Dual-task gait training improves cognition and resting-state functional connectivity in Parkinson's disease with postural instability and gait disorders

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ABSTRACT

Objectives. To assess whether dual-task gait/balance training with Action Observation Training (AOT) and Motor Imagery (MI) ameliorates cognitive performance and resting-state (RS) brain functional connectivity (FC) in Parkinson's disease (PD) patients with postural instability and gait disorders (PIGD).

Methods. 21 PD-PIGD patients were randomized into 2 groups: i) DUAL-TASK+AOT-MI group performed a 6-week training consisting of AOT-MI combined with practicing observed-imagined gait and balance exercises; ii) DUAL-TASK group performed the same exercises combined with landscape-videos observation. At baseline and after training, all patients underwent a computerized cognitive assessment, while 17 patients had also RS-fMRI scans. Cognitive and RS-FC changes (and their relationships) over time within and between-groups were assessed.

Results. After training, all PD-PIGD patients improved accuracy in a test assessing executive-attentive (mainly dual-task) skills. DUAL-TASK+AOT-MI patients showed increased RS-FC within the Anterior Salience Network (aSAL), and reduced RS-FC within the anterior Default Mode Network (aDMN), right Executive Control Network and Precuneus Network. DUAL-TASK patients showed increased RS-FC within the Visuospatial Network, only. Group x Time interaction showed that, compared to DUAL-TASK group, DUAL-TASK+AOT-MI cases had reduced RS-FC within the aDMN, which correlated with higher accuracy in a dual-task executive-attentive test.

Conclusions. In PD-PIGD patients, both trainings promote cognitive improvement and brain functional reorganization. DUAL-TASK+AOT-MI training induced specific functional reorganization changes of extra-motor brain networks, which were related with improvement in dual task performance.

Keywords: Parkinson's disease; action observation; motor imagery; dual-task; fMRI; cognition.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder associated with a loss of dopaminergic neurons, which is clinically defined by the presence of bradykinesia as cardinal motor symptom together with rigidity and rest tremor. In addition to motor alterations, PD patients present with a multitude of non-motor features, such as cognitive deficits, autonomic dysfunction, sleep disorders, mood alterations and smell impairment.[1] To date, a pharmacological treatment that could prevent PD progression is not yet available and, although the available drugs are efficient at the symptomatic level, they can lead to several side effects.[2] For this reason, the identification of additional non-pharmacological interventions (including motor and cognitive trainings) is crucial to improve patients' quality of life.[3]

Gait and posture control are abilities that, once learned, should be automatic, meaning that attentional control is not required to accomplish movements. However, postural instability and gait disorders (PIGD) are common motor features in PD,[4] and patients with this clinical phenotype show difficulties in managing dual-task situations, due to a loss of automaticity.[5] A recent study from our research group showed increased activity of non-motor cerebellar areas to manage dual-task and even simple motor tasks in PD-PIGD patients, which can be interpreted as a compensatory mechanism for the functional failure of cerebellar motor areas and basal ganglia.[6]

Several studies highlighted the importance and efficacy of motor and cognitive trainings in PD patients, also shedding light on brain plasticity mechanisms.[7, 8] Recent studies reported improvements in attentive skills and walking speed, and reduced number of falls in PD patients after training. These changes were associated with brain plasticity mechanisms, such as decreased activation in frontal regions post-training,[7] or increased gray matter volume in brain regions involved in motor control, coordination and learning.[8]

Action Observation Training (AOT) and Motor Imagery (MI) are emerging mental practice approaches targeted to improving motor learning,[9] which heavily rely on the functioning of the Mirror Neuron System (MNS). In previous studies in PD, the combination of AOT and MI

modulated movements' amplitude during imitation of observed actions,[10] improved balance performance and walking speed by reducing postural sway, reduced freezing of gait and motor disability, and improved quality of life.[11] In addition, AOT and MI were associated with brain activation in regions involved in the execution of balance tasks,[12, 13] with clinical improvements after training that were maintained over one month follow-up.[11]

In a recent task-based functional MRI (fMRI) study, we showed that dual-task gait/balance training promotes functional reorganisation of brain areas involved in motor control tasks and dual-task in PD-PIGD patients, and was associated with an amelioration of executive-attentive functioning skills and long-lasting effects on dual-task mobility and balance.[14] However, in our previous work,[14] we did not investigate the post-training resting state functional connectivity (RS-FC), neither other post-training cognitive changes beside executive-attentive abilities. Compared to a task-based paradigm, which reports the functional activity of a single brain network associated with any given task, RS-fMRI allows to observe many networks at once. Furthermore, the relationship between post-training RS-FC of different networks and changes in multiple cognitive domains would offer a comprehensive overview of brain-function reorganization after training. Thus, in the present study we wished to investigate the effects of a 6-week dual-task gait/balance training with AOT and MI on RS-FC and on multiple-domain cognition, as well as their relationship, in PD-PIGD patients.

MATERIALS AND METHODS

Subjects and study design

Twenty-one idiopathic PD cases with PIGD who underwent a 6 weeks dual-task gait/balance training and who had available baseline (T0, before training) and longitudinal (W6, after training) clinical and computer-based cognitive assessments were retrospectively selected. From this group, 17 patients had also available structural and RS-fMRI scans before (T0) and after training (W6). These patients are a subsample of a larger PD-PIGD group from our previous

study,[14] where we described the beneficial effect of the dual-task gait/balance training in combination with AOT-MI on gait and mobility, as well as on motor and dual-task brain functional activity of patients during fMRI.

All patients were recruited at the Neurology Unit, IRCCS Ospedale San Raffaele, Milan, Italy according to the following inclusion criteria: no positive family history for PD and other movements disorders; Hoehn and Yahr (H&Y) score ≤ 4; PIGD phenotype, based on a score ≤0.9 at the Movement Disorders Society Unified Parkinson's disease Rating Scale (MDS-UPDRS) tremor-dominant/PIGD ratio;[15] stable dopaminergic medication for at least four weeks and without any changes during the observation period (6 weeks total); no dementia and Mini Mental State Examination (MMSE) ≥ 24; no significant head tremor. Eligibility for the dual-task gait/balance training was assessed through neurological, neuropsychological, and motor functional evaluations performed at study entry (T0, see details below). The same visits were also performed at the end of training (W6). In general, exclusion criteria were: the presence of medical illnesses or substance abuse that could interfere with cognition; any (other) major systemic, psychiatric, neurological, visual and musculoskeletal disturbances or other causes of walking inability; contraindications to undergo MRI examination; brain damage at routine MRI, including lacunae and extensive cerebrovascular disorders.

As described previously,[14] after screening, patients were equally randomized in two training groups (DUAL-TASK + AOT-MI [N=11] and DUAL-TASK [N=10] groups; see details in the next paragraph) by using minimization method. All neurological, motor and cognitive evaluations, and dual-task gait/balance treatment were performed in ON condition (i.e., under regular dopaminergic medication); neurological assessment was also performed in OFF state. The same blinded assessors performed evaluations at each time-point.

The same sample of 23 age- and sex-matched, right-handed, healthy controls described in our previous work[14] underwent a neuropsychological assessment at T0 (please refer to Table 1 and Supplementary Table 2 for socio-demographic and neuropsychological features of healthy

controls). Furthermore, an independent group of 33 young healthy controls (age: 24.9 ± 2.8 years; 14 [42%] women; education: 15.4 ± 3.1 years) was also recruited among students at the Vita-Salute San Raffaele University in Milan in order to generate independent components (ICs) networks of interest representing the functional connectivity of the human brain at rest (see details below). All controls were recruited based on the following criteria: no family history of neurodegenerative diseases and normal neurological and cognitive assessment.

Local ethical standards committee on human experimentation approved the study protocol and all subjects provided written informed consent prior to study participation.

Physiotherapy training

As reported elsewhere,[14] the dual-task gait/balance training lasted 6 weeks for both DUAL-TASK + AOT-MI and DUAL-TASK groups. The DUAL-TASK + AOT-MI group performed a gait/balance training consisting of AOT and MI in combination with observed-imagined exercises (specifically, 2 minutes of task observation, 5 minutes of task execution, 2 minutes of task imagination, 5 minutes of task execution). On the other hand, the DUAL-TASK group performed the same number of exercises combined with watching landscape videos instead of observation/imagination.

Neurological, motor and standard neuropsychological assessments

At T0 and W6, blinded and experienced neurologists and physiotherapists performed a neurological and a motor functional evaluation. Full details on these evaluations and results are reported elsewhere.[14] At T0, blinded and experienced neuropsychologists, performed a cognitive screening evaluation to both PD patients and the age- and sex-matched healthy controls. Details of the neuropsychological assessment are fully reported in the supplementary materials.

Cambridge Neuropsychological Test Automated Battery (CANTAB)

To detect cognitive changes related to dual-task gait/balance training, PD-PIGD patients were monitored using an electronic neuropsychological tablet-based assessment, the Cambridge Neuropsychological Test Automated Battery (CANTAB). CANTAB is a computer-based cognitive battery, which includes a range of cognitive tests assessing accuracy and reaction times in several domains. According to the CANTAB Cognitive Test Selector, we selected the most suitable tests suggested for detecting cognitive changes in PD and which are highly sensitive to disease progression. Specifically, we selected the following sub-tests (https://www.cambridgecognition.com/cantab/test-batteries/parkinsons-disease/): Motor Screening Test (MOT), Attention Switching Task (AST), One Touch Stockings of Cambridge (OTS), Spatial Recognition Memory (SRM), and Spatial Working Memory (SWM). The overall assessment lasted about 40 minutes. Since the time interval between T0 and post-training visit was 6 weeks, our patients were administered parallel and randomized versions for each sub-test in order to avoid learning effects. A description of each selected CANTAB sub-test is reported in Supplementary Table 1. Supplementary Figure 1 reports a scheme for each sub-test. For further details relatively to the CANTAB subtests, please refer to: http://www.cambridgecognition.com/cantab/cognitive-tests/.

MRI acquisition

Using a 3.0 Tesla scanner (Ingenia CX, Philips Medical Systems, Best, The Netherlands) MRI scans were obtained during OFF time (i.e., at least 12 hours after their regular evening dopaminergic therapy administration), to mitigate the pharmacological effects on neural activity. RS-fMRI scans were obtained at T0 and the day after the end of training (W6), with a tolerance of 3 days. RS-fMRI was obtained using a T2* weighted echo planar imaging sequence with the following parameters: echo time (TE) = 35 ms, repetition time (TR) = 1572 ms, flip angle = 70°, field of view (FOV) = 240 × 240 mm, matrix = 96 × 94, 48 contiguous axial sections, thickness = 3 mm, acquisition time = 3 min and 57 sec, voxel reconstruction 2.5 x 2.5 x 3 mm. Before starting the RS-fMRI scanning, the technician talked with the participants through their earphones instructing

them to remain motionless, to keep their eyes closed, not to fall asleep, and not to think about anything in particular. At the end of the RS-fMRI scanning, the technician talked again with the participants asking whether they remained awake during the sequence.

The following structural MRI sequences were also acquired at baseline and after training: i) 3D T1-weighted: TR = 7.1 ms, TE = 3.2 ms, flip angle = 9°, 204 contiguous sagittal sections, thickness = 1 mm, FOV = 256 mm x 240 mm, matrix = 256 x 240, voxel reconstruction = 1 mm x 1 mm x 1 mm; ii) 3D T2-weighted: TR = 2500 ms, TE = 330 ms, flip angle = 90°, 192 contiguous sagittal sections, thickness = 1 mm, field of view (FOV) = 256 mm x 256 mm, matrix = 256 x 258, voxel reconstruction = 0.9 mm x 0.9 mm x 1 mm. iii) 3D FLAIR (only at baseline): TR = 4800 ms, TE = 269 ms, flip angle = 40°, 192 contiguous sagittal sections, thickness = 1.5 mm, FOV = 256 mm x 256 mm, matrix = 256 x 256, voxel size 1 x 1 x 1.

MRI analysis

MRI preprocessing and analysis was performed at the Neuroimaging Research Unit, IRCCS Scientific Institute San Raffaele, Milan, Italy, by researchers who were blind to patient group allocation.

Resting-state fMRI pre-processing

RS-fMRI data processing of patients and young controls was carried out using the FMRIB software library (FSLv5.0) as described previously.[16] The first four volumes of the RS-fMRI data were removed to reach complete magnet signal stabilization. The following FSL-standard preprocessing pipeline was applied: (1) motion correction using MCFLIRT; (2) high-pass temporal filtering (lower frequency: 0.01 Hz); (3) spatial smoothing (Gaussian Kernel of FWHM 6 mm); (4) single-session independent component (IC) analysis-based automatic removal of motion artifacts (ICA_AROMA) in order to identify those ICs representing motion-related artifacts.

RS-fMRI data set (‘clean’ from motion-related ICs) were co-registered to the participant’s 3D T1-weighted image using affine boundary-based registration as implemented in FLIRT and

subsequently transformed to the MNI 152 standard space with 4 mm isotropic resolution using non-linear registration through FNIRT. Pre-processed RS-fMRI data for each subject from the young control group were temporally concatenated across participants to create a single 4D data set. This RS-fMRI data set was then decomposed into ICs with a free estimate of the number of components using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC). The resulting young group-IC maps were spatially correlated with a referent atlas of functional regions of interest (ROIs) (http://findlab.stanford.edu/functional_ROIs.html), in order to support the visual classification of the most representative functional networks of the brain at rest (i.e., anterior and posterior salience [SAL], anterior and posterior default mode network [DMN], auditory, sensorimotor, primary and associative visual, basal ganglia, precuneus, visuo-spatial, left and right executive control network [ECN]).[17] In order to identify the subject-specific temporal dynamics and spatial maps associated with each group IC, a dual regression analysis was applied for all PD-PIGD patients.[18] Finally, spatial maps of all participants were collected into single 4D files for each original IC (network) and were ready for the statistical analyses at T0. To assess RS-FC changes after training in PD-PIGD patients, delta RS-FC maps for each IC (network) were obtained by subtracting follow-up (W6) subject-specific spatial maps (in MNI standard space) from baseline (T0) maps.

Statistical analysis

Demographic, clinical and cognitive data

At T0, sociodemographic and standard neuropsychological data were compared between all PD-PIGD patients and age- and sex-matched healthy controls, as well as clinical and CANTAB subtest differences between PD-PIGD groups using the Mann-Whitney U tests. Longitudinal CANTAB subtest changes after training were evaluated within PD-PIGD groups using linear mixed-effects models. Such models were adjusted for the baseline value of each considered variable and for baseline variable-by-time interaction. Furthermore, in order to adjust for longer reaction

times (and therefore motor impairment as a confounding variable in our cohort), those CANTAB variables that indicated response latencies (in AST, MOT and SRM) were adjusted for baseline MOT mean response latency values. Extreme outlier values (i.e., data points that fall more than three times below the first quartile or above the third quartile of the interquartile range) were investigated and removed from the analysis. P values were Bonferroni-corrected for multiple comparisons at $p<0.05$. All statistical analyses were performed using R Statistical software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria).

Network-based functional connectivity: Independent component analysis (ICA)

We performed: a) between-group (DUAL-TASK + AOT-MI vs DUAL-TAST groups) RS-FC comparisons within each IC (network) of interest at baseline; b) within-group analysis of each IC (network) changes after 6-week training; c) Group x Time interaction of RS-FC changes after training; d) correlations between RS-FC changes within each IC (network) of interest and significant measures of CANTAB that changed over time. All analyses were carried out using general linear models (GLMs) in FSL (FSLv5.0), including 4D maps (for T0 analyses [a]) or delta RS-FC maps (for longitudinal analyses [b, c, d]) for each IC (network) of each group of PD-PIGD patients as dependent variables. In the Group x Time interaction (d), the delta RS-FC maps for each IC (network) of each patient group were compared between groups. The relationship between cognitive and RS-FC changes [d] was assessed in the sub-sample of 17 PD-PIGD patients (please refer to Supplementary Table 3 for socio-demographic and cognitive performance at the computer-based battery at baseline), who had availability of both baseline and longitudinal cognitive assessments and RS-FC scans. In this model (d), we included delta RS-FC maps for each original IC (network) of each group of patients, separately, as dependent variable, and delta cognitive changes as covariates of interest. For all GLMs, nonparametric permutation tests (5000 permutations) were used, and analyses were restricted within the spatial RS-networks of interest using binary masks obtained by thresholding the corresponding Z map images ($Z>2.3$). Family-wise

error (FWE) correction for multiple comparisons was performed, implementing the threshold-free cluster enhancement using a significance threshold of $p<0.05$.

RESULTS

Socio-demographic, clinical and cognitive results at baseline

At T0, all PD-PIGD patients were matched with healthy controls for age and sex and were similar for educational levels (Table 1), but they performed slightly worse in almost all cognitive tests (Supplementary Table 2). Furthermore, the DUAL-TASK + AOT-MI and DUAL-TASK patient groups were similar in terms of sociodemographic and clinical features, both in ON and OFF states (Table 1), presented with a similar number of MCI cases (Table 1), and performed similarly in all cognitive and behavioural tests at the standard neuropsychological assessment (Supplementary Table 2) as well as at all CANTAB sub-tests (Table 2). In addition, no significant differences in terms of socio-demographic, clinical and cognitive variables were retrieved comparing the DUAL-TASK + AOT-MI and DUAL-TASK subgroups with available longitudinal RS-fMRI (Supplementary Table 3).

Longitudinal cognitive changes (CANTAB assessment)

After training, both DUAL-TASK+AOT-MI and DUAL-TASK patients ameliorated in terms of accuracy in the AST in set-shifting (incongruent) condition (Table 2). No other significant post-training changes were revealed in the other CANTAB sub-tests. Group x Time interaction did not show significant differences between the two patient groups.

Network-based functional connectivity: ICA

Within-group longitudinal analysis

At T0, we did not observe differences between DUAL-TASK + AOT-MI and DUAL-TASK groups in any IC (network) of interest. After the 6-week training, DUAL-TASK + AOT-MI patients

showed reduced RS-FC of the right anterior prefrontal cortex within the anterior DMN (aDMN) and left precuneus within both the right ECN and Precuneus Network, and increased RS-FC of the left anterior prefrontal cortex and left superior temporal regions within the anterior SAL (aSAL) (Table 3, Figure 1a, upper part). On the other hand, DUAL-TASK group showed increased RS-FC of the right superior parietal gyrus within the Visuospatial Network (Table 3, Figure 1a, lower part).

Between-groups longitudinal analysis

Group x Time interaction analyses showed that, after training, compared to DUAL-TASK group, the DUAL-TASK + AOT-MI group showed increased RS-FC of the left anterior prefrontal cortex within the aSAL and reduced RS-FC of the right anterior prefrontal cortex within the aDMN (Table 3, Figure 1b).

Correlation analyses: RS-FC and cognitive changes after training

In the DUAL-TASK + AOT-MI group, reduced RS-FC of the right anterior prefrontal cortex within the aDMN was correlated with improved accuracy in the AST set-shifting condition after training (Table 3; Figure 1c). No other relationships were observed.

DISCUSSION

To our knowledge, this is the first study which aimed to assess whether dual-task gait/balance training combined or not with AOT-MI is associated with both cognitive and RS-FC changes in a well characterized sample of PD-PIGD patients.

In our study, we observed that both groups undergoing dual-task gait/balance exercises ameliorated over 6 weeks in terms of accuracy in a task relying on set-shifting. This improvement was specific for the attentive-executive domain, despite different other domains, such as spatial recognition and spatial working memory, were assessed. In this specific test from a computer-based battery (i.e., the CANTAB), an arrow is displayed on either side of the screen (left or right) and can point in either direction (left or right). Participants must select the left or right button on the screen according to “the side on which the arrow appeared” or the “direction in which the arrow was

pointing", shifting from one request to the other by paying attention to suppress irrelevant stimuli (e.g., arrow appears on the right, but the correct answer is 'left'). A few studies demonstrated that a dual-task training positively improves some aspects of cognition, such as mental flexibility and processing speed.[19, 20] In our study, the positive changes that we observed after a dual-task training in both groups might be explained by a better functioning of PD patients to focus on the required task, to process parallel information at multiple levels, and to inhibit irrelevant information. In our previous study,[14] we observed improvements in motor performance in both groups but with substantial changes especially in the DUAL-TASK + AOT-MI. In the present work, we could not detect a specific effect of AOT-MI on cognition. Such a finding could be related to the type or the duration of training, which may be more specific to induce motor changes rather than cognitive improvement. Future studies should further investigate this aspect.

Despite a similar cognitive improvement in the two PD groups after training, distinct brain functional connectivity changes occur according to the rehabilitative protocol. Compared to the other group, DUAL-TASK + AOT-MI patients showed more substantial brain functional changes, with reduced RS-FC in anterior prefrontal regions within the aDMN. In line with previous findings,[14, 21] in the DUAL-TASK + AOT-MI group, we observed that such a frontal functional reorganization was associated with better accuracy in set-shifting. Reduced connectivity in brain frontal areas, specifically in anterior prefrontal regions, can be explained as a patient's more efficient and optimal motor control, together with lower reliance on attentive resources.[7] In fact, due to the loss of automaticity and motor control typical of PD patients, the activation of frontal areas is generally increased in dual-task or complex situations for monitoring needs.[22, 23] Previous findings demonstrated that the combination of AOT and MI might compensate for decreased automaticity and restore motor function, therefore reducing the need of attentive control performed by frontal regions in more complex conditions.[21] Thus, we can hypothesize that this type of training might improve executive functioning and eventually reduce the attentive overload.

Furthermore, compared to the DUAL-TASK group, DUAL-TASK + AOT-MI patients showed decreased RS-FC of the precuneus within the ECN and the Precuneus networks. This finding is in line with previous results, which showed reduced RS-FC of these circuits in association with motor and cognitive improvements after AOT training in multiple sclerosis patients.[24] Specifically, the precuneus belongs to the medial prefrontal-middle parietal neural network (which partially overlaps with the MNS) and has connections with lateral parietal regions and the supplementary motor area.[25] The anterior portions of the precuneus have been linked to mental and visuo-spatial imagery,[26] in particular in setting-up spatial attributes and in the generation of spatial information for imagined movements.[27] Furthermore, a possible role of the precuneus in internally guided attention and manipulation of mental images, which occurs also during MI practice, has been observed.[28] We observed a substantial reduced RS-FC of this brain region after AOT-MI training, which has been observed in previous functional imaging studies in healthy subjects when they were required to actually execute goal-directed actions.[28, 29]

Finally, compared to the DUAL-TASK group, the DUAL-TASK + AOT-MI patients showed, after training, increased RS-FC of the anterior prefrontal and superior temporal cortices within the aSAL. Previous findings reported that, in highly demanding cognitive situations, an anti-correlated coupling occurs between the SAL and the DMN; while the first RS-FC network is activated, the latter shows the opposite pattern, with reduced activity.[30] These patterns of activation have been associated with optimal cognitive performance in healthy subjects;[30] thus, we suppose that AOT can boost executive functioning skills in our patients by training them focusing on relevant salient stimuli, therefore reducing the attentional control performed by anterior prefrontal brain regions.

On the contrary, we observed only a few brain functional connectivity changes after training in the DUAL-TASK group, specifically in extra-motor areas of the visuospatial network, which are associated with sensorimotor integration and are usually hyperactivated in dual-task situations.[31] Even though DUAL-TASK group patients improved over time in attentive-executive tasks as well

as the DUAL-TASK + AOT-MI group, their functional reorganization occurred in a single network only, therefore suggesting a lower grade of training generalization for other motor and cognitive functions.

The present study has some limitations: first, the patient sample is small, mainly when groups were split according to different trainings, thus reducing the statistical power of our analyses. However, our patient groups were well characterized and similar at baseline in terms of socio-demographic, clinical (i.e., disease duration, disease staging and motor severity), neuropsychological and RS-FC profiles. In addition, only few cases had an MCI status and, compared to healthy controls, our patients showed only slightly worse cognitive performance. Concerning the small sample size, we must acknowledge that this study reflects a secondary analysis of an already published paper [14] in which the primary outcome was the dual-task gait performance. The sample size was calculated on that primary outcome and not on brain RS-FC changes. Second, we did not compare the RS-FC of patients with that of a group of healthy controls; for this reason, we were unable to establish whether (and how much) the RS-FC of patients was different from controls at baseline and whether these potential differences reduced after training. Third, we did not test several (other) aspects of the training, such as different total duration or week frequency, which are relevant for a comprehensive definition of the intervention. Furthermore, longer training periods and/or follow-up observations are needed to verify whether motor learning approaches, such as AOT and MI, have long-lasting effects, which are crucial in these patient cohorts. Finally, since we did not collect genetic data from our patients, we cannot exclude a genetic modulation of the observed changes.

To conclude, in our study, we observed that both DUAL-TASK and DUAL-TASK + AOT-MI promote cognitive improvement and brain functional reorganization processes. Dual-task gait/balance training + AOT-MI could be useful for obtaining functional reorganization of brain areas involved in motor control and executive-attentive abilities.

Statements and Declarations

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Declaration of interests

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Ethical standards

This study has been approved by the local ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.
All persons gave their informed consent prior to their inclusion in the study.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Table 1. Sociodemographic and clinical variables of PD-PIGD patients and heathy controls at baseline.

Socio-demographic variables	**HC**	**All PD-PIGD**	***p* all PD-PIGD *vs* HC**	**DUAL-TASK group**	**DUAL-TASK+ AOT-MI group**	***p* PD DUAL-TASK *vs* PD DUAL-TASK+AOT-MI**
N	23	21	-	10	11	-
Age [years]	63.75 ± 8.69 (52.05-80.08)	66.09 ± 8.31 (48.07-82.71)	0.32	63.43 ± 9.95 (48.07-79.44)	68.50 ± 5.95 (60.42-82.71)	0.12
Sex [M/F]	10/13	12/9	0.37	6/4	6/5	0.57
Education [years]	12.14 ± 3.80 (5-18)	11.48 ± 4.76 (5.00-20.00)	0.62	11.20 ± 5.24 (5.00-20.00)	11.72 ± 4.52 (5.00-17.00)	0.83
PD duration [years]	-	8.19 ± 4.09 (2.00-16.00)	-	8.10 ± 3.84 (2.00-13.00)	8.27 ± 4.50 (2.00-16.00)	0.91
LEDD [mg]	-	675.71 ± 414.94 (76.00-1867.00)	-	549.60 ± 232.26 (204.00-901.00)	790.36 ± 515.20 (76.00-1867.00)	0.24
H&Y [ON state]	-	2.36 ± 0.39 (2.00-3.00)	-	2.30 ± 0.35 (2.00-3.00)	2.41 ± 0.43 (2.00-3.00)	0.59
H&Y [OFF state]	-	2.43 ± 0.39 (2.00-3.00)	-	2.40 ±0.39 (2.00-3.00)	2.45 ± 0.41 (2.00-3.00)	0.76
UPDRS-II	-	12.05 ± 5.34	-	12.90 ± 5.62	11.27 ± 5.22	0.48

		(4.00-24.00)		(7.00-24.00)	(4.00-20.00)	
UPDRS-III [ON state]	-	29.21 ± 8.71 (13.00-51.00)	-	30.20 ± 8.35 (13.00-41.00)	28.32 ± 9.33 (14.00-51.00)	0.36
UPDRS-III [OFF state]	-	36.71 ± 11.22 (16.00-62.00)	-	35.60 ± 11.21 (16.00-56.00)	37.73 ± 11.67 (23.00-62.00)	0.77
MCI [yes/no]	-	7/14	-	3/7	4/7	1.00

Values are mean ± standard deviation in the first row, and minimum-maximum values in the second row. Categorical variables are reported as frequency. *p* values refer to Mann-Whitney U test_or Fisher's exact test for categorical variables. ***Abbreviations:*** *AOT-MI=action observation and motor-imagery; HC=healthy controls; H&Y=Hoehn and Yahr score; LEDD=levodopa equivalent daily dose; MCI=Mild Cognitive Impairment; M/F=male/female; mg=milligrams; N=number; PD=Parkinson's Disease; PIGD=postural instability and gait disorders; UPDRS=Unified Parkinson's Disease Rating Scale.*

Table 2. CANTAB performances of PD-PIGD patients at baseline (T0) and changes over time after dual-task training (T0-W6).

		DUAL-TASK group (N=10)			**DUAL-TASK + AOT-MI group (N=11)**			**DUAL-TASK + AOT-MI group *vs* DUAL-TASK group**	
		Mean ± SD	**Median (1^{st};3^{rd} quartiles)**	**p* T0-W6**	**Mean ± SD**	**Median (1^{st};3^{rd} quartiles)**	**p* T0-W6**	**p# T0**	**p^ T0-W6**
AST, percent total correct trials [%]	T0	82.62 ± 13.22	85.31 (79.06; 90.93)	**<0.001**	83.18 ± 14.78	87.50 (75.93; 94.37)	0.16	0.57	1.00
	Delta T0-W6	-6.44 ± 3.02	-6.87 (-7.97;-5.15)		-4.62 ± 6.43	-1.25 (-6.87; -0.15)			
AST, percent total correct trials (simple condition) [%]	T0	87.50 ± 12.33	90.62 (82.81; 95.93)	0.056	94.62 ± 5.03	95.00 (92.81; 98.44)	1.00	0.29	0.65
	Delta T0-W6	-4.62 ± 5.40	-3.12 (-8.43; 0.00)		-0.97 ± 7.94	1.25 (-5; 2.5)			
AST, percent total correct trials (set-shifting condition) [%]	T0	82.08 ± 6.28	80.00 (77.50; 87.50)	**<0.001**	73.98 ± 22.04	80.00 (59.37; 90.62)	**0.04**	0.88	1.00
	Delta T0-W6	-8.33 ± 3.06	-10.00 (-10.00;-6.25)		-6.5 ± 6.86	-4.37 (-8.43;-2.5)			
AST, mean response latency (simple condition) [msec]	T0	820.90 ± 172.12	821.49 (671.77; 898.94)	1.00	720.77 ± 140.43	673.24 (650.78; 761.51)	0.07	0.26	0.22
	Delta T0-W6	62.67 ± 152.38	59.16 (1.86; 133.67)		-47.45 ± 67.61	-43.35 (-75.09;-6.51)			
AST, mean response latency (set-shifting condition) [msec]	T0	908.81 ± 212.74	857.08 (735.04;1018.99)	1.00	813.24 ± 188.78	800.56 (653.57; 876.87)	0.69	0.48	0.37
	Delta T0-W6	80.85 ± 185.38	93.50 (7.44; 159.50)		-49.18 ± 161.38	-59.61 (-135.72; 43.48)			
MOT, mean response latency [msec]	T0	789.52 ± 202.47	735.50 (637.92; 946.40)	0.77	707.42 ± 126.14	708.40 (614.90; 775.00)	0.69	0.60	0.94
	Delta T0-W6	-21.42 ± 236.85	18.80 (-129.90; 96.87)		-31.13 ± 188.21	6.55 (-95.82; 109.72)			

OTS, first choice [errors]	T0	5.20 ± 3.01	6.00 (3.00; 7.75)	0.21	4.83 ± 3.29	4.00 (3.50; 7.25)	0.23	0.59	0.057
	Delta T0-W6	1.30 ± 2.71	0.50 (0.00; 3.50)		-1.54 ± 3.36	-2.00 (-2.50; 0.00)			
SRM, percent total correct trials [%]	T0	72.00 ± 13.16	77.50 (65.00; 80.00)	0.24	67.27 ± 12.91	70.00 (60.00; 72.50)	0.39	0.27	0.95
	Delta T0-W6	4.44 ± 14.02	10.00 (-10.00; 10.00)		5.00 ± 22.11	10.00 (-2.50; 13.75)			
SRM, mean response latency [msec]	T0	2986.64 ± 846.50	2741.28 (2634.61; 3190.03)	0.93	2757.01 ± 855.96	2650.98 (2005.47; 3368.30)	0.06	0.72	0.12
	Delta T0-W6	32.79 ± 1247.41	-285.86 (-478.93; 645.73)		5.00 ± 22.11	10.00 (-2.50; 13.75)			
SWM [total errors]	T0	24.60 ± 6.40	23.50 (22.00; 28.75)	1.00	18.00 ± 11.48	22.00 (9.50; 26.50)	0.06	0.40	1.00
	Delta T0-W6	-1.66 ± 8.88	1.00 (-3.00; 4.00)		-3.80 ± 6.26	-5.00 (-7.50;-2.25)			
SWM, strategy [accuracy score]	T0	18.20 ± 2.30	18.50 (16.50; 19.75)	0.68	17.09 ± 3.59	17.00 (15.00; 20.00)	0.09	0.70	1.00
	Delta T0-W6	-2.44 ± 6.19	-0.00 (-2.00; 1.00)		-2.10 ± 2.02	-2.50 (-3.75;-0.25)			

Values are mean ± standard deviation in the first row and median (first quartile; third quartile) in the second row. All the variables at T0 were compared between groups using the Mann-Whitney U test (p#). Longitudinal changes (T0-W6) were assessed in both PD groups using linear mixed-effects models (p*). Using the same models, a group-by-time interaction was assessed to evaluate longitudinal between-group differences (T0-W6) (p^). Statistical significance was accepted for values of p <0.05, Bonferroni-corrected for multiple comparisons. Bold faced values refer to significant p values < 0.05. ***Abbreviations:*** *AOT-MI= Action Observation Training-Motor Imagery; AST= Attention Switching Task; CANTAB= Cambridge Neuropsychological Test Automated Battery; MOT= Motor Screening Task; msec=milliseconds; OTS= One Touch Stockings of Cambridge; SD=standard deviation; SRM= Spatial Recognition Memory; SWM= Spatial Working Memory.*

Table 3. Significant RS-FC differences between and within groups over time, and relationships between RS-FC and CANTAB changes after training.

	RSN	Side	Brain regions (BA areas)	MNI coordinates	N of voxels	Intensity (Index)
Within-groups changes						
DUAL-TASK + AOT-MI						
Increased RS-FC	aSAL	L	Anterior PFC (BA10)	x -26; y 58; z 12	10	5.78
		L	Superior temporal gyrus (BA22)	x -58; y -26; z 0	5	5.78
Reduced RS-FC	aDMN	R	Anterior PFC (BA10)	x 2; y 70; z 4	1	4.47
		R	Anterior PFC (BA10)	x 18; y 70; z -4	1	4.87
	Right ECN	L	Precuneus (BA7)	x -6; y -74; z 52	7	5.04
	Precuneus Network	L	Precuneus (BA7)	x -2; y -62; z 56	14	4.58
DUAL-TASK						
Increased RS-FC	Visuospatial Network	R	Superior parietal gyrus (BA7)	x 26; y -62; z 56	3	4.53
Group x Time interaction						
DUAL-TASK + AOT-MI > DUAL-TASK	aSAL	L	Anterior PFC (BA10)	x -26; y 58; z 12	9	6.24
DUAL-TASK + AOT-MI < DUAL-TASK	aDMN	R	Anterior PFC (BA10)	x 2; y 74; z 4	6	4.05
		R	Anterior PFC (BA10)	x 6; y 70; z -8	3	4.71
		R	Anterior PFC (BA10)	x 18; y 70; z -4	1	4.4
Relationships between RS-FC and CANTAB changes after training						
DUAL-TASK + AOT-MI						
Reduced RS-FC *vs* better accuracy in AST, total correct trials in set-shifting condition	aDMN	R	Anterior PFC (BA10)	x 2; y 74; z 8	2	2.98

Coordinates (x, y, z) are in Montreal Neurological Institute (MNI) space. Results are shown at $p<0.05$ FWE corrected for multiple comparisons.

Abbreviations: *AOT-MI=action observation and motor-imagery; BA=Brodmann area; aDMN=Anterior Default Mode Network; aSAL=Anterior Salience Network; AST=Attention Switching Task; CANTAB=Cambridge Neuropsychological Test Automated Battery; ECN=Executive Control Network; L=left; MNI=Montreal Neurological Institute; N=number; PFC=Prefrontal Cortex; R=right; RS-FC=resting-state functional connectivity; RSN=resting-state network.*

FIGURE LEGEND

Figure 1. (a) Within-group resting state functional connectivity (RS-FC) changes after training in DUAL-TASK+AOT-MI (upper section) and DUAL-TASK (lower section) groups; **(b)** Between-groups RS-FC changes after training in the DUAL-TASK + AOT-MI > DUAL-TASK group; **(c)** Cognitive-fMRI correlations within the anterior Default Mode Network (aDMN) in the DUAL-TASK + AOT-MI group. Results are overlaid on the Montreal Neurological Institute (MNI) standard brain in neurological convention and displayed at $p<0.05$ Family-wise error corrected for multiple comparisons. Only significant results are reported. Coloured bar represents p values. Blue to green coloured bar reflects increased brain connectivity; red to yellow coloured bar reflects decreased brain connectivity. *Abbreviations:* AST = Attention Switching Task.