0.001** | | **<0.001** |
| Physical activity (walking) | | | | | |
| Continues[a] | | 0.93(0.89,0.97) | **<0.001** | 0.93(0.89,0.98) | **0.002** |
| Q1 (0,297) | 97,582 | Ref | | Ref | |
| Q2 (297,693) | 125,460 | 0.96(0.86,1.07) | 0.459 | 0.97(0.87,1.08) | 0.594 |
| Q3 (693,1,386) | 88,853 | 0.91(0.80,1.02) | 0.111 | 0.92(0.81,1.04) | 0.184 |
| Q4 (1,386,4,158) | 73,380 | 0.79(0.69,0.91) | **<0.001** | 0.81(0.71,0.93) | **0.002** |
| P-trend | | | **<0.001** | | **<0.001** |

Model 1: Adjusted for age, sex. Model 2: Adjusted for age, sex, race, Townsend deprivation index, BMI, smoke, alcohol, cardiovascular disease, diabetes, fish, fruit, vegetable, processed meat, unprocessed meat, fish oil supplement.
[a]Continuous expressed as change per 1,000 units.
Bold represents $p$-value <0.05.

TABLE 2 Associations of fish oil supplementation use with incident PD ($n$ = 385,275).

| | Number | Model 1 | | Model 2 | |
|---|---|---|---|---|---|
| | | HR (95%CI) | $p$-value | HR (95%CI) | $p$-value |
| Fish oil supplement use | | | | | |
| No | 263,747 | Ref | | Ref | |
| Yes | 121,528 | 0.88(0.80,0.96) | **0.004** | 0.89(0.82,0.98) | **0.016** |

Model 1: Adjusted for age, sex. Model 2: Adjusted for age, sex, race, Townsend deprivation index, BMI, smoke, alcohol, cardiovascular disease, diabetes, fish, fruit, vegetable, processed meat, unprocessed meat, sum of PA. Bold represents $p$-value <0.05.

with various fish oil supplement statuses resembled that of the total population. In contrast, in the female population, no significant associations were observed for PA levels and PD risk in either those using or not using fish oil supplements ($P$ for overall >0.05).

## 3.5 Association of fish oil supplementation use or PA with PD risk in participants with different genetic susceptibilities for PD

In this analysis, we did not find a significant interaction between fish oil supplementation use and genetic risk on PD risk (for interaction $p$ = 0.2701) (Supplementary Table S7). Also, we did not find a significant interaction between PA and genetic risk on PD risk (for interaction $p$ > 0.05) (Supplementary Table S8).

## 3.6 Sensitivity analysis

We further analyzed the cohort after excluding participants who developed PD within 2 years of study. The combined effect of fish oil supplement use and PA on the risk of PD, both in the total population

TABLE 3 Joint associations of fish oil supplementation and PA with incident PD ($n$ = 385,275).

| | Category | | | | | Continues | |
|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | *P* for interaction | Per1000 Met increase | *P* for interaction |
| Physical activity (sum) | | | | | | | |
| Using fish oil supplement | | | | | | | |
| Yes | Ref | 0.83(0.68,1.01) 0.059 | **0.63(0.51,0.78) < 0.001** | **0.62(0.50,0.76) < 0.001** | **0.0275** | **0.93(0.90,0.96) < 0.001** | **0.0105** |
| No | Ref | 0.95(0.82,1.10) 0.523 | 0.89(0.77,1.04) 0.132 | **0.84(0.72,0.97) 0.021** | | **0.98(0.96,1.00) 0.044** | |
| Physical activity (walking) | | | | | | | |
| Using fish oil supplement | | | | | | | |
| Yes | Ref | 0.87(0.72,1.05) 0.134 | 0.85(0.69,1.04) 0.116 | **0.64(0.50,0.81) < 0.001** | 0.0683 | **0.88(0.81,0.95) < 0.001** | **0.0289** |
| No | Ref | 1.03(0.90,1.18) 0.683 | 0.96(0.82,1.12) 0.579 | 0.91(0.77,1.08) 0.293 | | 0.97(0.92,1.02) 0.183 | |
| Physical activity (moderate) | | | | | | | |
| Using fish oil supplement | | | | | | | |
| Yes | Ref | 0.83(0.68,1.01) 0.058 | **0.63(0.51,0.78) < 0.001** | **0.59(0.48,0.73) < 0.001** | **0.0089** | **0.87(0.82,0.93) < 0.001** | **0.0151** |
| No | Ref | 0.88(0.77,1.02) 0.096 | **0.84(0.72,0.99) 0.032** | **0.86(0.74,0.99) 0.039** | | 0.96(0.92,1.00) 0.066 | |
| Physical activity (vigorous) | | | | | | | |
| Using fish oil supplement | | | | | | | |
| Yes | Ref | 1.01(0.86,1.19) 0.916 | 1.04(0.91,1.19) 0.539 | **0.85(0.73,0.99) 0.041** | 0.3278 | **0.92(0.86,0.99) 0.017** | 0.2631 |
| No | Ref | 0.93(0.75,1.16) 0.541 | 0.84(0.70,1.01) 0.060 | **0.72(0.59,0.89) 0.002** | | 0.97(0.92,1.01) 0.165 | |

Model: Adjusted for age, sex, race, Townsend deprivation index, BMI, smoke, alcohol, cardiovascular disease, diabetes, fish, fruit, vegetable, processed meat, unprocessed meat. Bold represents $p$-value <0.05.

and in the male as well as female populations, was mostly consistent with the results observed in the entire cohort (Supplementary Tables S9–S11).

## 4 Discussion

This prospective cohort study of 385,275 participants in the United Kingdom found that fish oil supplement use and all PA intensities (total, moderate, vigorous, and walking) were associated with a lower risk of developing PD. Notably, we found a significant interaction between PA levels and fish oil supplement use, with fish oil supplement use enhancing the effect of PA on PD risk. This effect was more pronounced in males than females. In addition, based on the interaction test, we observed that PD genetic risk did not affect the effect of fish oil supplementation use or PA on PD incidence.

Consistent with previous studies (Abbott et al., 2003; de Lau et al., 2005; Gao et al., 2007; Denny Joseph and Muralidhara, 2015; Hernando et al., 2019), our study found a similarly strong association between fish oil supplement use and a reduced risk of PD. Fish oil supplements, particularly omega-3 polyunsaturated fatty acids, are essential lipid nutrients in the human diet and play a key role in cell membrane structure. Omega-3 fatty acids have been shown to inhibit microglial cell activity and neuroinflammation, protect astrocyte function, and produce neurotrophic factors that improve neurodegeneration and normalize neurotransmission (McCarty and Lerner, 2020). Recent studies have also shown that omega-3 fatty acids improve PD by inhibiting pro-inflammatory cytokine release, restoring mitochondrial function and membrane fluidity, and reducing levels of oxidant production (Wu et al., 2021). The omega-3 fatty acid docosahexaenoic acid increases dopamine synthesis in striatal motor areas by phosphorylating the restrictive catecholamine synthase tyrosine hydroxylase in a manner dependent on second messenger-linked protein kinases (PKA and PKC), thus preventing deficits in postural stability, gait integrity, and dopamine neurochemistry (Chitre et al., 2020).

PA protection against PD was first suggested in 1992 (Sasco et al., 1992). Those authors found that increasing levels of PA were associated with a progressively lower risk of PD. Since that initial report, several subsequent epidemiological studies have confirmed this putative relationship (Kyrozis et al., 2013). Our findings are consistent with theirs. A meta-analysis of over half a million adults showed that a higher PA level—particularly moderate to vigorous PA—was associated with a lower risk of PD (Fang et al., 2018). While the importance of moderate to vigorous PA in reducing the risk of PD is widely recognized, little is known about its impact on walking activity.

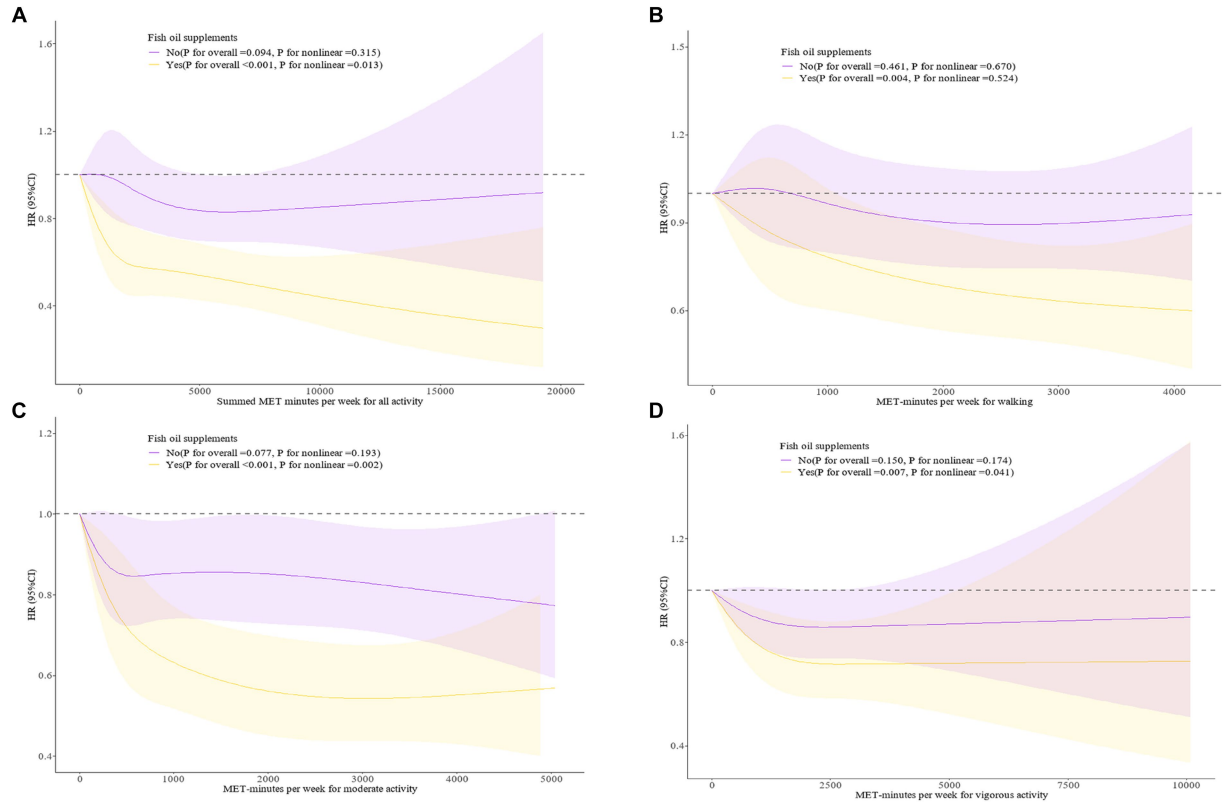


FIGURE 1
**(A)** Dose–response associations between Summed MET-minutes per week for all activity and fish oil supplement with PD incidence. **(B)** Dose–response associations between MET minutes per week for walking and fish oil supplement with PD incidence. **(C)** Dose–response associations between MET minutes per week for moderate activity and fish oil supplement with PD incidence. **(D)** Dose–response associations between MET minutes per week for vigorous activity and fish oil supplement with PD incidence. The analysis was performed after adjusting for age, sex, race, Townsend deprivation index, BMI, smoke, alcohol, cardiovascular disease, diabetes, fish, fruit, vegetable, processed meat, unprocessed meat. 95%CI, 95% confidence interval; HR, hazard ratio; MET, metabolic equivalent task.

To our knowledge, this was the first study to directly support the protective effect of walking activity on PD development in the general population. In addition, the results of the Cox proportional hazards model revealed that the risk of PD was significantly lower in the group with moderate physical activity above the lowest quartile (120 MET-min/week) compared to the group with moderate physical activity below the lowest quartile. Therefore, adults with limited self-directed time can benefit from a protective effect by choosing moderate exercise at a reduced duration. Several mechanisms have been proposed to explain the neuroprotective effects of PA. For example, PA has been shown to upregulate the production of various growth factors and receptors, maintain dopaminergic function, and reduce cellular inflammation and oxidative stress in animal models of PD (LaHue et al., 2016). Moreover, PA reduces damage to dopaminergic neurons in motor circuits, preserves striatal dopamine levels after treadmill activity in a rodent model of lesion-induced PD, and increases the loss of dopaminergic neurons after forced non-use of the contralateral forelimb (Tillerson et al., 2002).

This study was the first to prospectively and systematically examine the joint association of fish oil supplement use and PA with PD incidence. By using a longitudinal design, fish oil supplement use and PA measurements, and a large sample, this study provided direct and strong evidence of this association. Our findings showed a significant interaction between PA and fish oil supplement use, suggesting that fish oil supplement use improves the protection against and prevention of PD incidence by PA. Increasing the PA level resulted in a significantly lower PD incidence in fish oil supplement users than non-users. At certain activity levels, a significant association between PA and PD was observed only in those using fish oil supplements. These results emphasize the importance of promoting public health strategies of any PA intensity to combat the risk of PD. Given the high prevalence of PD, interventions that include adequate fish oil supplement intake should be particularly emphasized and encouraged for physically inactive people. Protection can also be achieved by consuming fish oil supplements and performing low-intensity or low-frequency PA. Those tolerating high-intensity or high-frequency exercise can be protected even more by consuming fish oil supplements. In summary, the current study highlights the potential benefits of combining fish oil supplement use and PA in PD prevention.

Interestingly, we also found that the combined association of fish oil supplement use and PA with PD incidence differed among the sexes. Our findings showed that males increased the protective effect of PA on PD incidence by consuming fish oil supplements, while females did not. Previous studies have shown a significant association between PA and reduced PD incidence in males but not females (Llamas-Velasco et al., 2021). Furthermore, sex also affects the

responsiveness to omega-3 fatty acid supplementation, with higher increases in plasma docosahexaenoic acid and lower triglycerides in males than females (Caslake et al., 2008; Danthiir et al., 2018). A meta-analysis showed that lower total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels were associated with PD development (Hong et al., 2022). Maybe it would help to suggest why it happens in males and not females. Our study had multiple strengths. The combined relationship between fish oil supplements and PA and PD onset has been inadequately studied. Our study provided new insights into this joint relationship. The study was based on a prospective cohort from the UK Biobank. This cohort is well-suited for studying exposure-disease relationships because of its large size, long follow-up, detailed and comprehensive information.

Our study had several limitations that should be acknowledged. First, although the questionnaire is widely used to quantify PA, it is a self-report measure that might be subject to reporting bias. Second, the dietary information in the UK Biobank study is self-reported, limited in scope, and might not provide a thorough picture of the overall healthy eating behavior. Third, despite controlling for multiple covariates, residual confounders cannot be completely excluded. Fourth, this study was conducted based on UK Biobank, the main participants of which were from a white ethnic background, so further research is still needed to validate the applicability of the findings of this study to other ethnic populations. Finally, although UK Biobank recruited a sample over 500,000, it actually had a low response rate (5.5%) and there may have been selection bias (Allen et al., 2012). However, by comparison with other studies, risk factor associations in UK Biobank seem to be generalizable (Batty et al., 2020). Future studies, e.g., clinical trials, are needed to confirm and determine the causal relationship of the associations observed in our study.

## 5 Conclusion

In this UK population-based study, we found that fish oil supplementation and PA reduce the incidence of PD, irrespective of genetic risk. Besides, fish oil supplement use additionally improved the protective effect of PA against PD incidence.

## Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: the study protocol is publicly available on the UK Biobank website (http://www.ukbiobank.ac.uk/).

## Ethics statement

The studies involving humans were approved by the North West Multi-Centre Research Ethics Committee approved the UK Biobank study, and all participants provided written informed consent to participate. The study protocol is publicly available on the UK Biobank website (http://www.ukbiobank.ac.uk/). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

FL: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Visualization. YS: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. JZ: Formal analysis, Methodology, Writing – original draft. YL: Data curation, Investigation, Writing – original draft. XuaC: Data curation, Software, Writing – original draft. XZ: Writing – review & editing. YH: Conceptualization, Writing – original draft. KC: Writing – review & editing. YZ: Writing – review & editing. QY: Writing – review & editing. XiaC: Writing – review & editing, Supervision. XinC: Writing – review & editing, Supervision. YW: Writing – review & editing, Supervision. GC: Writing – review & editing, Supervision, Funding acquisition, Resources.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1304629/full#supplementary-material

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# The effects of transcranial magnetic stimulation for freezing of gait in Parkinson's disease: a systematic review and meta-analysis of randomized controlled trials

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**Background:** Freezing of gait (FOG) is one of the most disabling gait disturbances in Parkinson's disease (PD), affecting mobility and balance severely, thereby leading to an increased risk of falls.

**Objectives:** The purpose of this systematic review and meta-analysis was to investigate the effects of transcranial magnetic stimulation on FOG in PD.

**Methods:** Based on PRISMA guidelines, we searched the databases of MEDLINE (PubMed), Cochrane Library, PEDro, Embase, and Web of Science. Studies of the English language published up to July 2023 were searched. We retrieved for studies of randomized controlled trials (RCTs) of transcranial magnetic stimulation to treat FOG after PD and screened by inclusion and exclusion criteria. Risk of bias was assessed using the Cochrane Collaboration's tool (Revman5.30). Characteristics of RCTs were extracted. The heterogeneity of the trials was measured by $I^2$ statistic. The effect size was expressed by a standardized mean difference (SMD) with a 95% confidence interval (CI).

**Results:** A total of 488 articles were screened, after screening sixteen RCTs involved in 408 patients were included in the qualitative analysis, and 15 RCTs were included in meta-analysis. The outcome measures included FOG-Q, walking time, TUG, and UPDRS. Six studies used FOG-Q as outcome measure, six studies used walking time, four studies used TUG, and six studies used UPDRS. Compared with placebo treatment, transcranial magnetic stimulation has positive significant effects in improving gait status with increased walking speed (SMD = −0.41, 95% CI = −0.75 to −0.06, $I^2$ = 7% $p$ = 0.02), FOG-Q scores (SMD = −0.55, 95% CI = −0.89 to −0.21, $I^2$ = 29%, $p$ = 0.002), UPDRS scores (SMD = −1.08, 95% CI = −1.39 to −0.78, $I^2$ = 49%, $P$ < 0.001) and the time of TUG (SMD = −0.56, 95% CI = −0.88 to −0.23, $I^2$ = 25%, $p$ = 0.02) decreased.

**Conclusion:** Transcranial magnetic stimulation could significantly improving gait conditions in PD patients with FOG.

**Systematic review registration:** https://www.crd.york.ac.uk/PROSPERO/#recordDetails, CRD42023434286.

KEYWORDS

transcranial magnetic stimulation, freezing of gait, Parkinson's disease, meta-analysis, TMS

# Introduction

Parkinson's disease (PD) is a progressive neurodegenerative illness of the central nervous system that usually affects middle-aged and elderly people (Frisardi et al., 2016), and is reported to increase in prevalence with ages (Hirsch et al., 2016). The main neural mechanism of PD is decreased in dopamine levels in the basal nucleus, namely the dorsal striatum. Epidemiological studies have shown that the prevalence of PD is 1 to 2% in persons 65 years of age or older (Weintraub et al., 2008), 4% in persons 80–89 years of age (Noyes et al., 2006). Parkinson's disease is clinically characterized by non-motor symptoms such as mood and affective disorders and sleep disorders, as well as motor symptoms such as resting tremor, bradykinesia, rigidity, postural instability and gait disturbances (Bloem et al., 2021). Freezing of gait (FOG) is one of the most disabling gait impairments in PD. A study included 990 patients with PD presented that the incidence of FOG was 32% (Giladi et al., 1992). FOG is an episodic phenomenon defined as a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk (Nutt et al., 2011). FOG usually occurs in situations where the gait is erratic, such as when turning a corner or going through a narrow passageway (Snijders et al., 2008). FOG seriously affects mobility, leads to increased risk of fall (Latt et al., 2009; Kerr et al., 2010) and poor quality of life (Moore et al., 2007; Rahman et al., 2008).

The most common treatment for motor symptoms in PD is dopamine-based pharmacologic treatments, and other treatments such as deep brain stimulation (Xie et al., 2017) and magnetic resonance imaging-guided focused ultrasound (Xu et al., 2021) have also been used. In addition, a variety of exercise interventions may improve motor symptoms to varying degrees (Gilat et al., 2021). The efficacy of pharmacologic treatments decreases over time, and adverse effects become apparent and other treatments' therapeutic effect is limited to some extent. The treatment of FOG is difficult, and despite the optimal pharmacologic and nonpharmacologic interventions are used, the majority of patients will still develop FOG (Bloem et al., 2015).

Transcranial magnetic stimulation (TMS) is a valuable non-invasive neuromodulation technique for modulating brain activity in a specific, distributed, cortico-subcortical network (Fregni and Pascual-Leone, 2007). High frequency TMS (≥5 Hz) could enhance motor cortex excitability (Gilio et al., 2002), whereas low frequency TMS (≤1 Hz) could downregulate cortical excitability (Chen et al., 1997). In recent years, TMS has been shown to be as a potential treatment for improving motor signs in PD (Elahi et al., 2009; Chou et al., 2015; Zhu et al., 2015; Chung and Mak, 2016). Some previous studies have demonstrated that TMS has a beneficial effect on FOG in PD (Xie et al., 2020; Deng et al., 2022). However, the studies referenced in the previous systematic reviews included crossover studies in addition to randomized control studies (RCTs), and the limited number of RCTs failed to provide sufficient evidence.

Therefore, we aimed to conduct a systematic review and meta-analysis of the RCTs assessing the efficacy of TMS on FOG in PD to offer an evidence-based basis for clinical treatment. Previous meta-analyses included studies that were not all RCTs; in recent years, studies have been updated, and our systematic review will only include all RCTs to improve the quality of evidence from our study.

# Materials and methods

## Protocol and registration

This systematic review was designed and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guideline (Page et al., 2021). The study has been registered with Prospero (registration number: CRD42023434286).

## Search strategy

Five large databases which included MEDLINE (PubMed), Cochrane Library, PEDro, Embase, and Web of Science were searched from inception through July 2023. In the process of searching for studies, we only considered studies in English. The studies were retrieved by using the keywords "Parkinson's disease" OR "disease of Parkinson" OR "Parkinson disorders" AND "freezing of gait" OR "gait disturbances" OR "gait" AND "transcranial magnetic stimulation" OR "TMS." Furthermore, we also manually retrieved for studies that appeared in other systematic reviews that might be related to our study.

## Eligibility criteria

The studies were included if they met the PICOS criteria as follows: (1) population (P): all of patients were adults older than 18 years diagnosed with freezing of gait in PD; (2) intervention (I): TMS; (3) control (C): placebo stimulation or no intervention was considered as the control; (4) outcomes (O): freezing of gait questionnaire (FOG-Q) as primary outcome and walking time, Unified Parkinson's Disease Rating Scale (UPDRS) and Time up and Go (TUG) as secondary outcomes; and (5) study design (S): RCTs.

The exclusion criteria were as follows: (1) duplicate studies, (2) case-controlled trials, (3) full article was not available, and (4) fail to extract the valid outcome data.

## Study selection and data acquisition

Firstly, the retrieved studies were imported into the EndnoteX20 document management system, and repeated records were deleted.

Secondly, two reviewers (LZC and WX) independently screened the title and abstract of the identified studies and excluded those that were not relevant. The full texts of the potentially relevant studies were further reviewed strictly according to the predesigned eligibility inclusion. Afterwards, we confirmed the final included studies after reviewing the full text. The inconsistencies of study selection were settled by discussion with another reviewer (LHY).

Two investigators (LZC and XXY) independently extracted the following information from each included study: subject characteristics, treatment methods, outcome measures, treatment duration, main parameters of TMS using a standardized extraction form. Discrepancies of data extraction were resolved by discussion with another researcher (TC).

## Quality assessment

The quality of the included randomized controlled studies was assessed by two authors (WX and LYY) independently using the Cochrane Collaboration's tool (Revman5.30) (Jørgensen et al., 2016). Risk bias assessment contains seven aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Disagreements were resolved by intragroup discussion or with another experienced researcher (LHY).

## Statistical analysis

All statistical analyses were performed using the Review Manager (RevMan 5.3) software. A Chi square test evaluated the statistical significance of heterogeneity. The $I^2$ was reported as a measure of heterogeneity, $I^2 > 50\%$ was interpreted as substantial heterogeneity, the random-effect model was applied to describe the center of the distribution of intervention effects. For quantitative synthesis, pooled-effect estimates were obtained by comparing the changes from baseline to the post-intervention across groups or directly comparing the post-intervention scores of each group. The effect size was expressed by a standardized mean difference (SMD) with a 95% confidence interval (CI).

# Results

## Literature search

We initially searched 3,030 records from five electronic databases. After deleting duplicates, screening the titles and abstracts, 36 records remained for further assessment. After reviewing the full text, we excluded 16 articles for the following reasons: and full-text not available ($n = 6$) and non-RCTs ($n = 10$). Eventually, 16 RCTs were included in the qualitative analysis and 15 RCTs in meta-analysis. Figure 1 summarized the inclusion process.

## Study characteristics

Sixteen RCTs with a total of 408 patients were included in our systematic review. The included studies were published between 2003 and 2021, and the sample size of the included studies ranged from 13 to 50. The average age of the patients ranged from 54.3 to 71.6, and the average duration of disease ranged from 3.5 to 13.8 years. A study (Benninger et al., 2011) used intermittent theta-burst stimulation (iTBS), a study used deep TMS, and the remaining studies used conventional rTMS as the intervention. The treatment time of TMS ranged from 1 session to 24 sessions. The outcome measures included FOG-Q, walking time, TUG, and UPDRS. Six studies (Benninger et al., 2012; El-Tamawy et al., 2013; Lee et al., 2014; Ma et al., 2019; Mi et al., 2019; Lench et al., 2021) used FOG-Q as outcome measure, six (Khedr et al., 2003, 2006; Lomarev et al., 2006; del Olmo et al., 2007; Benninger et al., 2011, 2012) studies used walking time, four studies (Yang et al., 2013; Lee et al., 2014; Cohen et al., 2018; Lench et al., 2021) used TUG, and six studies used UPDRS (Khedr et al., 2003; Arias et al., 2010; Benninger et al., 2012; Chung et al., 2020; Li et al., 2020; Lench et al., 2021). The detailed characteristics of the included studies were summarized in Tables 1, 2 (Khedr et al., 2003, 2006; Lomarev et al., 2006; del Olmo et al., 2007; Benninger et al., 2011, 2012; El-Tamawy et al., 2013; Yang et al., 2013; Lee et al., 2014; Jørgensen et al., 2016; Cohen et al., 2018; Ma et al., 2019; Mi et al., 2019; Li et al., 2020; Lench et al., 2021; Page et al., 2021).

## Result of quality assessment

The methodological quality and bias risk assessment according to the Cochrane risk of bias tool (Revman5.30) for included study were presented in Figures 2, 3. In general, the risk of bias for the included studies was relatively low.

## Results of analysis

The meta-analysis results showed that the FOG-Q scores for the rTMS group were better than those for the control group (fixed effects model, SMD = −0.55, 95% CI = −0.89 to −0.21, $I^2 = 29\%$, $p = 0.002$, Figure 4). Besides, our study found that real rTMS treatment had a significantly beneficial effects on accelerating walking speed (expressed in walking time) compared to placebo-controlled treatment (fixed effects model, SMD = −0.41, 95% CI = −0.75 to −0.06, $p = 0.02$) with low heterogeneity ($I^2 = 7\%$, Figure 5). Four studies evaluated TUG (expressed in walking time) and were included in the quantitative analysis. rTMS had a significantly beneficial effect on the mean changes in the difference from baseline to post-intervention compared with sham stimulation (fixed effects model, SMD = −0.56, 95% CI = −0.88 to −0.23, $I^2 = 25\%$, $p = 0.02$, Figure 6). In addition, Figure 7 represented the post-intervention effects of rTMS for the scores of UPDRS, our meta-analysis indicated that rTMS had significantly beneficial post-intervention effects compare with control group (fixed effects model, SMD = −1.08, 95% CI = −1.39 to −0.78, $I^2 = 49\%$, $p < 0.001$, Figure 7).

For subgroup analysis, TMS treatment of 1 Hz, 5 Hz, 10 Hz, and 25 Hz have significant efficacy (SMD = −0.79, 95% CI = −1.04 to −0.54, Figure 8), and the stimulation frequency of 25 Hz had a better effect size than the other frequencies (SMD = −0.91, 95% CI = −1.50 to −0.31, Figure 8). However, no statistically significant difference was found between different stimulation frequency ($I^2 = 0\%$, $p = 0.81$, Figure 8). The result of subgroup analysis based on stimulation site showed that the effect size of primary motor cortex (M1) was the

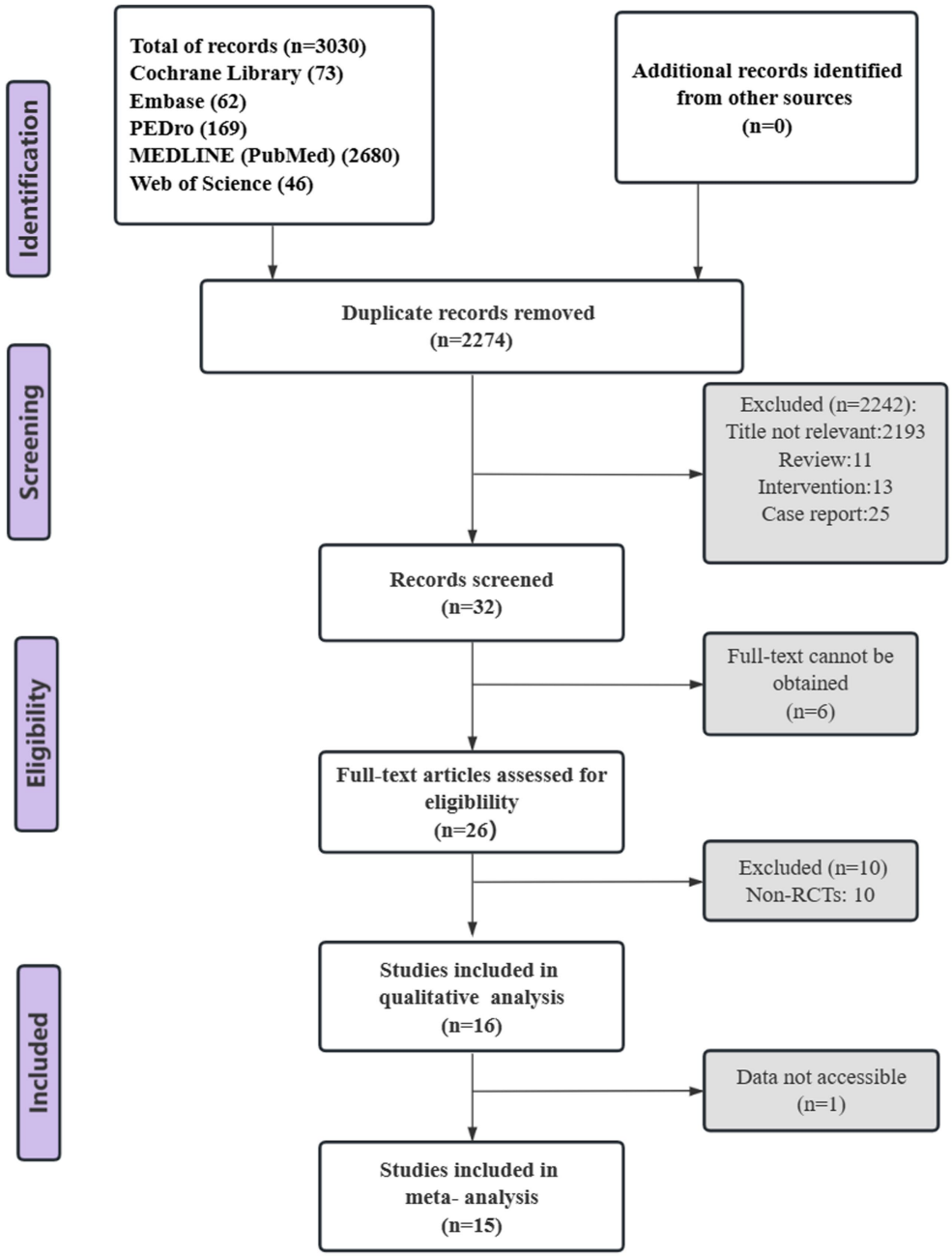


FIGURE 1
PRISMA flow diagram.

largest (SMD = −0.83, 95% CI = −1.12 to −0.53, Figure 9), and TMS of the dorsolateral prefrontal cortex (DLPFC) has no significant effect on FOG in PD patients (fixed effects model, SMD = −0.40, 95% CI = −1.25 to −0.45, $I^2 = 0\%$, $p = 0.36$, Figure 9). Furthermore, there was no significant difference between different subgroups based on stimulation sites ($I^2 = 0\%$, $p = 0.45$, Figure 9).

## Discussion

The purpose of our study was attended to investigate the effectiveness of TMS combined or not with other treatments on FOG after PD. In this systematic review and meta-analysis, we reviewed 16 RCTs of TMS in patients with FOG after PD. Our study discovered that real TMS intervention was more effective than placebo treatment for improvement of gait condition with accelerated walking speed after a period of intervention. This result was consistent with that of previous studies (Xie et al., 2020; Deng et al., 2022).

Overall, TMS is a relatively safe treatment. Out of 16 studies, only six reported adverse effects in patients during stimulation (Khedr et al., 2006; Benninger et al., 2011; El-Tamawy et al., 2013; Lee et al., 2014; Cohen et al., 2018; Li et al., 2020). Frequent adverse reactions are headache, dizziness, nausea, local pain and discomfort, which are mostly transient. Furthermore, no studies have reported

TABLE 1 Characteristics of participants included in studies.

| Study | Patients (M/F) | Age (years) | Disease duration (years) | H&Y stage | Interventions |
|---|---|---|---|---|---|
| Khedr et al. (2003)<br>Egypt | EG: 19 (14/5)<br>CG: 17 (10/7) | EG: 57.8 ± 9.2<br>CG: 57.5 ± 8.4 | EG: 3.45 ± 2.3<br>CG: 3.05 ± 2.1 | 2–3 | EG: real-rTMS<br>CG: sham-rTMS |
| Lomarev et al. (2006)<br>USA | EG: 9 (7/2)<br>CG: 9 (8/1) | EG: 63 ± 10<br>CG: 66 ± 10 | EG: 13.8 ± 6.8<br>CG: 10.8 ± 3.1 | 2–4 | EG: real-rTMS<br>CG: sham-rTMS |
| Khedr et al. (2006)<br>Egypt | EG: 10<br>CG: 10 | EG: 60.2 ± 9.48<br>CG:60.6 ± 10.6 | EG: 3.5 ± 0.7<br>CG: 3.8 ± 0.9 | 3–5 | EG: real-rTMS<br>CG: Occupational stimulation |
| del Olmo et al. (2007)<br>Spain | EG: 8<br>CG: 5 | 61.7 ± 5.22 | 8.0 ± 5.0 | 1–3 | EG: real-rTMS<br>CG: sham-rTMS |
| Arias et al. (2010)<br>Spain | EG: 9<br>CG: 9 | Not reported | Not reported | 2–4 | EG: real-rTMS<br>CG: sham-rTMS |
| Benninger et al. (2011)<br>Switzerland | EG: 13 (7/6)<br>CG: 13 (11/2) | EG: 62.1 ± 6.9<br>CG: 65.6 ± 9.0 | EG: 10.8 ± 7.1<br>CG: 6.5 ± 3.4 | EG: 2.6 ± 0.2<br>CG: 2.5 ± 0.1 | EG: real-iTBS<br>CG: sham-iTBS |
| Benninger et al. (2012)<br>Switzerland | EG: 13 (11/2)<br>CG: 13 (9/4) | EG: 55.8 ± 9.1<br>CG:54.3 ± 12.5 | EG: 8.6 ± 4.1<br>CG: 9.3 ± 6.8 | EG: 2.7 ± 0.3<br>CG: 2.9 ± 0.6 | EG: real-rTMS<br>CG: sham-rTMS |
| El-Tamawy et al. (2013)<br>Egypt | 16 (11/5) | 67.0 ± 7.32 | Not reported | 3.1 ± 0.6 | EG: real-rTMS<br>CG: sham-rTMS |
| Yang et al. (2013)<br>China | EG: 10 (5/5)<br>CG: 10 (7/3) | EG: 65.2 ± 11.1<br>CG: 67.0 ± 13.2 | EG: 6.4 ± 2.7<br>CG: 6.4 ± 3.6 | EG: 2.3 ± 0.4<br>CG: 2.4 ± 0.4 | EG: real-rTMS<br>CG: sham-rTMS |
| Lee et al. (2014)<br>Korea | 20 (13/7) | 71.6 ± 8.6 | 4.7 ± 2.6 | 3.4 ± 0.5 | EG: real-rTMS<br>CG: sham-rTMS |
| Cohen et al. (2018)<br>Israel | EG: 21 (17/4)<br>CG: 21 (15/6) | EG: 66.4 ± 4.8<br>CG: 66.8 ± 8.1 | EG: 4.7 ± 3.4<br>CG: 5.6 ± 3.7 | 2–4 | EG: real-rTMS<br>CG: sham-rTMS |
| Ma et al. (2019)<br>China | EG: 18 (8/10)<br>CG: 10 (5/5) | EG: 59.9 ± 9.2<br>CG: 66.0 ± 8.6 | EG: 8.9 ± 5.5<br>CG: 7.5 ± 4.7 | Not reported | EG: real-rTMS<br>CG: sham-rTMS |
| Mi et al. (2019)<br>China | EG: 20 (9/11)<br>CG: 10 (5/5) | EG: 62.7 ± 10.6<br>CG: 65.6 ± 8.7 | EG: 9.2 ± 5.8<br>CG: 7.4 ± 4.8 | EG: 2.6 ± 0.9<br>CG: 2.4 ± 0.9 | EG: real-rTMS<br>CG: sham-rTMS |
| Chung et al. (2020)<br>China | EG1: 17 (10/7)<br>EG2: 17 (9/8)<br>CG: 16 (7/9) | EG1:62.7 ± 6.8<br>EG2: 62.1 ± 5.7<br>CG: 62.1 ± 5.7 | EG1: 5.2 ± 3.4<br>EG2: 7.5 ± 4.9<br>CG: 6.9 ± 3.3 | EG1: 2.2 ± 0.3<br>EG2: 2.2 ± 0.4<br>CG: 2.3 ± 0.3 | EG1: 25 Hz-rTMS<br>EG2: 1 Hz-rTMS<br>CG: sham-rTMS |
| Li et al. (2020)<br>China | EG: 24 (16/8)<br>CG: 24 (16/8) | EG: 61.7 ± 6.9<br>CG: 61.5 ± 8.4 | EG: 5.5 ± 3.7<br>CG: 6.5 ± 5.1 | EG: 1.9 ± 0.6<br>CG: 1.8 ± 0.6 | EG: real-rTMS<br>CG: sham-rTMS |
| Lench et al. (2021)<br>USA | EG:12 (7/5)<br>CG: 8 (7/1) | EG: 66.6 ± 7.5<br>CG: 64.5 ± 8.9 | EG: 8.7 ± 7.1<br>CG: 8.0 ± 5.6 | EG: 2.3 ± 0.4<br>CG: 2.3 ± 0.3 | EG: real-rTMS<br>CG: sham-rTMS |

USA, the United States of America; EG, experimental Croup; CG, control Croup; M, male; F, female; rTMS, repetitive transcranial magnetic stimulation; iTBS, intermittent theta burst stimulation.

serious adverse effects during stimulation. A study by Rossi et al. (2021) reported that induction of seizures is the most serious adverse effect of TMS, whereas risk of TMS to induce seizures is certainly very low. Similarly, seizures were not reported in any of the sixteen studies included in our study. The stimulation parameters of transcranial magnetism may be an important factor affecting safety. The stimulus intensity applied in the study was at 80–110% RMT, and this range of stimulus intensity is also considered to be safer. Flitman et al. (1998) reported an episode of a generalized tonic clonic seizure in a healthy subject using parameters of 120% of MT, 15 Hz, train duration of 0.75 s, and with variable intervals between trials. This may be due to intervals that are too short or the intensity of the stimulus. To ensure safety, consider reducing the stimulation duration and increasing the intervals when the stimulation frequency and intensity are high.

The result of our meta-analysis based on frequency demonstrated that TMS of 1 Hz, 5 Hz, 10 Hz, and 25 Hz had significant effects in improving gait status when compared to the sham stimulation. Furthermore, subgroup analyses also revealed that TMS of 25 Hz has a greater effect size in comparison to other frequencies. Chung et al. (2020) compared the effects of 1 Hz, 25 Hz and sham stimulation on gait and motor performance. It was found that 1 or 25 Hz TMS prior to treadmill training enhanced and prolonged the effects of training on gait and motor performance compared to sham stimulation. However no significant treatment difference was found between 1 Hz and 25 Hz stimulation. In addition to this, there are no other studies directly comparing the effects of different frequencies of TMS on FOG in patients with PD. A randomized, double-blinded, cross-over study by Kim et al. (2015) reported that 10 Hz rTMS over the M1 area of the dominant

TABLE 2 Main parameters of TMS.

| Study | Coil type | rTMS site | Frequency | Intensity | No. of pulse *session | Trains | Treatment duration | Post-evaluation | Outcomes |
|---|---|---|---|---|---|---|---|---|---|
| Khedr et al. (2003) | F8 | M1 + DLPFC | 5 Hz | 120%MT | 2000*10 | Not reported | 10 days | 10 days; $Post_{1m}$ | Time of the 25-m walk; motor section of the UPDRS |
| Lomarev et al. (2006) | F8 | M1 + DLPFC | 25 Hz | 100%MT | 1200*8 | Not reported | 4 weeks | 4 weeks; $Post_{1m}$ | Time of the 10-m walk |
| Khedr et al. (2006) | F8 | M1 | 10 Hz | 100%MT | 3000*6 | 20 tps of 5 s | 6 days | 6 days | Time of the 25-m walk |
| del Olmo et al. (2007) | F8 | DLPFC | 10 Hz | 90%RMT | 450*10 | 15 tps of 1 s | 10 days | 1 day | Walking time |
| Arias et al. (2010) | C | M1 | 1 Hz | 90%RMT | 600*10 | 50 tps | 10 days | 10 days | Motor section of the UPDRS |
| Benninger et al. (2011) | C | M1 + DLPFC | iTBS (50 Hz) | 80%AMT | 600*8 | 20 tps of 2 s | 2 weeks | 2 weeks; $Post_{1m}$ | Time of the 10-m walk |
| Benninger et al. (2012) | C | M1 | 50 Hz | 80%AMT | 1000*8 | Not reported | 2 weeks | 2 weeks; $Post_{1m}$ | Time of the 10-m walk; FOG-Q; motor section of the UPDRS III |
| El-Tamawy et al. (2013) | F8 | M1 | 1 Hz | 90%MT | 500*12 | 10 tps of 50 s | 4 weeks | 4 weeks | FOG-Q |
| Yang et al. (2013) | F8 | M1 | 5 Hz | 100%RMT | 1200*12 | 24 tps of 10 s | 4 weeks | 4 weeks | TUG |
| Lee et al. (2014) | double-cone; F8 | M1; SMA; DLPFC | 10 Hz | 90%RMT | 1000*1 | 20 tps of 5 s | 1 day | 1 days | FOG-Q; TUG |
| Cohen et al. (2018) | H-coil | M1 + PFC | 1/10 Hz | 110/100%MT | 900/800*24 | 40 tps of 2 s | 3 months | 3 months | TUG |
| Ma et al. (2019) | F8 | SMA | 10 Hz | 90%RMT | 1000*10 | 20 tps of 5 s | 10 days | 12 days, $Post_{1m}$ | FOG-Q |
| Mi et al. (2019) | F8 | SMA | 10 Hz | 90%RMT | 1000*10 | 20 tps of 5 s | 10 days | 12 days, $Post_{1m}$ | FOG-Q |
| Chung et al. (2020) | double-cone | bilateral M1 | 1 Hz, 25 Hz | 80%RMT | 1200*12 | Not reported | 3 weeks | 1 day, $Post_{1m}$ | TUG; MDS-motor section of the UPDRS III |
| Li et al. (2020) | F8 | M1 | 20 Hz | 80%RMT | 2000*5 | 20 tps of 5 s | 1 week | 1 week | MDS- motor section of the UPDRS III |
| Lench et al. (2021) | F8 | SMA | 1 Hz | 110%RMT | 1200*10 | Not reported | 10 days | 10 days | FOG-Q; motor section of the UPDRS-III |

F8, figure-eight coil; M1, primary motor cortex; DLPFC, dorsolateral prefrontal cortex; MT, motor threshold; $Post_{1m}$, 1 month postintervention; tps, trains per session; UPDRS: Unified Parkinson's Disease Rating Scale; rTMS, repetitive transcranial magnetic stimulation; RMT, resting motor threshold; C, circular; AMT, active motor threshold; FOG-Q, freezing of gait questionnaire; TUG, time up and go; SMA, supplementary motor area; PFC, prefrontal cortex; MDS-UPDRS III, Movement Disorder Society–Unified Parkinson's Disease Rating Scale motor score Part III.

hemisphere for 5 sessions in a week has significant improvements. A study by Benninger et al. (2012) concluded that 50 Hz rTMS over theM1 area could not improve motor performance and functional status in patients with PD. As far as current study is concerned, there is no agreement on the optimal stimulation frequency for TMS for FOG in patients with PD.

The results also showed that TMS of DLPFC had no significant benefit in improving gait status when compared to the sham stimulation group. The results of our study are similar to those of del Olmo et al. and different from those of Lee et al. A study by del Olmo et al. (2007) indicated that rTMS of the DLPFC not have a significant benefit on the performance of motor tasks in PD patients. Lee et al.

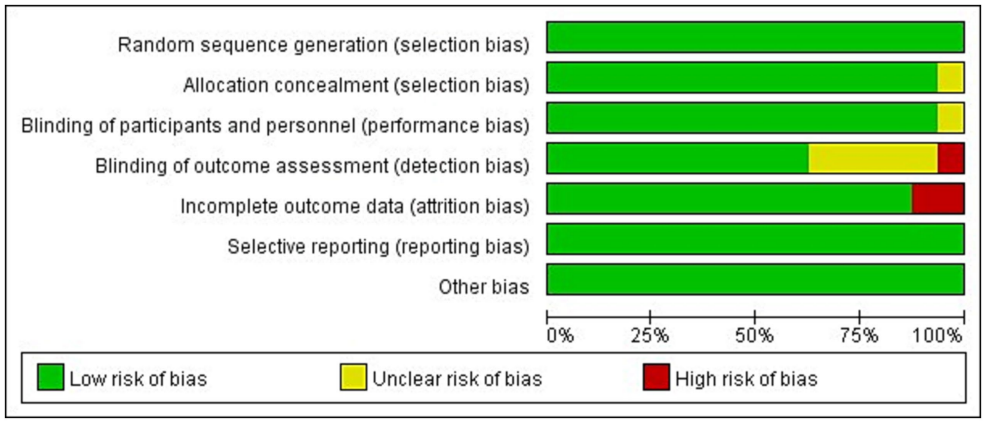


FIGURE 2
Risk of bias items shown as percentages across the included studies.

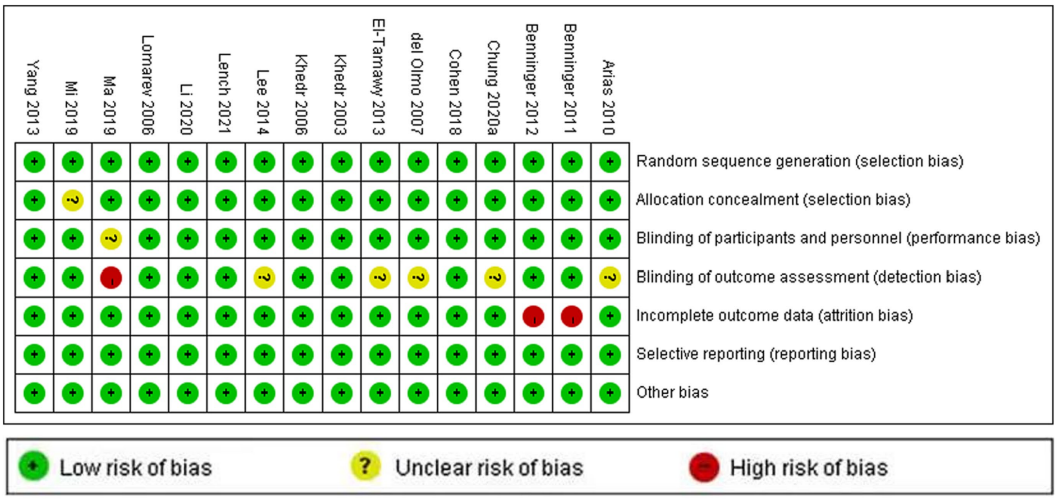


FIGURE 3
Risk of bias assessment for 16 RCTs.

(2014) demonstrated that there was a positive effect of 10 Hz rTMS over the DLPFC on FOG and gait function. Dagan et al. (2017) reported that deep rTMS over the middle prefrontal cortex (mPFC) could not impact the severity of FOG. Additionally, the result of subgroup analysis showed greater effect sizes for TMS therapy over M1, SMA, or M1 combined with DLPFC compared to placebo treatment. However, there were no significant differences between stimulation of the M1 vs. the SMA vs. M1 combined with DEPLFC. Seven studies (Khedr et al., 2006; Arias et al., 2010; Benninger et al., 2012; El-Tamawy et al., 2013; Yang et al., 2013; Chung et al., 2020; Li et al., 2020) have targeted the M1 region for TMS stimulation. A study by El-Tamawy et al. (2013) though rTMS over the M1 may have a therapeutic effect in on-freezers with advanced PD. Chung et al. (2020) found that 1 and 25 Hz rTMS groups produced a greater improvement in fastest walking speed at post-intervention than the sham group. These studies also reported that TMS over the M1 have positive effects on the improvement of gait performance (Khedr et al., 2006; Arias et al., 2010; Maruo et al., 2013; Yang et al., 2013; Li et al., 2020). A fMRI study noted that 25 Hz rTMS to the bilateral M1 increased functional connectivity between the SMA and prefrontal areas during complex motor tasks (González-García et al., 2011). However, Benninger et al. (2012) proposed that 50 Hz rTMS of the M1 did not improve gait in PD. Fricke et al. (2019) hypothesized that TMS may activate subpopulations of neurons in the hypothalamic nucleus through direct projections from cortical neurons to different cortical areas (e.g., primary motor cortex), and that abnormal amplitude activity in the hypothalamic nucleus may be associated with motor symptoms in PD. The abnormal amplitude activity of the hypothalamic nucleus may be related to the motor symptoms of PD, and TMS stimulation may be able to change these abnormal amplitudes. They hypothesized that persistent decoupling of hypothalamic nucleus neurons could be improved by combined two-site TMS. They performed combined two-site TMS on dorsal premotor cortex and primary motor cortex in 20 patients with PD, and the results showed that combined two-site TMS had no clinically meaningful beneficial effects on motor symptoms in PD.

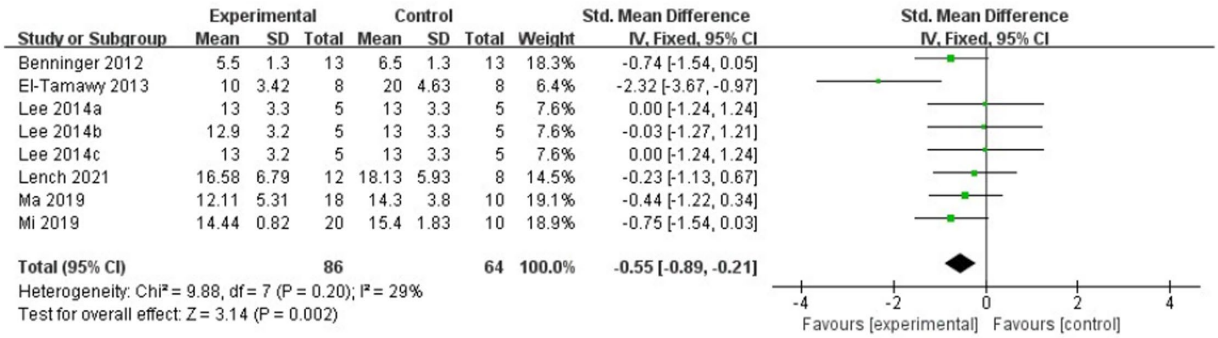


FIGURE 4
Forest plot for FOG-Q comparison, standard mean difference.

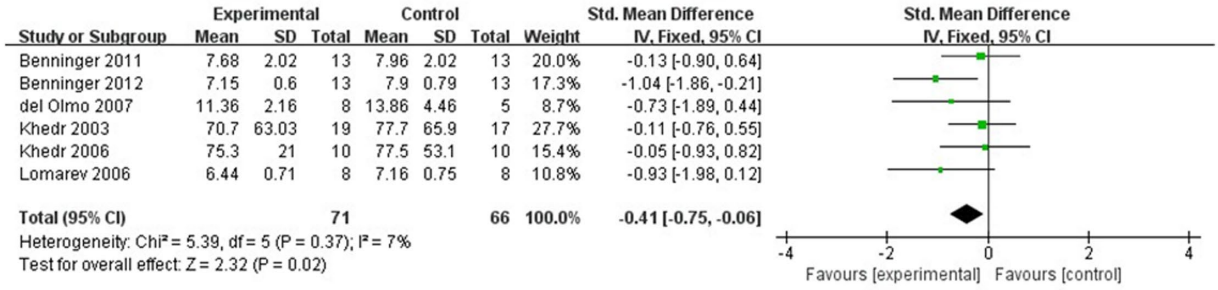


FIGURE 5
Forest plot for walking time comparison, standard mean difference.

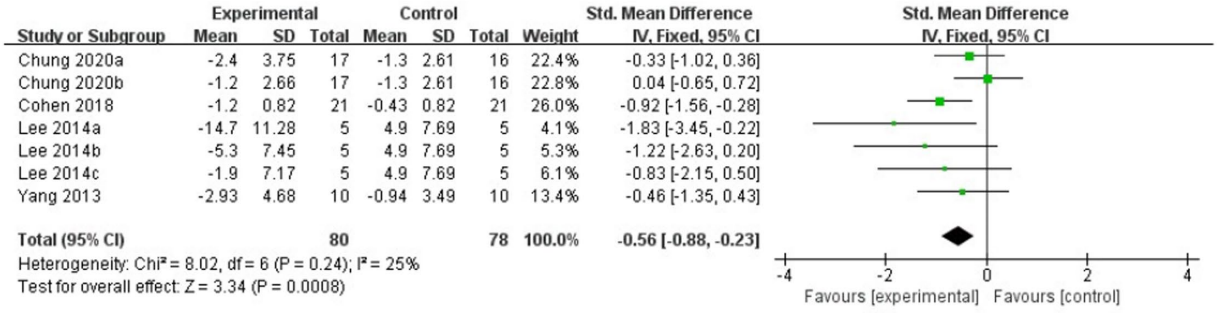


FIGURE 6
Forest plot for TUG comparison, standard mean difference.

Three studies (Ma et al., 2019; Mi et al., 2019; Lench et al., 2021) have targeted the SMA area for TMS stimulation. The results of these studies all found that rTMS over the SMA could improve the gait performances in patients with PD, which was consistent with our findings. The SMA is located anterior to the M1 leg area, and the SMA is important in several types of motor processes and is activated before movement initiation (Nachev et al., 2008). Therefore, Kim et al. suggest that SMA stimulation is a more-appropriate target in PD patients with FOG (Kim et al., 2018). Three studies (Khedr et al., 2003; Lomarev et al., 2006; Benninger et al., 2011) have targeted the M1 combined with DLPFC regions for TMS stimulation. The findings of these previous studies indicated that rTMS has a positive effect on improvement in motor performance in FOG after PD, which was in agreement with our results. A study by Lee et al. (2014) noted rTMS over the M1, SMA, and DLPFC all induced greater effects than placebo treatment and rTMS over the M1, DLPFC have a greater effect compare to SMA, but no significant differences were found between the M1 and the DLPFC stimulation. However, Kim et al. (2018) believed that SMA is a more appropriate target than the MI area for brain stimulation when treating PD patients with FOG, which was not consistent with the results of Lee et al. Another randomized cross-over pilot study compared the effects of 10 Hz rTMS over the MI and DLPFC on the patients with FOG after PD, they concluded that no significant effect of rTMS over the DLPFC and M1 on FOG, but has a

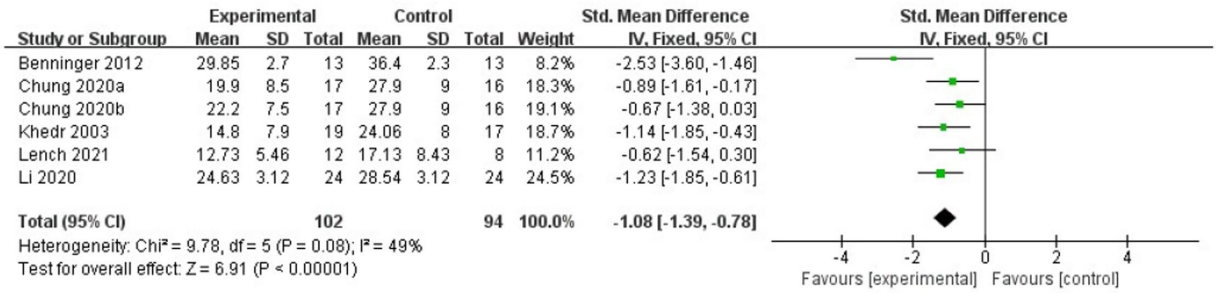


FIGURE 7
Forest plot for UPDRS comparison, standard mean difference.

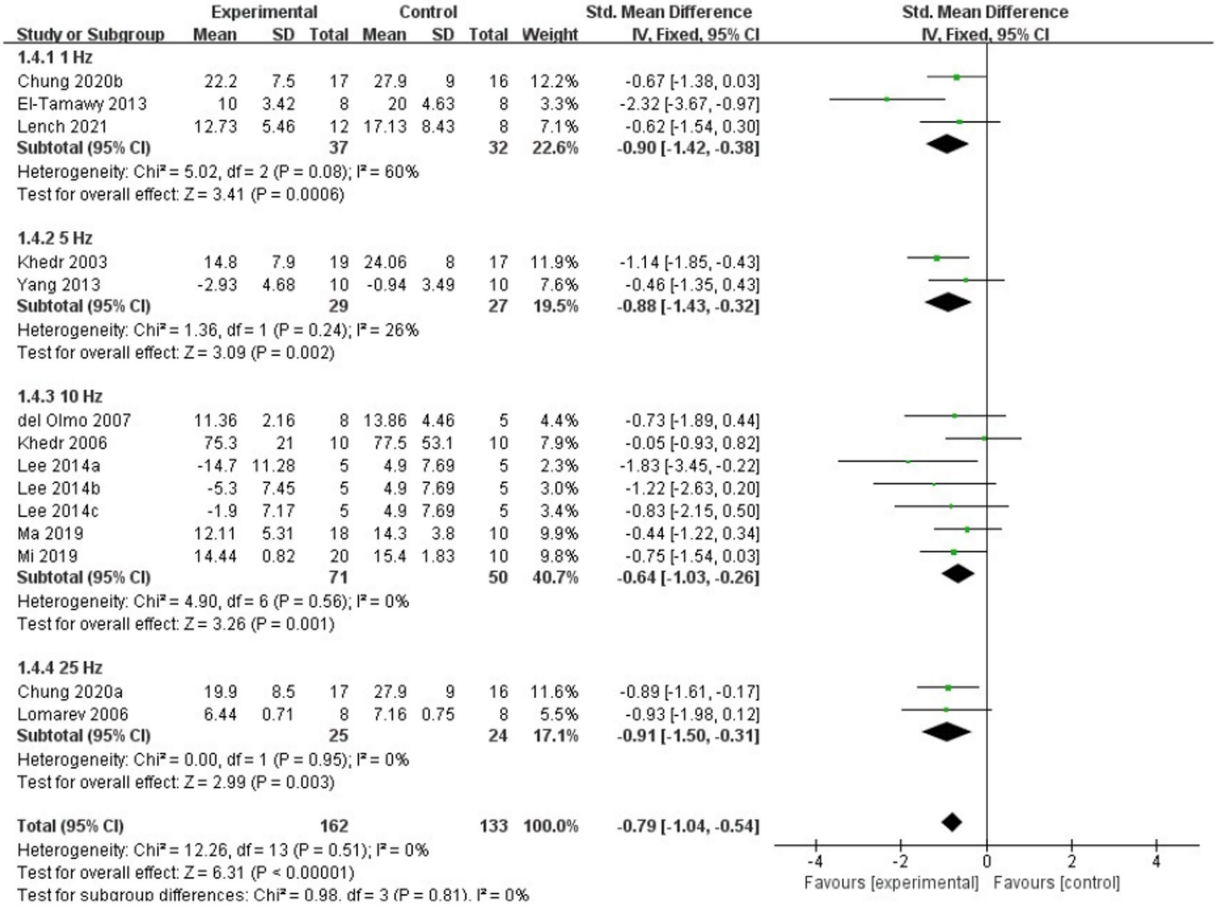


FIGURE 8
Forest plot for subgroup analysis according to the stimulation frequency.

trend toward improvement of the Stroop test interference after rTMS over the DLPFC (Rektorova et al., 2007). In addition, a previous study also suggested that theta burst stimulation (TBS) over the cerebellar does not improve FOG in patients with PD (Janssen et al., 2017). As far as current research is concerned, there is no agreement on the optimal brain stimulation target for TMS for FOG in patients with PD.

However, there are several limitations to this study. First of all, the studies included in quantitative analysis were dissimilar regarding the severity of symptoms, disease duration, and the time of TMS therapies. In addition, the sample size of these studies was relatively small. Therefore, the final results should be carefully interpreted. Furthermore, the risk of bias in some areas was not clear due to incomplete data in a few studies, which limited the results.

## Conclusion

TMS therapy presented some significant benefits on improvement of gait and motor performance. However, the results of subgroup analyses based on different frequencies and different brain stimulation targets did not show significant differences. Further large studies are required in the future to investigate the optimal

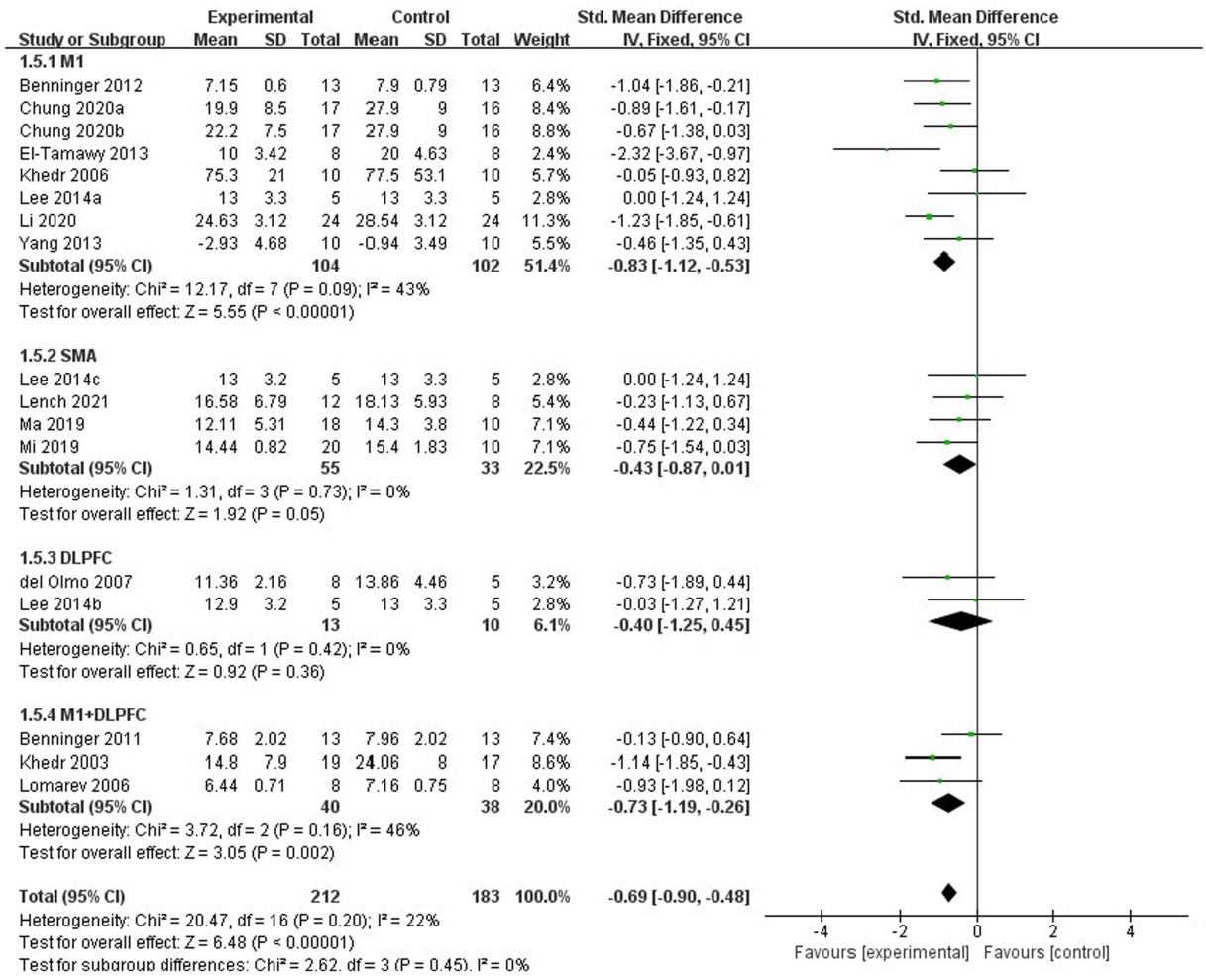


FIGURE 9
Forest plot for subgroup analysis according to the stimulation site.

stimulation parameters for TMS in patients with FOG in PD. Although it has been reported that TMS may cause side effects such as headache, dizziness, nausea, and malaise, these adverse stimuli are mostly transient, and TMS can be considered a relatively safe treatment. In conclusion, TMS had a positive and significant effect in improving gait such as increased walking speed, FOG-Q score, UPDRS score and reduced TUG time compared to placebo treatment. This suggests that transcranial magnetism is an effective treatment modality for FOG in PD.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

ZL: Conceptualization, Writing – original draft, Writing – review & editing, Data curation, Resources. XW: Investigation, Methodology, Software, Writing – original draft. XX: Data curation, Formal analysis, Project administration, Writing – original draft. YL: Data curation, Formal analysis, Supervision, Writing – original draft. CT: Project administration, Resources, Supervision, Validation, Writing – original draft. SK: Writing – review & editing, Writing – original draft, Funding acquisition. HL: Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Effects of non-invasive vagus nerve stimulation on clinical symptoms and molecular biomarkers in Parkinson's disease

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Non-invasive vagus nerve stimulation (nVNS) is an established neurostimulation therapy used in the treatment of epilepsy, migraine and cluster headache. In this randomized, double-blind, sham-controlled trial we explored the role of nVNS in the treatment of gait and other motor symptoms in Parkinson's disease (PD) patients. In a subgroup of patients, we measured selected neurotrophins, inflammatory markers and markers of oxidative stress in serum. Thirty-three PD patients with freezing of gait (FOG) were randomized to either active nVNS or sham nVNS. After baseline assessments, patients were instructed to deliver six 2 min stimulations (12 min/day) of the active nVNS/sham nVNS device for 1 month at home. Patients were then re-assessed. After a one-month washout period, they were allocated to the alternate treatment arm and the same process was followed. Significant improvements in key gait parameters (speed, stance time and step length) were observed with active nVNS. While serum tumor necrosis factor- α decreased, glutathione and brain-derived neurotrophic factor levels increased significantly ($p<0.05$) after active nVNS treatment. Here we present the first evidence of the efficacy and safety of nVNS in the treatment of gait in PD patients, and propose that nVNS can be used as an adjunctive therapy in the management of PD patients, especially those suffering from FOG.

**Clinical trial registration:** identifier ISRCTN14797144.

KEYWORDS

**vagus nerve stimulation, Parkinson's disease, gait, neuroinflammation, oxidative stress**

## Introduction

For more than 20 years, surgically implanted vagus nerve stimulation (VNS) has been recognized as an adjuvant neuromodulation therapy for epilepsy (Terry, 2009). Additionally, it has proven effective in treating depression, cluster headache, and migraine (Mauskop, 2005). The *nucleus tractus solitarius* and *locus coeruleus* are believed to be the primary targets of VNS, although the precise mechanisms are still mostly unknown (Kraus et al., 2007; Oshinsky et al., 2014). Handheld non-invasive VNS (nVNS) devices have recently been developed, simplifying

this technique of treatment (Yuan and Silberstein, 2016). The capacity to test the intervention in a variety of medical conditions without running the risk of surgical or post-operative complications (Ben-Menachem et al., 2015) is only one benefit of this strategy. According to several studies, VNS may have anti-inflammatory effects in addition to its impact on central neural networks (Corcoran et al., 2005; Majoie et al., 2011). As a result, possible uses have been suggested for a variety of inflammatory diseases, such as rheumatoid arthritis, sepsis, diabetes, and cardiovascular conditions (Bonaz et al., 2016). It is interesting to note that neuroinflammation has been connected to the pathophysiology of Parkinson's disease (PD) and several other neurodegenerative diseases (Akiyama et al., 2000).

The most widespread and second most common neurodegenerative movement disorder, PD is characterized by bradykinesia, resting tremor, rigidity, and postural instability (Sveinbjornsdottir, 2016), which are the syndrome defining clinical features; however, other phenotypic subtypes (and phenotype-genotype associations) are recognized (Dulski et al., 2022). Patients with PD struggle to walk at a normal pace and rhythm (Mondal et al., 2019a). When PD is at advanced stages, patients experience freezing of gait (FOG), feeling "glued to the ground" for seconds or minutes (Giladi et al., 2001). These symptoms are incapacitating and eventually worsen because of progressive degeneration within the nigrostriatal system (Riederer and Wuketich, 1976). Inflammation along with oxidative stress and altered cellular metabolism are undoubtedly the key participants in the pathophysiology of PD (Beal, 2003). Upregulation of neuroinflammatory mediators has been found in PD patients by our team (Chatterjee et al., 2020) and others (Wang et al., 2015). In order to slow the progression of the disease, inflammatory modulators have been thoroughly investigated (Klegeris et al., 2007); however, the results to date have been inconclusive.

It has recently been reported that VNS can improve mobility in a rat model of PD (Farrand et al., 2017) and two preclinical studies have shown that a single cervical nVNS application can improve gait in individuals with PD (Morris et al., 2019; Mondal et al., 2019a). There is mounting evidence that VNS can lower oxidative stress, regulate inflammatory cytokines, and strengthen anti-oxidative mechanisms (Chen et al., 2016). Whilst the anti-inflammatory effects of VNS could have important disease modifying actions in PD (Johnson and Wilson, 2018), these mechanisms are unlikely to account for the single dose effects of nVNS. Although the precise mechanisms by which VNS exerts its effects in PD remain largely unknown (Sun et al., 2013; Mondal et al., 2019b), the immediate improvements seen after a single application of nVNS in pilot studies are more likely to be the result of indirect activation of central neural circuitry, including noradrenergic projections from the *locus coeruleus* (Johnson and Wilson, 2018), a brain region implicated in the aetiopathogenesis of FOG (Ono et al., 2016). Despite the positive results of pilot studies of nVNS in PD, it is not apparent if or to what extent continuous stimulation might have long-lasting benefits (Hays et al., 2013; Lewine et al., 2019).

We investigated the effectiveness of cervical nVNS (gammaCore, ElectroCore, Inc., NJ, United States) as an addition to standard treatment for PD patients with FOG in a randomized double-blind sham-controlled cross-over trial. In order to evaluate the impact of chronic nVNS on neuroinflammation and neuroplasticity in PD patients, we also evaluated serum levels of specific indicators of inflammation and oxidative stress as well as brain derived neurotrophic factor (BDNF) in a subgroup of patients. Our results confirm that treatment with nVNS three times per day for 1 month improves gait and inflammatory biomarkers in blood in patients with PD.

# Methods

We recruited 33 PD patients of both sexes, aged 30–80, from the movement disorders outpatient clinic at a tertiary care hospital in Eastern India who had FOG. Only patients who were able to turn 180 degrees on the spot and walk continuously for at least 30 meters without assistance were included in the trial. Patients with baseline scores of 2 on both items 2.13 and 3.11 of the MDS- UPDRS rating scale, which are specific to FOG were included in the analysis. These patients were diagnosed in accordance with UK Brain Bank Criteria (Meara et al., 1999).

We excluded patients with i) early atypical parkinsonism (such as supranuclear gaze palsy), ii) vision impairment iii) concurrent local or systemic disorders (such as osteoarthritis or other neurological conditions) that could have an impact on gait, iv) deep brain stimulation surgery, v) implanted cardiac pacemaker, vi) metallic implants close to the stimulation site (such as fusion of cervical vertebrae), vii) uncontrolled hypertension, viii) recent myocardial infarction, or ix) known or suspected cardiovascular disease.

## Study methodology

Each patient underwent four assessments during the 12-week study period (consort diagram; Figure 1). Prior to randomization, patients were evaluated for eligibility at the screening appointment based on a set of criteria, including a review of their medical history and current medications. Within 7 days following the consent process, patients were asked to come in for baseline evaluations before receiving their devices. This included an extensive neurological evaluation as part of a general physical examination. Clinical measures were used to evaluate the motor and non-motor symptoms of PD (see section below). On the same day, tests of cognition and gait were also conducted. Following an overnight L-dopa-free interval, all assessments were conducted in the OFF state. The patients were randomly assigned to either active nVNS or sham nVNS first (explained in the Treatment section). Patients and carers were instructed to apply the therapy at home for a month after receiving training on how to administer nVNS. After 4 weeks (the first treatment period), the patients came back for their follow-up appointment. Patients from the same cohort returned for a second follow-up appointment after a washout period of 4 weeks, when they were then assigned the alternative intervention for the second phase of the trial (second treatment period). At each of the four visits, the same set of evaluations were conducted.

A small number of patients only took part in the biomarker investigation. For the redox marker, serum samples from 14 patients in the active nVNS arm and 12 patients in the control arm were collected. Seven patients provided paired samples for the calculation of inflammatory biomarkers and for BDNF determination. Six subjects had their blood drawn twice (at the

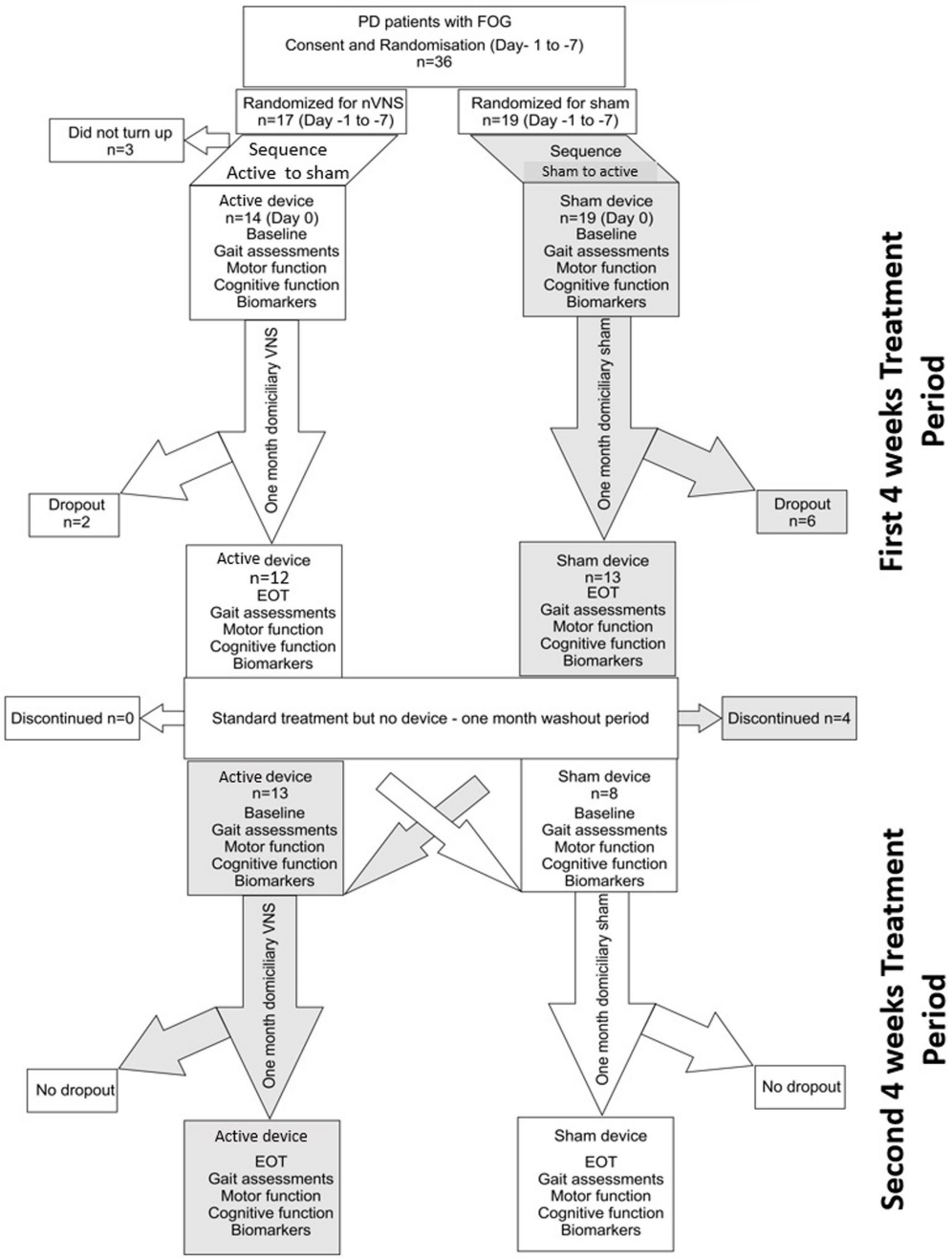


FIGURE 1
Consort diagram for the randomized cross over controlled trial comparing active non-invasive VNS (nVNS) and sham nVNS. PD, Parkinson's disease; FOG, freezing of gait; n, number/sample size; VNS, Vagus nerve stimulation; EOT, End of treatment visit.

beginning and end of each treatment period). The samples from the remaining subjects, which were unpaired samples, only covered one treatment session.

## Randomization

Allocation of active and sham nVNS of devices was blinded and randomized in a 1:1 ratio. Simple randomization was done using a computer-generated list of random numbers (Random Allocation version 2.0). Active and sham nVNS devices could only be distinguished by their serial numbers. The commercial sponsor (electroCore, Inc.) sent the unblinded trial oversight committee (not involved in patient recruitment or evaluation) a comprehensive list of serial numbers and the stimulation mode of each device (sham or active) and its serial number. The distribution of devices was not disclosed to the researchers, site coordinators, or participants until the experiments were completed.

## Treatment

A proprietary frequency-modulated electrical stimulus (5 kHz sine wave stimuli of 1 ms duration at 25 Hz) was produced by the active nVNS device (electroCore, Inc.) at low voltage (24 V) and a maximum current output of 60 mA. The stimulation was applied to the neck near the vagus nerve using two stainless steel contact surfaces coated with conductive gel. The sham device (also provided by electroCore, Inc.) was identical in terms of appearance, weight, and user interface, and while it delivered detectable electrical stimulation to the skin (with a maximum output of 14 V and 24 mA), the sham stimulator's proprietary low-frequency (0.1 Hz biphasic DC) delivery was specifically engineered not to activate the vagus nerve. Using the medial borders of the sternocleidomastoid muscle and the carotid pulse as anatomical landmarks, the treatment consisted of two, 2-min stimulation intervals delivered 5–10 min apart to the left vagus nerve to reduce any potential cardiac side effects (cardiac vagal efferents typically travel in the right vagus nerve). The intensity of the electrical stimulation was individualized based on the pain threshold of the patient. The maximum intensity was selected just below the pain threshold of the patient. For each participant, the identical stimulus intensity was applied throughout the entire investigation. We inquired about any adverse nVNS-related incidents. Every day, the intervention was given at three predetermined times: immediately after waking up, 6 to 8 h after the first treatment, and again 6 to 8 h after the second treatment.

## Assessments

At each visit, a set of clinical rating measures and gait analysis tools were used to evaluate PD-related motor and non-motor symptoms in each patient.

Gait analysis, the MDS-UPDRS scale (Pedersen et al., 2008), the freezing of gait questionnaire (Giladi et al., 2000), (FOG-Q) and the falls efficacy scale (Hauer et al., 2010) were used to evaluate motor function. Gait was evaluated using the Timed Up and Go test (Christopher et al., 2021) and an instrumented walkway (GaitRite, United States) (Webster et al., 2005). In addition to the questionnaire on freezing of gait (FOGQ), *post hoc* video gait evaluations were carried out to gauge the degree of FOG. The Mattis Dementia Rating Scale (Bezdicek et al., 2015) and the Mini Mental State Examination (Folstein et al., 1975) were two of the non-motor functional assessments. The rapid eye movement sleep behavior disorder (RBD) (Folstein et al., 1975; Stiasny-Kolster et al., 2007) screening questionnaire was one of the non-motor functional tests for cognition and sleep. In a smaller subset of individuals, serum biomarkers were assessed (see above). The Supplementary material contains a description of the assessment protocols in detail.

TNF-α, IL-6, IL-10, and BDNF were quantified in serum using ELISA kits that are available commercially (Abcam, United States). Using an iMark Microplate Reader (BIORAD, United States), serum levels of reduced glutathione and superoxide dismutase, two indicators of oxidative stress, were examined. The Supplementary material describes certain procedures in detail.

## Estimation of sample size

Patients were recruited to this pilot study from the movement disorders clinic for a total of 36 months. As a pilot study and without prior knowledge of the predicted treatment impact (and variability) of a month of nVNS a formal power calculation was not considered necessary.

## Security and adherence

Through the reporting of adverse events and subsequent causality analyses using set WHO-UMC standards, patient safety was evaluated. At each appointment, sitting blood pressure and pulse were recorded for each patient. The patients were instructed to fill out a paper diary to note negative incidents.

## Statistical analysis

For parametric data, the mean (and standard deviation) and for nonparametric data, the median (and interquartile range) were used to present clinical and demographic information. The Shapiro–Wilk test (as well as distribution histograms) were used to determine whether the data were normal. Percentages were used to depict categorical data. Left and right gait characteristics were pooled and averaged if there was no side-to-side difference. Using the Wilcoxon Sign Rank test, differential carryover effects between the two sequences were investigated. Because each intervention in the study was only for 1 month, period effects were not anticipated (Karl et al., 2020). The percentage change of the outcome variables from each period was combined, regardless of the order in which the devices were allocated. The Wilcoxon signed rank test was used to assess changes in absolute values of outcome measures (such as biomarkers and clinical rating scores) following the application of active or sham nVNS. The Wilcoxon signed rank test for paired samples was used to examine the percentage change in outcomes from baseline between the active nVNS and sham groups. Fisher's Exact Test was used to compare categorical variables. The threshold for statistical significance was defined as a $p$ value of 0.05. The Benjamini Hochberg correction for multiple comparison method was used (Benjamini and Hochberg, 1995). Statistical analysis was completed using SPSS version 20 (IBM, United States).

# Results

Thirty six participants were enrolled in this cross-over trial; 17 were initially randomized to receive active nVNS and 19 received sham nVNS. Three patients withdrew from the study after the initial screening and randomization procedures. Twenty-one patients successfully completed both arms of the cross-over trial and had thus received both active and sham nVNS by the end of the study (Figure 1 – consort diagram). All participants who finished one or both periods were included in the pre-post analysis. At the conclusion of the study, there were twenty-five pairs of pre-post data for sham nVNS and twenty-one pairs for active nVNS. The 21 patients who finished both arms of the cross-over study were also subjected to an inter-group comparison of the primary outcome measures.

Between sham nVNS and active nVNS, the mean UPDRS III score did not differ at baseline (40.3 vs. 38.5, $p = 0.328$). Table 1 displays the baseline summary scores contrasting the two groups. Table 1 also includes information on demographics, gait measures,

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TABLE 1 Comparing the baseline characteristics of demographics, clinical characteristics and serum biomarkers between active and sham nVNS groups.

| | Both groups Mean (SD) | Baseline – Sham Group Mean (SD) | Baseline – Active Group Mean (SD) | Group Comparisons (*p* value) |
|---|---|---|---|---|
| Demography | | | | |
| Age (years) | 62.5 ± 10.3 | 60.8 ± 14.4 | 62.26 ± 10.5 | 1.0 |
| Sex (n) (female) | 3 (10.2%) | 3 (11.5%) | 2 (8.7%) | 1.0 |
| Gait | | | | |
| Velocity (cm/s) | 64.5 ± 20.6 | 66.9 ± 19.4 | 61.9 ± 20.3 | 0.13 |
| Average Step Length (cm) | 25 ± 20.5 | 36.8 ± 10.4 | 36.2 ± 10.3 | 0.3 |
| Average Stance time (s) | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.03 |
| Clinical scores | | | | |
| MDS-UPDRS I | 15.9 ± 7.3 | 15.6 ± 6.8 | 16.3 ± 8.1 | 0.67 |
| MDS-UPDRS II | 21.4 ± 5.5 | 20.8 ± 5.8 | 22.1 ± 5.1 | 0.14 |
| MDS-UPDRS III | 39.5 ± 11.6 | 40.3 ± 12.7 | 38.5 ± 10.4 | 0.33 |
| H & Y | 2.4 ± 0.6 | 2.4 ± 0.5 | 2.3 ± 0.7 | 0.26 |
| TUG (s) | 42 ± 55.2 | 39.2 ± 77.5 | 45.4 ± 67.5 | 0.71 |
| FES | 55.2 ± 10.6 | 54.2 ± 12.8 | 56.4 ± 7.3 | 0.25 |
| MMSE | 26.4 ± 3.9 | 25.9 ± 3.8 | 26.5 ± 3.8 | 0.92 |
| RBDSQ | 4.7 ± 2.9 | 4.9 ± 2.9 | 5.2 ± 2.9 | 0.27 |
| FOGQ1 | 2.9 ± 0.5 | 2.9 ± 0.53 | 2.9 ± 0.54 | 1.00 |
| FOGQ2 | 2.7 ± 0.5 | 2.6 ± 0.5 | 2.8 ± 0.5 | 0.16 |
| FOGQ3 | 3.2 ± 0.7 | 3.1 ± 0.6 | 2.5 ± 0.7 | 0.26 |
| FOGQ4 | 2.5 ± 0.9 | 2.4 ± 0.8 | 2.6 ± 0.9 | 0.61 |
| FOGQ5 | 2.3 ± 0.9 | 2.3 ± 0.8 | 2.4 ± 1.1 | 0.88 |
| FOGQ6 | 2.4 ± 0.9 | 2.3 ± 0.8 | 2.5 ± 0.9 | 0.45 |
| Total FOG-Q score | 14.7 ± 5.4 | 15.5 ± 3.1 | 13.9 ± 6.9 | 0.38 |
| Biomarkers | | | | |
| Serum TNF-α (pg/ml) | 25.6 ± 4.1 | 23.2 ± 2.2 | 28.1 ± 4.1 | 0.1 |
| Serum reduced glutathione (pg/ml) | 6.4 ± 0.7 | 6.7 ± 0.7 | 6.0 ± 0.6 | 0.3 |
| Serum BDNF (pg/ml) | 1945.2 ± 256.6 | 1943.7 ± 348.1 | 1943.7 ± 146.4 | 0.3 |

The differences were assessed by Wilcoxon Sign Rank Test for numerical variables and Fisher's exact test for categorical variables (e.g., sex); $p < 0.05$ (*) was considered significant. [SD, Standard Deviation; MDS-UPDRS, MDS-Unified Parkinson's disease Rating Scale; H&Y, Hoehn and Yahr scale; TUG, Timed Up and Go test; FES, Falls Efficacy Scale; MMSE, Mini Mental State Examination; RBD-Q, REM Sleep Behavior Disorder Questionnaire; FOG-Q, Freezing of Gait Questionnaire; TNF-α, Tumor Necrosis Factor-α; BDNF, Brain Derived Neurotrophic Factor].

clinical traits, and serum marker levels, none of which at baseline differed significantly across groups. Table 2 compares the differences between individual outcome measures (gait parameters and clinical features) for the two groups before and after intervention (active and sham nVNS).

According to a pairwise pre-post analysis, velocity increased by 16% ($p = 0.018$), step length increased by 11% ($p = 0.021$) and step time decreased by 16% ($p = 0.003$) in the active nVNS group, whereas changes in velocity (2.3%, $p = 1.0$), step length and step time (1.7%, $p = 0.708$) were not significant for the sham nVNS group. With active nVNS but not sham nVNS, velocity ($p = 0.018$), step time ($p = 0.012$), and step length ($p = 0.021$) all significantly improved.

Clinical outcome measures improved considerably in both groups when we evaluated the change in clinical scores before and after therapy in the two groups independently. Both groups showed a significant improvement in the UPDRS II, III, the falls efficacy scale score, and the FOGQ score.

Less than one-third of individuals with FOG experienced freezing episodes while having their gait evaluated (recorded on camera simultaneously). The average length of freezing episodes when walking around the laboratory gait assessment circuit (see Supplementary Figure S1A) decreased from 21 ± 47 to 15 s ± 37 s in the active nVNS group ($p = 0.042$) but did not change significantly after the sham nVNS intervention (27 ± 67 to 72 ± 268 s; $p = 0.575$). However, neither group had a clinically significant change as a result of the average difference in freezing time. The overall amount of time needed to complete the laboratory gait assessment circuit did not differ substantially between the sham nVNS group (128 ± 130 to 159 ± 299 s; $p = 0.968$) and the active nVNS group (116 ± 55 to 94 ± 32 s; $p = 0.007$). The baseline average times for the active nVNS and sham nVNS groups to complete the laboratory gait assessment circuit were 130 and 116 s; ($p = 0.897$), respectively.

Among the biochemical parameters, TNF-α levels were significantly decreased from baseline in patients receiving active

TABLE 2 Pre-post differences in clinical profile and gait characteristics for active nVNS and sham nVNS groups.

| Clinical outcome variables | Baseline (Pre for nVNS) Mean (SD) | Post-intervention nVNS Mean (SD) | *p* value pre-post nVNS | Baseline (Pre for sham) Mean (SD) | Post-intervention sham Mean (SD) | *p* value pre-post sham |
|---|---|---|---|---|---|---|
| Gait outcome variables | | | | | | |
| Velocity | 61.9 ± 20.3 | 72 ± 19.1 | 0.003* | 66.6 ± 20.3 | 68.31 ± 18.2 | 0.689 |
| Step length | 36.2± 10.3 | 40.3±10.15 | 0.007* | 36.8 ± 10.4 | 37.2 ± 10 | 0.797 |
| Swing time variability | 0.04±0.02 | 0.03 ± 0.02 | 0.085 | 0.04 ± 0.03 | 0.04 ± 0.03 | 0.432 |
| Step time | 0.6±0.10 | 0.57 ± 0.08 | 0.003* | 0.57 ± 0.099 | 0.55 ± 0.08 | 0.059 |
| Swing time | 0.37±0.06 | 0.38 ± 0.07 | 0.970 | 0.36 ± 0.07 | 0.35 ± 0.07 | 0.338 |
| Stance time | 0.83±0.17 | 0.75 ± 0.12 | 0.001* | 0.77 ± 0.16 | 0.74 ± 0.12 | 0.304 |
| Stride velocity variability | 6.4±3.2 | 6.9 ± 3.4 | 0.846 | 6.9 ± 2.55 | 6.9±2.43 | 0.841 |
| Step length variability | 3.9±1.5 | 4. ± 2.3 | 0.440 | 4.1 ± 1.2 | 4.3 ± 1.3 | 0.543 |
| Step time variability | 0.05 ± 0.03 | 0.04 ± 0.02 | 0.114 | 0.05 ± 0.03 | 0.05 ± 0.035 | 0.920 |
| Step time asymmetry | 0.04 ± 0.04 | 0.02 ± 0.02 | 0.056 | 0.03 ± 0.03 | 0.03 ± 0.04 | 0.819 |
| Step length asymmetry | 3.1 ± 2.4 | 2.5 ± 2.2 | 0.149 | 2.7 ± 2.4 | 2.7 ± 2.1 | 0.808 |
| Step width | 11± 2.9 | 10.7± 2.9 | 0.357 | 10.8 ± 2 | 10.7 ± 3.7 | 0.424 |
| Clinical characteristics | | | | | | |
| MDS-UPDRS I | 16 ± 8 | 13 ± 7 | 0.004* | 16 ± 7 | 13 ± 8 | 0.030 |
| MDS-UPDRS II | 22 ± 5 | 18± 5 | 0.001* | 21 ± 6 | 17 ± 7 | 0.009* |
| MDS-UPDRS III | 39 ± 10 | 32 ± 12 | 0.002* | 40 ± 1 | 33 ± 1 | 0.002* |
| H & Y | 2 ± 0.7 | 2 ± 0.7 | 0.083 | 2 ± 0.5 | 2 ± 0.5 | 0.655 |
| TUG (s) | 45± 67 | 35± 47 | 0.033 | 39 ± 77 | 42 ± 101 | 0.098 |
| FES | 56 ± 7 | 50± 8 | 0.001* | 54 ± 13 | 48 ± 13 | 0.003* |
| MMSE | 26 ± 4 | 27 ± 3 | 0.195 | 26 ± 4 | 25 ± 6 | 0.905 |
| RBDSQ | 5.2 ± 2.9 | 4.1 ± 3 | 0.036 | 4 ± 2.8 | 3.6 ± 2.9 | 0.177 |
| Total FOG-Q score | 16.5 ± 3.5 | 13.2 ± 3.9 | 0.001* | 15.5 ± 3 | 11.9 ± 4.3 | 0.001* |
| DRS Total | 124.8 ± 14.8 | 120.6 ± 28.9 | 0.727 | 120 ± 18.4 | 114 ± 31.6 | 0.819 |

The differences were assessed by Wilcoxon Sign Rank Test; $p < 0.05$ (*) was considered significant after correction for multiple comparisons [SD, Standard Deviation; MDS-UPDRS, MDS-Unified Parkinson's disease Rating Scale; H&Y, Hoehn and Yahr scale; TUG, Timed Up and Go test; FES, Falls Efficacy Scale; MMSE, Mini Mental State Examination; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; FOG-Q, Freezing of Gait Questionnaire].

nVNS (28.1 to 23.5 pg./mL; $p = 0.028$) but not in those receiving the sham nVNS intervention (23.2 to 24.7 pg./mL; $p = 0.499$; Figures 2A,B). As demonstrated in Figures 2C,D, the reduced glutathione concentration rose following active nVNS (6.1 to 6.8 pg./mL, p = 0.02) but remained relatively unchanged following sham nVNS stimulation (6.7 to 6.1 pg./mL, $p = 0.05$). The active nVNS intervention significantly raised BDNF levels (1946.7 to 2204.1 pg./mL, $p = 0.028$), but decreased with sham nVNS stimulation (1943.7 to 1682.7 pg./mL, $p = 0.028$) as demonstrated in Figures 2E,F. Between groups, there were no appreciable variations in the concentrations of IL-6 ($p = 0.128$), IL-10 ($p = 0.108$), or the specific activity of superoxide dismutase ($p = 0.058$).

Figure 3 displays percentage changes in gait parameters relative to the starting point. Between the active and sham nVNS groups, we discovered significant changes in step length ($p = 0.017$), stance duration ($p = 0.006$) and the percentage change in velocity ($p = 0.014$).

In Figure 4 we compared the percentage change in clinical scores between the active and sham nVNS treatments. The percentage change in the clinical ratings did not significantly differ across the groups.

Unexpected results emerged from patient perceptions of their experiences with freezing and their fear of falling as measured by the FOGQ and the falls efficacy scales, respectively. The six gait-freezing questionnaire items and the mean score significantly decreased in both groups. In the sham nVNS and active nVNS groups, the overall FOGQ scores decreased by 26.3% ($p = 0.001$) and 21% ($p = 0.001$), respectively. Following active and sham nVNS the mean falls efficacy scale scores decreased by 10.7% ($p = 0.001$) and 12% ($p = 0.003$) respectively.

Between the two groups, there was a comparable percentage change in cognitive scores (Figure 4C). For each group independently calculated, the difference between the raw scores before and after the treatment was not statistically significant.

With either intervention, there was no carry over effect (Supplementary Tables S1, S2).

## Discussion

This is the first randomized, double-blind, sham-controlled study to attest to the efficacy of cervical nVNS as an adjunctive treatment for PD. After receiving active nVNS treatment for a month, there were noticeable improvements in gait. The central neuronal networks

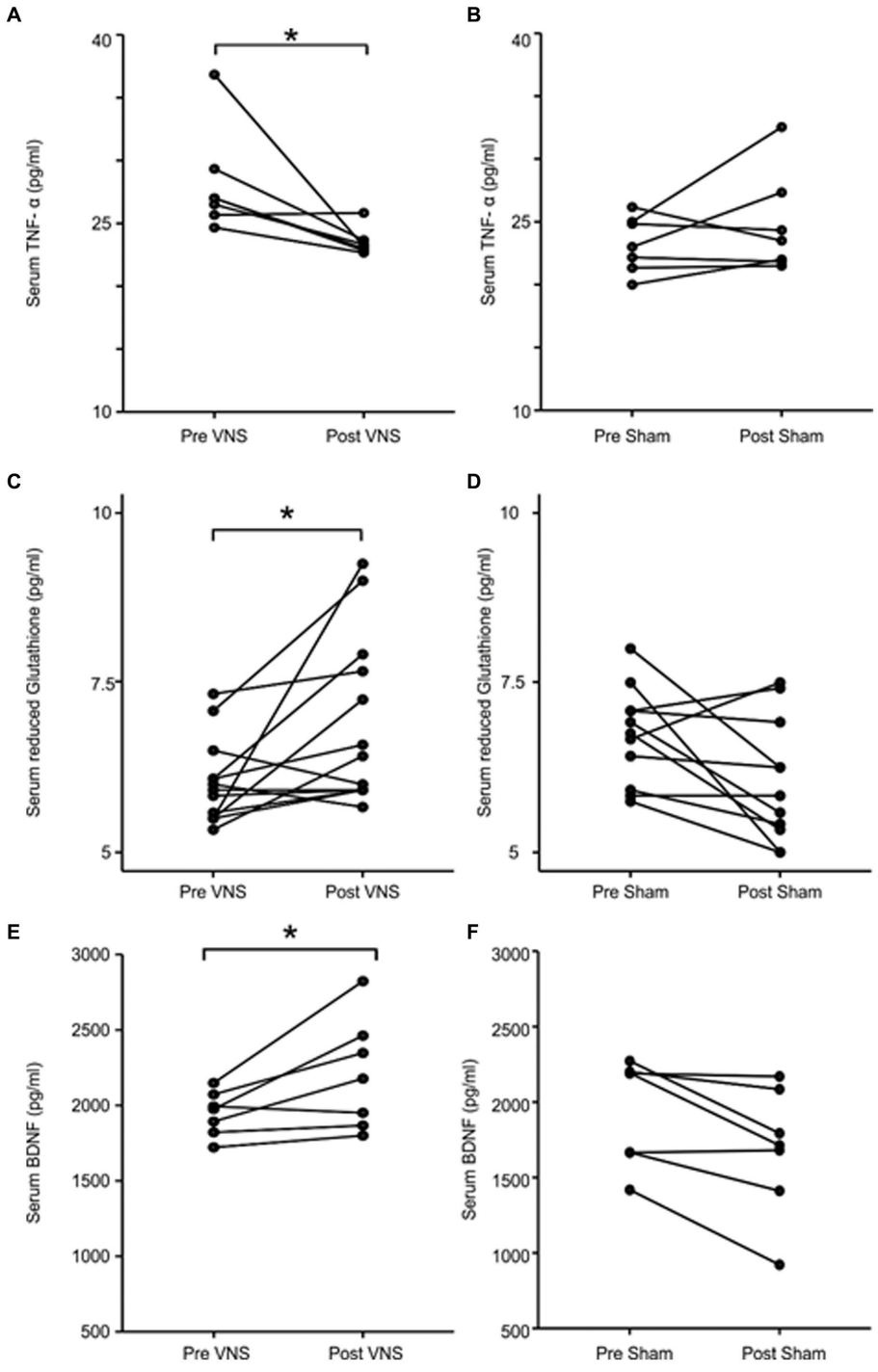


FIGURE 2
Comparing levels of serum biomarkers before and after intervention in the active and sham nVNS groups. **(A,C,E)** The change in serum TNF-$\alpha$, reduced glutathione and BDNF concentration after active nVNS compared to baseline. **(B,D,F)** The change in serum TNF-$\alpha$, reduced glutathione and BDNF concentration after sham nVNS compared to baseline. Statistical differences were assessed using the Wilcoxon Sign Rank Test, where $p < 0.05$ (*) was considered significant.

controlling gait are modulated immediately by nVNS (Figure 5A) but less obvious are the mechanisms by which the long-term effects of nVNS emerge. While the rise in serum BDNF would seem to suggest that neuroplasticity plays a role, the ability of nVNS to reduce pro-inflammatory cytokines such as TNF-α hints at an anti-inflammatory action. The changes in antioxidant levels may also point to disease-modifying effects.

As shown in Figure 5, previous investigations in animals have demonstrated that VNS largely exerts its effects through afferent inputs to the *nucleus tractus solitarius* and subsequent sequential activation of the *locus coeruleus* (Engineer et al., 2011). A noradrenergic nucleus, the *locus coeruleus* projects broadly to cortical and subcortical regions (Frangos and Komisaruk, 2017). If there is direct brain activation through excitatory neurotransmitters such as

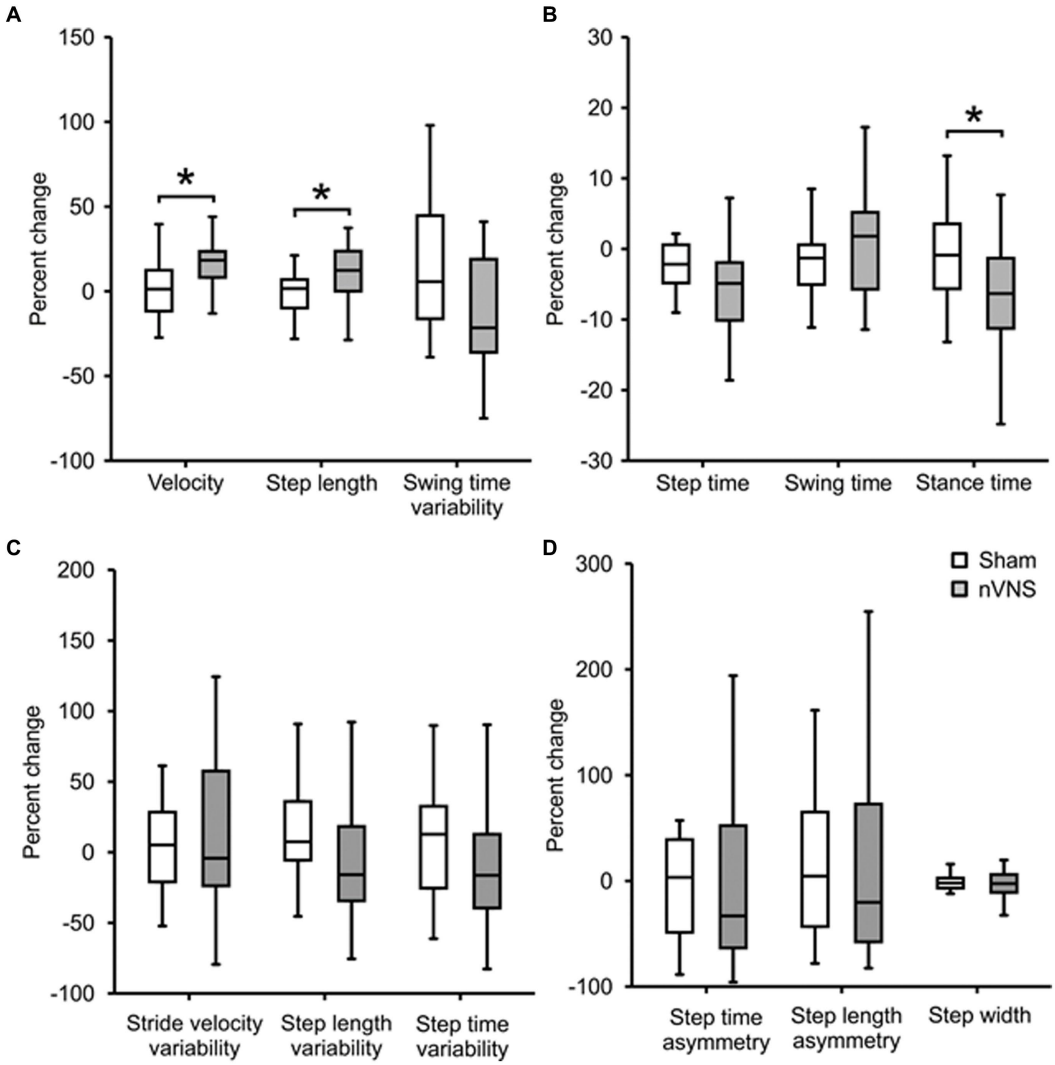


FIGURE 3
Comparing the percentage change in gait parameters between active and sham nVNS groups. Representative gait parameters are presented. **(A)** Percentage change (from baseline) in gait parameters from the 'pace' domain between the active nVNS and sham nVNS groups. **(B)** Percentage change (from baseline) in gait parameters from the "rhythm" domain for active and sham nVNS groups. **(C)** Percentage change (from baseline) in gait parameters from the 'variability' domain. **(D)** Percentage change (from baseline) in gait parameters from the 'asymmetry' and 'postural control' domains. Differences were assessed statistically using the Wilcoxon Sign Rank Test, where $p < 0.05$ (*) was considered significant.

noradrenaline (Grimbergen et al., 2009), improvements in postural instability and gait in PD would be anticipated. Since the *locus coeruleus* receives afferent input from the forebrain cholinergic *nucleus basalis* of Meynert, which projects cholinergic fibers widely throughout the cerebral cortex, hence cortical cholinergic tone is also likely to be enhanced by nVNS (Engineer et al., 2011). It is interesting to note that deficits of walking speed in PD patients have been linked to diminished cortical cholinergic tone (Rochester et al., 2012). In this study, a walkway with built-in pressure sensors was used to measure the parameters of two-dimensional gait in detail. Based on principal component analysis of gait data from PD patients, gait parameters are often divided into five categories (pace, rhythm, asymmetry, variability, and postural control) (Lord et al., 2014). With nVNS therapy, we saw significant gains in velocity and step length (in the pace domain) and a decrease in stance time (in the rhythm domain), showing that PD patients were walking more quickly and more rhythmically. Other gait metrics significantly improved from baseline, specifically after active nVNS therapy, in all five gait domains, indicating that nVNS improves gait quality across the board for PD patients. The timed up and go test, another quantitative surrogate measure of gait speed, also showed considerable improvement.

Mixed results were obtained from the video-based assessment of gait freezing, one of the key outcome metrics. Although only the active nVNS group experienced a significant decrease in the average length of freezing episodes while moving around the gait assessment circuit in the lab, both groups experienced a significant decline from baseline in the patients' perceptions of the disability brought on by FOGQ and fear of falling. Therefore, the clinical significance of the changes in freezing duration is unclear. Given the methodological limitations of video-based assessment of gait freezing, this clinically marginal outcome is not wholly surprising. Less than one-third of our patients

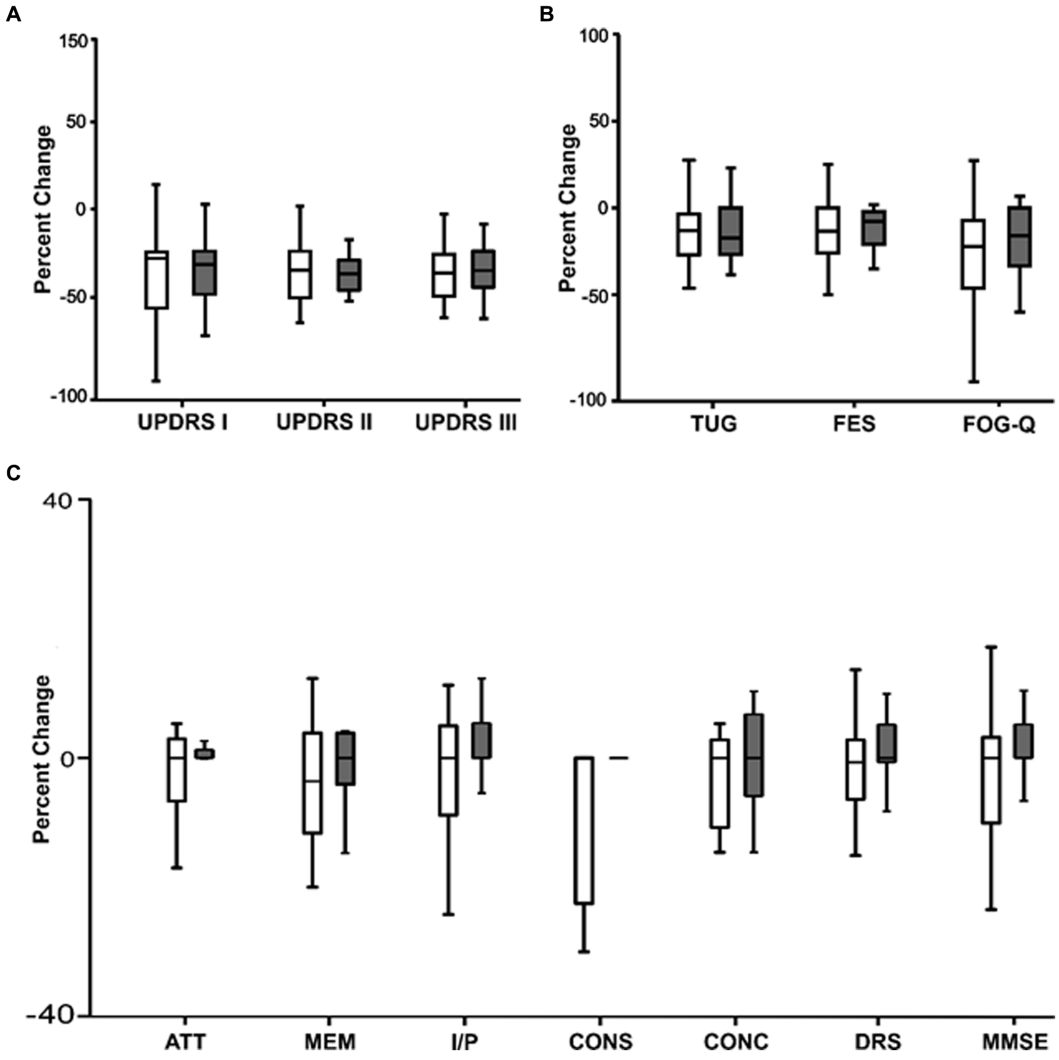


FIGURE 4
Comparing the percentage change (from baseline) in clinical characteristics between active and sham nVNS groups. **(A)** Percentage change (from baseline) in MDS – Unified Parkinson's disease Rating Scale (UPDRS Part I, II, III) between active and sham nVNS groups. **(B)** Percentage change (from baseline in time taken for Timed Up and Go Test TUG, Falls Efficacy Scale) (FES score, and Freezing of Gait Questionnaire (FOG-Q) score between active and sham nVNS groups. **(C)** Percentage change (from baseline) in total Dementia Rating Scale (DRS) score and scores in specific domains (ATT, MEM, I/P, CONS, CONC) and Mini Mental State Examination (MMSE) score between active and sham nVNS groups [AAT, Attention; MEM, Memory; I/P, Initiation and Perseveration; CONS, Construction; CONC, Conceptualisation]. Statistical differences were assessed using the Wilcoxon Sign Rank Test, where $p < 0.05$ (*) was considered significant.

experienced freezing episodes during video recording, as the severity of freezing can alter over the course of a single clinic visit (Nieuwboer and Giladi, 2008). Additionally, we avoided using methods that would cause FOGQ while we were filming. Gait freezing should ideally be measured over a longer examination time, with covert video capture. This might be done with a wearable monitoring device or by examining extensive domiciliary video records. Such methods might be used in nVNS interventional trials in the future.

We evaluated two crucial non-motor characteristics, cognition and sleep (especially RBD), both of which are worse in PD patients as the disease advances. In order to maintain healthy cognition, basal forebrain cholinergic neurons are critical for controlling attention (Sarter and Bruno, 2004). Additionally, medications that improve cholinergic transmission are frequently used to treat cognitive impairment (Ellis, 2005). One could have anticipated an increase in cognitive performance in the nVNS group as the putative mechanism the putative mechanism is the cholinergic effects of nVNS via *nucleus basalis* of Meynert (Johnson and Wilson, 2018). While there have been conflicting findings on how VNS affects cognition (Rizzo et al., 2003), the majority of studies have failed to show any appreciable improvements in cognition in patients receiving VNS as a supplementary therapy for epilepsy (Dodrill and Morris, 2001). The main drawback of such research is the short follow-up period; with less than a year of continuous treatment, it is challenging to detect meaningful cognitive gain (or a slower rate of deterioration/progression). Given the relatively brief duration of nVNS treatment, the lack of improvement in

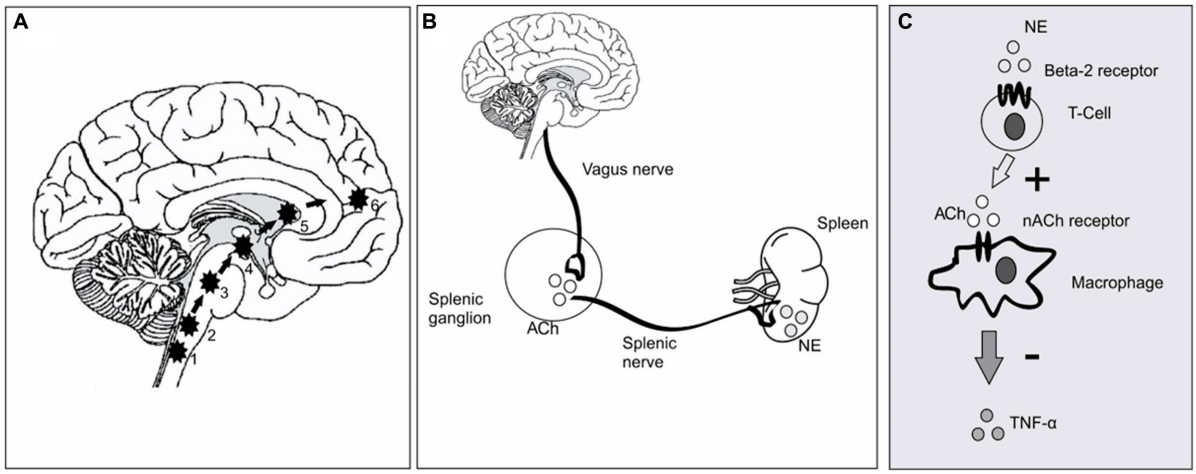


FIGURE 5
Putative mechanism of nVNS action at circuit level and cellular level. **(A)** The pathway of direct stimulation of brain regions. 1&2, Dorsal motor nucleus of the vagus and *nucleus tractus solitarius*; 3, *Locus coeruleus*; 4&5, Basal ganglia and thalamus; 6, forebrain cholinergic nucleus (including *nucleus basalis* of Meynert). **(B)** Inflammatory reflex through vagus nerve showing the efferent limb. Vagus nerve stimulation leads to secretion of ACh in the splenic ganglion. ACh in turn stimulates the splenic nerve, which provides direct adrenergic innervation to the spleen [Ach, Acetyl Choline; NE, Norepinephrine/Noradrenaline]. **(C)**. The cellular and molecular environment inside the spleen. NE secreted by splenic nerve stimulates T cells (cholinesterase positive to secrete Ach). The secreted neurotransmitter binds with the 7-α subunit of nicotinic ACh receptors on the surface of macrophages and inhibits secretion of TNF-$\alpha$.

cognitive tests in our group of patients is therefore not wholly unexpected. With nVNS, RBD might likewise be anticipated to improve, especially in light of findings pointing to the *locus coeruleus* as a significant anatomical substrate of RBD (García-Lorenzo et al., 2013). Even though we discovered no impacts of nVNS in our study, future research using polysomnography may want to revisit the effects of nVNS on RBD.

Evidence also points to a reflex mechanism (Figures 5B,C) (Tracey, 2009) through which vagal afferent stimulation activates vagal efferent fibers, which in turn trigger splenic T-cells to produce acetylcholine. Consequently, less cytokine is secreted as a result of ACh binding to nicotinic receptors (7-subunit) on the surface of macrophages in and around the spleen. Therefore, as part of this crossover study, we also examined a number of molecular biomarkers of inflammation and redox dysregulation, which have been shown to be upregulated in the serum and cerebrospinal fluid of PD patients (Müller et al., 1998) and to correlate in some studies with the degree of motor dysfunction and the degree of neurodegeneration in PD, raising the possibility that PD is an inflammatory disease (Adams et al., 2019). Despite the fact that we did not track the impact of nVNS on circulating T-cell subsets, we were able to demonstrate that it markedly decreased TNF-α levels and elevated reduced glutathione concentrations. Superoxide dismutase activity and IL-6 and IL-10 levels did not show any appreciable alterations. This might be connected to the stimulation settings (Tsaava et al., 2020). These could be further optimized to have an improved anti-inflammatory impact.

As a peripheral biomarker of neuroplasticity in numerous neurodegenerative illnesses, including PD, BDNF has received immense attention in research. PD patients have considerably lower serum levels of BDNF than age-matched controls and it has been shown that the concentration is negatively correlated with the severity of the disease (Scalzo et al., 2010). It is therefore interesting to note that BDNF is also closely linked to inflammation, suggesting that it may act as a link between neuroplasticity and inflammation (Calabrese et al., 2014). Peripheral BDNF concentration has been employed as a surrogate measure for interventional effects on neuroplasticity in a variety of neurostimulation investigations (Zhao et al., 2019). Following VNS, BDNF expression was increased in rat brain, indicating a potential neuro-modulatory/neuroprotective impact (Follesa et al., 2007). We assessed peripheral BDNF in a subset of patients from our dataset in order to translate this finding and found that BDNF concentration considerably increased following active nVNS.

Overall, our findings offer the first proof that nVNS decreases key pro-inflammatory cytokines, enhances both BDNF and reduced glutathione levels in PD patients, and that nVNS may even have disease-modifying effects in PD. Along with improvements in motor symptoms in PD patients, additional biomarkers, including BDNF, TNF-α, and reduced glutathione may be useful for optimizing nVNS treatment regimens for PD.

The main goals of this study were to ascertain whether a novel intervention could treat PD symptoms that are in general very challenging to treat and, if successful, to bring a potentially useful therapeutic technology to the clinic. Importantly, the treatment should be secure and simple to use. We therefore monitored adverse events to evaluate the safety of nVNS. Fortunately, neither interventional group reported any clinically significant negative device-related effects. Every patient had their blood pressure and pulse tested at each appointment, and there was no significant variation from baseline for either of these vital signs. The effects of

stimulating the right vagus nerve on heart rate are negligible and did not pose an additional risk of adverse cardiac effects, despite the fact that we advised patients to stimulate the left vagus nerve to avoid the theoretical risk of adverse cardiac effects (Yamakawa et al., 2014). According to recent research, therapy can be administered safely on either side (Spuck et al., 2008). With the exception of two patients who needed help from their carer to administer nVNS, most patients were happy with the treatment and could self-administer the therapy at the required frequency. Three patients who reported severe discomfort at the lowest stimulator settings withdrew from the study. Two patients who could not tolerate sham stimulation also withdrew from the study. Other participants who also withdrew from the study did so for reasons that had nothing to do with the research equipment or side effects of the intervention.

Although our results are highly encouraging, there are nevertheless some limitations, not least of which is the fact that after correcting for multiple comparisons, we observed no significant difference between groups. This was predicted because the experiment was intended to serve as a pilot study where findings would inform the power calculation for a subsequent trial. Other limitations will also need to be addressed before embarking upon a larger trial of nVNS in PD. These include the measurement of molecular biomarkers in every trial participant, if possible and using ambulatory monitoring devices to overcome the shortcomings of video-based assessment of FOG (as described above). Finally, practical concerns about the delivery of nVNS in elderly populations may need to be addressed in future generations of the device, regardless of whether a carer is required (see above).

This study has offered preliminary proof that nVNS is safe and effective for treating both motor and non-motor symptoms of PD. Future research on nVNS for PD should first determine how long treatment benefits (and potential neuroprotective effects) persist before noticeable motor symptoms reappear in order to optimize treatment parameters in the future. We hope that our promising results will provoke interest and encourage stakeholders to consider collaborating on a larger, definitive multi-center studies of nVNS in PD.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by Institute of Neurosciences Kolkata Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

BM: Formal analysis, Methodology, Project administration, Writing – original draft. SC: Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. RB: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. AR: Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing. KC: Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing. PB: Methodology, Project administration, Writing – review & editing. RS: Methodology, Project administration, Writing – review & editing. SH: Methodology, Project administration, Writing – review & editing. SS: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. SB: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. MB: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. HK: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1331575/full#supplementary-material

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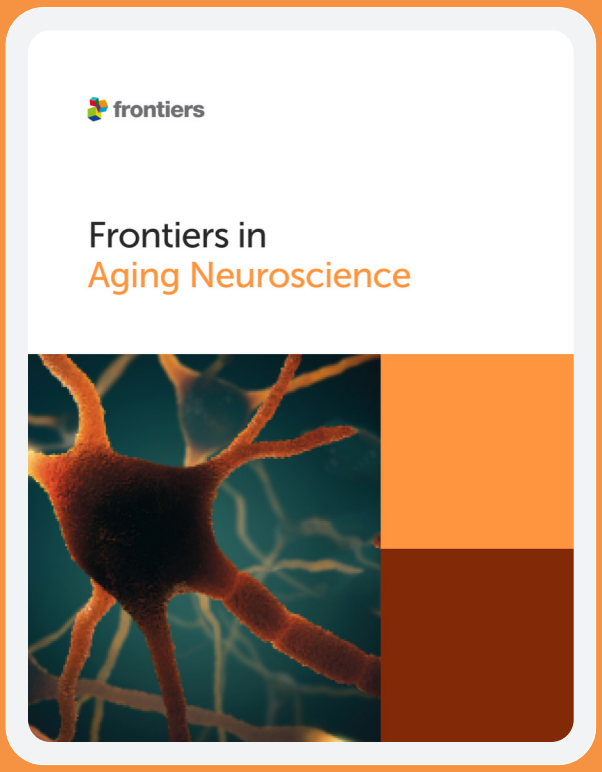


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