

AWARD NUMBER: W81XWH-18-1-0665

TITLE: Investigating Exercise-Induced Neuroplasticity and Its Mechanisms in Parkinson's Disease: Targeting Executive Function and Brain Circuitry

PRINCIPAL INVESTIGATOR: Giselle Petzinger MD

CONTRACTING ORGANIZATION: University of Southern California
Dept. Contracts & Grants
1640 Marengo St., 7th floor, Site 701,
Los Angeles, CA, 90033-9263

REPORT DATE: JANUARY 2024

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland, 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release.
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE JANUARY 2024		2. REPORT TYPE Final Report		3. DATES COVERED 15SEPT2018 - 14SEPT2023	
4. TITLE AND SUBTITLE Investigating Exercise-Induced Neuroplasticity and Its Mechanisms in Parkinson's Disease: Targeting Executive Function and Brain Circuitry				5a. CONTRACT NUMBER W81XWH-18-1-0665	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Giselle Petzinger E-Mail: petzinge@med.usc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Southern California 1640 Marengo St., 7 th floor, Suite 701 Los Angeles, CA 90033-9263				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release, Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Parkinson's disease (PD) is the second most frequent neurodegenerative disorder of old age. A common problem in PD is cognitive impairment. There are no effective treatments. Impairment in executive function (EF) is the most common subtype of cognitive impairment and leads to challenges in daily function, including decision making, multi-tasking, and quality of life. Exercise studies in the aging field and preliminary studies in our PD animal work support the role of high intensity skill practice and high motor fitness in promoting greater EF performance compared to aerobic exercise, and cardiovascular fitness (e.g. V02max). While a wide range of exercise modalities have shown to improve motor performance in PD patients, investigations of the relationship between exercise and EF in PD and mechanisms of neuroplasticity remain a significant gap in knowledge. This application will address this gap through complementary translational studies in humans and animals. The purpose of this 18-month longitudinal clinical study is to examine the association between EF related cognitive performance and fitness levels, specifically cardiovascular and motor fitness, as well as exercise intensity. We hypothesize that high intensity regular exercise as well as High level of Motor fitness will be associated with greater level of cognitive EF performance over the 18-month period, than High levels of Cardiovascular Fitness or low intensity exercise. We also hypothesize that EF related brain circuitry and connectivity will have a greater association with high level motor fitness than cardiovascular fitness and mediate the association between higher level of cognitive EF performance and motor fitness seen at 18 months.					
15. SUBJECT TERMS Executive Function; Parkinson's disease; Motor Fitness; Cardiovascular Fitness; Exercise					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 103	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4-26
4. Impact	28
5. Changes/Problems	30
6. Products	30
7. Participants & Other Collaborating Organizations	33
8. Special Reporting Requirements	34
9. Appendices	34

1. INTRODUCTION:

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder of old age. A common problem in PD is cognitive impairment. There are no effective treatments. Impairment in executive function (EF) is the most common subtype of cognitive impairment and leads to challenges in daily function, including decision making, multi-tasking, and quality of life. Exercise studies in the aging field and preliminary studies in our PD animal work support the role of high intensity skill practice and high motor fitness in promoting greater EF performance compared to aerobic exercise, and cardiovascular fitness (e.g., V02max). While a wide range of exercise modalities have shown to improve motor performance in PD patients, investigations of the relationship between exercise and EF in PD and mechanisms of neuroplasticity remain a significant gap in knowledge. This application addressed this gap through complementary translational studies in humans and animals. The focus of this clinical study application was to investigate the long-term (18 month) association between motor (MF) and cardiovascular (CF) fitness and cognitive performance, focused on EF. This study also examined the association of MF and CF and cognitive circuitry in PD at baseline and 18 months to determine whether strength of connectivity in these cognitive circuits mediates the relationship between high MF and high cognitive performance. Finally, we investigated the role of high-intensity exercise on EF and cognitive circuitry in PD through the use of weekly wearable accelerometer device used every three months and its association with cognitive performance and strength of cognitive circuitry at baseline and 18 months. Complementary animal studies examining the molecular mechanisms, cognitive gains and enhanced cognitive circuitry (blood flow analysis) underlying motor skilled vs aerobic exercise were conducted in parallel with Dr. Holschneider PI Award number W81XWH18-1-0666.

2. KEYWORDS:

Cognitive Function; Circuitry; Parkinson's disease; Motor Fitness; Cardiovascular Fitness; Physical Activity

3. ACCOMPLISHMENTS:

3.1. What were the major goals of the project?

Aim 1: Exercise Intensity and Cognitive Function: This objective tested the hypothesis that exercise intensity (avg METS/hr/wk over 18-months) is significantly associated with EF. We will further test the hypothesis that moderate/high intensity exercise (HIE) versus low intensity exercise (LIE) over 18-months will have significantly greater association with EF at 18 months.

Aim 2: Fitness and Cognitive Function: This objective tested the hypothesis that motor and cardiovascular fitness are differentially associated with EF at 18-months. We also will test whether high compared to low motor fitness level has a significantly stronger association with EF at 18-months. Finally, we examined whether motor fitness mediates the association between exercise intensity and level of EF.

Aim 3: Neuroimaging: MRI was collected to measure EF (cognitive/attention) network activity at baseline and 18 months. This objective tests the hypothesis that changes in brain activity in networks sub-serving EF mediate the association between exercise intensity and EF at 18-months. We will also examine whether changes in brain activity in networks sub- serving EF mediate the association between motor fitness and EF.

3.2. What was accomplished under these goals?

3.2.1. Enrollment and Retention of Study Participants over Covid-19 Pandemic

At USC, 50 participants were enrolled in the study. After a pause due to COVID-19, the study was completed, and a total of 42 participants completed the study, with 8 dropping out of the study (Table 1). Twenty-one of

50 total participants at USC had a baseline MRI, and 19 individuals completed a follow-up MRI at USC (Table 1). The demographic information from these participants is listed in Table 2.

At UCSD/VMRF, 49 participants were enrolled in the study. The total enrollment was stopped at 49 participants due to the COVID-19 pandemic. After a pause due to COVID-19, the study was completed, and a total of 35 participants completed the study, with 13 dropping (Table 1). Thirty of the 49 enrolled participants completed baseline MRI at UCSD, 23 of 49 participants completed a follow-up MRI at UCSD (Table 1). The demographic information from these participants is listed in Table 2.

Due to COVID-19 pandemic and physical closure of clinics, the study was interrupted, thus extending the expected duration of the study. Once, restrictions were lifted we were able to resume our physical interaction with patients and bring them into the clinic to complete the study. Thus, the average time between baseline and follow-up visit for an individual participant was approximately 24 months, 23.63 months. (average of 709 days with SD of 118 days).

Study Drop Out Reasons:

1. Health issues including injury or other illness or DBS implantation (7)
2. Relocation/move (5)
3. Failed physical task or refused to complete a task at second visit (3)
4. Unreachable at follow up (3)
5. No longer interested in participating (3)
6. Passed away (1)

8 USC drop out

3 for #1, 3 for #2, 1 for #3, 1 for #6

14 UCSD drop out

4 for #1, 2 for #2, 2 for #3, 3 for #4, 3 for #5

A total of 99 PD individuals enrolled in the study and 77 remained in the study and had a second follow up visit approximately 2 years later. Covid pandemic extended the length of the study, and we believe effected the ability to retain patients in the study.

Dropout Numbers by Mild Cognitive Impairment (MCI) Status at Baseline. Upon enrollment patients underwent neuropsychological testing. Determination of presence or absence of MCI was conducted through consensus between Drs. Schiehser and Petkus at VAMRF and UCSD, respectively. We noted that of those patients who failed to complete the study, there were a higher proportion of patients with Mild Cognitive Impairment (MCI) at baseline compared to non-MCI patients at baseline that did not complete a follow up evaluation.

	MCI at baseline (50 total)	No MCI at baseline (49 total)
2 Visits	68% (34 individuals)	87.75% (43 individuals)
1 Visit	32% (16 individuals)	12.2% (6 individuals)

Table 1: Study Retention Characteristics.

	Whole Group		Imaging Subset	
	Baseline	Follow-Up	Baseline	Follow-Up
USC	50	42	21	19
UCSD/VMRF	49	35	30	23

USC: University of Southern California.

UCSD/VMRF: University of California San Diego/Veterans Medical Research Foundation

3.2.2. Study Participant Characteristics : At both study sites, cognitive function was evaluated with a thorough neuropsychological battery at (i) baseline (ii) follow-up (approximately 2 years). The average and standard deviation for each period is listed in Table 3.

Table 1: Study Demographic Information listed as Mean (Standard Deviation).

	Baseline	Follow-Up
Age	66.77 (8.70)	
Sex	57 M/42 F	
Years of Education	16.79 (2.05)	
Years of PD Diagnosis	4.78 (4.84)	
UPDRS I-IV	43.89 (16.25)	51.15 (21.14)
UPDRS III (motor)	23.89 (10.52)	27.22 (14.29)
LED	562.29 (384.30)	725.10 (498.73)

UPDRS: Unified Parkinson's disease rating scale; LED: levodopa equivalent dosage (mg)

Table 2: Cognitive Test Scores for study population at baseline and follow up in PD individuals. Scores listed as Mean (Standard Deviation).

	Baseline	Follow-Up
Adaptive Digit Ordering Digit Sequencing Score	6.52 (2.26)	5.75 (2.32)
Adaptive Digit Ordering Digit Forward Score	8.03 (2.05)	8.01 (2.35)
D-KEFS Color Naming Score	33.00 (6.38)	34.91 (8.68)
D-KEFS Word Reading Score	23.96 (5.30)	24.74 (6.05)
D-KEFS Inhibition Score	63.17 (15.65)	65.55 (22.96)
D-KEFS Inhibition/Switching Score	69.19 (32.80)	74.55 (33.64)
D-KEFS Verbal Fluency-Letter Fluency	41.63 (12.50)	44.14 (13.60)
D-KEFS Verbal Fluency-Category Fluency	38.97 (9.60)	38.84 (8.99)
D-KEFS Verbal Fluency-Switching Fluency	13.21 (3.33)	12.90 (3.27)
Boston Naming Test Spontaneous and Semantic Cue Score	56.83 (3.46)	57.12 (2.60)
CVLT-II Total Score Trials 1-5	40.81 (9.96)	40.61 (10.29)
CVLT-II Short Delay Free Recall	7.97 (3.33)	7.60 (3.45)
CVLT-II Long Delay Free Recall	8.14 (3.61)	7.73 (3.90)
Brief Visuospatial Memory Test Trials 1-3 Total Score	16.44 (6.96)	17.42 (7.18)
Brief Visuospatial Memory Test Long-Delay Total Score	7.21 (3.07)	7.51 (3.15)
Judgement of Line Orientation Total Score	23.83 (4.39)	23.82 (4.14)
Hooper Visual Organization Test Total Score	24.67 (3.11)	24.47 (2.85)
Wisconsin Card Sorting Test-64 Perseverative Responses	9.70 (7.57)	8.73 (5.88)

D-KEFS: Delis-Kaplan Executive Function System; CVLT-II: California Verbal Learning Test-II

3.2.3 Aims and Results :

Aim 1: Exercise Intensity and Cognitive Function

Study participants were provided hip-worn accelerometer devices to wear over the course of one week and throughout the duration of the study, approximately every 3-4 months. Average minutes/day spent in either (i) light physical activity (ii) moderate to vigorous intensity physical activity or (iii) sedentary behavior were calculated from established cut points for accelerometers and level of physical activity in older adults. Baseline and last follow up time point for these outcome measures are listed in Table 3.

Table 3: Data on amount of physical activity of various intensities derived from actigraph devices at baseline and follow up (2 years) and average over 2 years in PD individuals. Data are minutes per day, listed as mean (standard deviation), unless stated.

	Baseline min/day	Follow-Up min/day	2-year Avg min/wk
Sedentary Time	591.23 (91.75)	596.43 (95.87)	4154 mins/wk (584.8)
Light Physical Activity	266.91 (91.51)	250.96 (90.23)	1791 mins/wk (571.8)
Moderate to Vigorous Physical Activity	17.74 (15.06)	15.59 (17.35)	119 mins/wk (109.3)

1A. Cross-Sectional Baseline Findings Physical Activity:

Participants wore an accelerometer for seven days during waking hours and data was aggregated to estimate minutes spent in either (i) Moderate to Vigorous or (ii) Light intensity physical activity. Participants also completed a seven-day exercise log to record daily exercise habits including start and stop time of types of exercise (e.g walking, running, yoga). From the log, exercise parameters of interest were derived including (i) duration (average mins/day) 62.63 mins/day (SD = 34.58) (ii) frequency (total number of exercise sessions/week) 8.70 exercise sessions per week (SD = 4.81); and (iii) variety (different types of exercise/week) 3.26 different types of exercise per week (SD = 1.65).

Overall findings, after controlling for age, sex MDS-UPRDSR motor score and self-reported exercise duration, there was a significant association of time spent in moderate to vigorous intensity physical activity and EF, Memory, visual spatial function, and global cognition (MOCA) (see Table 4). Findings support that intensity of PA is more important than duration of exercise in association with cognitive performance in individuals with PD. **The overall findings** were (i) greater time spent in moderate to vigorous physical activity at baseline is significantly associated with better baseline cognitive performance including executive function, memory, and the global function, using the Montreal Cognitive Assessment (MoCA). See Table 5. There were no significant associations between light intensity physical activity and sedentary behavior and cognitive performance in individuals with PD.

Table 4: Cross-sectional associations between daily minutes of moderate to vigorous physical activity and baseline cognitive performance. Estimates in bold denote $p < .05$.

	β	95% Confidence Interval Lower, Upper	p
MoCA	.040	.003, .076	.034
Executive Function	.017	.003, .030	.014
Memory	.014	.002, .026	.021
Visuospatial Function	.020	.006, .034	.005
Attention	.011	-.004, .026	.143
Language	.014	.000, .028	.056

1.B. Longitudinal Findings Physical Activity:

Participants wore an accelerometer for seven days during waking hours and physical activity data was aggregated to estimate minutes spent in either (i) Moderate to Vigorous or (ii) Light intensity physical activity or (iii) sedentary behavior. Patients wore the accelerometers approximately every 3-4 month over the course of two years.

For this analysis an overall change in physical activity over the two years was compared to the overall change in cognitive function including EF, memory, language, attention, and visual spatial function (judgement of line and Hooper Orientation of line). The statistical analysis included calculating the slope of change in time spent (i) moderate to vigorous intensity physical activity (ii) light intensity physical activity and (iii)

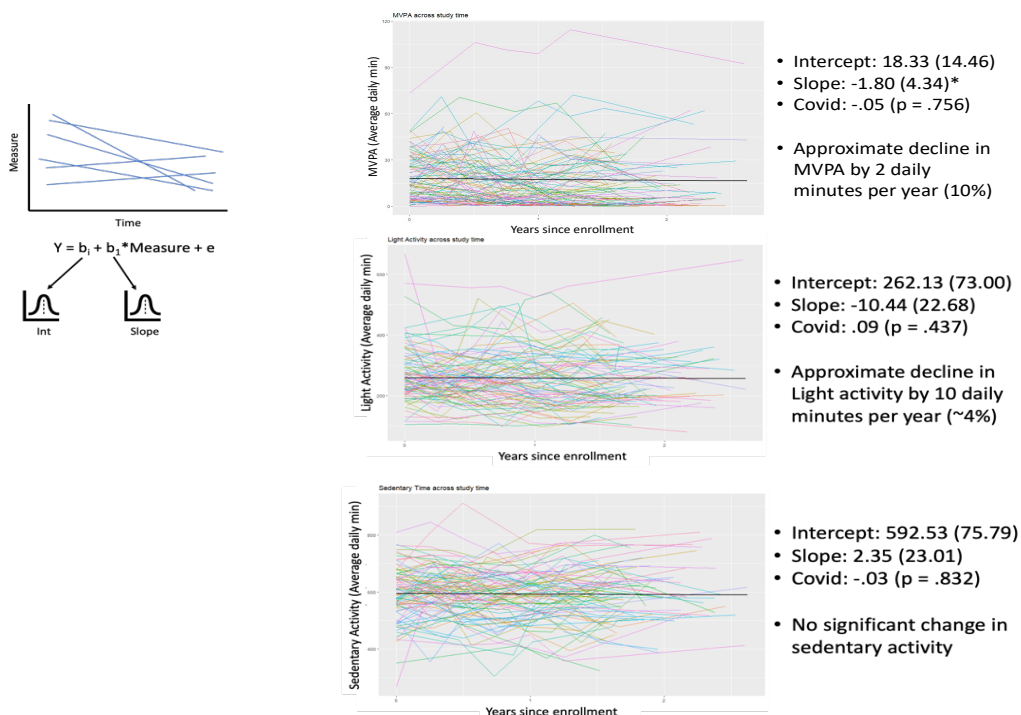
sedentary behavior over the study period for each study participant using a linear estimation model. This calculation provided a slope, , called bslope, and an intercept (extrapolated baseline), called bint. To compare change in physical activity with overall change in cognitive performance, a latent change in cognitive performance was calculated for EF, memory, attention, and language. Visual performance function only included two variables which were not enough parameters to calculate a latent change, and so were examined separately using average at baseline and follow up and the determined change. Change in PA was compared with change in cognitive performance. Co-variates included age, education, gender, UPDRS-motor, study site and covid-pandemic. All study participants were included in the analysis.

Overall findings, we found a 10% decline in time spent in Moderate to Vigorous (MVPA) and 4% decline in Light Intensity Physical Activity per year. We found no significant change in time spent in Sedentary Activity. Regarding cognitive change, we found a significant decline in Executive Function and a trend towards a decline in Attention over 2 years. There were no significant changes in memory, language, or visual spatial function (JLO, HVOT) over 2 years.

We found no significant association between change in MVPA and Cognitive Function over 2 years with any cognitive domains. We found no significant association between light intensity PA or sedentary behavior and any cognitive function domains. Possible limitations impacting our results may include the high dropout rate, the loss of MCI patients over time (therefore patients were more cognitively intact), and the small decline in cognitive performance in our PD population over 2 years.

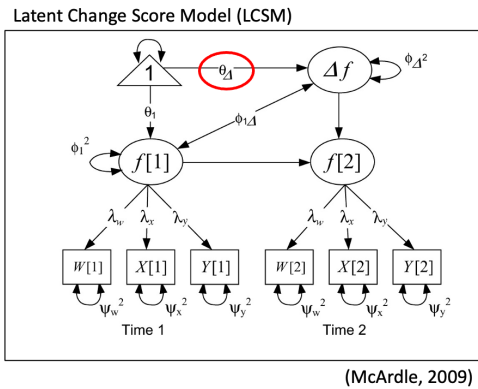
- (i) Longitudinal Parameterization of Physical Activity: To calculate a change in time spent in different levels of physical activity over 2 years an individual linear estimation model was used. The intercept represents the extrapolated avg. baseline value of time spent in physical activity and the slope represents change (in minutes) over 2 years. COVID-19 pandemic lockdown was included in the model and did not significantly impact any physical activity slopes.

Individual colored lines represents time spent in level PA of one study participant over the course of 2 years.



(ii) Parameterization of Cognitive Change

Calculation of Latent Change in Cognitive Performance for EF, attention, Memory, and Language domains



Steps

1. Construct latent variables at time 1 and time 2
2. Check for measurement invariance across time (metric)
3. Estimate latent change

Latent difference scores means and variances are presented in **Table 5**. Significant latent change was observed for executive function such that follow-up latent scores were 0.21 standard units lower than baseline executive function scores ($p = 0.033$). Point estimates of latent change across the other cognitive domains were not significant (language: $\Delta_{\text{cog}} = 0.12$, $p = 0.145$; memory: $\Delta_{\text{cog}} = 0.003$, $p = 0.938$), however a trend in change was detected with attention ($\Delta_{\text{cog}} = -0.69$, $p = 0.079$). Sample difference scores for the Hooper Visual Organization Test and Judgment of Line Orientation Test were -0.06 and -0.01 standard units across the study period respectively.

Table 5 : Change in Cognitive Function

Model	Mean Change		Change Variance	
	Est.	95% CI	Est.	95% CI
Language	0.12	[-0.04, 0.29]	0.04	[-0.12, 0.19]
Attention	-0.69 ⁺	[-1.45, 0.08]	0.30	[-0.10, 0.70]
Memory	0.003	[-0.06, -0.08]	0.06*	[0.01, 0.11]
Executive Function	-0.21*	[-0.40, -0.02]	0.24	[-0.07, 0.54]
Hooper Visual Organization Test	-0.06	-	-	-
Judgment of Line Orientation Test	-0.01	-	-	-

Note: Change in Hooper Visual Organization Test and Judgement of Line Orientation Test were calculated by subtracting subject Z-scores of each test at time 1 from Z-scores at time-2. Scores were then normalized relative to the number of years between test administrations.

*** $p < .001$, ** $p < .01$, * $p < .05$, + = trend

(iii) Results: Panels (Upper, Middle, and Lower)

Upper Panel: No significant association between change MVPA (intercepts and slopes) and change in cognition. **Middle Panel:** No significant association between change in Light PA (intercepts and slopes) and change in cognition. **Lower Panel:** No significant association between change in sedentary behavior (intercepts and slopes) and change in cognition. Higher sedentary baseline predicts less decline in one visuospatial subtest.

Legend: bint = extrapolated baseline, bslope = slope or change. A positive bint, bslope = positive association with cognitive performance. dark orange (P = 0.05-0.096), not significant, trends; yellow (P = 0.10 to 0.13) not significant. green (p = 0.015 to

MVPA																
	Base Model						Core Covariates						Full Model			
	bint	95% CI	p-val	bslope	95% CI	p-val	bint	95% CI	p-val	bslope	95% CI	p-val	bint	p-val	bslope	p-val
Language	0.05	[-.02, .11]	0.156	0.26	[-.15, .67]	0.209	0.04	[-.03, .11]	0.318	0.15	[-.27, .56]	0.482	0.04	0.309	0.19	0.392
Executive	0.09	[-.00, .18]	0.057	0.09	[-.46, .64]	0.741	0.03	[-.05, .11]	0.526	-0.07	[-.69, .54]	0.814	0.03	0.483	0.024	0.939
Attention	-0.05	[-.41, .31]	0.787	1.27	[-1.19, 3.7]	0.313	-0.16	[-.53, .20]	0.387	0.26	[-2.33, 2.86]	0.843	-0.156	0.389	0.54	0.669
Memory	-0.03	[-.13, .07]	0.602	-0.45	[-1.01, .09]	0.102	-0.06	[-.17, .06]	0.331	-0.46	[-1.06, .13]	0.127	-0.06	0.318	-0.51	0.11
VOT	-0.11	[-.38, .17]	0.434	1.04	[-.40, 2.47]	0.158	-0.26	[-.59, .07]	0.127	1.29	[-.38, 2.96]	0.13	-0.25	0.147	1.62	0.07
JLO	-0.26	[-.79, .27]	0.329	1.8	[-1.43, 5.03]	0.274	-0.03	[-.60, .53]	0.906	1.2	[-1.83, 4.23]	0.438	-0.05	0.86	0.08	0.958
MoCA	-0.19	[-.58, .20]	0.34	1.14	[-.78, 3.07]	0.249	-0.28	[-.74, .17]	0.224	1.07	[-1.03, 3.16]	0.319	-0.3	0.202	0.82	0.417
No Covariates						Age, Sex, Education, and UPDRS						COVID, Site				

Light																
	Base Model						Core Covariates						Full Model			
	bint	95% CI	p-val	bslope	95% CI	p-val	bint	95% CI	p-val	bslope	95% CI	p-val	bint	p-val	bslope	p-val
Language	0.01	[-.07, .09]	0.823	0.45	[-.08, .90]	0.096	-0.04	[-.13, .06]	0.41	0.44	[-.18, 1.06]	0.162	-0.04	0.397	0.43	0.164
Executive	0.03	[-.13, .19]	0.707	0.33	[-.46, 1.12]	0.417	-0.09	[-.29, .12]	0.398	0.21	[-.69, 1.12]	0.643	-0.09	0.367	0.2	0.649
Attention	0.32	[-.35, .98]	0.35	-0.46	[-3.49, 2.57]	0.766	-0.04	[-.75, .67]	0.915	-0.76	[-3.71, 2.19]	0.613	-0.05	0.896	-0.82	0.579
Memory	-0.02	[-.16, .12]	0.795	0.07	[-.55, .68]	0.836	-0.03	[-.19, .12]	0.68	0.07	[-.60, .74]	0.841	-0.03	0.684	0.07	0.844
VOT	-0.05	[-.50, .41]	0.848	0.91	[-.83, 2.65]	0.305	0.01	[-.47, .48]	0.911	0.47	[-1.42, 2.35]	0.627	0.02	0.951	0.46	0.627
JLO	-0.19	[-.67, .29]	0.441	-0.27	[-3.85, 3.31]	0.882	-0.11	[-.82, .60]	0.765	0.17	[-3.44, 3.77]	0.928	-0.18	0.62	0.26	0.882
MoCA	0.16	[-.35, .67]	0.53	0.42	[-1.58, 2.42]	0.683	0.03	[-.56, .61]	0.925	0.55	[-1.68, 2.79]	0.628	0.02	0.955	0.55	0.642
No Covariates						Age, Sex, Education, and UPDRS						COVID, Site				

Sedentary																
	Base Model						Core Covariates						Full Model			
	bint	95% CI	p-val	bslope	95% CI	p-val	bint	95% CI	p-val	bslope	95% CI	p-val	bint	p-val	bslope	p-val
Language	0	[-.08, .09]	0.983	-0.45	[-1.06, .16]	0.151	0.04	[-.04, .12]	0.307	-0.35	[-.91, .21]	0.224	0.04	0.301	-0.34	0.232
Executive	0.04	[-.11, .19]	0.628	-0.59	[-1.41, .24]	0.163	0.14	[-.01, .30]	0.074	-0.38	[-1.14, .39]	0.334	0.15	0.061	-0.33	0.385
Attention	-0.23	[-.77, .31]	0.396	0.4	[-2.17, 2.96]	0.763	0.05	[-.50, .59]	0.862	1.08	[-1.33, 3.49]	0.38	0.06	0.825	1.22	0.303
Memory	-0.02	[-.13, .10]	0.77	0.12	[-.46, .70]	0.685	-0.02	[-.13, .09]	0.768	0.09	[-.46, .65]	0.744	-0.02	0.767	0.09	0.746
VOT	-0.06	[-.49, .38]	0.802	0.14	[-1.75, 2.03]	0.886	-0.03	[-.50, .43]	0.893	0.61	[-1.51, 2.74]	0.572	-0.04	0.885	0.69	0.521
JLO	0.73	[.19, 1.28]	0.008	0.49	[-3.16, 4.14]	0.793	0.87	[.17, 1.58]	0.015	1.31	[-2.45, 5.07]	0.495	0.89	0.015	0.91	0.612
MoCA	-0.11	[-.66, .45]	0.713	-0.01	[-1.62, 1.89]	0.989	-0.04	[-.62, .54]	0.889	-0.12	[-2.03, 1.79]	0.904	-0.02	0.95	-0.19	0.858
No Covariates						Age, Sex, Education, and UPDRS						COVID, Site				

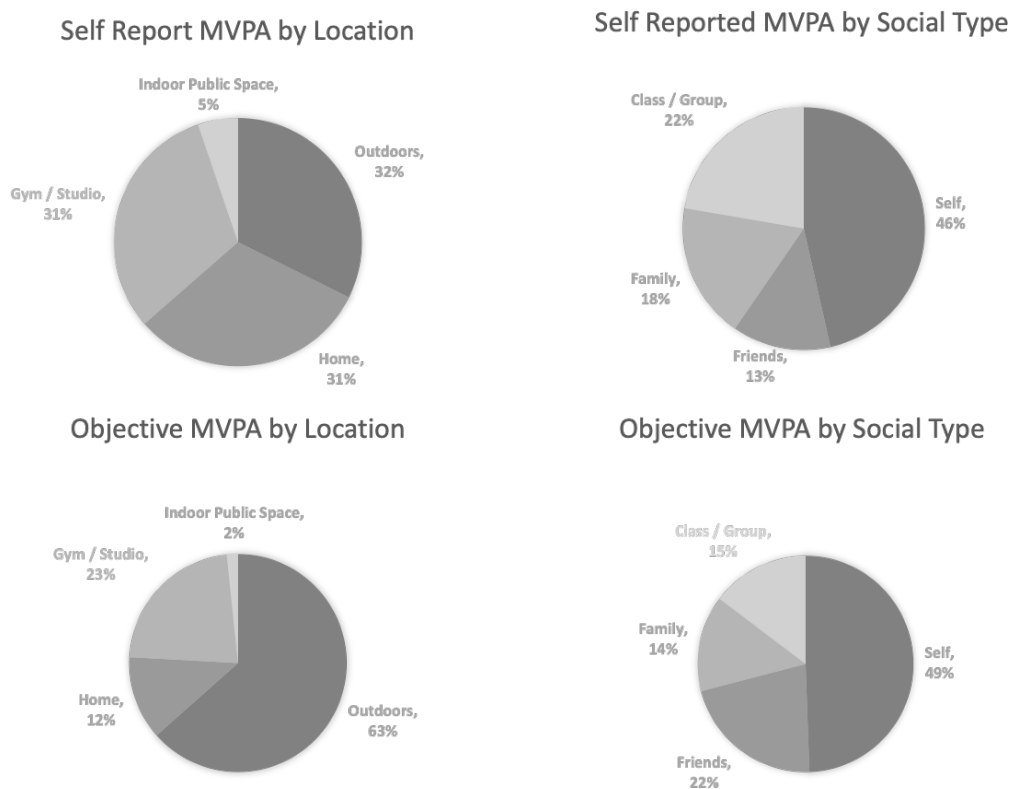
0.008) , significant at P < 0.05

1.C. Characteristics of time spent in Moderate to vigorous Physical Activity Intensity in PD :

The goal of this study was to determine where participants spent time conducting physical activity of moderate to vigorous physical intensity. We also examined with whom patients spent time in moderate to vigorous physical activity. Participants wore an accelerometer and completed exercise logs for seven days. For the seven-day exercise logs, participants were asked to record daily exercise habits including (i) start and stop time of exercise (e.g walking, running, yoga), and (ii) exercise location (e.g. home, gym, outdoors) and (iii) with whom they were exercising(e.g. class, family, self). Moderate to vigorous physical activity was captured (i) subjectively (self-rating) and (ii) objectively-accelerometers. First, participants were asked to subjectively **self-rate** how hard (e.g moderate or light intensity) they were exercising each time they were undergoing an exercise routine and for how long. Second, accelerometer data was used to capture objective levels of physical activity (moderate to vigorous) through established cut points of moderate to vigorous PA and using their exercise logs (start and stop time of each exercise routine), to estimate how many minutes of their actual exercise was spent in moderate to vigorous intensity physical activity.

Overall findings were that study participants exercised at a moderate intensity of exercise primarily in an (i) (where) outdoor setting (32 to 63%) and (ii) (with whom) when exercising by themselves (46-49%) These findings were particularly evident with the objective accelerometer data. See Figure A . We also found that compared to objective estimates of time spent in moderate to vigorous physical activity (light and medium grey bars) using accelerometers, PD individuals self-report frequently overestimated how much time they were working at a moderate intensity level (dark grey) during various exercise types. See Figure B.

Figure A



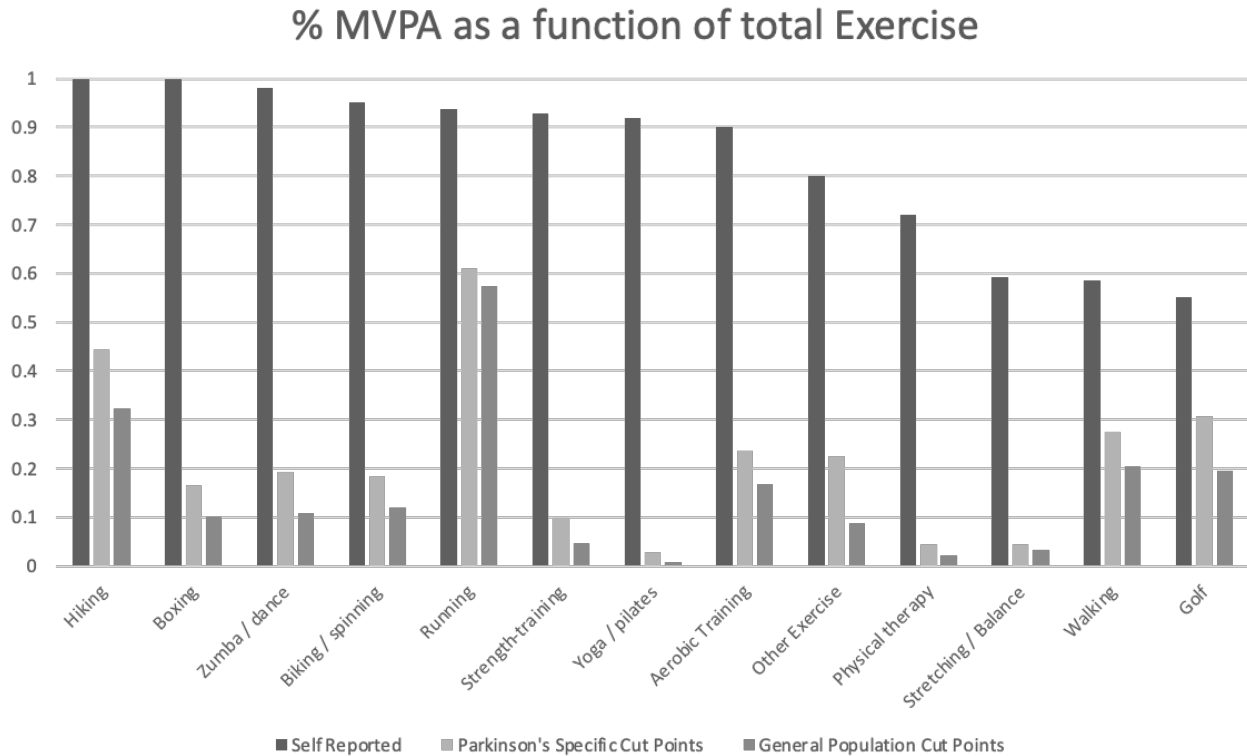
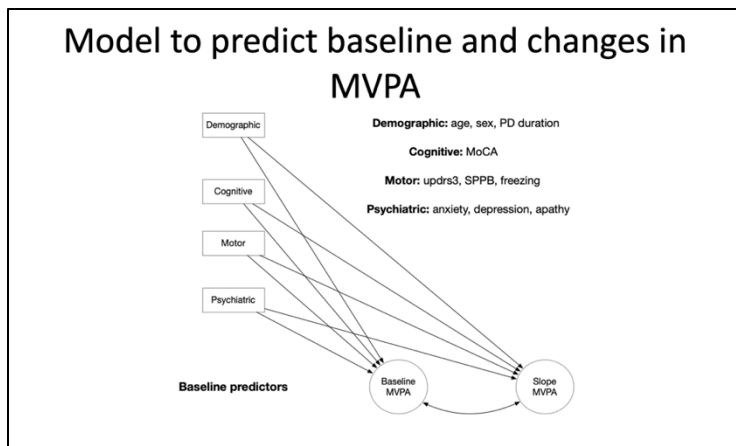


Figure B: 1 = 100% of exercise routine.

1.D. Predictors of time spent in moderate to vigorous physical activity in PD:

The goal of this study was to determine baseline predictors of time spent in moderate to vigorous physical activity (i) at baseline and (ii) over the 2 year study (longitudinal). Findings from this analysis may help identify interventions to help increase physical activity, particularly, moderate to vigorous intensity level in individuals with PD. Baseline measures included age, sex, UPDRS motor score, Freezing of gait, Motor performance level (short physical performance test), cognitive function (MOCA) and Psychiatric battery (Geriatric Depression scale, GDS, General Anxiety Scale, GAI and Apathy scale). Analysis included multiple linear regression including baseline parameters and association with estimated baseline and slope of MVPA (over time, 2 years).



Overall Findings: Individuals with PD, baseline features of being female, and having better cognitive performance (MOCA), better UPDRS motor score and motor performance, less freezing, less apathy and more anxiety predicted more time spent in MVPA at baseline. We also found that in individuals with PD, being male, having less motor symptoms, better cognitive performance, as well as less anxiety and more apathy at baseline predicted less

decline in time spent in moderate to vigorous physical activity over a two-year period. Covid pandemic, age, PD duration and depression did not predict decline in time spent in MVPA. **Our overall findings** suggest that gender, and better motor and cognitive performance were the strongest predictors of time spent in MVPA at baseline and overtime (two years). Anxiety and apathy had mixed effects on predicting MVPA.

1.C. Effect of Structured Exercise Parameters on Cognitive Change in Parkinson's Disease

Though exercise is noted to improve PD-cognition, little is known about how specific parameters of exercise, including duration, frequency, and variety impact cognitive function in PD. The purpose of this study was to identify the association between exercise (i) duration, (ii) frequency and (iii) variety in our PD cohort and cognitive performance and to examine the association between changes in these exercise parameters over time and cognitive decline individuals with PD. Structured exercise parameters were collected by a seven-day daily exercise log that was completed by study participants every three to five months throughout the study period. From the log, exercise parameters of interest were derived including (i) duration, (ii) frequency and (iii) variety. All exercise logs were collected, and parameters extracted throughout the 2 years study for each individual participant. .

To examine change in these parameters over time, a Multi-level structural equation models (SEMs) and multiple-indicator latent difference score SEMs were used to characterize individual trajectories of structured exercise and latent cognitive change scores respectively across the study period. Variation in exercise trajectory intercepts and slopes were then used to predict latent different scores for cognition. Seventy-seven individuals of the original ninety-nine enrolled individuals with mild to moderate PD completed a comprehensive neuropsychological battery assessing cognitive function at baseline and two years.

Results: Participants (N=99) were on average 66.77 (SD = 8.70) years old at baseline, mostly male (58% male), with 16.79 years of education (SD = 2.05), and an average MDS-UPDRS motor (part III) score of 23.89 (SD = 10.52) (Table 1). Participants had a baseline average duration of 428.69 minutes per week (SD = 246.13), frequency of 8.52 sessions per week (SD = 4.91) , and a variety of 3.22 types per week (SD = 1.67) (Table 2). At follow-up (mean days between visits = 708.82 ± 117.46), participants had an average duration of 392.49 minutes per week (SD = 279.36), frequency of 8.12 sessions per week (SD = 5.08), and a variety of 2.75 types per week (SD = 1.33). Types of exercise commonly reported included: walking, strength training, boxing, biking/spinning, aerobic training, yoga/Pilates, physical therapy, stretching, Zumba/dance, running, hiking, swimming, and golf.

Multilevel Structural Equation Analysis of Longitudinal Structured Exercise: Slopes and intercept parameter means for structured exercise are reported in Table 6. The latent intercept represents an extrapolated baseline parameter. The latent slope represents the change over time for the exercise parameter. Longitudinal models of exercise duration revealed a significant decline of 17.34 weekly minutes per year in the study ($p < 0.001$). Similarly, exercise frequency significantly declined at a rate of 0.49 exercise sessions per year ($p < 0.001$). We did not find evidence for any significant decline in exercise variety across the study period ($b = 0.03$; $p = 0.651$). All models accounted for the co-occurrence of the COVID-19 pandemic when estimating individual rates of change in structured exercise variables.

Latent difference scores means and variances are presented in Table 5. Significant latent change was observed for executive function such that follow-up latent scores were 0.21 standard units lower than baseline executive function scores ($p = 0.033$). Point estimates of latent change across the other cognitive domains were not significant (language: $\Delta_{\text{cog}} = 0.12$, $p = 0.145$; memory: $\Delta_{\text{cog}} = 0.003$, $p = 0.938$), however a trend in change was detected with attention ($\Delta_{\text{cog}} = -0.69$, $p = 0.079$). Sample difference scores for the Hooper Visual Organization Test and Judgment of Line Orientation Test were -0.06 and -0.01 standard units across the study period respectively.

Table 6: Group level trajectory estimates for subjective exercise variables with time-varying Covid-19 adjustment.

Model	Latent Intercept		Latent Slope	
	Est.	95% CI	Est.	95% CI
Duration	434.22***	[431.22, 437.16]	-17.34***	[-20.40, -14.22]
Frequency	8.62***	[8.32, 8.94]	-0.49***	[-.54, -.45]
Variety	3.25***	[2.86, 3.63]	0.03	[-.11, .17]

*** $p < .001$, ** $p < .01$, * $p < .05$

Duration measured as minutes per week, Frequency and Variety measured as instances per week. N=96. Corrected for age, sex, years of education, MDS UPDRS, COVID-19 Lockdown

Overall Findings:

(i) Regarding estimated baseline exercise performance (intercept) there was a significant association between baseline **greater frequency** of exercise practice and better language performance and visual spatial function, as measured by Hooper Visual Organization Task. There was also a significant association between baseline **greater variety** of exercise practice and language performance and a trend to better executive function. There was also a significant relationship between greater exercise frequency at baseline and worse Judgement of Line Orientation. Covariates that were included in the model were age, sex, education, and UPDRS motor score. There were no association between baseline exercise duration and cognition.

(ii) Regarding estimated change in exercise performance (slope) there was a significant association between change in **frequency** of exercise practice and visual spatial function. Specifically, **less decline in exercise frequency** was associated with less decline in visual spatial function, as measured by Hooper Visual Organization Task. There was also an association between change (slope) in exercise variety and language, and visual spatial function whereby **less decline in exercise variety** was significantly associated with less decline in visual spatial (JLO) and language performance. There was a significant inverse relationship between change in exercise change in variety and memory and attention. Covariates that were included in the model were age, sex, education, and UPDRS motor score.

(iii) There was no association between **baseline or change in exercise duration** and cognitive performance.

Conclusions: Exercise frequency and variety are associated with less cognitive decline, particularly in visuospatial function and language, over two years in PD. Frequency and variety of exercise can be included in exercise prescription to improve PD-related cognitive symptoms.

1.D. Exercise Type and Mood in PD

The goal of the study was to examine the association of structured exercise types (walking, aerobic and skilled exercise) and mood-related symptoms of PD. In comparing both exercise practice and mood, it can bring attention to which exercise type may help to maintain mental well-being for those with PD. 97 study

participants who completed mood assessments and a seven-day exercise log at baseline were included in the analysis.

Structured Exercise Categories: Subjects were requested to complete a seven-day exercise log and record all exercise activities, including type and duration, performed daily for seven consecutive days. For each study participant exercise type and time spent were assigned to 3 broad structured exercise categories based on their shared characteristics including (i) aerobic exercise, (ii) skilled exercise, and (iii) walking. Aerobic Exercise is defined as any activity that strengthens the cardiovascular system and its distribution of oxygen to cells responsible for energy expenditure. This included activities that increase heart and respiratory rates such as aerobic training, Zumba/dance, running, and biking/spinning. Skilled Exercise included activities that required exhibiting proper “form” including strength training, boxing, yoga/Pilates, physical therapy, stretching, swimming, hiking, and golf. Though Aerobic Exercise and Skilled Exercise contain multiple SE types, there was enough people who walked as a form of exercise ($n = 80$) to warrant its own category. People who did not report Aerobic Exercise, Skilled Exercise, or Walking were not included. The total minutes participants spent in each category were calculated by combining all types of SE per category. Exercise categories and their respective SE types can be viewed in Figure 1.

Mood Assessments: All mood assessments were obtained at the study visit baseline. *Anxiety* was measured with the Geriatric Anxiety Inventory (GAI). This is a 20-item dichotomous scale where participants respond either yes (0) or no (1) to indicate if they experienced anxiety symptoms over the past week. The GAI has been shown to effectively measure anxiety in patients with PD. Higher scores indicate greater anxiety. *Depression* was measured with the Beck Depression Inventory-II (BDI-II). This is a 21-item 4-point Likert scale where participants selected an option between 0 (e.g. “I am not discouraged about my future”) and 3 (e.g. “I feel my future is hopeless and will only get worse”) for each item. All items are summed for a total score. The BDI-II has been validated in PD with two subgroups based on Stohlman assessment. The first groups are Somatic which are the physical manifestations and include crying, indecisiveness, loss of energy, changes in sleep patterns and appetite, difficulty concentrating, fatigue, and loss of interest in sex. The second group is Affective which are the internal and behavioral manifestations and include sadness, pessimism, past failure, loss of pleasure, guilty feelings, punish feelings, self-dislike, self-criticalness, suicidal thoughts, agitation, loss of interest, worthlessness, and irritability. Higher scores indicate greater depressive symptoms. *Apathy* was measured with the Starkstein Apathy Scale (SAS). This is a 14-item 4-point Likert scale where participants selected an option between 0 (“not at all”) and 3 (“a lot”) for each item. Participants were asked to answer each item as it relates to their apathy over the course of 4 weeks. The items are summed for a total score. Higher scores indicate greater apathy.

Statistical analysis included a correlation analysis comparing time spent (i) aerobic exercise, (ii) skilled exercise, and (iii) walking with severity of depression (BDI-II), anxiety (GAI) and apathy (SAS). Linear regression analysis was completed for those associations showing significant correlations. Demographic data is shown in Table 7. Total time in each category can be found in Table 8.

Overall findings indicated that aerobic exercise was significantly correlated with less depression. BDI-II scores were then divided into two subscales (somatic and affective) and both subscales were found to be negatively correlated with *Aerobic Exercise*. Linear regression analysis showed that greater time spent greater time spent in aerobic exercise was significantly associated with less depression, after adjusting for age, sex, education, disease duration and UPDRS motor score. Table 9. There was no significant association between walking or skilled exercise and depression, anxiety, and apathy in PD.

Table 7: Demographic profile (n = 97)

Characteristic	Mean (SD)	Range
Age, years	66.5 (8.8)	45 - 83
Female/male, No. (% male)	42 / 55 (57%)	
Education, years	16.8 (2.1)	12 - 20
Disease duration, years	4.8 (4.9)	0 - 35
UPDRS Total	108.6 (4.9)	37 - 84
UPDRS-III Motor	56.8 (10.4)	67 - 154
BDI-II	7.9 (5.3)	0 - 22
GAI	3.1 (3.6)	0 - 15
SAS	10.4 (5.5)	0 - 24

Figure 1: Summary of Exercise Type at Baseline.

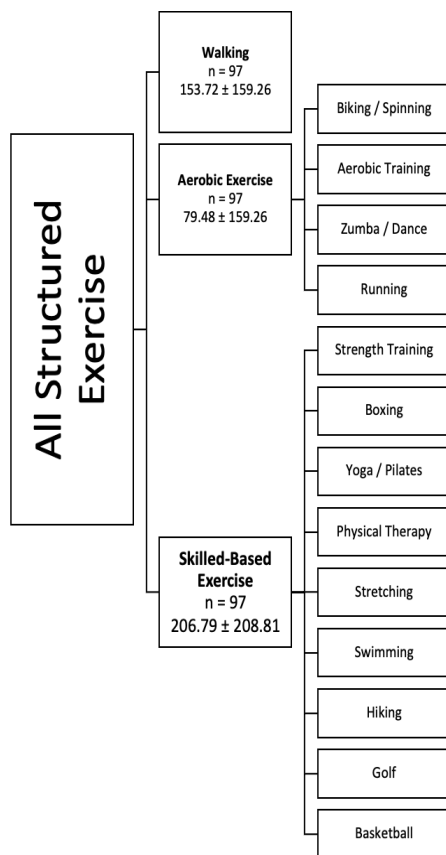


Table 8: Time spent in walking, aerobic and skilled exercise.

Self-Reported Exercise	Minutes Per Week
Walking	153.72 ± 159.26
Aerobic	79.48 ± 124.96
Skilled	206.79 ± 208.81

Table 9: Linear regression, adjusted for age, sex, education, disease duration and UPDRS motor.

	Aerobic			
	β	95% CI (Lower, Upper)	<i>p</i>	<i>R</i> ² change
Age	-.246	-.361, -.131	<.001	
Sex	-.613	-2.572, 1.347	.536	
Yrs of Education	.407	-.047, .861	.078	
Disease Duration	.101	-.093, .295	.302	
UPDRS Motor	.210	.109, .312	<.001	
BDI	-.012	-.020, -.005	.002	

Aim 2: Motor Fitness and Cognitive Function

We examined whether higher motor fitness, (termed physical performance), at baseline was associated with better cognitive performance at baseline (cross-sectional analysis), and whether higher physical performance at baseline predicted higher cognitive function over two years in individuals with PD. Motor fitness (physical performance) measures were collected at baseline and included (i) the Physical Performance Test, PPT, (ii) Short physical Performance Battery, SPPB, (iii) Time Up and Go, TUG, and (iv) the Ten Step Test, TST. Cognitive function including executive function, attention, language, memory, and visual spatial domains were evaluated at baseline and 2 years follow up. Mild cognitive impairment (MCI) status was determined through consensus at baseline based on established published criteria. A latent factor estimating “physical performance” was generated using structural equation modeling (SEM). The four indicators that loaded onto this physical functioning latent factor were the PPT, SPPB, TUG, and TST. All four factor loadings were estimated while the mean of the latent factor was set to zero with a variance set to one. Multivariable linear regressions were used to estimate associations between the physical functioning latent factor and cognitive function including general cognition (MoCA) and executive function (EF), attention, memory, language, and visual spatial function (JLO and HVOT). All effects were adjusted for age, sex, education, MDS-UPDRS Part III, freezing of gait, and study site.

Results: Cross-Sectional and Longitudinal Assessment of Motor Fitness (physical performance) and Cognitive function.

The overall findings from our study show that better physical performance at baseline was associated with significantly better global cognitive performance. This effect was largely driven by significantly better performance on tests of executive function followed by attention and language ability. Results of the structure equation model examining associations between the physical performance and risk of PD-MCI revealed that better physical performance. (per 1 SD) was associated with lower risk of being classified with PD-MCI (OR = .277; 95% confidence interval (CI) = .090, .852; B-H $p = .025$). Furthermore, physical performance declined on average .12 standard deviations over the two-year follow-up period. Individuals with larger declines in physical performance experienced larger concurrent declines in executive function, aspects of visuospatial ability, and marginally significant declines to attention, language, and global cognitive performance. All observed associations were not explained by difference in age, sex, motor-severity of PD, freezing of gait, or education.

Our overall findings are the first to demonstrate cross-sectional and longitudinal associations between higher motor fitness (physical performance) and better cognitive function in individuals with PD. Higher baseline motor fitness was associated with better baseline cognition across multiple domains (**Table 10**), including attention, language, executive function, and MoCA. Further, less decline in motor fitness over 2 years was associated with less decline in cognitive function over the study period (**Table 11**), particularly in executive function and the visuospatial task (judgement of line orientation).

Limitations: Due to COVID pandemic we were unable to complete aerobic fitness assessments in our participants and were not able to complete this analysis.

Table 10: Cross-sectional associations between baseline physical performance and baseline cognitive function. Estimates in bold denote $p < .05$.

Outcome ^a	Effect of physical function per 1 SD	95% Confidence Interval		p
	Estf ^b	Lower	Upper	
Montreal Cognitive Assessment	.91	.36	1.46	<.01
Attention	.37	.15	.60	<.01
Language	.24	.02	.46	.06
Hooper visual organization	-.04	-.26	.19	.74
Judgement of line orientation	.14	-.07	.36	.26
Executive functioning	.33	.11	.55	<.01
Episodic memory	.10	-.12	.31	.43

^a all domain-specific latent factors of cognitive performance, the Hooper visual organization test, and Judgement of line orientation were z-score standardized (mean = 0; standard deviation = 1).

^b parameter estimates were adjusted for age, sex, years of education, Unified Parkinson's Disease Rating Scale Motor Score, and Freezing of Gait Questionnaire total score.

Table 11: Associations between changes in physical performance and concurrent changes in cognitive function. Estimates in bold denote $p < .05$.

Outcome: declines in cognitive performance ^a	Effect of decline in physical performance (per SD)	95% Confidence Interval		p
	Estf ^b	Lower	Upper	
Montreal Cognitive Assessment	.54	-.08	1.16	.09

Attention	.22	-.01	.44	.06
Language	.23	-.03	.49	.08
Hooper visual organization	.19	-.07	.46	.15
Judgement of line orientation	.27	.01	.52	.04
Executive functioning	.30	.04	.55	.03
Episodic memory	.21	-.05	.49	.11

^a all domain-specific latent factors of cognitive performance, the Hooper visual organization test, and Judgement of line orientation were z-score standardized (mean = 0; standard deviation = 1).

^b parameter estimates were adjusted for age, sex, years of education, Unified Parkinson's Disease Rating Scale Motor Score, and Freezing of Gait Questionnaire total score.

Aim 3: Neuroimaging

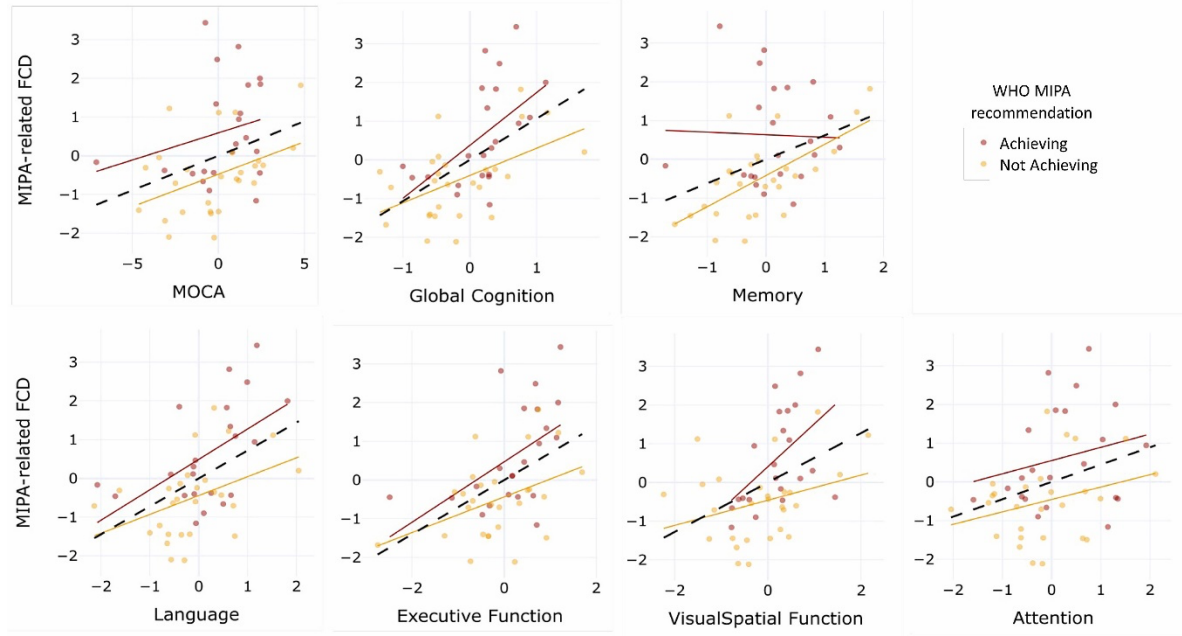
3.A. Resting State MRI: A cross-sectional evaluation revealed that achieving 150 minutes of moderate to vigorous intensity physical activity per week (in alignment with the World Health Organization recommendation) is associated with increased functional connectivity during resting state MRI imaging within the following regions:

- (i) the bilateral precuneus, a region within the posterior parietal lobe,
- (ii) the marginal part of cingulate sulcus, a region in the cingulate cortex,
- (iii) the brain stem,
- (iv) the hippocampus,
- (v) the middle posterior part of the cingulate gyrus and sulcus, a region in the cingulate cortex,
- (vi) and the middle frontal sulcus, a region within the frontal lobe.

Further, the combined functional connectivity of these regions is associated with better performance in global cognition and across multiple cognitive domains, including executive function, language, and visual spatial function (Figure A; black dashed line).

Our overall findings support that achieving 150 minutes of moderate to vigorous physical activity per week moderated the relationship between brain connectivity and global cognition, EF, language, and visual spatial function (Figure A, red and yellow lines).

Figure A (next page): Black dashed line represents the relationship between increased functional connectivity at baseline and cognition at baseline. Red and yellow lines represent those achieving and not achieving 150 minutes of moderate to vigorous physical activity, respectively, and show that this threshold has a moderating effect on the association between functional connectivity and cognition in some domains.



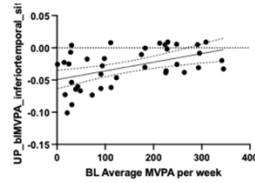
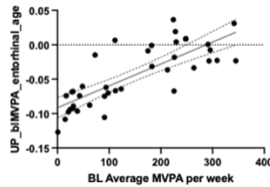
3. B. Volumetric Analysis: For this study, we examined the association between (i) baseline time spent in moderate to vigorous physical activity (MVPA) and (ii) average time spent in MVPA over 2 years and change in CNS volume fraction over 2 years. There were 42 PD individuals with structural MRI at baseline and follow-up and included in this study. Brain volumetric fraction change was obtained for 34 cortical and 6 subcortical regions of interest using the Desikan-Kilany Atlas and FreeSurfer subcortical segmentation.

Correlational analysis was performed showing significant correlation between the Accumbens and Average MVPA over 2 years (Table A). There was also significant correlation between entorhinal cortex and baseline MVPA and average MVPA over 2 years, and a significant correlation between inferior temporal cortex and baseline MVPA. For those CNS regions with significant correlation, we then conducted, Multiple linear regression followed by backward elimination of non-significant covariates were performed. Co-variates included in the analysis were age, gender, PD duration, site, days between visits.

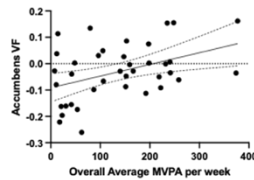
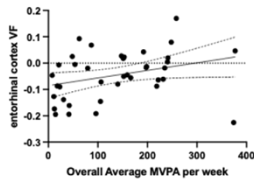
Overall Findings: There was a significant association between baseline MVPA and the entorhinal cortex and the inferior temporal lobe (See Figure below). Greater time spent in MVPA at baseline predicted greater volume size in the entorhinal cortex and the inferior temporal lobe over 2 years. There was also a significant association between average MVPA over 2 years and change in volume fraction of the (i) entorhinal cortex and (ii) nucleus accumbens (see Figure below). Greater average time spent MVPA over 2 years was associated with less decline in volume of the entorhinal cortex and nucleus accumbens.

	Baseline Average MVPA		Overall Average MVPA	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Thalamus	-0.001	0.993	-.059	0.719
Caudate	-0.244	0.129	-0.241	0.140
Putamen	0.005	0.973	0.082	0.618
Pallidum	0.183	0.259	0.182	0.267
Accumbens	0.270	0.092	0.361	0.024
Hippocampus	-0.249	0.121	-0.140	0.396

Baseline MVPA		
	Standardized β	<i>p</i>
Entorhinal Cortex (Left Panel Below)	0.349	0.029*
Inferior Temporal (Right Panel Below)	0.340	0.024 *



Average MVPA		
	Standardized β	<i>p</i>
Entorhinal Cortex (Left Panel Below)	0.318	0.052
Accumbens (Right Panel Below)	0.421	0.009 *



3. C. Microstructural Analysis: Microstructural results were calculated from diffusion MRI and T1-weighted images at the voxel-wise level for 20 participants that passed baseline and follow-up quality control checks. 6 microstructural metrics of interest were generated, anisotropic and isotropic intracellular signal, representing axons and small processes and cellularity respectively, extracellular free water, T1/T2 ratio, aggregate axonal g-ratio, and aggregate axonal conduction velocity. The mean within each of 7 bilateral subcortical ROIs (the Amygdala, Caudate, Hippocampus, Nucleus Accumbens, Pallidum, Putamen, and Thalamus) and the cholinergic basal forebrain areas 1, 2, 3, and 4, was calculated and was compared to the behavioral or cognitive metric of interest controlling for subject sex, age, years after PD diagnosis, brain volume, days between baseline and follow-up scan, and scanning site. After Benjamini & Hochberg multiple comparison correction

5 subcortical gray matter regions had significant positive associations between intracellular isotropic signal and Global Executive Scores in the Caudate, Nucleus Accumbens, Pallidum, Putamen, and Thalamus, all at the $p < 0.05$ level. Additional significant positive associations were found after multiple comparison correction between Global Attention Scores and intracellular isotropic signal in the Caudate, and between Language Scores and intracellular isotropic signal in the Caudate and Nucleus Accumbens, all at the $p < 0.05$ level. Global Cognitive Scores were significantly positively associated with intracellular isotropic signal in the Caudate, Nucleus Accumbens, Putamen, and Thalamus, with an additional positive association between intracellular anisotropic signal in the Nucleus Accumbens, all at the $p < 0.05$ level. No other microstructural metric or metric of interest survived multiple comparison testing. When Motor Fitness was examined in the cholinergic basal forebrain however it was found to have a significant positive association with intracellular isotropic ($p < 0.01$) and anisotropic signal ($p < 0.05$) in area 4. The only cholinergic basal forebrain relationship with a cognitive metric was between intracellular anisotropic signal and MOCA score ($p < 0.01$) also in area 4.

Overall findings suggest that cellular degeneration in core subcortical GM structures, particularly the caudate the nucleus accumbens, may drive cognitive changes, while degeneration in cholinergic basal forebrain area 4 may be more closely associated with less Motor Fitness and worse general cognition as measured by the MOCA.

3.D. Analysis of Perivascular Spaces (PVS) Changes with cognition in Parkinson's disease (PD)

The glymphatic system has several functions in healthy individuals, including (i) transport of neurotransmitters and nutrients (e.g., glucose and lipids), (ii) immune surveillance, and, as previously mentioned, (iii) clearance of cellular debris, metabolic waste products, and protein aggregates. Perivascular spaces (PVS) are an essential component of this system, encompassing the fluid filled spaces surrounding penetrating arteries and veins in the brain. These spaces serve as the primary influx and efflux pathways for essential nutrients and waste products, alike. PVS are found in both white matter and grey matter, including in the basal ganglia. PVS enlargement, quantified on MRI as either greater PVS volume or higher incidence of dilated PVS, is thought to indicate glymphatic system dysfunction, quantified in several studies as higher PVS volume or greater incidence of dilated PVS on MRI.

Two previous studies have linked basal ganglia PVS to general decline of cognitive function in PD. The first associated higher basal ganglia PVS in PD individuals with conversion from normal cognition to mild cognitive impairment (MCI) or from MCI to dementia over five years. The second associated higher PVS in the basal ganglia in PD with greater decline in Montreal Cognitive Assessment (MoCA) scores over three years. While these studies support an association between PVS and decline in general cognition in PD, the relationship between PVS and decline in specific cognitive domains has yet to be examined.

The primary goal of this study was to evaluate PVS volume, expressed as volume fraction, within white matter and the basal ganglia and its association with baseline cognitive function and cognitive decline within specific cognitive domains. PVS volume was determined from T1w magnetic resonance imaging (MRI) using a previously described method to obtain volume fraction measurements. Along with white matter within the centrum semiovale, prefrontal white matter regions (medial orbitofrontal, rostral middle frontal, superior frontal) were also selected for analysis due to their known role in PD-cognition. A full neuropsychological assessment was collected on study participants at baseline and two years.

3.D.1. Cross-Section PVS and Cognition in PD

Perivascular spaces (PVS), fluid-filled compartments surrounding brain vasculature, are an essential component of the glymphatic system responsible for transport of waste and nutrients. Glymphatic system

impairment may underlie cognitive deficits in Parkinson's disease (PD). Studies have focused on the role of basal ganglia PVS with cognition in PD, but the role of white matter PVS is unknown. This study examined the relationship of white matter and basal ganglia PVS with domain-specific and global cognition in individuals with PD. Fifty individuals with PD underwent 3T T1w magnetic resonance imaging (MRI) to determine PVS volume fraction, defined as PVS volume normalized to total regional volume, within (i) centrum semiovale, (ii) prefrontal white matter (medial orbitofrontal, rostral middle frontal, superior frontal), and (iii) basal ganglia. A neuropsychological battery included assessment of global cognitive function (Montreal Cognitive Assessment, and global cognitive composite score), and cognitive-specific domains (executive function, memory, visuospatial function, attention, and language). Higher white matter rostral middle frontal PVS was associated with lower scores in both global cognitive and visuospatial function. In the basal ganglia higher PVS was associated with lower scores for memory with a trend towards lower global cognitive composite score.

Overall Findings: While previous reports have shown that greater amount of PVS in the basal ganglia is associated with decline in global cognition in PD, our findings suggest that increased white matter PVS volume may also underlie changes in cognition.

	Mean (SD)
N (# of females)	50 (23)
Age	65.36 (8.98)
Education (years)	16.68 (2.20)
Body Mass Index (kg/m ²)	25.89 (4.75)
MDS-UPDRS part III score	20.84 (9.08)
Years of Parkinson's diagnosis	4.52 (5.34)
Cardiovascular Risk Score	0.56 (0.73)
Levodopa Equivalent Daily Dose (mg)	575.57 (394.01)

	β	95% CI Lower, Upper	<i>p</i>
MoCA			
centrum semiovale PVS	-73.84	-288.02, 140.34	0.982
rostral middle frontal PVS	-318.40*	-613.85, -22.95	0.047
superior frontal PVS	-80.33	-205.88, 45.22	0.408
basal ganglia PVS	-109.02	-321.32, 103.28	0.306
Global Cognition			
rostral middle frontal PVS	-112.22*	-192.59, -31.84	0.028
basal ganglia PVS	-84.65*	-147.55, -21.76	0.030
Visuospatial Function			
rostral middle frontal PVS	-124.79*	-229.90, -19.67	0.042
Memory			
centrum semiovale PVS	-16.22	-88.46, 56.01	0.653
rostral middle frontal PVS	-51.43	-151.76, 48.91	0.307
superior frontal PVS	1.26	-42.91, 45.42	0.954
basal ganglia PVS	-57.99	-127.88, 11.90	0.153

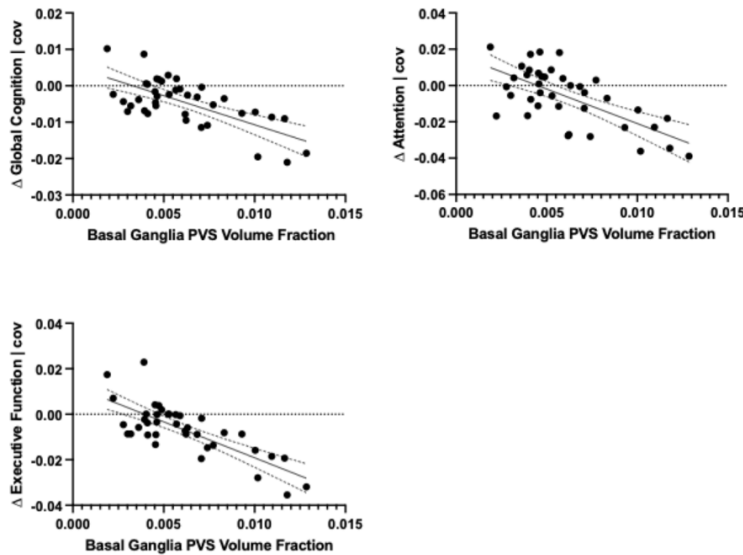
PVS: perivascular space

Significant regressions in **bold**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

3.D.2. Baseline PVS Predicts Cognitive Decline in PD

Pathologic perivascular spaces (PVS), the fluid-filled compartments surrounding brain vasculature, may underlie cognitive decline in Parkinson's disease (PD). However, whether this impacts specific cognitive domains have not been investigated. This study examined the relationship of PVS volume at baseline with domain-specific and global cognitive change over two years in PD individuals. Thirty-nine individuals with PD underwent 3T T1w MRI to determine PVS volume fraction (PVS volume normalized to total regional volume) within (i) centrum semiovale, (ii) prefrontal white matter (medial orbitofrontal, rostral middle frontal, superior frontal), and (iii) basal ganglia. A neuropsychological battery included assessment of cognitive domains and global cognitive function at baseline and two years. Higher basal ganglia PVS at baseline was associated with greater decline in attention, executive function, and global cognition scores.

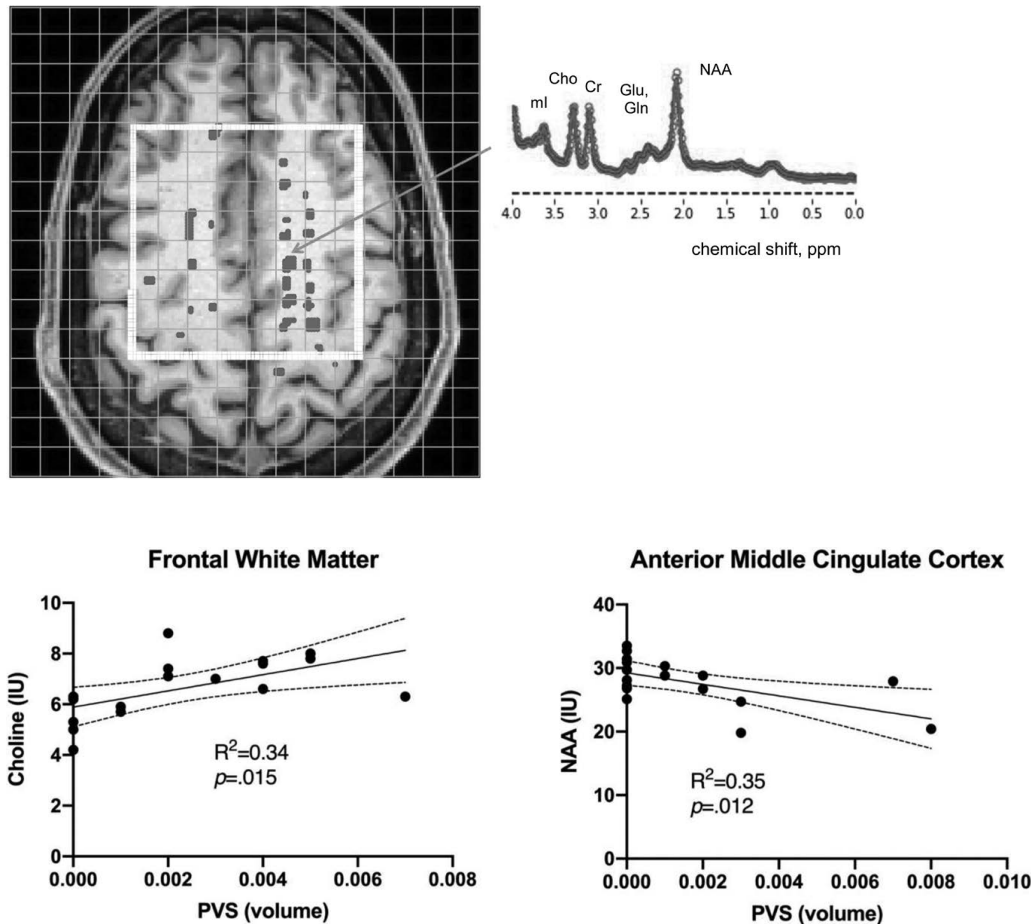
Overall Findings : While previous reports have associated elevated PVS volume in the basal ganglia is associated with decline in global cognition in PD, our findings show such decline may affect the attention and executive function domains.



3.D.3. Magnetic Resonance Spectroscopy (MRS) Analysis of PVS in Parkinson's disease.

Objective Higher volume fraction of perivascular space (PVS) has recently been reported in Parkinson's disease (PD) and related disorders. Both elevated PVS and altered levels of neurometabolites, assayed by proton magnetic resonance spectroscopy (MRS), are suspected indicators of neuroinflammation, but no published reports have concurrently examined PVS and MRS neurometabolites. Methods In an exploratory pilot study, we acquired multivoxel 3-T MRS using a semi-Localization by Adiabatic SElective Refocusing (sLASER) pulse sequence (repetition time/echo time = 2810/60 ms, voxels $10 \times 10 \times 10$ mm³) from a 2D slab sampling bilateral frontal white matter (FWM) and anterior middle cingulate cortex (aMCC). PVS maps obtained from high resolution ($0.8 \times 0.8 \times 0.8$ mm³) T1-weighted MRI were co-registered with MRS. In each MRS voxel, PVS volume and neurometabolite levels were measured. Results Linear regression accounting for age, sex, and BMI found greater PVS volume for higher levels of choline-containing compounds (Cho; $P = 0.047$) in FWM and lower PVS volume for higher levels of N-acetyl compounds (NAA; $P = 0.012$) in aMCC. Since (putatively) higher Cho is associated with inflammation while NAA has anti-

inflammatory properties, these observations add to evidence that higher PVS load is a sign of inflammation. Additionally, lower Montreal Cognitive Assessment scores were associated with lower NAA in aMCC ($P = 0.002$), suggesting that local neuronal dysfunction and inflammation contribute to cognitive impairment in PD. **Overall Findings:** These exploratory findings indicate that co-analysis of PVS and MRS is feasible and may help elucidate the cellular and metabolic substrates of glymphatic and inflammatory processes in PD.

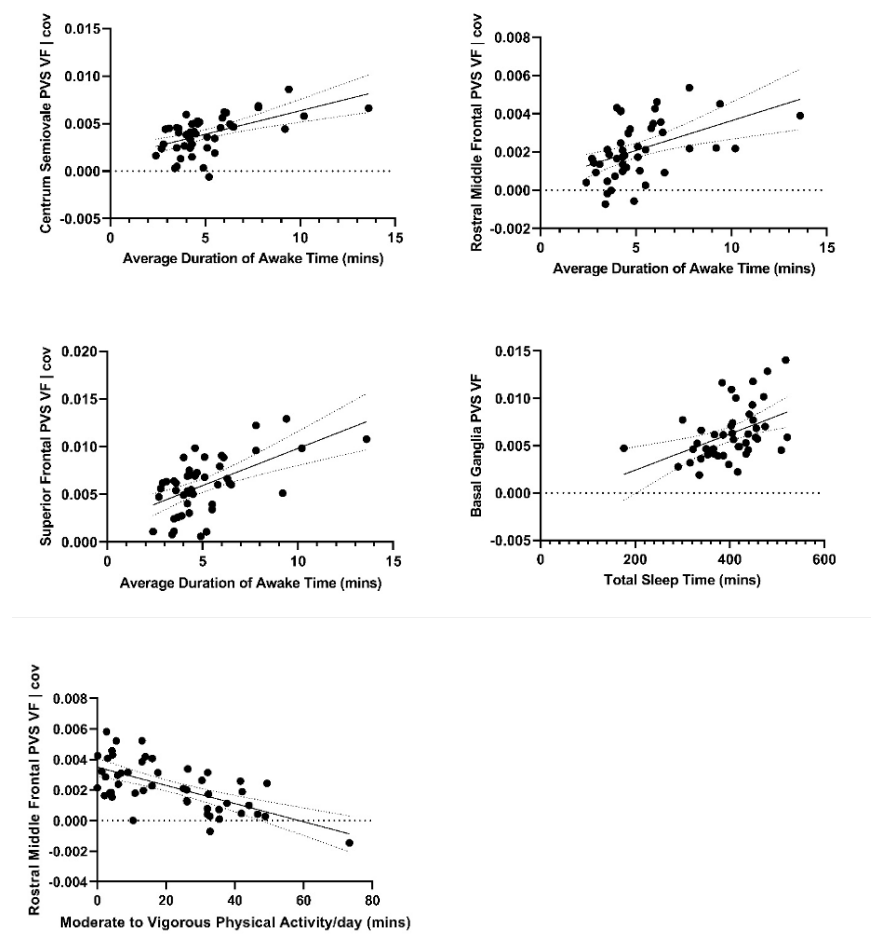


3.D.4. The relationship between PVS, Sleep, and Physical Activity in PD

Impairment of the glymphatic system, including enlarged perivascular spaces (PVS), has been associated with neurodegenerative disorders, including Parkinson's disease (PD). Lifestyle factors, including proper sleep and ample physical activity can also impact neurodegenerative disorders, although the relationship between PVS and lifestyle in PD has not yet been established. This study examined the relationship between lifestyle factors, including parameters of sleep quality and physical activity, and white matter and basal ganglia PVS volume in PD. Forty-eight individuals with PD wore accelerometers on the hip and wrist for seven days to assess sleep (wrist) and physical activity (hip). Participants underwent 3T T1w magnetic resonance imaging (MRI) to determine PVS volume fraction, defined as PVS volume normalized to white matter volume, within the (i) centrum semiovale, (ii) prefrontal white matter (medial orbitofrontal, rostral middle frontal, superior frontal), and (iii) basal ganglia. Longer average time awake during sleep was associated with higher centrum

semiovale, rostral middle frontal, and superior frontal PVS volume. Longer total sleep time was associated with higher basal ganglia PVS volume. Greater minutes per day of moderate to vigorous physical activity were associated with less rostral middle frontal PVS volume.

Overall Findings: Lifestyle factors, including sleep quality and physical activity, may impact the glymphatic system in PD.



END OF RESEARCH ACCOMPLISHMENTS SECTION

What opportunities for training and professional development has the project provided?

(i) Throughout the entire granting and NCE periods (including throughout the COVID-19 lockdown and limited pandemic access periods), the entire research group conducted a weekly Zoom meeting consisting of all principal investigators, researchers, coordinators, staff, graduate, and undergraduate students. These meetings included all research sites (USC, UCSD, UCLA, U, Virginia). Each meeting included an agenda based on project progression, data assessment, design and writing of research manuscripts, discussion of the scientific literature (journal club) as well as discussions of barriers and challenges during the research project. A critical component of this research groups was to mentor junior researchers as well as students to improve their understanding of translational research as well as developing impactful research outcomes.

(ii) The entire clinical research team meet weekly with the preclinical research teams lead by D. Holschneider and M. Jakowec. These meetings provided an opportunity to develop effective translational perspectives and to discuss and develop research experiments bridging preclinical studies in animal models and to develop an understanding of how these relationships are established.

(iii) The research team included several graduate students that were supported by this research program (A. Lundquist, E. Donahue, D. Phillips, R. Foreman, M. Pilgrim). These students were in the doctoral students in the USC Neuroscience graduate program, USC LONI, U. Virginia Data Science Program.

(iv) The research team included many undergraduate students (typically 6 at USC at any time) and this [project provided them with an outstanding opportunity to be directly engaged in both clinical and preclinical studies to develop their understanding of translational research. Many of these students have gone on the advanced training in medical school, graduate school, and pharmacy as well as career positions. The skills they developed in data collection from first principles, through data analyses, and manuscript preparation and writing, which is reflected in their inclusion as co-authorship on the manuscripts that emerged from this research program were instrumental in supporting their successful applications.

(v) The participants in this research project carried out several retreats in Los Angeles and at the Lake Arrowhead Conference center. The Los Angeles retreats were single day events to allow researchers to discuss progress, data outcomes, and present research findings. These were attended by senior and junior researchers as well as graduate and undergraduate students. Work from this project was used to apply for and receive university funds to support an annual conference entitled “Metaplasticity and Megaplasticity: Changing the Brain from Synapse to Community” which was held at Lake Arrowhead, CA from 12/6-8/2019 and 11/18-20/2022. These events provided a mentoring opportunity where students presented their research in a lecture format.

How were the results disseminated to communities of interest?

Several unique opportunities were made possible through this research program to share outcomes measures and to educate a wide spectrum of different groups and communities.

(i) Research from this project has been used to develop and implement community programs at the Plaza de la Raza community center, which is a few blocks from the UCS Medical Center and is the oldest Latino Community Center in the United States. Investigators hosted several evening events to educate community members on Parkinson’s disease, cognition, and dementia in the aging population, and to develop an environment to promote discussion, question/answer, and resources available. These events were attended by 150 community members.

(ii) As an extension of the events held at the Plaza de la Raza addition collaborative bridges were developed with Physical Therapy and Occupational Therapy programs at USC to develop classes for the community in dance, theater, exercise, and cooking. The goals of these classes are to improve cognitive resilience in the aging Latino/Latina population in the aging population and in individuals with Parkinson's disease. Currently these classes are attended by 20 to 30 individuals per class. The initial launching of these programs made possible in this granting mechanisms have attracted additional grant support from private foundations as well as the USC Good Neighbors Community Program.

(iii) Members of this research program have disseminated the findings to the community through a number of local and national as well as international symposia designed for individuals with Parkinson's disease, their caregivers, and families. Similar events included Grand Rounds and professional presentation to clinicians and academic faculty.

(iv) Researchers in this program participate several academic classes at the graduate and undergraduate levels. Findings from this program are shared with students to provide state-of-the-art knowledge of Parkinson's disease and new and novel therapeutic strategies. Classes include medical and neuroscience students but also involve a range of non-medical students. For example, the PI of this research program is a founding faculty member of a new class in the USC Kaufman School of Dance DANC195, which teaches students about Parkinson's disease and with faculty shows them how exercise and physical activity can provide a foundation in evidence-based medicine to treat individuals with Parkinson's disease. The initial class enrolled 33 students who as part of their curriculum worked with patients in using dance as a means to improve motor skills and cognition.

(v). During this DOD grant, Dr Petzinger was awarded an internal USC diversity grant to study the promotion of Physical Activity in Latinas with chronic neurological disease. At completion of this grant, Dr Petzinger was able to demonstrate that using a FitBit activity device and app in combination with behavioral coaching (Lifestyle redesign), Latinas with chronic neurological disorders could achieve the recommended level of moderate to vigorous physical activity after 4 months of coaching. Achieving this level of PA was associated with less visits to the hospital and overall improved quality of life. This project was completed in collaboration with USC occupational therapy and a local Latino wellness community center within Los Angeles County.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

This proposal helped elucidate the effects of exercise intensity and motor fitness levels on cognitive function important for EF, the most common form of cognitive impairment in PD.

We conducted a longitudinal naturalistic (observational) 2-year study monitoring physical activity intensity and fitness level along with exercise logs. This approach is novel since it examines "real world" practices using objective measures (accelerometers and motor fitness) along with exercise logs to help translate into direct exercise prescription guidelines for patients that can be performed in the community. Findings from this study demonstrate that 150 mins per week of moderate to vigorous intensity physical activity is important for cognitive function in individuals with PD and are associated with increased connectivity in various brain regions including those subserving executive function. Additionally, this study demonstrates that greater motor fitness is associated with better cognitive function, less risk for MCI and better cognitive function over time. Our structural imaging studies support that greater motor fitness may be associated with brain regions

important for frontal lobe function, including cholinergic neurons of the nucleus basalis. Importantly findings from this study identify parameters of exercise activity, including intensity and fitness level, as well as exercise variety and frequency that can be monitored and help guide neurorehabilitation and exercise practices and policies for patients with PD and stake holders.

Complementary Animal studies conducted simultaneously by Dr. Holschneider (PI- Award number W81XWH18-1-0666) provided critical synergistic and complementary insights by helping to identify potential underlying mechanisms for exercise benefits on cognitive circuits in PD. The overall impact of the study is to provide evidence-based medicine to support a cognitive rehabilitation program for individuals through an exercise prescription.

Additionally, a major impact of this grant has been to be one of the first studies to identify the role of the glymphatic system, and in particular changes in perivascular space volume (PVS) within the white matter and basal ganglia and its association with PD cognition. Our studies are also one of the first studies to demonstrate how PVS may be impacted by lifestyle factors such as sleep and moderate to vigorous physical activity in PD. This grant allowed for new imaging approaches to investigate mechanisms of cognitive change in PD that are novel to the field.

What was the impact on other disciplines?

Though the results are not directly applicable to populations not evaluated in this study, the development of exercise prescription to provide cognitive benefit to PD individuals may benefit multiple aging populations, including individuals with dementias, other age-related neurodegenerative disorders, and healthy aging individuals. Cognitive decline in aging populations is difficult to address and ameliorate, and since exercise provides benefit to PD cognition, this may suggest that other fields should explore exercise prescription as a way to modify cognitive function across a spectrum of age-related diseases.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Understanding how lifestyle, specifically exercise/physical activity impacts cognition in individuals with PD and in aging is essential in a world with a large aging population. By researching and understanding how physical activity benefit PD individuals, behavioral practice for larger populations may be informed in such a way that the benefits of lifestyle modification reach diverse populations. Further, physical activity and other lifestyle modifications are available to members of every socio-economic status, increasing the potential impact of our study results.

Latino Community Outreach: Findings from these studies have been used to help develop exercise and dance classes within the surrounding Latino community of University of Southern California (USC), Keck School of Medicine to help serve our Latino PD and aging population. We have a weekly exercise and dance class held at the oldest Latino cultural center in the country, called Plaza de la Raza. Our instructors include USC physical and occupational therapy and USC dance instructors. We also hold a weekly cooking and theater class. The program was created by Dr. Giselle Petzinger and in Collaboration with the community directors of Plaza de La Raza and is currently funded by a community outreach grant and a family foundation. Findings from this DOD study supported the rational and importance of creating regular exercise classes for individuals with PD, including individuals from diverse communities.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Due to COVID-19 precautions, and the fact that patients were not able to come in for follow up study visit assessments for a period of time both at USC and UCSD, we requested and obtained a second One year No Cost Extension until 9-15-2023 to complete the longitudinal analysis of our study. The study period for scheduling a follow up visit for patients was longer than 18 months and was approximately 2 years on average due to the COVID-19 pandemic. We were not able to complete the cardiovascular fitness of the exam due to the COVID-19 pandemic and not having access to the equipment needed to complete this assessment.

Actual or anticipated problems or delays and actions or plans to resolve them

The no cost extension obtained to complete the study following the COVID-19 research shut down allowed us to successfully complete the study.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Not Applicable.

Significant changes in use of biohazards and/or select agents

Not Applicable.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

1. Donahue EK, Venkadesh S, Bui V, Tuazon AC, Wang RK, Haase D, Foreman RP, Duran JJ, Petkus A, Wing D, Higgins M, Holschneider DP, Bayram E, Litvan I, Jakowec MW, Van Horn JD, Schiehser DM, **Petzinger GM**. Physical Activity Intensity is Associated with Cognition and Functional Connectivity in

Parkinson's disease, 2022. *Parkinsonism Relat Disord*. 2022 Sep 24;104:7-14. doi: 10.1016/j.parkreldis.2022.09.005. Online ahead of print. PMID: 36191358

2. Donahue EK, Bui V, Foreman RP, Duran JJ, Venkadesh S, Choupan J, Van Horn JD, Alger JR, Jakowec MW, **Petzinger GM**, O'Neill J. Magnetic resonance spectroscopy shows associations between neurometabolite levels and perivascular space volume in Parkinson's disease: a pilot and feasibility study. *NeuroReport*. 2022 May; 33(7): 291-6.
3. Donahue EK, Foreman RP, Duran JJ, Jakowec MW, Petkus AJ, O'Neill J, Holschneider DP, Choupan J, Van Horn JD, Bayram E, Litvan I, Schiehser DM, **Petzinger GM**. High baseline perivascular space volume in basal ganglia is associated with attention and executive function decline in Parkinson's disease. (Accepted *Brain and Behavior*).
4. Donahue EK, Foreman RP, Duran JJ, Jakowec MJ, O'Neill J, Petkus AJ, Holschneider DP, Choupan J, Van Horn JD, Bayram E, Litvan I, Schiehser DM, **Petzinger GM**. Increased perivascular space volume in white matter and basal ganglia is associated with cognition in Parkinson's disease. (Under second review in *Brain Imaging and Behavior*).
5. Donahue EK, Pilgrim M, Foreman RP, Burke J, Wang R, Haase D, Kikkawa K, Figueroa K, Hong E, Duran JJ, Bayram E, Litvan I, Petkus AJ, Schiehser DM, **Petzinger GM**. Maintenance of exercise frequency and variety parameters over 24 months is associated with less cognitive decline in Parkinson's disease. (In preparation).
6. Donahue EK, Foreman RP, Duran JJ, Jakowec MW, Wing D, Higgins M, O'Neill J, Holschneider DP, Choupan J, Van Horn JD, Bayram E, Litvan I, Schiehser DM, **Petzinger GM**. Increased sleep quality and physical activity are associated with decreased perivascular space volume in Parkinson's disease. (In preparation).
7. Petkus, A, Donahue ER, Jakowec, M, Bayram ece, Van Horn, JD, Litvan, I, **Petzinger G**, Schiehser, D. Data-driven Sequence of cognitive Decline in people with Parkinson's Disease (submitted *J. Neurology, Neurosurgery and Psychiatry*).
8. Petkus AJ, Jakowec MW, Schiehser DM, Van Horn JD, **Petzinger GM**. Greater Motor Fitness predicts and Better Cognitive Function in Parkinson's disease. (In preparation).
9. Microstructural and volumetric analysis of exercise and cognition in Parkinson's disease (In Preparation)

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

Posters:

- "Physical Activity Intensity is Associated with Cognition and Connectivity in Parkinson's Disease" Parkinson's Foundation Centers of Excellence Clinical Leadership Conference. May 2022. Boston, MA.
- "Associations Between Physical Activity, Functional Connectivity, and Cognition in Parkinson's Disease." Organization for Human Brain Mapping Annual Conference. June 2022. Glasgow, Scotland.

- “White Matter PVS in Parkinson’s Disease is Associated with Decreased Cognition and Interrupted Sleep, and Less Physical Activity.” Movement Disorders Society World Congress. September 2022. Madrid, Spain.
- “Perivascular spaces in Parkinson’s disease are associated with decreased cognition, interrupted sleep, less physical activity, and blood levels of mmp9” Society for Neuroscience Conference. November 2022. San Diego, CA.
- “Physical activity intensity is associated with cognition, connectivity, and blood solutes in Parkinson’s disease.” Society for Neuroscience Conference. November 2022. San Diego, CA.
- “Administration of the Humanin-Like Mitochondrial Peptides SHLP2 and SHLP2-K4R Provides Neuroprotection Against the Dopamine-Depleting Neurotoxin MPTP.” Society for Neuroscience Conference. November 2022. San Diego, CA.
- “Astrocytic Regulation of Dopamine Neurotransmission in the Basal Ganglia through Lactate Shuttling.” Society for Neuroscience Conference. November 2022. San Diego, CA.
- “The role of astrocytes and L-lactate in Parkinson’s disease and exercise-induced neuroplasticity.” Cold Spring Harbor. December 2022. Cold Spring Harbor, NY.

Presentations:

-
- “Perivascular Space in Parkinson’s Disease.” START Parkinson’s Support Group Meeting. February 2021.
- “Perivascular spaces and inflammation in Parkinson’s disease.” University of Southern California Neuroscience Graduate Program Symposium. Annual Meeting May 2021. Los Angeles, CA.
- “Perivascular spaces in Parkinson’s disease.” University of Southern California Neurology Resident Didactic lecture. April 2022. Los Angeles, CA.
- “Sleep in Parkinson’s disease.” Metaplasticity and Megaplasticity: Changing the Brain from Synapse to Community. Annual Meeting November 2022. Lake Arrowhead, CA.
- “Impact of Exercise on PD?” Metaplasticity and Megaplasticity: Changing the Brain from Synapse to Community. Annual Meeting November 2022. Lake Arrowhead, CA.
- “The Contribution of Perivascular Spaces to Parkinson’s Disease Pathophysiology.” University of Southern California Department of Neurology Grand Rounds. April 2023. Los Angeles, CA.
- “Impact of Exercise on Cognition in PD.” Parkinson Society British Columbia Global Symposium. University of British Columbia, Canada. March 2023.
- “Impact of Exercise on Cognition in PD: Building Resilience.” Parkinson Wellness Recovery Annual Retreat. June 2023. Scottsdale, AZ.
- “Impact of Exercise on Cognition in PD.” California Physical Therapy Association San Jose District Meeting. San Jose, CA. July 2023.
- “Mechanisms of Exercise in Parkinson’s Disease and its Models” University of Southern California Department of Neurology Grand Rounds. July 2023. Los Angeles, CA.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

University of Southern California

Name: Giselle Petzinger, MD
Project Role: PI
Research Identifier: N/A
Nearest person month worked: 0.67 mo
Contribution to the project: No change.

Name: Jennifer Hui, MD
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 0.03 mo
Contribution to the project: No change.

Name: Andrew Petkus, Ph.D.
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 0.6 mo
Contribution to the project: No change.

Name: Vy Bui, BS
Project Role: Research coordinator
Research Identifier: N/A
Nearest person month worked: 2.4 months
Contribution to the project: No change.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

University of California San Diego/VA San Diego

Name: Dawn Schiehser, PhD
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 0.45 calendar months
Contribution to Project: No change.

Name: Deyran Paredes
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.89 calendar months
Contribution to Project: Deryan is a research assistant who aids in patient recruitment and communication, cognitive behavioral assessment, fitness evaluation/monitoring, and neuroimaging.

Name: David Wing
Project Role: Collaborator
Research Identifier: N/A
Nearest person month worked: 0.3 months
Contribution to the project: No change.

Name: Michael Higgins
Project Role: Research Associate
Research Identifier: N/A
Nearest person month worked: 0.75 months
Contribution to the project: No change.

University of Virginia:

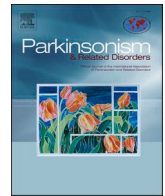
Name: Jack Van Horn, Ph.D. (Moved from USC to University of Virginia)
Project Role: Co-Investigator
Research Identifier: N/A
Nearest person month worked: 0.36mo
Contribution to the project: No change.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

9. APPENDICES:

pdf Files of Published and Submitted manuscripts including:



Physical activity intensity is associated with cognition and functional connectivity in Parkinson's disease

Erin K. Donahue^{a,b,#}, Siva Venkadesh^{c,#}, Vy Bui^a, Angelie Cabrera Tuazon^{d,e}, Ryan K. Wang^a, Danielle Haase^a, Ryan P. Foreman^a, Jared J. Duran^a, Andrew Petkus^a, David Wing^{d,f}, Michael Higgins^{d,f}, Daniel P. Holschneider^{b,g}, Ece Bayram^h, Irene Litvan^h, Michael W. Jakowec^a, John Darrell Van Horn^{c,i}, Dawn M. Schiehser^{d,e,1}, Giselle M. Petzinger^{a,1,*}

^a Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA, 90089, USA

^b Neuroscience Graduate Program, University of Southern California, Los Angeles, CA, 90089, USA

^c Department of Psychology, University of Virginia, Charlottesville, VA, 22904, USA

^d Veterans Administration San Diego Healthcare System (VASDHS), San Diego, CA, 92161, USA

^e Department of Psychiatry, University of California, San Diego, CA, 92093, USA

^f Herbert Wertheim School of Public Health, University of California San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0811, USA

^g Department of Psychiatry & the Behavioral Sciences, University of Southern California, Los Angeles, CA, 90089, USA

^h Parkinson and Other Movement Disorder Center, Department of Neurosciences, University of California San Diego, California, 92092-0886, USA

ⁱ School of Data Science, University of Virginia, Charlottesville, VA, 22904, USA

ARTICLE INFO

Keywords:

Global cognition
Resting-state fMRI
WHO recommendation
Actigraphy

ABSTRACT

Background: Cognitive impairment is common in Parkinson's disease (PD) and often leads to dementia, with no effective treatment. Aging studies suggest that physical activity (PA) intensity has a positive impact on cognition and enhanced functional connectivity may underlie these benefits. However, less is known in PD. This cross-sectional study examined the relationship between PA intensity, cognitive performance, and resting state functional connectivity in PD and whether PA intensity influences the relationship between functional connectivity and cognitive performance.

Methods: 96 individuals with mild-moderate PD completed a comprehensive neuropsychological battery. Intensity of PA was objectively captured over a seven-day period using a wearable device (ActiGraph). Time spent in light and moderate intensity PA was determined based on standardized actigraphy cut points. Resting-state fMRI was assessed in a subset of 50 individuals to examine brain-wide functional connectivity.

Results: Moderate intensity PA (MIPA), but not light PA, was associated with better global cognition, visuospatial function, memory, and executive function. Individuals who met the WHO recommendation of ≥ 150 min/week of MIPA demonstrated better global cognition, executive function, and visuospatial function. Resting-state functional connectivity associated with MIPA included a combination of brainstem, hippocampus, and regions in the frontal, cingulate, and parietal cortices, which showed higher connectivity across the brain in those achieving the WHO MIPA recommendation. Meeting this recommendation positively moderated the associations between identified functional connectivity and global cognition, visuospatial function, and language.

Conclusion: Encouraging MIPA, particularly the WHO recommendation of ≥ 150 min of MIPA/week, may represent an important prescription for PD cognition.

* Corresponding author. Department of Neurology, 1333 San Pablo St., MCA-243, University of Southern California, Los Angeles, CA, 90033, USA.

E-mail address: petzinge@med.usc.edu (G.M. Petzinger).

indicates co-first authors.

¹ indicates co-senior authors.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects motor and nonmotor features including cognition [1]. Cognitive impairment is common in PD and may include deficits in executive function, visual spatial function, memory, attention, and language. Cognitive impairment in PD can range from mild to dementia and has been shown to be associated with decrements in quality of life [2]. Lower functional connectivity is shown to underlie cognitive impairment and decline [3]. Studies in aging suggest that physical activity (PA) may play an important role in facilitating cognitive function [4] and that these benefits may be due to changes in the brain's structure and enhanced functional connectivity [5–7]. PA is defined as any non-sedentary activity that increases metabolic expenditure above rest and may be characterized by level of intensity (light, moderate, and vigorous) [8]. Studies in aging support that intensity of PA, more than duration, may have the greatest effect on cognitive performance [9,10]. In concordance with these reports, the World Health Organization (WHO) provides specific recommendations that PA should achieve a level of moderate-to-vigorous intensity to gain cognitive benefits. Specifically, individuals should achieve at least 150 min of accumulated time spent in moderate intensity PA, 75 min of accumulated time spent in vigorous PA, or an equivalent combination of the two [11].

Compared to aging, less is known about the association of intensity of PA and cognition in PD. Loprinzi and colleagues (2018) reported an association between increased minutes of moderate to vigorous PA and improved cognition, as evaluated by the Montreal Cognitive Assessment (MoCA), in individuals with mild to moderate PD [12]. However, gaps in knowledge remain, including the role of light intensity PA and its association with cognition, as well as the effect of intensity of PA on individual cognitive domains. In addition, little is known about the impact of PA on brain-wide functional connectivity in PD, and whether intensity of PA may influence the association between functional connectivity and cognitive performance.

The purpose of this study was to examine the associations between the intensity of PA and cognition and functional connectivity in PD. Examining lifestyle factors that may impact cognitive performance could improve quality of life and reduce progression to dementia. Intensity of PA was examined using a seven-day wearable accelerometer device to capture time spent in both light and moderate intensity PA. The primary goal was to examine the association between intensity of PA and global cognition and individual cognitive domains in PD. In addition, we examined whether meeting the WHO guidelines of 150 min of accumulated time spent per week in moderate intensity PA (MIPA) is associated with greater cognitive benefits in PD. The second goal was to explore whether the association between brain wide functional connectivity and cognitive performance is influenced by the intensity of PA. Using a predictive model approach, specific brain regions for examining brain wide functional connectivity and their association with cognitive performance were first identified by determining their ability to predict an individual meeting the WHO recommendation of 150 min per week of accumulated time spent in moderate intensity PA. These regions were utilized to examine the relationship between functional connectivity and cognitive performance, and the possible moderation of PA on this relationship.

2. Methods

2.1. Participants

Participants ($n = 96$) with idiopathic PD were enrolled in a multi-site (University of Southern California, USC; Veterans Affairs San Diego Healthcare System, VASDHS/University of California San Diego, UCSD) observational study. PD diagnosis was neurologically confirmed using UK Brain Bank criteria [13]. Inclusion criteria were as follows: (i) willing and able to provide informed consent; (ii) medically eligible for MRI;

and (iii) Hoehn and Yahr score ≤ 3 . Exclusion criteria included (i) history of severe psychiatric illness and (ii) dementia based on MDS-PD dementia criteria. Institutional Review Boards (IRB) from each site approved all study procedures. Demographic data included age, sex, years of education, years since diagnosis, MDS-UPDRS motor score (part III), and levoaopa equivalent daily dose (LEDD) [14].

2.2. Objective physical activity assessment

Objective assessment of PA was measured using the ActiGraph GT3X+ accelerometer (ActiGraph, LLC; Pensacola, FL). A seven-step algorithm identified as best-practice was used for the collection, processing, and summarization of PA accelerometer data collected with wearable system [15]. Participants were asked to wear the ActiGraph for seven days during all activities except water-based activities and while sleeping [16].

The GT3X+ provides both raw acceleration data and transformed summaries ("counts") aggregated across different time periods ("epochs", e.g., 1s, 10s, 1min). Non-wear time was excluded from analysis and was defined as ≥ 90 min of consecutive zero counts and a spike tolerance of 2 min with a 30-min window of zero counts upstream and downstream of each observed spike [17]. For this study, minute-by-minute estimates of activity were categorized into intensity-weighted summaries of PA based on metabolic equivalent of task (METs) using previously validated calibration thresholds based on counts per minute (CPM), including Light PA (1.5–2.99 METs, 100–1951 CPM), Moderate PA (3.0–5.99 METs, 1952–5723 CPM), and vigorous PA (6.0+ METs, >5724 CPM) [18]. All data analysis was performed using ActiLife software. Time spent in MIPA was determined using the following calculation: moderate PA minutes + (2 x vigorous PA minutes) [19].

2.3. Self-reported structured exercise log

Participants completed a seven-day exercise log to record daily exercise habits, including start and stop time and types of exercise (e.g., walking, running, yoga). From the log, exercise parameters of interest were derived, including (i) duration (average minutes/day); (ii) frequency (total number of exercise sessions/week); and (iii) variety (different types of exercise/week).

2.4. Neuropsychological assessment

The neuropsychological battery measured global cognitive performance and performance across the cognitive domains of attention, language, visuospatial function, memory (episodic), and executive function. Language was measured by Letter Fluency and Category Fluency subtests from the Verbal Fluency Test of the Delis Kaplan Executive Function System (D-KEFS) and the total score from the Boston Naming Test. Attention was measured by the Color Naming (Condition 1) and Word Reading (Condition 2) subtests from the D-KEFS Color Word Interference Test (CWIT), Forward Digit Span condition of the Adaptive Digit Ordering Test (DOT-A), and the Digit Sequencing condition of the DOT-A. Visuospatial function was measured by the Judgment of Line Orientation Test and the Hooper Visual Organization Test. Memory (episodic) was measured by the Total Trials 1–5, Short Delay Free Recall, and Long Delay Free Recall conditions of the California Verbal Learning Test (2nd Edition; CVLT-II) and the Immediate Recall and Delayed Recall conditions of the Brief Visuospatial Memory Test (BVMPT). Executive function was measured using the number of perseverative responses on the Wisconsin Card Sorting Test (WCST), Inhibition (Condition 3) and Inhibition/Switching (Condition 4) subtests from the D-KEFS CWIT, and Verbal Fluency Switching subtest (Total Score) from the D-KEFS Verbal Fluency Test. Domain scores were calculated by averaging z-scores from individual tests within a domain. Global cognition was assessed using a mean z-score of the five cognitive

domains (global cognition). The total score of the MoCA was used as a general cognition screen.

2.5. Statistical analysis

2.5.1. Physical activity intensity and structured exercise parameter association with cognition

Preliminary analyses examined the distribution of key variables to assess their characteristics (means, standard deviations, skewness, and kurtosis) to provide descriptive statistics of the study population. To reduce comparisons, correlation analysis was conducted prior to regression analysis to select comparisons of interest. Bivariate unadjusted Pearson correlations were performed between general cognition (MoCA), global cognition, and each cognitive domain (language, attention, visuospatial function, memory, executive function) and light PA or MIPA. Bivariate unadjusted Pearson correlations coefficients were obtained between MoCA, global cognition, and each cognitive domain and exercise log parameters (minutes of exercise, frequency of exercise, and variety of exercises). Where statistically significant correlations were identified, hierarchical linear regression analyses were conducted to examine associations between global cognitive function and each cognitive subdomain and PA, adjusting for age, sex, MDS-UPDRS motor score, and total self-reported duration of structured exercise.

2.5.2. Achieving WHO recommendation for MIPA and association with cognition

Participants underwent a categorical split based on whether they achieved or did not achieve the 150-min recommendation of MIPA per week. Demographic information for the two groups is presented in [Supplementary Table 1](#). Age and MDS-UPDRS motor score were significantly different between groups and were included as covariates in the statistical analysis. A general linear model (GLM) was used to examine the association between the WHO categorical split and MoCA and global cognition and each cognitive domain, adjusting for age, sex, MDS-UPDRS motor score, and total self-reported duration of structured exercise.

Levodopa equivalent daily dose was not significantly correlated with global cognition or any of the cognitive domains and were excluded from analyses. Study site was explored as a covariate but not included in the final model because including it as a covariate did not alter any statistical outcome. Self-reported total duration of structured exercise was added as a covariate in all analyses to ensure effect was due to intensity, rather than time spent in exercise [20]. Statistical analyses were conducted using SPSS, Version 28.0.1. Statistical significance was set at $p \leq 0.05$.

2.6. Functional MRI processing and connectivity analysis

2.6.1. Image acquisition

Resting-state functional MRI (fMRI) scans were obtained using Siemens PRISMA (USC) or GE 3T MRI (UCSD) scanners from 50 participants (420 vol, TR = 800.0, TE = 30.0). Scans were corrected for head-motion using MCFLIRT. Three translational and three rotational parameters were estimated frame-by-frame to calculate mean framewise displacement (MFD) for the entire duration of the scan. The average MFD was 0.12 mm ([Table 1](#)). No exclusion criteria were applied based on head-motion since MFD did not show any significant relationship with MIPA minutes ($p = 0.74$), age ($p = 0.22$), sex ($p = 0.11$), MDS-UPDRS motor scores ($p = 0.73$), MoCA ($p = 0.76$), global cognition ($p = 0.82$), memory ($p = 0.85$), language ($p = 0.73$), attention ($p = 0.99$), executive function ($p = 0.80$), or visual-spatial function ($p = 0.78$).

2.6.2. Image preprocessing

Scans were co-registered to anatomical T1-weighted MRI (TR = 2500.0, TE = 20.0), and the cortical gyri and sulci were segmented

Table 1

Study demographic information. ^a Minutes of light (less than 3 METs) physical activity per day as captured by ActiGraph monitor; ^b Minutes of moderate intensity (greater than or equal to 3 METs, with vigorous double weighted) physical activity per day as captured by ActiGraph monitor; ^c Minutes of self-reported structured exercise per day as reported on a seven-day exercise diary; ^d Frequency (number of incidences per week) of self-reported structured exercise per week as reported on a seven-day exercise diary; ^e Variety (number of different types of exercise) of self-reported structured exercise per week as reported on a seven-day exercise diary. Abbreviations: MIPA: moderate intensity physical activity (as recorded by wearable device); MDS-UPDRS: Movement Disorder Society Unified Parkinson's Disease Rating Scale.

	Total	Resting-state fMRI Subset	p-values
Demographics			
N (Female)	96 (41)	50 (23)	.704
Age	66.76 (8.60)	65.52 (9.13)	.876
Years Since Diagnosis	4.72 (4.81)	4.46 (5.36)	.766
Years of Education	16.81 (2.05)	16.68 (2.20)	.723
MDS-UPDRS Motor Score	23.99 (10.33)	21.16 (9.43)	.108
Dopamine Equivalents	568.51 (383.41)	579.57 (390.07)	.870
Resting-State Movement			
Mean Framewise Displacement (mm)	..	0.12 (0.06)	
Physical Activity (ActiGraph)			
Light ^a (Mins/Day)	263.71 (97.01)	267.94 (80.55)	.792
Moderate (Mins/Day)	18.74 (16.17)	21.96 (18.55)	.280
Exercise (Self-Reported Log)			
Duration ^c (Mins/Day)	62.63 (34.54)	71.76 (37.88)	.145
Frequency ^d (Per Week)	8.70 (4.81)	9.24 (4.99)	.526
Variety ^e (Per Week)	3.26 (1.65)	3.46 (1.57)	.481

according to the parcellation scheme in Destrieux et al. which defines 148 anatomical brain regions [21]. In addition, the BOLD signals for the subcortical amygdala, caudate, putamen, nucleus accumbens, hippocampus, pallidum, thalamus, cerebellum, and the brain stem were extracted [22]. Subcortical region inclusion was based on the graph-representation and analysis of brain anatomical connectivity in previous studies [23,24]. The fMRI BOLD signals were band-pass filtered in the range [0.01, 0.125] Hz, linear trends were removed, and signals were standardized to zero-mean and unit variance. Preprocessing was carried out using the Nilearn package in Python [25].

2.6.3. Functional connectivity estimation

Functional connectivity between brain regions were characterized using the frequencies and phase differences (lags) of the peak magnitudes of coherence extracted from the fMRI signal cross spectra [26]. For each region, we computed the degree (or number) of connections with lags less than or equal to 1 s to identify synchronized activations among spatially distributed regions. A 1 s delay in coherent activations was allowed between regions to account for regional variabilities in hemodynamic responses [27]. The number of anatomical regions that were coherent with a certain region over the scan duration denoted the degree of functional connectivity.

2.6.4. Statistical analysis involving functional connectivity

Identification of regional functional connectivity that are associated with MIPA using a sequential feature selection and logistic regression (LR) was undertaken ([Supplementary Figure 1](#)). The region identification and validation proceeded as follows: at each iteration, one of the n subjects was left out for validation and the remaining $n-1$ subjects were considered in a feature-search step. An LR model was trained to classify a subset (90%) of subjects into one of two categories: achieving or not achieving the WHO MIPA recommendation. Model performance on the

training set was evaluated using a 5-fold cross-validation. This process was repeated for $k = 100$ iterations, and the number of iterations in which a region was selected for inclusion in the model denoted its selection frequency. Then, the anatomical regions which were selected in at least one iteration were used to train a model on the entire $n-1$ subjects, and this model's prediction accuracy was evaluated on the left-out subject. Thus, the subject under evaluation was never included in the feature-selection method. This feature selection approach has been previously shown to reliably select the functional connectivity features using a sample size comparable to the present study [28]. Finally, a new, composite variable was derived by linearly combining the connectivity degrees of identified regions using their LR coefficients computed on the entire dataset. This composite variable, which is referred to as "MIPA-related functional connectivity," was used in a GLM analysis to investigate its association with global cognition and cognitive domains, adjusting for age, sex, and MDS-UPDRS motor scores. Demographic information for those achieving versus not achieving the WHO recommendations and who underwent neuroimaging is presented in [Supplementary Table 2](#). Age was significantly different between groups and was included as a covariate in the statistical analysis. The moderation effect of PA intensity was then examined by comparing the GLM model coefficients associating functional connectivity and cognitive performance between those participants achieving versus not achieving the WHO MIPA recommendation (see Robinson and colleagues [29]). All statistical analyses involving functional connectivity were performed in Python using scikit-learn and statsmodels packages [25,30].

3. Results

3.1. Participant and demographic characteristics

The total number of participants in this study were 96, age = 66.76 years old ($SD = 8.60$) years of education = 16.81 ($SD = 2.05$), 57% male, MDS-UPDRS motor score = 23.99 ($SD = 10.33$), years of PD diagnosis = 4.72 ($SD = 4.81$), average LEDD = 568.51 mg ($SD = 383.41$). Participants had on average 263.71 min of light PA per day ($SD = 97.01$) and 18.74 min of MIPA per day ($SD = 16.17$) and on average 6.81 days ($SD = 1.07$). Participants reported on average 62.63 min of exercise per day ($SD = 34.54$), 8.70 exercise sessions per week ($SD = 4.81$), and 3.26 different types of exercise per week ($SD = 1.65$) ([Table 1](#)). The only significant difference between the total PD group and the neuroimaging subgroup was the proportion of females was higher in the latter ([Table 1](#)).

3.2. Relationship between linear time spent in physical activity and cognitive performance

Correlational analysis revealed that time spent in MIPA was positively associated with all cognitive domains, including global cognition ($r(94) = 0.398, p < 0.001$), executive function ($r(94) = 0.305, p = 0.003$), memory ($r(94) = 0.299, p = 0.003$), visuospatial function ($r(94) = 0.386, p < 0.001$), attention ($r(94) = 0.298, p = 0.003$), and language ($r(94) = 0.248, p = 0.015$) as well as the MoCA ($r(94) = 0.295, p = 0.004$) ([Supplementary Table 3](#)). In addition, correlational analysis showed that light PA was positively associated with MoCA ($r(94) = 0.275, p = 0.007$), global cognition ($r(94) = 0.232, p = 0.023$), executive function ($r(94) = 0.227, p = 0.026$), and memory ($r(94) = 0.268, p = 0.008$), but not visuospatial function ($r(94) = 0.196, p = 0.056$), attention ($r(94) = 0.061, p = 0.554$), or language ($r(94) = 0.147, p = 0.152$) ([Supplementary Table 3](#)). Alternatively, self-reported exercise duration, frequency, or variety was not significantly correlated with MoCA, global cognition, or any cognitive domain (all p 's > 0.324) ([Supplementary Table 3](#)).

After controlling for age, sex, MDS-UPDRS motor score, and self-reported exercise duration, MIPA remained significantly associated with MoCA (unstandardized $\beta = 0.040$, 95% CI [0.003, 0.076], $\Delta R^2 =$

0.04, $p = 0.034$), global cognition (unstandardized $\beta = 0.015$, 95% CI [0.005, 0.025], $\Delta R^2 = 0.068, p = 0.003$), visuospatial function (unstandardized $\beta = 0.020$, 95% CI [0.006, 0.034], $\Delta R^2 = 0.07, p = 0.005$), executive function (unstandardized $\beta = 0.017$, 95% CI [0.003, 0.030], $\Delta R^2 = 0.049, p = 0.014$), and memory (unstandardized $\beta = 0.014$, 95% CI [0.002, 0.026], $\Delta R^2 = 0.036, p = 0.021$), but not with attention (unstandardized $\beta = 0.011$, 95% CI [-0.004, 0.026], $\Delta R^2 = 0.021, p = 0.143$) or language (unstandardized $\beta = 0.014$, 95% CI [0.001, 0.028], $\Delta R^2 = 0.035, p = 0.056$) ([Table 2](#), [Supplementary Fig. 2](#)). After controlling for age, sex, MDS-UPDRS motor score, and self-reported exercise duration, there was no association between light PA and MoCA (unstandardized $\beta = 0.003$, 95% CI [-0.002, 0.009], $\Delta R^2 = 0.011, p = 0.262$), global cognition (unstandardized $\beta = 0.000$, 95% CI [-0.002, 0.002], $\Delta R^2 = 0.000, p = 0.884$), executive function (unstandardized $\beta = 0.000$, 95% CI [-0.002, 0.002], $\Delta R^2 = 0.000, p = 0.885$), or memory (unstandardized $\beta = 0.000$, 95% CI [-0.002, 0.002], $\Delta R^2 = 0.001, p = 0.789$) ([Table 2](#), [Supplementary Fig. 2](#)).

3.2.1. Relationship between categorical time spent in MIPA and cognitive performance

37 of the 96 study participants met the WHO recommendation of 150 min of accumulated time in MIPA. Individuals meeting this criteria had higher global cognition ($F(1, 94) = 6.218, p = 0.014$), executive function ($F(1, 94) = 4.257, p = 0.042$), visuospatial function ($F(1, 94) = 5.616, p = 0.020$), and trending towards higher memory ($F(1, 94) = 3.185, p = 0.078$), than those who did not meet this recommendation, after controlling for age, sex, MDS-UPDRS motor score, and self-reported exercise duration. There was no significant difference between groups in MoCA ($F(1, 94) = 1.266, p = 0.263$), attention ($F(1, 94) = 2.766, p = 0.100$), or language ($F(1, 94) = 1.920, p = 0.169$) ([Table 3](#)).

3.2.2. Identification of Physical Activity Intensity-related brain regions

Key brain regions for examining functional connectivity and their association with cognitive performance were first identified using a predictive model approach. Specifically, from a total of 148 brain regions considered (see above), seven brain regions were selected based on their ability to predict whether an individual was classified as meeting or not meeting the WHO MIPA recommendation. These brain regions included (i) the bilateral precuneus (PrCun), a region within the posterior parietal lobe, (ii) the marginal part of cingulate sulcus (CgSMarp), a

Table 2

Adjusted associations between average number of minutes engaged in moderate intensity physical activity (greater than or equal to 3 METs, vigorous double weighted; top) or light physical activity (less than 3 METs; bottom) per day and domain-specific cognitive performance that were significant in correlations ($N = 96$). All domain-specific cognitive performance were z-score standardized and associations represent the effect of 1 min increase in moderate intensity physical activity per day on z-score standardized cognitive performance adjusted for age, sex, MDS-UPDRS motor score, and average minutes of self-reported structured exercise per day as reported on a seven-day exercise diary.

	β	95% CI Lower, Upper	P
Moderate Intensity Physical Activity			
MoCA	.040*	.003, .076	.034
Global Cognition	.015*	.005, .025	.003
Executive Function	.017*	.003, .030	.014
Memory	.014*	.002, .026	.021
Visuospatial Function	.020**	.006, .034	.005
Attention	.011	-.004, .026	.143
Language	.014	.000, .028	.056
Light Physical Activity			
MoCA	.003	-.002, .009	.262
Global Cognition	.000	-.002, .002	.885
Executive Function	.000	-.002, .002	.885
Memory	.000	-.002, .002	.789

Table 3

Adjusted associations between physical activity minutes per day categorized as achieving or not achieving WHO moderate intensity physical activity recommendation (see text for details) and domain-specific cognitive performance (N = 96). All domain-specific cognitive performance were z-score standardized and associations represent the effect of 1 min increase in moderate intensity physical activity per day on z-score standardized cognitive performance adjusted for age, sex, MDS-UPDRS motor score, and average minutes of self-reported structured exercise per day as reported on a seven-day exercise diary.

	F	p
MoCA	1.266	.263
Global Cognition	6.218*	.014
Executive Function	4.257*	.042
Memory	3.185	.078
Visuospatial Function	5.616*	.020
Attention	2.766	.100
Language	1.920	.169

region in the cingulate cortex, (iii) the brain stem (BSt), (iv) the hippocampus (Hip), (v) the middle posterior part of the cingulate gyrus and sulcus (MPosCgG/S), a region in the cingulate cortex, (vi) and the middle frontal sulcus (MFS), a region within the frontal lobe (Supplementary Fig. 3A). These regions showed an accuracy of 0.66 ($p = 0.015$, permutation test) in predicting whether a subject had achieved the WHO MIPA recommendation. All identified regions showed a higher degree of functional connectivity in the those who achieved this recommendation compared to those who did not (Supplementary Fig. 3B). The functional connectivity of all seven brain regions were combined into one composite variable for further analysis with cognitive performance and the moderation effects of PA intensity.

3.2.3. Association between functional connectivity of PA intensity-related brain regions and cognitive performance

There were significant positive associations between functional

connectivity of these combined brain regions and the following: MoCA ($\beta = 0.18$, 95% CI [0.03, 0.33], $p = 0.021$), global cognition ($\beta = 1.07$, 95% CI[0.58, 1.55], $p < 0.001$), executive function ($\beta = 0.70$, 95% CI [0.33, 1.07], $p < 0.001$), memory ($\beta = 0.62$, 95% CI [0.15, 1.09], $p = 0.01$), visuospatial function ($\beta = 0.64$, 95% CI [0.23, 1.05], $p = 0.002$), attention ($\beta = 0.45$, 95% CI [0.06, 0.84], $p = 0.02$), and language ($\beta = 0.72$, 95% CI [0.35, 1.1], $p < 0.001$) (Fig. 1). Importantly, the coefficients of these associations were significantly higher in the group achieving the WHO recommended MIPA minutes for global cognition, language, and visuospatial function. Thus, the categorical MIPA positively moderated the relationship between functional connectivity of these combined brain regions and global cognition ($\beta_{\text{achieving}} = 1.4$, $\beta_{\text{notachieving}} = 0.57$, $p = 0.023$), visuospatial function ($\beta_{\text{achieving}} = 1.27$, $\beta_{\text{notachieving}} = 0.16$, $p = 0.002$), and language ($\beta_{\text{achieving}} = 0.82$, $\beta_{\text{notachieving}} = 0.38$, $p = 0.047$). There were no significant moderation effects with MoCA ($\beta_{\text{achieving}} = 0.14$, $\beta_{\text{notachieving}} = 0.16$, $p = 0.593$), memory ($\beta_{\text{achieving}} = -0.18$, $\beta_{\text{notachieving}} = 0.69$, $p = 0.981$), or attention ($\beta_{\text{achieving}} = 0.33$, $\beta_{\text{notachieving}} = 0.30$, $p = 0.457$), but a trend with executive function ($\beta_{\text{achieving}} = 0.77$, $\beta_{\text{notachieving}} = 0.41$, $p = 0.085$) (Fig. 1).

4. Discussion

Major findings from this cross-sectional study examining the associations between intensity of PA, resting-state functional connectivity, and cognitive functioning in PD include: (1) accumulated time spent in moderate intensity PA (MIPA), but not light intensity PA was associated with higher MoCA, global cognition, executive function, memory, and visuospatial function; neither light, nor moderate PA was associated with attention or language; (2) achieving 150 min of accumulated time in MIPA per week (with respect to the recommendations of the WHO) is associated with higher global cognition, executive function, visuospatial function, and memory (trend), but not MoCA, attention, or language; and (3) intensity of PA, defined categorically as achieving 150 min of

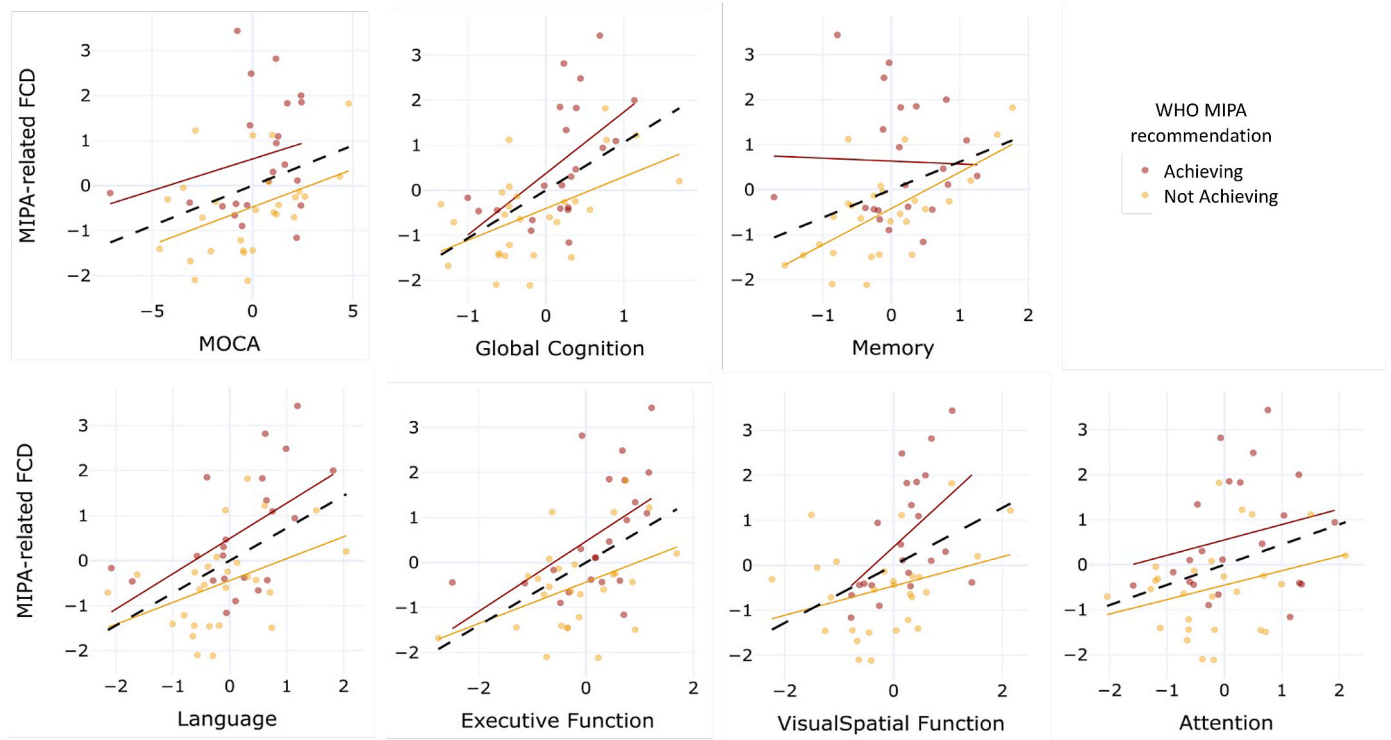


Fig. 1. Associations between moderate intensity physical activity (MIPA)-related functional connectivity degree (FCD) and cognitive performances after accounting for age, sex, MDS-UPDRS motor score, and average minutes of self-reported structured exercise per day (Cov). The coefficient of association between FCD and cognition is significantly higher in the WHO MIPA-yes group for global cognition, language, and visual spatial scores. Dotted lines represent overall associations.

accumulated time in MIPA per week, moderated the relationship between functional connectivity and global cognition, visuospatial function, language, and trend for executive function.

Taken together, these findings suggest that intensity of PA may be important for improving cognitive performance in PD.

PA intensity has been reported to reduce the risk of PD [31], and one study illustrated moderate to vigorous PA was associated with higher MoCA scores in PD patients [12]. The present study demonstrated that objectively measured MIPA had broad effects on multiple cognitive domains except for attention and language. These findings are consistent with observational studies in aging demonstrating the impact of MIPA on many facets of cognition [32]. Although self-reported parameters of structured exercise, such as duration, frequency, and variety, were not a primary aim of this study, we also found that none of these self-reported exercise parameters were significantly associated with cognition. Further analysis demonstrated that this effect of MIPA on cognition remained significant even after controlling for duration of structured exercise, thus highlighting the relative importance of intensity of PA over duration. Furthermore, given that objective measures of PA capture both non-exercise and exercise activities supports that this benefit of intensity of PA may come from a variety of daily activities.

While the WHO recommends at least 150 min per week of MIPA, 75 min per week of vigorous intensity PA, or an equivalent combination of both, to maintain cognitive health in the normal aging population [11, 33], the importance of this recommendation for cognitive health in PD is unknown. Thirty seven of our 96 participants achieved this recommendation, and these individuals (i.e., those who achieved the WHO recommendation of ≥ 150 min of MIPA per week) performed better on measures of global cognition, executive function, visuospatial function, and memory (the latter of which was trend) compared to those who did not. In agreement with this cross-sectional study, an interventional study by Uc and colleagues (2014) found that individuals who achieved the same WHO recommendation performed better on a test of inhibition, which is a component of executive function [34]. Collectively, the results of this study suggest that while moderate intensity PA may be important, meeting the recommendation of 150 min of accumulated time per week in MIPA is a useful and beneficial prescription for improving cognitive performance in PD. Moderate intensity PA includes activities that induce a light sweat within 10 min, and increases breathing and heart rate, such it becomes somewhat difficult to have a conversation and heart rate increases to 64–76% maximal heart rate (calculated as $220 - \text{age in years}$) [35]. Examples of moderate intensity PA include activities such as brisk walking, dancing, or raking the yard [35].

Previous studies in aging individuals have suggested that the benefits of PA on cognitive performance may be due to improved functional connectivity changes in cortical and subcortical regions [5]. In the present study, functional connectivity was examined by combining those brain regions that were predictive of achieving 150 min of MIPA (including the middle frontal lobe, the bilateral posterior parietal lobe, two regions in the cingulate cortex, the hippocampus, and the brain stem). These brain regions showed higher functional connectivity degrees in the group achieving 150 min of MIPA per week than the group not achieving 150 min of MIPA, and their composite connectivity was positively associated with all cognitive domains assessed. Note that the selection process for the brain regions considered the magnitudes of differences in their connectivity between the two groups and did not consider any directionality of those differences (i.e., higher, or lower connectivity in a group was not a criterion for region selection) (see also Section 2.6.4). Additionally, the observed positive correlations between functional connectivity and cognitive performance showed higher coefficients in the group achieving 150 min of MIPA per week for global cognition, visuospatial function, and language. Taken together, these observations suggest that the identified regions maybe important for mediating the effects of PA intensity on cognition.

A possible explanation for the influence of intensive PA on both

functional connectivity and cognition could be due to changes in blood flow, along with an increase in metabolites and neurotrophic factors, which may help to support synaptic integrity. For example, a recent study examining transcript expression in brains of healthy individuals reported that people with moderate or high PA had upregulation of genes involved in synaptic integrity in regions that included the hippocampus, substantia nigra, and prefrontal cortex [36]. The same genes were also found to be downregulated in neurodegeneration, including PD [36]. Work from our lab has also demonstrated that intensive PA is associated with synaptogenesis in a PD animal model [37]. Therefore, one mechanism by which PA could alter function connectivity to enhance cognitive performance may be through enhanced synaptic function in the context of PD.

4.1. Limitations and strengths

There are several limitations to the present study. Due to the cross-sectional nature of this study, a causal relationship between PA and cognition cannot be determined, such that better cognitive function may drive higher levels of PA, rather than higher amounts of intensive PA impacting better cognitive function. Furthermore, due to the mild-moderate severity of the PD in our study population, these results may not be generalizable to individuals with more advanced PD. However, strengths of this study include the objective nature by which PA was captured, allowing a more complete assessment of all daily activities. Importantly, due to the observational nature of this study, real-world, community-based activities were captured, and the results are likely to generalize to other individuals with PD who are ambulatory and independent.

5. Conclusions

The findings of this study support that moderate intensity physical activity may be important for promoting cognitive function in PD. Particularly, achieving the WHO recommendation of ≥ 150 min of MIPA per week was associated with better cognition and higher degrees of functional connectivity that are brain-wide in nature. Consequently, increased time spent in MIPA, which may be incorporated into daily activities, may represent an important and feasible prescription for promoting cognitive performance in mild to moderate PD.

Funding sources

This work was supported by grants from the US Department of Defense (U.S. Army, CDMRP) #W81XWH18-1-0666 (DPH), #W81XWH19-1-0443 (MWJ), #W81XWH18-1-0665 (GP), the Parkinson's Foundation (GP). Special thanks to friends of the USC Parkinson's disease research group, including George and Marylou Boone, Walter and Susan Doniger, and the family of Don Roberto Gonzalez Barrera.

Author roles

EKD, SV, MWJ, and GMP were responsible for doing the literature search. MWJ, JDVH, DMS, and GMP were responsible for designing the study. SV, VB, ACT, RKW, DH, RPF, JJD, AP, BTL, DW, MH, EB, IL, DS, and GMP were responsible for the data collection. EKD and SV were responsible for analyzing the data. EKD, SV, JDVH, MWJ, DMS, and GMP were responsible for interpreting the data. EKD, SV, and AP were responsible for the figures. EKD, SV, VB, MWJ, and GMP were responsible for writing the manuscript. EKD, SV, VB, ACT, RKW, DH, RPF, JJD, AP, BTL, DW, MH, DPH, EB, IL, MWJ, JDVH, DMS, and GMP were responsible for critically reviewing the manuscript. VB, ACT, DH, and GMP accessed and verified the underlying data. EKD was responsible for submitting the study for publication.

Financial disclosure of authors

Authors report no competing interests outside of published work.

Data sharing

Data is available by request to GMP.

Declaration of competing interest

The authors report no conflicts of interest or disclosures.

Acknowledgements

The authors gratefully acknowledge the discussion and help from colleagues not included in the authorship list, including Zhuo Wang and Derek Phillips. The authors also gratefully acknowledge the participation of our patients, without whom this research would not have been possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parkreldis.2022.09.005>.

References

- [1] D. Aarsland, B. Creese, M. Politis, K.R. Chaudhuri, D.H. Ffytche, D. Weintraub, C. Ballard, Cognitive decline in Parkinson disease, *Nat. Rev. Neurol.* 13 (2017) 217–231, <https://doi.org/10.1038/nrneurol.2017.27>.
- [2] R.A. Lawson, A.J. Yarnall, G.W. Duncan, T.K. Khoo, D.P. Breen, R.A. Barker, D. Collerton, J.P. Taylor, D.J. Burn, Severity of mild cognitive impairment in early Parkinson's disease contributes to poorer quality of life, *Park. Relat. Disord.* 20 (2014) 1071–1075, <https://doi.org/10.1016/j.parkreldis.2014.07.004>.
- [3] M. Hassan, L. Chato, P. Benquet, A. Delval, C. Leroy, L. Plomhause, A.J. H. Moonen, A.A. Duits, A.F.G. Leentjens, V. van Kranen-Mastenbroek, L. Defebvre, P. Derambure, F. Wendling, C. Dujardin, Functional connectivity disruptions correlate with cognitive phenotypes in Parkinson's disease, *Neuroimage Clin* 14 (2017) 591–601, <https://doi.org/10.1016/j.nicl.2017.03.002>.
- [4] N.J. Kirk-Sanchez, E.L. McGough, Physical exercise and cognitive performance in the elderly: current perspectives, *Clin. Interv. Aging* 9 (2014) 51–62, <https://doi.org/10.2147/CIA.S39506>.
- [5] F. Herold, A. Töpel, L. Schega, N.G. Müller, Functional and/or structural brain changes in response to resistance exercises and resistance training lead to cognitive improvements - a systematic review, *Eur. Rev. Aging Phys. Act. Off. J. Eur. Group Res. Elder. Phys. Act.* 16 (2019) 10, <https://doi.org/10.1186/s11556-019-0217-2>.
- [6] M.A. Gogniat, C.M. Mewborn, T.L. Robinson, K.R. Jean, L.S. Miller, The relations between physical activity level, executive function, and white matter microstructure in older adults, *J. Phys. Activ. Health* 18 (2021) 1286–1298, <https://doi.org/10.1123/jpah.2021-0012>.
- [7] J.C. Smith, K.A. Nielson, J.L. Woodard, M. Seidenberg, S. Durgerian, P. Antuono, A. M. Butts, N.C. Hantke, M.A. Lancaster, S.M. Rao, Interactive effects of physical activity and APOE-ε4 on BOLD semantic memory activation in healthy elders, *Neuroimage* 54 (2011) 635–644, <https://doi.org/10.1016/j.neuroimage.2010.07.070>.
- [8] D. Thivel, A. Tremblay, P.M. Genin, S. Panahi, D. Rivière, M. Duclos, Physical activity, inactivity, and sedentary behaviors: definitions and implications in occupational health, *Front. Public Health* 6 (2018) 288, <https://doi.org/10.3389/fpubh.2018.00288>.
- [9] Z.Z. Zlatar, S. Godbole, M. Takemoto, K. Crist, C.M.C. Sweet, J. Kerr, D. E. Rosenberg, Changes in moderate intensity physical activity are associated with better cognition in the multilevel intervention for physical activity in retirement communities (MIPARC) study, *Am. J. Geriatr. Psychiatr.* 27 (2019) 1110–1121, <https://doi.org/10.1016/j.jagp.2019.04.011>.
- [10] H. Makizako, T. Liu-Ambrose, H. Shimada, T. Doi, H. Park, K. Tsutsumimoto, K. Uemura, T. Suzuki, Moderate-intensity physical activity, hippocampal volume, and memory in older adults with mild cognitive impairment, *J. Gerontol. Ser. A* 70 (2015) 480–486, <https://doi.org/10.1093/gerona/glu136>.
- [11] World Health Organization, WHO Guidelines on Physical Activity and Sedentary Behaviour, World Health Organization, 2020. <https://www.ncbi.nlm.nih.gov/books/NBK566045/>.
- [12] P.D. Loprinzi, M.M. Danzl, E. Ulanowski, C. Paydo, A pilot study evaluating the association between physical activity and cognition among individuals with Parkinson's disease, *Disabil. Health J.* 11 (2018) 165–168, <https://doi.org/10.1016/j.dhjo.2017.05.004>.
- [13] A.J. Hughes, S.E. Daniel, L. Kilford, A.J. Lees, Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases, *J. Neurol. Neurosurg. Psychiatry* 55 (1992) 181–184, <https://doi.org/10.1136/jnnp.55.3.181>.
- [14] Parkinson's Disease Measurement, PwP, surveys, trials, analysis. <https://www.parkinsonsmeasurement.org/toolbox/levodopaEquivalentDose.htm>.
- [15] D.P. Heil, S. Brage, M.P. Rothney, Modeling physical activity outcomes from wearable monitors, *Med. Sci. Sports Exerc.* 44 (2012) S50–S60, <https://doi.org/10.1249/MSS.0b013e3182399dce>.
- [16] C.E. Matthews, M. Hagströmer, D.M. Pober, H.R. Bowles, Best practices for using physical activity monitors in population-based research, *Med. Sci. Sports Exerc.* 44 (2012) S68–S76, <https://doi.org/10.1249/MSS.0b013e3182399e5b>.
- [17] L. Choi, Z. Liu, C.E. Matthews, M.S. Buchowski, Validation of accelerometer wear and nonwear time classification algorithm, *Med. Sci. Sports Exerc.* 43 (2011) 357–364, <https://doi.org/10.1249/MSS.0b013e3181ed61a3>.
- [18] P. Freedson, H.R. Bowles, R. Troiano, W. Haskell, Assessment of physical activity using wearable monitors: recommendations for monitor calibration and use in the field, *Med. Sci. Sports Exerc.* 44 (2012) S1–S4, <https://doi.org/10.1249/MSS.0b013e3182399b7e>.
- [19] CDC, Move More; Sit Less, Cent. Dis. Control Prev. (2022). <https://www.cdc.gov/physicalactivity/basics/adults/index.htm>.
- [20] M. Angevaeren, L. Vanhees, W. Wendel-Vos, H.J.J. Verhaar, G. Aufdemkampe, A. Aleman, W.M.M. Verschuren, Intensity, but not duration, of physical activities is related to cognitive function, *Eur. J. Cardiovasc. Prev. Rehabil. Off. J. Eur. Soc. Cardiol. Work. Groups Epidemiol. Prev. Card. Rehabil. Exerc. Physiol.* 14 (2007) 825–830, <https://doi.org/10.1097/HJR.0b013e3282ef995b>.
- [21] C. Destrieux, B. Fischl, A. Dale, E. Halgren, Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature, *Neuroimage* 53 (2010) 1–15, <https://doi.org/10.1016/j.neuroimage.2010.06.010>.
- [22] B. Fischl, D.H. Salat, E. Busa, M. Albert, M. Dieterich, C. Haselgrove, A. van der Kouwe, R. Killiany, D. Kennedy, S. Klaveness, A. Montillo, N. Makris, B. Rosen, A. M. Dale, Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain, *Neuron* 33 (2002) 341–355, [https://doi.org/10.1016/S0896-6273\(02\)00569-x](https://doi.org/10.1016/S0896-6273(02)00569-x).
- [23] A. Irimia, J.D. Van Horn, Systematic network lesioning reveals the core white matter scaffold of the human brain, *Front. Hum. Neurosci.* 8 (2014) 51, <https://doi.org/10.3389/fnhum.2014.00051>.
- [24] A. Irimia, M.C. Chambers, C.M. Torgerson, J.D. Van Horn, Circular representation of human cortical networks for subject and population-level connectomic visualization, *Neuroimage* 60 (2012) 1340–1351, <https://doi.org/10.1016/j.neuroimage.2012.01.107>.
- [25] A. Abraham, F. Pedregosa, M. Eickenberg, P. Gervais, A. Mueller, J. Kossaifi, A. Gramfort, B. Thirion, G. Varoquaux, Machine learning for neuroimaging with scikit-learn, *Front. Neuroinf.* 8 (2014) 2825–2830, <https://doi.org/10.3389/fninf.2014.00014>.
- [26] P. Welch, The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms, *IEEE Trans. Audio Electroacoust.* 15 (1967) 70–73, <https://doi.org/10.1109/TAU.1967.1161901>.
- [27] A. Mitra, A.Z. Snyder, C.D. Hacker, M.E. Raichle, Lag structure in resting-state fMRI, *J. Neurophysiol.* 111 (2014) 2374–2391, <https://doi.org/10.1152/jn.00804.2013>.
- [28] A. Vo, W. Sako, K. Fujita, S. Peng, P.J. Mattis, F.M. Skidmore, Y. Ma, A.M. Uluğ, D. Eidelberg, Parkinson's disease-related network topographies characterized with resting state functional MRI, *Hum. Brain Mapp.* 38 (2017) 617–630, <https://doi.org/10.1002/hbm.23260>.
- [29] C.D. Robinson, S. Tomek, R.E. Schumacker, Tests of moderation effects: difference in simple slopes versus the interaction, *Term* 39 (2013) 9.
- [30] S. Seabold, J. Perktold, Statsmodels: Econometric and Statistical Modeling with Python, Austin, Texas, 2010, pp. 92–96, <https://doi.org/10.25080/Majora-92bf1922-011>.
- [31] X. Fang, D. Han, Q. Cheng, P. Zhang, C. Zhao, J. Min, F. Wang, Association of levels of physical activity with risk of Parkinson disease: a systematic review and meta-analysis, *JAMA Netw. Open* 1 (2018), e182421, <https://doi.org/10.1001/jamanetworkopen.2018.2421>.
- [32] K.I. Erickson, C. Hillman, C.M. Stillman, R.M. Ballard, B. Bloodgood, D.E. Conroy, R. Macko, D.X. Marquez, S.J. Petruzzello, K.E. Powell, FOR 2018 physical activity guidelines advisory committee*, physical activity, cognition, and brain outcomes: a review of the 2018 physical activity guidelines, *Med. Sci. Sports Exerc.* 51 (2019) 1242–1251, <https://doi.org/10.1249/MSS.0000000000001936>.
- [33] M. Menai, V.T. van Hees, A. Elbaz, M. Kivimaki, A. Singh-Manoux, S. Sabia, Accelerometer assessed moderate-to-vigorous physical activity and successful ageing: results from the Whitehall II study, *Sci. Rep.* 8 (2017), 45772, <https://doi.org/10.1038/srep45772>.
- [34] E.Y. Uc, K.C. Doerschug, V. Magnotta, J.D. Dawson, T.R. Thomsen, J.N. Kline, M. Rizzo, S.R. Newman, S. Mehta, T.J. Grabowski, J. Bruss, D.R. Blanchette, S. W. Anderson, M.W. Voss, A.F. Kramer, W.G. Darling, Phase I/II randomized trial of aerobic exercise in Parkinson disease in a community setting, *Neurology* 83 (2014) 423–425, <https://doi.org/10.1212/WNL.0000000000000644>.
- [35] B.R. MacIntosh, J.M. Murias, D.A. Keir, J.M. Weir, What is moderate to vigorous exercise intensity? *Front. Physiol.* 12 (2021), 682233, <https://doi.org/10.3389/fphys.2021.682233>.
- [36] J.A. Santiago, J.P. Quinn, J.A. Potashkin, Physical activity rewires the human brain against neurodegeneration, *Int. J. Mol. Sci.* 23 (2022) 6223, <https://doi.org/10.3390/ijms23116223>.
- [37] W.A. Toy, G.M. Petzinger, B.J. Leyshon, G.K. Akopian, J.P. Walsh, M.V. Hoffman, M.G. Vucković, M.W. Jakowec, Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-

1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease,
Neurobiol. Dis. 63 (2014) 201–209, <https://doi.org/10.1016/j.nbd.2013.11.017>.

Magnetic resonance spectroscopy shows associations between neurometabolite levels and perivascular space volume in Parkinson's disease: a pilot and feasibility study

Erin K. Donahue^{a,b}, Vy Bui^a, Ryan P. Foreman^a, Jared J. Duran^a, Siva Venkadesh^c, Jeiran Choupan^d, John D. Van Horn^{c,e}, Jeffry R. Alger^f, Michael W. Jakowec^a, Giselle M. Petzinger^{a,*} and Joseph O'Neill^{g,*}

Objective Higher volume fraction of perivascular space (PVS) has recently been reported in Parkinson's disease (PD) and related disorders. Both elevated PVS and altered levels of neurometabolites, assayed by proton magnetic resonance spectroscopy (MRS), are suspected indicators of neuroinflammation, but no published reports have concurrently examined PVS and MRS neurometabolites.

Methods In an exploratory pilot study, we acquired multivoxel 3-T MRS using a semi-Localization by Adiabatic SElective Refocusing (sLASER) pulse-sequence (repetition time/echo time = 2810/60 ms, voxels $10 \times 10 \times 10 \text{ mm}^3$) from a 2D slab sampling bilateral frontal white matter (FWM) and anterior middle cingulate cortex (aMCC). PVS maps obtained from high-resolution ($0.8 \times 0.8 \times 0.8 \text{ mm}^3$) T1-weighted MRI were co-registered with MRS. In each MRS voxel, PVS volume and neurometabolite levels were measured.

Results Linear regression accounting for age, sex, and BMI found greater PVS volume for higher levels of choline-containing compounds (Cho; $P = 0.047$) in FWM and lower PVS volume for higher levels of N-acetyl compounds (NAA; $P = 0.012$) in aMCC. Since (putatively) higher Cho is associated with inflammation while NAA has anti-inflammatory properties, these observations add to evidence that higher PVS load is a sign of inflammation. Additionally, lower Montreal Cognitive Assessment

scores were associated with lower NAA in aMCC ($P = 0.002$), suggesting that local neuronal dysfunction and inflammation contribute to cognitive impairment in PD.

Conclusion These exploratory findings indicate that co-analysis of PVS and MRS is feasible and may help elucidate the cellular and metabolic substrates of glymphatic and inflammatory processes in PD.

NeuroReport 33: 291–296 Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

NeuroReport 2022, 33:291–296

Keywords: anterior middle cingulate cortex, choline-compounds, frontal white matter, Montreal Cognitive Assessment, N-acetyl-aspartate, neuroinflammation, Virchow-Robin spaces

^aDepartment of Neurology, University of Southern California, ^bNeuroscience Graduate Program, University of Southern California, Los Angeles, California, ^cDepartment of Psychology, University of Virginia, Charlottesville, Virginia, ^dLaboratory of Neuroimaging, USC Stevens Neuroimaging and Informatics Institute, University of Southern California, Los Angeles, California, ^eSchool of Data Science, University of Virginia, Charlottesville, Virginia, ^fDepartment of Neurology, University of California, Los Angeles and ^gDivision of Child Psychiatry, UCLA Semel Institute for Neuroscience, Los Angeles, California, USA

Correspondence to Michael W. Jakowec, PhD, Department of Neurology, University of Southern California, 1333 San Pablo St, MCA241, Los Angeles, CA 90089, USA
Tel: +1 323 442 3367; e-mail: mjakowec@surgery.usc.edu

*Giselle M. Petzinger and Joseph O'Neill are co-senior authors.

Received 23 January 2022 Accepted 16 February 2022

Introduction

Recently, we [1] and others [2,3] have documented increased perivascular space (PVS; also known as Virchow-Robin space) in Parkinson's disease (PD). Increased PVS is also observed in other neurodegenerative disorders, such as Alzheimer's disease [4]. PVS is associated with elevated inflammatory serum proteins, infiltration of leukocytes, and proliferation of reactive astrocytes, indicating that increased PVS may represent one or more aspects of a normal or pathological inflammatory response in several conditions [5].

Neuroinflammation may be associated with cognitive decline in the elderly and is a likely contributor to PD pathogenesis [6]. Proton magnetic resonance spectroscopy (MRS) neurometabolites are potential markers of

both inflammatory and metabolic changes that can impact cognition, including in PD. Proinflammatory states and diminished cognition are seen with elevated brain levels of the MRS neurometabolites glutamine (Gln), choline-containing compounds (Cho), creatine + phosphocreatine (Cr), and *myo*-inositol (mI), which are more abundant in astrocytes than in neurons [7–9]. In contrast, anti-inflammatory states and improved cognition are linked to higher levels of NAA, a neurometabolite that is highly abundant in, and nearly exclusive to, neurons [10]. Glutamate (Glu), found abundant in both neurons and glia, can be increased or decreased in inflammation. Numerous findings have implicated altered MRS neurometabolites levels in PD, including in connection with inflammation [11].

To the best of our knowledge, no published study has examined the co-occurrence of PVS and MRS-detected neurometabolites in any condition, including PD. The present study tested the feasibility of co-analysis of PVS and MRS endpoints in a small sample of PD patients in whom both structural MRI (PVS quantification) and MRS (neurometabolite quantification) had been collected. MRI was acquired with a whole-brain high-resolution T1-weighted scan while MRS was acquired from a 2D multivoxel array ('slab') sampling bilateral frontal white matter (FWM) and frontal midline cortices, both of which are implicated in cognitive impairment in PD [12]. We anticipated detecting appreciable PVS in FWM of PD patients, based on our prior study and on FWM forming part of the centrum semiovale, a predilection site for increased PVS (along with basal ganglia and midbrain) [1]. Although PVS in cortical gray matter has been observed, it is relatively understudied compared to PVS in white matter. Therefore, we also assayed PVS in the frontal midline cortical gray matter regions. Finally, we examined the relationship between PVS-associated neurometabolite levels and the Montreal Cognitive Assessment score (MoCA), a widely used instrument for measuring global cognitive performance in PD [13].

Methods
Participants

This pilot study comprised 17 individuals with idiopathic PD recruited from the movement disorders clinic at the University of Southern California (USC). All participants had a diagnosis of PD per UK Brain Bank (G.M.P.) criteria and met the following additional inclusion criteria: (1) willing and able to provide informed consent; (2) age 50–85 years; (3) medically eligible for MRI imaging; (4) score between Hoehn and Yahr II and III; and (5) English as primary language. Exclusion criteria were: (1) contraindication to MRI (e.g. metal implants, severe claustrophobia); (2) history of severe psychiatric illness; (3) PD dementia based on MDS-PD dementia criteria. The USC Institutional Review Board approved all study procedures. Table 1 lists age, sex, years of education (self-report), years since diagnosis, Unified Parkinson's Disease Rating Scale motor score (part III), and MoCA scores.

MRI acquisition

Structural MRI and MRS were acquired together in a 60-min session on a 3-T Siemens Prisma with 32-channel phased-array head coil at the Center for Image Acquisition (CIA) on the Health Science Campus at USC. Participants were scanned in the 'ON' state, typically 1–2 hours after taking medications. Whole-brain T1w MRI was acquired with a Siemens magnetization-prepared rapid gradient-echo pulse-sequence (flip 8°, repetition-time/echo-time/inversion-time = 2400/2.22/1000ms, voxels 0.8×0.8×0.8mm³; 1 NEX, acceleration factor 2, 6:38min). Water-suppressed (WS) [¹H]-MRS was acquired in a 16×16 2D array ('slab') using the Center for Magnetic Resonance Research

Table 1 Demographics of study participants

N(F)	17 (7)
Age (years)	64.5 (10.6)
Education (years)	17.3 (1.9)
Duration of PD (years)	5.5 (8.0)
UPDRS motor (points)	54.2 (9.9)
BMI (kg/m ²)	25.1 (4.3)
ICV (mm ³)	1 608 982 (166 435)
MoCA (points)	26.9 (2.1)

Data are represented as mean ± SD.
ICV, intracranial volume; MoCA, Montreal Cognitive Assessment; UPDRS, Unified Parkinson's Disease Rating Scale.

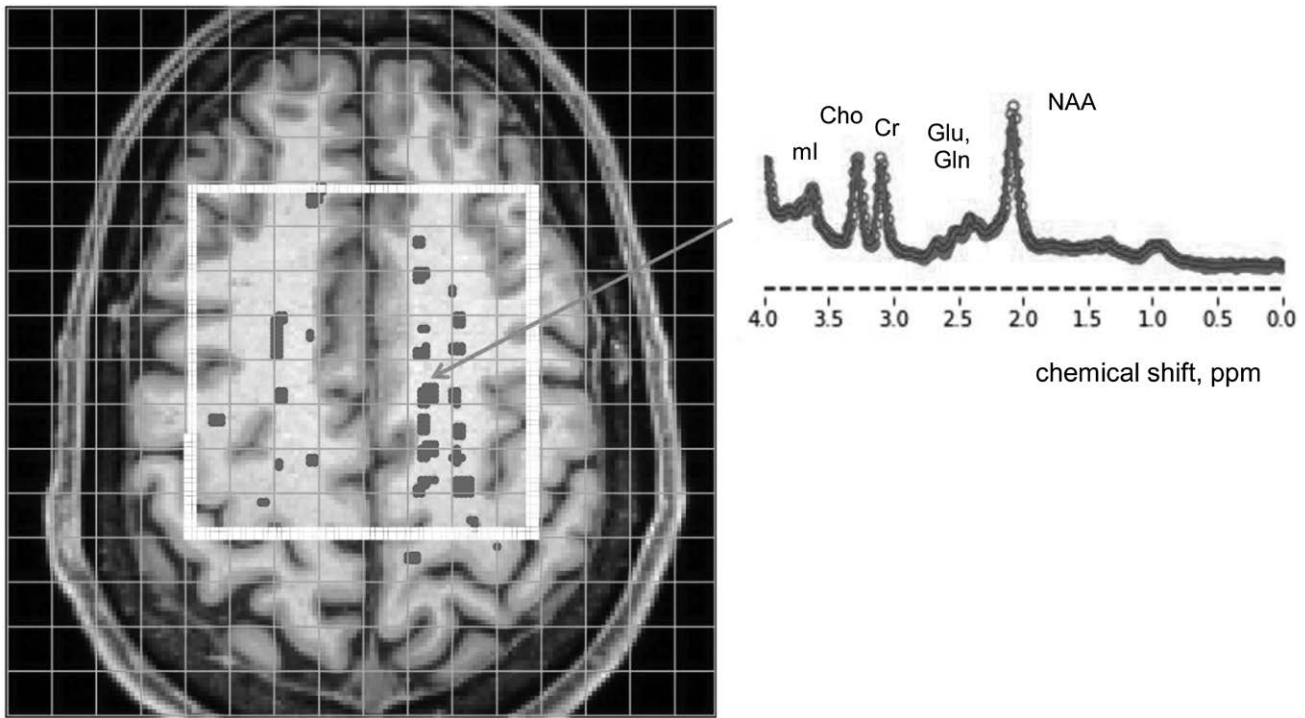
semi-Localization by Adiabatic SElective Refocusing (sLASER) [14] pulse-sequence (repetition-time/echo-time = 2810/60ms, 4 NEX, voxels 10×10×10mm³). The slab was positioned as in our prior work [15]. Briefly, it was oriented dorsally tangent to the genu corpus callosum as seen in the sagittal plane. The 8×8 subarray at the center of the slab constituted an 'excitation box' (Fig. 1) from which usable spectra could be obtained. Rostro-caudally, the box sampled bilateral frontal midline cortex (FMC) from pregenual anterior cingulate (pACC) to premotor regions. Mesio-laterally the box spanned the longitudinal fissure symmetrically to sample bilateral supraventricular FWM. Identical non-WS (NWS) MRS (1 NEX) was acquired immediately beforehand.

MRI post-processing and perivascular space mapping

T1w MRI underwent preprocessing and regional parcellation with FreeSurfer v5.3.0. Preprocessing included motion correction, intensity normalization, and skull stripping. Parcellation yielded binary masks of left and right rostral anterior cingulate, caudal anterior cingulate, and superior frontal cortex (SFC) per the Desikan-Killiany atlas, the three major constituents of FMC in our MRS slab. Rostral anterior cingulate and caudal anterior cingulate correspond to pACC, and anterior middle cingulate cortex (amCC) in the Vogt nomenclature. FWM consisted of the unparcelled moiety of white matter from the FreeSurfer output that was colocalized with the MRS slab. All brain regions were manually checked by study personnel.

PVS maps were generated from whole-brain T1w MRI as described [1]. Briefly, T1w MRI was enhanced using an adaptive nonlocal mean filtering technique applied only on high-frequency spatial noise using a moving patch with a radius of one voxel removing the noise at a single-voxel level and preserving signal intensities that are spatially repeated. Subsequently, a Frangi filter was applied using the Quantitative Imaging Toolkit. The Frangi filter estimated a 'vesselness' measure for each voxel from eigenvectors of the Hessian matrix of the image. The parameter c was set to half the value of the maximum Hessian norm. Frangi filters estimate vesselness at different scales and provide a maximum likelihood. The scale was set to a range of 0.1–5 voxels to maximize vessel inclusion. The output of this step was a brain-wide quantitative map of vesselness using

Fig. 1



(a) Axial-oblique (parallel to dorsum of genu corpus callosum) 3-T T1w MRI of a Parkinson's patient. Superposed is the sLASER magnetic resonance spectroscopy (MRS; TR/TE = 2810/60 ms, voxels $10 \times 10 \times 10 \text{ mm}^3$) 16×16 ($160 \times 160 \text{ mm}^2$) 2D voxel grid (light gray). The 8×8 sLASER excitation volume is bordered in bold white. Gray dots depict brain regions with non-zero perivascular spaces (PVS). (b) raw (light gray) and fit (dark gray) MR spectra from left frontal white matter (FWM) voxel indicated by gray arrow. Principal resonances are labeled for *N*-acetyl-compounds (NAA), glutamate (Glu) and glutamine (Gln), creatine + phosphocreatine (Cr), choline-containing compounds (Cho), and *myo*-inositol (ml). sLASER, semi-Localization by Adiabatic SElective Refocusing.

the maximum value across scales. A vesselness threshold of 0.0002 was used to obtain the PVS mask. All T1w MRI were manually checked for misalignment and post-processing failure. White-matter signal hyperintensities were eliminated by manual inspection of each T1w volume by trained operators, as this step significantly improves the specificity of the technique to PVS [16].

Magnetic resonance spectroscopy post-processing

sLASER MRS was post-processed as described by Alger *et al.* [17]. Briefly, MRS and MRI were reviewed by experts (J.R.A., J.O.N.). Studies of poor quality (e.g. excess head motion, inaccurately prescribed slabs) were excluded. Each T1w MRI was tissue-segmented into gray matter, white matter, and CSF subvolumes using FSL (<https://fsl.fmrib.ox.ac.uk/fsl>). These operations were performed without spatial transform, so the subvolumes would be aligned with the T1w ('native space'). pACC, aMCC, and SFC masks were brought into native space with the FreeSurfer `mri_convert` module. MRS reconstruction and coil combination were performed with Siemens software. This yielded an 8×8 grid of 'voxel spectra' for each WS and NWS study. Further processing was done with SVFit2016 together with a series of purpose-written software tools.

Operations performed by SVFit2016 included time-domain filtering and non-linear least-squares spectral fitting to determine neurometabolite levels for each MRS voxel in the excitation box (excluding edges). SVFit2016 uses the Levenberg–Marquardt implementation of the Gauss-Newton method to fit spectra in the frequency domain. The fitting routine is a modified version of MPFIT (<http://purl.com/net/mpfit>). Fits for NWS used a model spectrum that included only water. Fits for WS spectra included lactate, NAA, NAAG, Glu, Gln, GABA, Cr, Cho, mI, numerous low-level neurometabolites, residual water, lipids, and macromolecules. Model spectra were simulated in Versatile Simulation, Pulses and Analysis (<https://scion.duhs.duke.edu/vespa/project>). Fit quality for all spectra was reviewed by experts (J.R.A., J.O.N.). Poor fits were refit with alternative parameters. Voxel spectra that showed poor fits after multiple retries were excluded. Spectra with signal-to-noise ratio < 5 were excluded, as were spectra with B_0 inhomogeneity > 0.045 ppm.

Perivascular space magnetic resonance spectroscopy co-registration

Spatial coordinates of the T1w, PVS maps, regional masks, and MRS were read from the corresponding file

headers. This enabled co-alignment of gray matter, white matter, CSF, cortical region, and PVS mask with the MRS voxels. In co-alignment, the subvolumes were used to estimate the tissue content of each MRS voxel. This was used for CSF correction of neurometabolite levels and to calculate PVS volume in each MRS voxel. This permitted co-analysis of PVS and neurometabolite levels. MRS voxels from left and right hemispheres passing quality control and containing >60% white matter were assigned to FWM for analysis; voxels with >30% pACC, aMCC, or SFC (combined left and right) were assigned to the respective cortical region. Cross-voxel averaging was performed for each subject to yield values for PVS volume in each brain region. Additionally, neurometabolite levels were averaged to a mean within each brain region.

Statistical analysis

Bivariate unadjusted Pearson correlations were performed between PVS volume in each region (FWM, aMCC, pACC, SFC) and neurometabolite concentrations (NAA, Glu, Gln, Cr, Cho, ml) in the same regions. Given the modest sample size, alongside *r* we report Cohen's *d* (calculated per Borenstein *et al.*, 2009) [18] to indicate the effect size. For the correlations that were significant (*P* ≤ 0.05), linear regression analyses were then conducted to examine associations between the PVS and neurometabolites within that same region, while adjusting for the effects of age, sex, and BMI [19]. No adjustment was made for total intracranial volume as it did not correlate significantly with PVS volume in any region. All statistical analyses were conducted using SPSS, version 28.0.1.

Results

Higher frontal white matter perivascular space is associated with higher Cho

Table 2 lists group mean neurometabolite levels in each brain region. Correlation analysis showed a significant positive association between FWM PVS and FWM Cho (*r*(15) = 0.579, *d* = 0.309, *P* = 0.015; Fig. 2). Using a linear regression controlling for age, sex, and BMI, there remained a significant association between FWM PVS and FWM Cho [unstandardized β = 278.23, 95% confidence interval (CI) 4.18–552.28, *P* = 0.047]. No significant correlations were found between FWM PVS and any other FWM neurometabolite.

Higher anterior middle cingulate cortex perivascular space is associated with lower N-acetyl-compounds

Correlation analysis showed a significant negative association between aMCC PVS and aMCC NAA (*r*(15) = 0.592, *d* = 0.200, *P* = 0.012; Fig. 2). Using a linear regression controlling for age, sex, and BMI, there remained a significant association between aMCC PVS and aMCC NAA (unstandardized β = −939.43, 95% CI −1786.56 to −92.30, *P* = 0.033). No correlation was found between aMCC PVS and any other aMCC neurometabolite. No statistically significant correlations were observed in any other gray matter region (pACC, SFC).

Magnetic resonance spectroscopy neurometabolite levels and Montreal Cognitive Assessment scores

Correlation analysis showed a significant positive association between aMCC NAA and MoCA (*r*(15) = 0.686, *d* = 0.566, *P* = 0.002; Fig. 3). Using a linear regression controlling for age, sex, and BMI, there remained a significant association between aMCC NAA and MoCA (unstandardized β = 0.303, 95% CI 0.072–0.534, *P* = 0.014). Correlation analysis did not find a significant association between FWM Cho and MoCA (*r*(15) = 0.103, *P* = 0.695).

Discussion

The aim of this study was to investigate the co-occurrence of quantitative features of PVS and neurometabolites in individuals with PD. To our knowledge, we are the first to successfully combine the analyses of neurometabolites and PVS through multi-modal neuroimaging. We found that higher levels of FWM PVS were associated with higher levels of FWM Cho. Furthermore, we found that higher levels of aMCC PVS were associated with lower levels of aMCC NAA, and that higher aMCC NAA were associated with higher MoCA scores. Overall, these findings support that PVS and neurometabolites may be interrelated in PD.

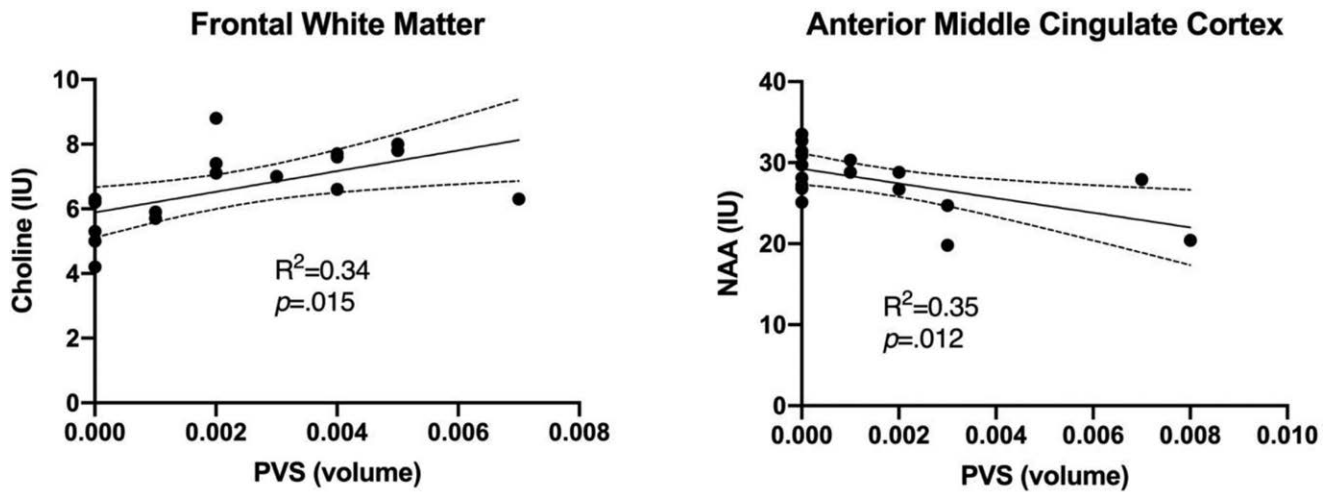
In FWM, higher Cho was associated with increased PVS. Cho is more abundant in astrocytes than in neurons [7]. One critical role of astrocytes is to form end-feet serving as the internal boundary wall of PVS and the structure and function of astrocytes are regulated by inflammation [20]. Therefore, higher Cho may be indicative of astrocyte activation and increased

Table 2 Levels of neurometabolites in white- and gray-matter regions

	FWM	aMCC	pACC	SFC
NAA (IU)	26.3 (1.8)	27.8 (3.8)	33.8 (6.0)	28.1 (3.1)
Glu (IU)	22.2 (2.0)	31.9 (4.8)	34.7 (6.3)	30.7 (4.4)
Gln (IU)	7.1 (1.3)	9.0 (1.6)	9.1 (3.0)	9.8 (3.1)
Cr (IU)	14.8 (1.1)	18.3 (2.4)	20.2 (3.5)	18.2 (2.1)
Cho (IU)	6.6 (1.2)	7.6 (1.5)	9.3 (2.5)	7.2 (1.4)
ml (IU)	11.4 (1.5)	14.8 (2.8)	16.4 (3.8)	14.5 (2.5)

aMCC, anterior middle cingulate cortex; Cho, choline-compounds; Cr, creatine+phosphocreatine; FWM, supraventricular frontal white matter; Gln, glutamine; Glu, glutamate; IU, institutional units; ml, *myo*-inositol; NAA, *N*-acetyl-compounds; pACC, pregenual anterior cingulate cortex; SFC, superior frontal cortex.

Fig. 2



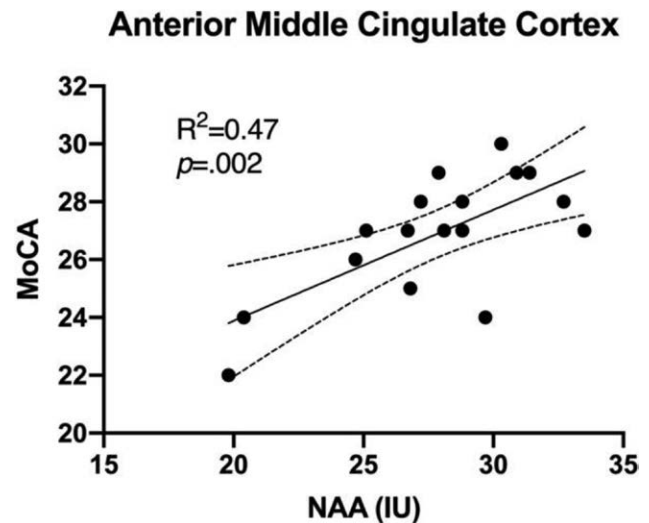
Levels of magnetic resonance spectroscopy (MRS) for (a) choline-containing compounds (Cho) as a function of the volume of perivascular space (PVS) in supraventricular frontal white matter (FWM) in PD individuals (dots). Higher Cho is associated with greater PVS. Both Cho and PVS have previously been related to neuroinflammation, and (b) *N*-acetyl compounds in anterior middle cingulate cortex (aMCC). Lower NAA is associated with greater PVS. Anti-inflammatory properties have been attributed to NAA which is generally considered an index of neuronal health. IU, institutional units; PD, Parkinson's disease; NAA, *N*-acetyl-compounds.

inflammation. Although the relationship between cognitive impairment and Cho is not evident in our small sample population, others have reported elevated Cho in the posterior cingulate of PD individuals with cognitive impairment [21]. Taken together, elevated Cho levels with increased PVS may be strong indicators of inflammation in PD pathogenesis.

In aMCC, lower NAA was associated with increased PVS. NAA is abundant in mature neurons and is widely used as a marker of neuronal density [22]. NAA may also have an anti-inflammatory role [10], consistent with the inverse relationship between NAA and PVS observed in this study. This finding aligns with a prior study, which saw decreased NAA in the occipital lobe of PD individuals [21]. Additionally, we found that lower NAA in aMCC was associated with lower global cognitive performance (MoCA), consistent with prior literature that showed decreased posterior cingulate NAA in individuals with PD-related cognitive impairment [21]. In concert, these findings suggest that gray matter-related PVS and neurometabolites may also play a role in PD-associated inflammation and cognitive decline, and merit further investigation.

There are several limitations to this study. These include small sample size and exclusion of individuals with advanced PD dementia. While this study was conducted using scans acquired at 3-T strength, higher spatial resolution, superior visualization of PVS, and better fitting of MR spectra are possible at ultra-high field (7-8T). We also used a cross-sectional design, which limits the interpretation of causality. Future studies should consider larger

Fig. 3



Montreal Cognitive Assessment (MoCA) score in individuals with PD as a function of NAA level in aMCC. Higher NAA, likely representing better neuronal health and possibly lower inflammation, is associated with higher MoCA score. aMCC, anterior middle cingulate cortex; NAA, *N*-acetyl-compounds; PD, Parkinson's disease.

sample sizes, incorporation of control groups, sampling of further brain regions, and longitudinal design.

Conclusion

This pilot study demonstrates that it is feasible to detect relationships between PVS and MRS neurometabolites,

such as Cho and NA, in both white matter and gray matter in PD individuals. This pilot study provides additional evidence for the role of neuroinflammation in PD, and future MRS-PVS studies are needed to better elucidate the role of neurometabolites in PVS.

Acknowledgements

We would like to thank Dawn Schiehser (UCSD) and Daniel Holschneider (USC) for their insightful discussions and Giuseppe Barisano for consultation on methodological approaches.

This work was supported by grants from the US Department of Defense (U.S. Army, CDMRP) #W81XWH19-1-0443 (M.W.J.), #W81XWH18-1-0665 (G.M.P.), the Parkinson's Foundation (G.M.P.), and the Don Roberto Gonzalez Family Foundation (G.M.P.).

Conflicts of interest

There are no conflicts of interest.

References

- Donahue EK, Murdos A, Jakowec MW, Sheikh-Bahaei N, Toga AW, Petzinger GM, Sepehrband F. Global and regional changes in perivascular space in idiopathic and familial Parkinson's disease. *Mov Disord* 2021; **36**:1126–1136.
- Shen T, Yue Y, Zhao S, Xie J, Chen Y, Tian J, et al. The role of brain perivascular space burden in early-stage Parkinson's disease. *NPJ Parkinsons Dis* 2021; **7**:12.
- Park YW, Shin NY, Chung SJ, Kim J, Lim SM, Lee PH, et al. Magnetic resonance imaging-visible perivascular spaces in basal ganglia predict cognitive decline in Parkinson's disease. *Mov Disord* 2019; **34**:1672–1679.
- Ramirez J, Berezuk C, McNeely AA, Scott CJ, Gao F, Black SE. Visible Virchow-Robin spaces on magnetic resonance imaging of Alzheimer's disease patients and normal elderly from the Sunnybrook Dementia Study. *J Alzheimers Dis* 2015; **43**:415–424.
- Satizabal CL, Zhu YC, Dufouil C, Tzourio C. Inflammatory proteins and the severity of dilated Virchow-Robin Spaces in the elderly. *J Alzheimers Dis* 2013; **33**:323–328.
- Stojkowska I, Wagner BM, Morrison BE. Parkinson's disease and enhanced inflammatory response. *Exp Biol Med (Maywood)* 2015; **240**:1387–1395.
- Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J Neurosci* 1993; **13**:981–989.
- Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci* 1993; **15**:289–298.
- Chang L, Munsaka SM, Kraft-Terry S, Ernst T. Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain. *J Neuroimmune Pharmacol* 2013; **8**:576–593.
- Rael LT, Thomas GW, Bar-Or R, Craun ML, Bar-Or D. An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells. *Biochem Biophys Res Commun* 2004; **319**:847–853.
- Yu CC, Chen MH, Lu CH, Huang YC, Chen HL, Tsai NW, et al. Altered striato-cerebellar metabolism and systemic inflammation in Parkinson's disease. *Oxid Med Cell Longev* 2016; **2016**:1810289.
- Hall JM, Ehgoetz Martens KA, Walton CC, O'Callaghan C, Keller PE, Lewis SJ, Moustafa AA. Diffusion alterations associated with Parkinson's disease symptomatology: a review of the literature. *Parkinsonism Relat Disord* 2016; **33**:12–26.
- Litvan I, Goldman JG, Tröster AI, Schmand BA, Weintraub D, Petersen RC, et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: movement Disorder Society Task Force guidelines. *Mov Disord* 2012; **27**:349–356.
- Oz G, Tkáč I. Short-echo, single-shot, full-intensity proton magnetic resonance spectroscopy for neurochemical profiling at 4 T: validation in the cerebellum and brainstem. *Magn Reson Med* 2011; **65**:901–910.
- O'Neill J, O'Connor MJ, Kalender G, Ly R, Ng A, Dillon A, et al. Combining neuroimaging and behavior to discriminate children with attention deficit-hyperactivity disorder with and without prenatal alcohol exposure. *Brain Imaging Behav* 2021; **16**:69–77.
- Sepehrband F, Barisano G, Sheikh-Bahaei N, Choupan J, Cabeen RP, Lynch KM, et al.; Alzheimer's Disease Neuroimaging Initiative. Volumetric distribution of perivascular space in relation to mild cognitive impairment. *Neurobiol Aging* 2021; **99**:28–43.
- Alger JR, O'Neill J, O'Connor MJ, Kalender G, Ly R, Ng A, et al. Neuroimaging of supraventricular frontal white matter in children with familial attention-deficit hyperactivity disorder and attention-deficit hyperactivity disorder due to prenatal alcohol exposure. *Neurotox Res* 2021; **39**:1054–1075.
- Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis*. Wiley and Sons Ltd; 2009.
- Barisano G, Sheikh-Bahaei N, Law M, Toga AW, Sepehrband F. Body mass index, time of day and genetics affect perivascular spaces in the white matter. *J Cereb Blood Flow Metab* 2021; **41**:1563–1578.
- Sofroniew MV. Astrocyte reactivity: subtypes, states, and functions in CNS innate immunity. *Trends Immunol* 2020; **41**:758–770.
- Nie K, Zhang Y, Huang B, Wang L, Zhao J, Huang Z, et al. Marked N-acetylaspartate and choline metabolite changes in Parkinson's disease patients with mild cognitive impairment. *Parkinsonism Relat Disord* 2013; **19**:329–334.
- Rae CD. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res* 2014; **39**:1–36.



Increased perivascular space volume in white matter and basal ganglia is associated with cognition in Parkinson's Disease

Erin Kaye Donahue¹ · Ryan Patrick Foreman¹ · Jared Joshua Duran¹ · Michael Walter Jakowec¹ · Joseph O'Neill² · Andrew J. Petkus¹ · Daniel P. Holschneider^{1,3} · Jeiran Choupan⁴ · John Darrell Van Horn^{5,6} · Siva Venkadesh⁵ · Ece Bayram⁷ · Irene Litvan⁷ · Dawn M Schiehser^{8,9} · Giselle Maria Petzinger¹

Accepted: 9 October 2023
© The Author(s) 2023

Abstract

Perivascular spaces (PVS), fluid-filled compartments surrounding brain vasculature, are an essential component of the glymphatic system responsible for transport of waste and nutrients. Glymphatic system impairment may underlie cognitive deficits in Parkinson's disease (PD). Studies have focused on the role of basal ganglia PVS with cognition in PD, but the role of white matter PVS is unknown. This study examined the relationship of white matter and basal ganglia PVS with domain-specific and global cognition in individuals with PD. Fifty individuals with PD underwent 3T T1w magnetic resonance imaging (MRI) to determine PVS volume fraction, defined as PVS volume normalized to total regional volume, within (i) centrum semiovale, (ii) prefrontal white matter (medial orbitofrontal, rostral middle frontal, superior frontal), and (iii) basal ganglia. A neuropsychological battery included assessment of global cognitive function (Montreal Cognitive Assessment, and global cognitive composite score), and cognitive-specific domains (executive function, memory, visuospatial function, attention, and language). Higher white matter rostral middle frontal PVS was associated with lower scores in both global cognitive and visuospatial function. In the basal ganglia higher PVS was associated with lower scores for memory with a trend towards lower global cognitive composite score. While previous reports have shown that greater amount of PVS in the basal ganglia is associated with decline in global cognition in PD, our findings suggest that increased white matter PVS volume may also underlie changes in cognition.

Keywords Virchow-Robinson space · Global cognition · Visuospatial function · Memory · Rostral middle frontal

Dawn M Schiehser and Giselle Maria Petzinger are Co-Senior Authors.

✉ Giselle Maria Petzinger
petzing@med.usc.edu

¹ Department of Neurology, Keck School of Medicine, University of Southern California, 1333 San Pablo St, MCA-243, Los Angeles, CA 90033, USA

² Division of Child Psychiatry, UCLA Semel Institute for Neuroscience, Los Angeles, CA 90024, USA

³ Department of Psychiatry & the Behavioral Sciences, University of Southern California, Los Angeles, CA 90033, USA

⁴ Laboratory of NeuroImaging, USC Stevens Neuroimaging and Informatics Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA

⁵ Department of Psychology, University of Virginia, Charlottesville, VA 22904, USA

⁶ School of Data Science, University of Virginia, Charlottesville, VA 22904, USA

⁷ Parkinson and Other Movement Disorder Center, Department of Neurosciences, University of California San Diego, La Jolla, CA 92093, USA

⁸ Veterans Administration San Diego Healthcare System (VASDHS), San Diego, CA 92161, USA

⁹ Department of Psychiatry, University of California, San Diego, CA 92093, USA

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting both motor and nonmotor functions including cognition (Aarsland et al., 2017). Mild cognitive impairment (MCI) is common in PD, and can affect multiple cognitive domains, including executive function, attention, memory, and visuospatial function (Aarsland et al., 2021). MCI often progresses to dementia. Pathophysiological changes underlying cognitive changes remain poorly elucidated one potential mechanism may be due to dysfunction of the glymphatic system, leading to decreased clearance of metabolic waste products and protein aggregates from the brain parenchyma (DeBette et al., 2019; Ding et al., 2017; Zhu et al., 2010).

In the healthy brain the glymphatic system serves several essential roles including (i) immune surveillance, (ii) transport of neurotransmitters and nutrients (e.g., glucose and lipids), and (iii) clearance of cellular debris, metabolic waste products, and protein aggregates (Jessen et al., 2015). A key component of the glymphatic system is the perivascular spaces (PVS), which are the fluid filled compartments surrounding perforating vasculature throughout the brain including the basal ganglia and white matter. PVS are bordered on one side by blood vessels and on the other side by astrocytic endfeet (Engelhardt & Ransohoff, 2012). Dysfunction of the glymphatic system has been reported as increased PVS volume or count, due to enlargement as determined by neuroimaging (Donahue et al., 2021).

Within the field of aging, studies have shown an association between greater amount of PVS and cognitive decline in both white matter and basal ganglia (MacLulich et al., 2004; Paradise et al., 2021). In PD, studies have also shown an association between increased basal ganglia PVS count and decreased cognitive performance. For example, higher PVS count is associated with worsening of global cognition as assessed by the Montreal Cognitive Assessment (MoCA). Additionally, in PD higher basal ganglia PVS count is associated with conversion from normal cognition to MCI, and from MCI to dementia (Chen et al., 2022; Park et al., 2019). While general cognition is associated with PVS in PD, the relationship between changes in PVS and deficits in specific cognitive domains has not been explored. Additionally, while there is an association between increased basal ganglia PVS and cognition, less is known about the relationship between changes in white matter PVS volume and cognition in PD.

The primary goal of this study was to evaluate PVS volume, defined as volume fraction, within overall and regional frontal white matter and the basal ganglia and its association with global and domain-specific cognitive performance. PVS volume was determined from T1w magnetic resonance

imaging (MRI) using an automated method that quantitates PVS volume relative to total white matter volume in the local volume-of-interest (volume fraction) (Sepehrband et al., 2019). This allowed assessment of PVS within the large centrum semiovale white matter region of interest, as well as in selective regions of the frontal white matter (medial orbitofrontal, rostral middle frontal, superior frontal white matter) and the basal ganglia. The centrum semiovale and basal ganglia are regions in which PVS has been routinely abundant in past studies. Frontal lobe regions were selected for analysis due to its known role in cognitive function in PD. A full neuropsychological assessment was collected on study participants, including global cognition (MoCA and global composite score) and executive function, episodic memory (memory) visuospatial function, attention, and language scores, as well as assessment of cognitive status.

Methods

Study participants

For this cross-sectional analysis, data of 50 participants with idiopathic PD were obtained. Participants were evaluated as part of a multi-site (University of Southern California (USC) and Veterans Affairs San Diego Healthcare System (VASDHS) / University of California San Diego (UCSD) study. PD diagnosis was based on the UK Brain Bank criteria (Hughes et al., 1992). Inclusion criteria included: (i) willing and able to provide informed consent; (ii) medically eligible/safe for MRI; and (iii) Hoehn and Yahr score ≤ 3 . Exclusion criteria included (i) history of severe psychiatric illness or other neurological disease, including prior stroke and (ii) dementia per criteria in Hoops et al., 2009 with MOCA < 18. Institutional Review Boards from each site approved all study procedures. Demographic data were collected, including age, sex, years of education, years of Parkinson's disease diagnosis, MDS-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III score (motor), body mass index (BMI; kg/m²), Levodopa Equivalent Dosage (LED; mg) (*Parkinson's Disease Measurement: PwP, Surveys, Trials, Analysis*, n.d.), and cardiovascular risk score (calculated by summing 0=no/1=yes for smoking, hypertension, high cholesterol, diabetes). Assessments were conducted in the 'ON' medication state.

MRI acquisition

Structural MRI was acquired on a Siemens Prisma (USC) or a GE MR 750 3T MRI (UCSD) 3T MRI scanner. Whole-brain T1w MRI was acquired with a magnetization-prepared rapid gradient-echo pulse-sequence. On the Prisma

the acquisition parameters were: flip 8°, repetition-time/echo-time/inversion-time = 2400/2.22/1000ms, voxels $0.8 \times 0.8 \times 0.8$ mm³; 1 NEX, acceleration factor 2, 6:38 min; on the MR 750 the acquisition parameters were: flip 8°, repetition-time/echo-time/inversion-time = 2852/3.548/1000ms, voxels $0.8 \times 0.8 \times 0.8$ mm³; 1 NEX, acceleration factor 2, 7:58 min. Study site was included as a covariate to account for any differences due to slight parameter changes.

Perivascular space mapping

T1w MRI underwent preprocessing and regional parcellation with FreeSurfer v5.3.0. Preprocessing included motion correction, non-uniform intensity normalization, Talairach transformation, and skull stripping. PVS was mapped from T1w MRI as previously described (Donahue et al., 2021). T1w MRI was enhanced using an adaptive nonlocal mean filtering technique applied only on high-frequency spatial noise using a moving patch with a radius of one voxel (Manjón et al., 2010). A Frangi filter was applied using the Quantitative Imaging Toolkit (Cabeen, 2020; Frangi et al., 1998). A ‘vesselness’ probability measure was estimated for each voxel from eigenvectors of the Hessian matrix (parameter *c* set to half the value of the maximum Hessian norm) of the image at different scales (0.1–5 voxels) to provide a maximum likelihood/maximize vessel inclusion. This results in a brain-wide quantitative map of vesselness. A vesselness threshold of 0.0002 was used to obtain the PVS mask.

All T1w MRI were manually checked for misalignment, post-processing failure, lacunes, white-matter hyperintensities, which were corrected on manual inspection (Sepehrband et al., 2021; Benjamin et al., 2018). Brain volume and centrum semiovale, basal ganglia and frontal regional masks were derived using the Desikan-Killiany atlas (Desikan et al., 2006), using the *recon-all* module of FreeSurfer. Centrum semiovale PVS was calculated by summing caudal middle frontal, inferior parietal, pars opercularis, pars orbitalis, pars triangularis, post central, precentral, rostral middle frontal, superior frontal, superior parietal, supramarginal, and unsegmented white matter not defined in the Desikan-Killiany atlas from both hemispheres. Because pathophysiological changes in the basal ganglia and frontal lobe are thought to be involved in early cognitive changes in PD, we also included these regions in our analysis (Farina et al., 2000; Lewis et al., 2003; Owen et al., 1995; Taylor et al., 1990). Frontal white matter regions included the medial orbitofrontal, the rostral middle frontal, and the superior frontal regions (note - rostral middle frontal and superior frontal are subregions of centrum semiovale). PVS volume measurements within each region were normalized to the total white matter volume (or total basal ganglia volume) of the same region to provide an internal control for possible

PD-related changes in white matter volume. This produced a “volume fraction” that represents the fraction of the region of interest that is composed of PVS. All PVS results are expressed as volume fraction.

Neuropsychological assessment

The neuropsychological battery measured global cognitive performance and performance across cognitive domains including executive function, memory, visuospatial function, attention, and language. Executive function was measured by (i) Wisconsin Card Sorting Test-64 card version (WCST) perseverative responses, (ii) Inhibition (condition 3) and Inhibition/Switching (condition 4) subtests from the Delis Kaplan Executive Function System (D-KEFS) Color Word Interference Test (CWIT), and (iii) D-KEFS Verbal Fluency Switching subtest. Memory was measured by (i) California Verbal Learning Test (2nd Edition; CVLT-II) Total Trials 1–5, Short-Delay Free Recall, and Long-Delay Free Recall, (ii) Brief Visuospatial Memory Test (BVMT) Immediate- and Delayed-Recall conditions. Visuospatial function was measured by the total scores on the (i) Judgment of Line Orientation Test and (ii) Hooper Visual Organization Test. Attention was measured by (i) D-KEFS CWIT Color Naming (Condition 1) and Word Reading (Condition 2) subtests and (ii) Adaptive Digit Ordering Test Forward Digit Span and Digit Sequencing conditions. Language was measured by (i) D-KEFS Verbal Fluency Letter Fluency and Category Fluency subtests and (ii) the Boston Naming Test. Domain scores were calculated by averaging the z-scores of all components. Global cognition was assessed using a mean of the five cognitive domain z-scores (Global Cognition). The MoCA score was used as a general cognition screen.

Statistics

All statistical analyses were conducted using SPSS, Version 28.0.1 (Armonk, NY: IBM Corp). To be retained as significant ($p \leq 0.05$), potential relationships between cognitive scores and regional PVS volume fraction had to survive both screening with a simple correlation analysis and a follow-up linear regression. A bivariate unadjusted Pearson correlation was performed between each cognitive domain score, and Global Cognition and MoCA scores, and the PVS volume fraction in each brain region (centrum semiovale, medial orbitofrontal, rostral middle frontal, superior frontal, and basal ganglia). If a correlation was significant, a hierarchical linear regression analysis using backwards elimination was applied (Lilja & Linse, 2022) to increase the power of comparisons, using: age, sex, years of education, years of Parkinson’s disease diagnosis, MDS-UPDRS part

Table 1 Study demographics

	Mean (SD)
N (# of females)	50 (23)
Age	65.36 (8.98)
Education (years)	16.68 (2.20)
Body Mass Index (kg/m ²)	25.89 (4.75)
MDS-UPDRS part III score	20.84 (9.08)
Years of Parkinson's diagnosis	4.52 (5.34)
Cardiovascular Risk Score	0.56 (0.73)
Levodopa Equivalent Daily Dose (mg)	575.57 (394.01)
MDS-UPDRS: Movement Disorder Society-Unified Parkinson's Disease Rating Scale	

III score, BMI, LED, cardiovascular risk score, and study site (1 = USC/Siemens scanner, n = 21, and 2 = UCSD/GE scanner, n = 30). Regression p-values are Benjamini-Hochberg False Discovery Rate corrected within each predictor variable, to account for multiple comparisons (*False Discovery Rate Online Calculator | Tools*, n.d.). Any individual beyond three standard deviations in either the independent or dependent variable was considered an extreme outlier and was excluded from analyses for that comparison (3 removed from medial orbitofrontal, 1 removed from basal ganglia).

Results

Participants were on average 65.36 years of age (SD = 8.98) with 16.68 (SD = 2.20) years of education; 54% were male, with a mean MDS-UPDRS part III score (motor) of 20.84 (SD = 9.08), mean Body Mass Index (BMI) of 25.89 kg/m² (SD = 4.75), and a mean cardiovascular risk score of 0.56 (SD = 0.73) (Table 1). Mean scores for the individual cognitive tests used to calculate domain-specific z scores and MOCA scores are shown in Supplementary Table 1.

Rostral middle frontal PVS volume fraction was negatively correlated with (i) MoCA ($r(48) = -0.524, p < 0.001$), (ii) global cognition z score ($r(48) = -0.380, p = 0.007$), (iii) visuospatial function ($r(48) = -0.336, p = 0.017$), and (iv) memory ($r(48) = -0.490, p < 0.001$) (Supplementary Table 2). After adjusting for covariates in the linear regression, rostral middle frontal PVS volume fraction remained significantly associated with (i) MoCA (age, BMI; unstandardized $\beta = -318.40$, 95% CI [-613.85, -22.95], $\Delta R^2 = 0.061, p = 0.047$), (ii) global cognition (MDS-UPDRS part III score; unstandardized $\beta = -112.22$, 95% CI [-192.59, -31.84], $\Delta R^2 = 0.119, p = 0.028$), and (iii) visuospatial function (MDS-UPDRS part III score; unstandardized $\beta = -124.79$, 95% CI [-229.90, -19.67], $\Delta R^2 = 0.092, p = 0.042$), but not memory (age, sex, MDS-UPDRS part III score, site; unstandardized $\beta = -51.43$, 95% CI [-151.76, 48.91], $\Delta R^2 = 0.009, p = 0.307$) (Table 2; Fig. 1).

Basal ganglia PVS volume fraction was negatively correlated with (i) MoCA ($r(47) = -0.313, p = 0.029$), (ii) global cognition z-score ($r(47) = -0.311, p = 0.029$), and (iii) memory ($r(47) = -0.308, p = 0.031$) (Supplementary Table 2). After adjusting for covariates in the linear regression, basal ganglia PVS volume fraction remained significantly associated with global cognition z-score (age, sex, MDS-UPDRS part III score, years of education; unstandardized $\beta = -84.65$, 95% CI [-147.55, -21.76], $\Delta R^2 = 0.087, p = 0.030$) but not memory (age, sex, MDS-UPDRS part III score, site; unstandardized $\beta = -57.99$, 95% CI [-127.88, 11.90], $\Delta R^2 = 0.021, p = 0.153$) or MoCA (age, sex, BMI, site; unstandardized $\beta = -109.02$, 95% CI [-321.32, 103.28], $\Delta R^2 = 0.012, p = 0.306$) (Table 2; Fig. 1).

Centrum semiovale PVS volume fraction was negatively associated with MoCA ($r(48) = -0.358, p = 0.011$) and memory ($r(48) = -0.374, p = 0.007$). Associations did not remain significant after adjusting for covariates: centrum semiovale

Table 2 Adjusted associations between perivascular space volume fraction in centrum semiovale, rostral middle frontal, and superior frontal white matter and the basal ganglia

	β	95% CI Lower, Upper	p
MoCA			
centrum semiovale PVS	-73.84	-288.02, 140.34	0.982
rostral middle frontal PVS	-318.40*	-613.85, -22.95	0.047
superior frontal PVS	-80.33	-205.88, 45.22	0.408
basal ganglia PVS	-109.02	-321.32, 103.28	0.306
Global Cognition			
rostral middle frontal PVS	-112.22*	-192.59, -31.84	0.028
basal ganglia PVS	-84.65*	-147.55, -21.76	0.030
Visuospatial Function			
rostral middle frontal PVS	-124.79*	-229.90, -19.67	0.042
Memory			
centrum semiovale PVS	-16.22	-88.46, 56.01	0.653
rostral middle frontal PVS	-51.43	-151.76, 48.91	0.307
superior frontal PVS	1.26	-42.91, 45.42	0.954
basal ganglia PVS	-57.99	-127.88, 11.90	0.153

PVS: perivascular space

Significant regressions in bold. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

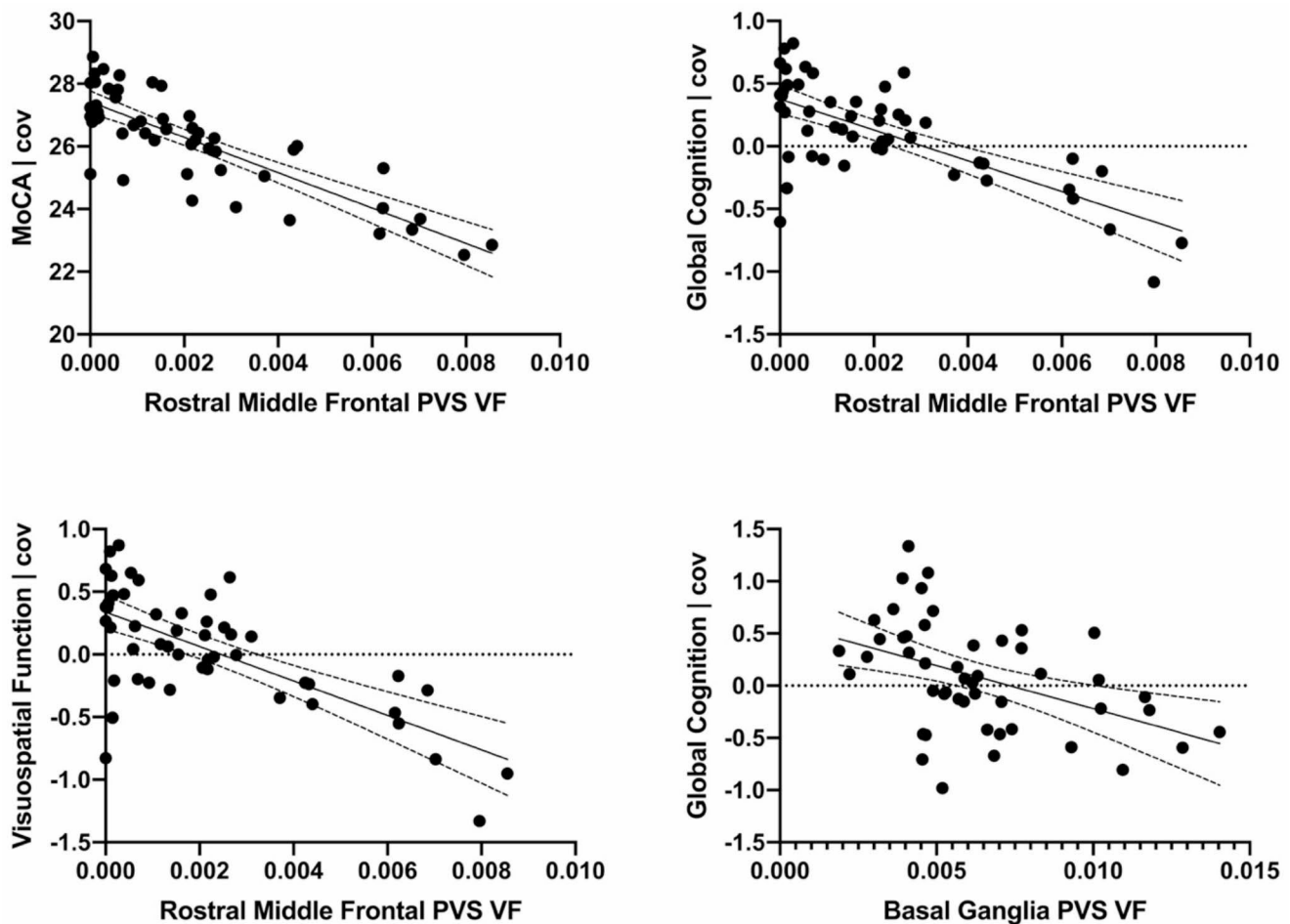


Fig. 1 Significant adjusted associations between rostral middle frontal and basal ganglia perivascular space volume fraction and cognition. See Methods for covariates. PVS: perivascular space; VF: volume fraction

PVS volume fraction-MoCA (age, BMI, site; unstandardized $\beta = -73.84$, 95% CI [-288.02, 140.34], $\Delta R^2 = 0.006$, $p = 0.982$), and centrum semiovale PVS volume fraction-memory (age, sex, MDS-UPDRS part III score, site; unstandardized $\beta = -16.22$, 95% CI [-88.46, 56.01], $\Delta R^2 = 0.002$, $p = 0.653$) (Table 2).

Superior frontal PVS volume fraction was negatively associated with MoCA ($r(48) = -0.337$, $p = 0.017$) and memory ($r(48) = -0.293$, $p = 0.039$). Associations did not remain significant after adjusting for covariates; superior frontal PVS volume fraction-MoCA (age, BMI, site; unstandardized $\beta = -85.33$, 95% CI [-205.88, 45.22], $\Delta R^2 = 0.021$, $p = 0.408$), and superior frontal PVS volume fraction-memory (age, sex, MDS-UPDRS part III score, site; unstandardized $\beta = 1.26$, 95% CI [-42.91, 45.42], $\Delta R^2 = 0.000$, $p = 0.954$) (Table 2).

No associations were observed between medial orbitofrontal PVS volume fraction and cognitive function. No associations were observed in any region between PVS volume fraction and language, attention, or executive function.

Discussion

The aim of this study was to investigate the association of increased PVS volume in white matter and basal ganglia with domain-specific and global cognitive performance in PD. This study identified a significant association between increased rostral middle frontal PVS volume and general cognition as measured by MoCA and a global cognitive composite score in individuals with PD. Given that our average MOCA score was 26.18 it is possible that our findings were driven by individuals with mild cognitive impairment and will be investigated in future studies with a larger cohort. In addition to rostral middle frontal volume, there was a significant association between basal ganglia PVS volume and a global cognitive composite score. The rostral middle frontal region is located within the prefrontal cortex, encompassing both the dorsolateral and anterior prefrontal cortex. While a few studies have demonstrated an association between basal ganglia PVS and global cognition in PD, our study is the first to demonstrate a relationship between

white matter PVS volume and both global and domain specific cognitive performance in PD (Chen et al., 2022; Park et al., 2019). Our methodological approach assessed change in large diameter PVS (considered > 3 mm) and excluded lacunes (small PVS) (Ramirez et al., 2022). Future studies will examine if both small and large diameter PVS are associated with cognition in PD (Ramirez et al., 2022). This association of global cognition with rostral middle frontal and basal ganglia PVS may be due to the known contribution of both brain regions in early PD-related cognitive changes (Lewis et al., 2003). We also observed a relationship between increased rostral middle frontal white matter PVS volume and worse visuospatial function. A previous study showed an association between centrum semiovale white matter PVS and changes in visuospatial function in healthy aging (MacLulich et al., 2004). Our study aligns with this finding in that PVS in the rostral middle frontal white matter, a segment of the centrum semiovale, was associated with our visuospatial function composite. Further, studies have demonstrated a relationship between the rostral middle frontal region and tests of visuospatial function. Specifically, fMRI studies have shown activation in rostral middle frontal areas, including the dorsolateral and anterior prefrontal cortex, in individuals conducting the Hooper visual organization task (HVOT; Moritz et al., 2004), which is a visuospatial test with an executive function component.

While a few studies have previously reported an association between basal ganglia PVS and cognition in PD, to the best of our knowledge, our study is the first to report that white matter PVS may also contribute to cognitive performance in PD (Chen et al., 2022; Park et al., 2019). One possible explanation for this discrepancy may be due to differences in methodology for examining PVS burden. In our study, PVS was assessed as volume (volume fraction), as opposed to PVS counts or a ranking system, as reported in other studies (Chen et al., 2022; Park et al., 2019). Volumetric quantification may capture a more complete picture of PVS involvement in disease pathophysiology given that a significant characteristic of PVS dysfunction is thought to be dilation (Zou et al., 2019). For example, PVS volume in white matter has been shown to be higher in PD relative to healthy controls (Donahue et al., 2021). Taken together, our results and previous studies suggest that PVS volume changes could contribute to PD pathophysiology, including cognitive performance.

Currently, the mechanisms leading to increased PVS amount, and cognitive decline are unknown. One suggestion is that PVS volume may reflect decreased flow in the glymphatic system resulting in either increased accumulation of metabolic waste products or failure to deliver needed metabolic substrates. For example, surgical block of glymphatic flow in rodents leads to the accumulation of

alpha-synuclein, a pathophysiological protein hallmark of PD (Zou et al., 2019). Glymphatic flow may also be disrupted by chronic inflammatory processes known to occur in many neurodegenerative disorders including PD (de Groot & Burgas, 2015; De Virgilio et al., 2016; Klein, 2000; Mogensen et al., 2021). Changes in PVS may also be associated with reduced integrity of the blood brain barrier (BBB) (Chen et al., 2021). For example, a recent study from our group showed an association between increased PVS volume in the frontal white matter and increased choline-containing compounds a potential marker of astrocyte gliosis (Donahue et al., 2022). Since astrocytes are critical component of BBB structure and function, these changes may reflect BBB breakdown or reduced neuroenergetic support by astrocytes, both impacting glymphatic integrity reflected by PVS pathology (Urenjak et al., 1993). Further studies are needed to identify the underlying mechanisms leading to increased PVS in disorders of the brain including PD and how such changes lead to disease state and progression.

Understanding the impact of changes in PVS in PD and its progression are important since studies have suggested a strong association between lifestyle factors (sleep, exercise) and glymphatic system function. Animal studies show that glymphatic flow increases during sleep (Xie et al., 2013). Clinical studies have demonstrated an association between increased PVS and obstructive sleep apnea and longer self-reported time in bed (Jia et al., 2021; Ramirez et al., 2021). Furthermore, voluntary running has also been shown to increase glymphatic flow and clearance of amyloid-beta protein aggregates in rodents (He et al., 2017; von Holstein-Rathlou et al., 2018). These reports highlight the potential targeting of the glymphatic systems through lifestyle that may impact disease progression in PD including cognitive decline.

Limitations and strengths

There are several limitations to this study. First, the absence of T2-weighted images precluded using the automated mapping pipeline to fully discern PVS from white matter hyperintensities and lacunes. Instead, all PVS masks were quality control checked to remove any white matter hyperintensities or lacune erroneously mapped as PVS. Second, the cross-sectional design of the study prevents the determination of causality in the relationship between PVS and cognition. Third, this study did not examine the independent contribution of cerebrovascular burden to cognition. However, given that cardiovascular risk profile is related to cerebrovascular burden, and our participants have very low cardiovascular risk profiles (mean of ~0.5 score on 5 yes/no questions) and no history of stroke, supports that PVS may be an important contributor to cognitive performance independent of

cerebrovascular burden. Future longitudinal studies will examine longitudinal relationships between PVS and cognition to determine directionality. There are several strengths of this study including (i) utilization of automated (objective) assessment of PVS, and (ii) determination of PVS volume rather than count may provide insight into mechanisms of glymphatic dysfunction.

Conclusions

This study supports that greater PVS volume in either white matter or basal ganglia is associated with worse cognitive performance in PD. Understanding PVS pathophysiology may provide insight into function of the glymphatic system and its impact on PD and its progression.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11682-023-00811-4>.

Author contributions EKD and GMP are responsible for preparation of this manuscript. EKD, RPF, and JJD are responsible for T1w MRI quality control. JC is responsible for PVS quantification. AJP and DMS are responsible for neuropsychological scoring and cognitive domain score calculations. EKD, JO, JDVH, DMS, GMP, and MWJ are responsible for statistical analysis. EB, IL, DMS, and GMP are responsible for conducting the study. EKD, RPF, JJD, SV, AJP, EB, IL, DPH, JC, JDVH, JO, DMS, MWJ, and GMP are responsible for reviewing and providing feedback on the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by SCEL, Statewide California Electronic Library Consortium. This work was supported by grants from the US Department of Defense (U.S. Army, CDMRP) #W81XWH18-1-0666 (DPH), # W81XWH19-1-0443 (MWJ), #W81XWH18-1-0665 (GP), the Parkinson's Foundation (GP). Open access funding provided by SCEL, Statewide California Electronic Library Consortium

Data availability Data available upon request to GMP.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Institutional Review Boards from each site (University of Southern California/Veterans Affairs San Diego Healthcare System/University of California San Diego) approved all study procedures.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended

use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

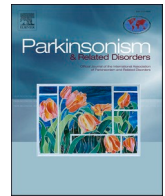
References

- Aarsland, D., Creese, B., Politis, M., Chaudhuri, K. R., Ffytche, D. H., Weintraub, D., & Ballard, C. (2017). Cognitive decline in Parkinson Disease. *Nature Reviews Neurology*, 13(4), 217–231. <https://doi.org/10.1038/nrneurol.2017.27>.
- Aarsland, D., Batzu, L., Halliday, G. M., Geurtsen, G. J., Ballard, C., Chaudhuri, R., K., & Weintraub, D. (2021). Parkinson disease-associated cognitive impairment. *Nature Reviews Disease Primers*, 7(1), 47. <https://doi.org/10.1038/s41572-021-00280-3>.
- Benjamin, P., Trippier, S., Lawrence, A. J., Lambert, C., Zeestraten, E., Williams, O. A., Patel, B., Morris, R. G., Barrick, T. R., MacKinnon, A. D., & Markus, H. S. (2018). Lacunar infarcts, but not perivascular spaces, are predictors of Cognitive decline in Cerebral Small-Vessel Disease. *Stroke*, 49(3), 586–593. <https://doi.org/10.1161/STROKEAHA.117.017526>.
- Cabeen, R. P. (2020, May 6). *Quantitative Imaging Toolkit: Software for Interactive 3D Visualization, Data Exploration, and Computational Analysis of Neuroimaging Datasets*. Ryan Cabeen. / about/publication/cabeen-2018-quantitative/.
- Chen, S., Shao, L., & Ma, L. (2021). Cerebral edema formation after Stroke: Emphasis on blood-brain barrier and the lymphatic drainage system of the brain. *Frontiers in Cellular Neuroscience*, 15, 716825. <https://doi.org/10.3389/fncel.2021.716825>.
- Chen, H., Wan, H., Zhang, M., Wardlaw, J. M., Feng, T., & Wang, Y. (2022). Perivascular space in Parkinson's Disease: Association with CSF amyloid/tau and cognitive decline. *Parkinsonism & Related Disorders*, 95, 70–76. <https://doi.org/10.1016/j.parkreldis.2022.01.002>.
- de Groot, N. S., & Burgas, M. T. (2015). Is membrane homeostasis the missing link between inflammation and neurodegenerative Diseases? *Cellular and Molecular Life Sciences: CMLS*, 72(24), 4795–4805. <https://doi.org/10.1007/s00018-015-2038-4>.
- De Virgilio, A., Greco, A., Fabbri, G., Inghilleri, M., Rizzo, M. I., Gallo, A., Conte, M., Rosato, C., Ciniglio Appiani, M., & de Vincentiis, M. (2016). Parkinson's Disease: Autoimmunity and neuroinflammation. *Autoimmunity Reviews*, 15(10), 1005–1011. <https://doi.org/10.1016/j.autrev.2016.07.022>.
- Debette, S., Schilling, S., Duperron, M. G., Larsson, S. C., & Markus, H. S. (2019). Clinical significance of magnetic resonance imaging markers of Vascular Brain Injury: A systematic review and Meta-analysis. *JAMA Neurology*, 76(1), 81–94. <https://doi.org/10.1001/jamaneurol.2018.3122>.
- Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage*, 31(3), 968–980. <https://doi.org/10.1016/j.neuroimage.2006.01.021>.
- Ding, J., Sigurðsson, S., Jónsson, P. V., Eiriksdóttir, G., Charidimou, A., Lopez, O. L., van Buchem, M. A., Guðnason, V., & Launer, L. J. (2017). Large perivascular spaces visible on magnetic resonance imaging, Cerebral Small Vessel Disease Progression, and risk of Dementia: The Age, Gene/Environment susceptibility-Reykjavik Study. *JAMA Neurology*, 74(9), 1105–1112. <https://doi.org/10.1001/jamaneurol.2017.1397>.
- Donahue, E. K., Murdos, A., Jakowec, M. W., Sheikh-Bahaei, N., Toga, A. W., Petzinger, G. M., & Sepehrband, F. (2021). Global and Regional changes in Perivascular Space in Idiopathic and

- Familial Parkinson's Disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, 36(5), 1126–1136. <https://doi.org/10.1002/mds.28473>.
- Donahue, E. K., Bui, V., Foreman, R. P., Duran, J. J., Venkadesh, S., Choupan, J., Van Horn, J. D., Alger, J. R., Jakowec, M. W., Petzinger, G. M., & O'Neill, J. (2022). Magnetic resonance spectroscopy shows associations between neurometabolite levels and perivascular space volume in Parkinson's Disease: A pilot and feasibility study. *Neuroreport*, 33(7), 291–296. <https://doi.org/10.1097/WNR.0000000000001781>.
- Engelhardt, B., & Ransohoff, R. M. (2012). Capture, crawl, cross: The T cell code to breach the blood-brain barriers. *Trends in Immunology*, 33(12), 579–589. <https://doi.org/10.1016/j.it.2012.07.004>.
- False Discovery Rate Online Calculator | Tools. (n.d.). Retrieved December 19, (2022). from <https://tools.carbocation.com/FDR>.
- Farina, E., Gattellaro, G., Pomati, S., Magni, E., Perretti, A., Cannatà, A. P., Nichelli, P., & Mariani, C. (2000). Researching a differential impairment of frontal functions and explicit memory in early Parkinson's Disease. *European Journal of Neurology*, 7(3), 259–267. <https://doi.org/10.1046/j.1468-1331.2000.00066.x>.
- Frangi, A. F., Niessen, W. J., Vincken, K. L., & Viergever, M. A. (1998). *Multiscale vessel enhancement filtering*. 130–137.
- He, X., Liu, D., Zhang, Q., Liang, F., Dai, G., Zeng, J., Pei, Z., Xu, G., & Lan, Y. (2017). Voluntary Exercise promotes glymphatic clearance of amyloid Beta and reduces the activation of astrocytes and microglia in aged mice. *Frontiers in Molecular Neuroscience*, 10, 144. <https://doi.org/10.3389/fnmol.2017.00144>.
- Hoops, S., Nazem, S., Siderowf, A. D., Duda, J. E., Xie, S. X., Stern, M. B., & Weintraub, D. (2009). Validity of the MoCA and MMSE in the detection of MCI and Dementia in Parkinson Disease. *Neurology*, 73(21), 1738–1745.
- Hughes, A. J., Daniel, S. E., Kilford, L., & Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's Disease: A clinico-pathological study of 100 cases. *Journal of Neurology Neurosurgery and Psychiatry*, 55(3), 181–184. <https://doi.org/10.1136/jnnp.55.3.181>.
- Jessen, N. A., Munk, A. S. F., Lundgaard, I., & Nedergaard, M. (2015). The Glymphatic System: A beginner's guide. *Neurochemical Research*, 40(12), 2583–2599. <https://doi.org/10.1007/s11064-015-1581-6>.
- Jia, Y., Liu, C., Li, H., Li, X., Wu, J., Zhao, Y., Xu, M., Yu, H., Guan, Z., Sun, S., Zhang, C., & Duan, Z. (2021). Enlarged Perivascular Space and its correlation with Polysomnography indicators of obstructive sleep apnea. *Nature and Science of Sleep*, 13, 863–872. <https://doi.org/10.2147/NSS.S305465>.
- Klein, J. (2000). Membrane breakdown in acute and chronic neurodegeneration: Focus on choline-containing phospholipids. *Journal of Neural Transmission (Vienna Austria: 1996)*, 107(8-9), 1027–1063. <https://doi.org/10.1007/s007020070051>.
- Lewis, S. J. G., Dove, A., Robbins, T. W., Barker, R. A., & Owen, A. M. (2003). Cognitive impairments in early Parkinson's Disease are accompanied by reductions in activity in frontostriatal neural circuitry. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 23(15), 6351–6356.
- Lilja, D. J., & Linse, G. M. (2022). *Linear Regression Using R: An Introduction to Data Modeling, 2nd Edition*. University of Minnesota Libraries Publishing. <https://doi.org/10.24926/8668/1301>.
- MacLulich, A. M. J., Wardlaw, J. M., Ferguson, K. J., Starr, J. M., Seckl, J. R., & Deary, I. J. (2004). Enlarged perivascular spaces are associated with cognitive function in healthy elderly men. *Journal of Neurology Neurosurgery & Psychiatry*, 75(11), 1519–1523. <https://doi.org/10.1136/jnnp.2003.030858>.
- Manjón, J. V., Coupé, P., Martí-Bonmatí, L., Collins, D. L., & Robles, M. (2010). Adaptive non-local means denoising of MR images with spatially varying noise levels. *Journal of Magnetic Resonance Imaging: JMRI*, 31(1), 192–203. <https://doi.org/10.1002/jmri.22003>.
- Mogensen, F. L. H., Delle, C., & Nedergaard, M. (2021). The Glymphatic System (En)during inflammation. *International Journal of Molecular Sciences*, 22(14), 7491. <https://doi.org/10.3390/ijms22147491>.
- Moritz, C. H., Johnson, S. C., McMillan, K. M., Haughton, V. M., & Meyerand, M. E. (2004). Functional MRI neuroanatomic correlates of the Hooper Visual Organization Test. *Journal of the International Neuropsychological Society: JINS*, 10(7), 939–947. <https://doi.org/10.1017/s1355617704107042>.
- Owen, A. M., Sahakian, B. J., Semple, J., Polkey, C. E., & Robbins, T. W. (1995). Visuo-spatial short-term recognition memory and learning after temporal lobe excisions, frontal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia*, 33(1), 1–24. [https://doi.org/10.1016/0028-3932\(94\)00098-a](https://doi.org/10.1016/0028-3932(94)00098-a).
- Paradise, M., Crawford, J. D., Lam, B. C. P., Wen, W., Kochan, N. A., Makkar, S., Dawes, L., Trollor, J., Draper, B., Brodaty, H., & Sachdev, P. S. (2021). Association of Dilated Perivascular Spaces with Cognitive decline and Incident Dementia. *Neurology*, 96(11), e1501–e1511. <https://doi.org/10.1212/WNL.00000000000011537>.
- Park, Y. W., Shin, N. Y., Chung, S. J., Kim, J., Lim, S. M., Lee, P. H., Lee, S. K., & Ahn, K. J. (2019). Magnetic resonance imaging-visible Perivascular spaces in basal Ganglia Predict Cognitive decline in Parkinson's Disease. *Movement Disorders: Official Journal of the Movement Disorder Society*, 34(11), 1672–1679. <https://doi.org/10.1002/mds.27798>.
- Parkinson's Disease Measurement: PwP, surveys, trials, analysis*. (n.d.). Retrieved June 12, (2022). from <https://www.parkinsonsmeasurement.org/toolBox/levodopaEquivalentDose.htm>.
- Ramirez, J., Holmes, M. F., Berezuk, C., Kwan, D., Tan, B., Beaton, D., Scott, C. J. M., Ozzoude, M., Gao, F., Yu, D., Swardfager, W., Lawrence-Dewar, J., Dowlatshahi, D., Saposnik, G., Boulos, M. I., Murray, B. J., Symons, S., Bartha, R., Black, S. E., & Lim, A. (2021). MRI-visible perivascular space volumes, sleep duration and daytime dysfunction in adults with Cerebrovascular Disease. *Sleep Medicine*, 83, 83–88. <https://doi.org/10.1016/j.sleep.2021.03.043>.
- Ramirez, J., Berberian, S. A., Breen, D. P., Gao, F., Ozzoude, M., Adamo, S., & ONDRI Investigators. (2022). Small and large magnetic resonance imaging-visible Perivascular spaces in the basal ganglia of Parkinson's Disease patients. *Movement Disorders*, 37(6), 1304–1309. <https://doi.org/10.1002/mds.29010>. PMID: 35403259.
- Sepehrband, F., Barisano, G., Sheikh-Bahaei, N., Cabeen, R. P., Choupan, J., Law, M., & Toga, A. W. (2019). Image processing approaches to enhance perivascular space visibility and quantification using MRI. *Scientific Reports*, 9(1), 12351. <https://doi.org/10.1038/s41598-019-48910-x>.
- Sepehrband, F., Barisano, G., Sheikh-Bahaei, N., Choupan, J., Cabeen, R. P., Lynch, K. M., Crawford, M. S., Lan, H., Mack, W. J., Chui, H. C., Ringman, J. M., & Toga, A. W. (2021). Volumetric distribution of perivascular space in relation to mild cognitive impairment. *Neurobiology of Aging*, 99, 28–43. <https://doi.org/10.1016/j.neurobiolaging.2020.12.010>. & Alzheimer's Disease Neuroimaging Initiative
- Taylor, A. E., Saint-Cyr, J. A., & Lang, A. E. (1990). Memory and learning in early Parkinson's Disease: Evidence for a frontal lobe syndrome. *Brain and Cognition*, 13(2), 211–232. [https://doi.org/10.1016/0278-2626\(90\)90051-o](https://doi.org/10.1016/0278-2626(90)90051-o).
- Urenjak, J., Williams, S. R., Gadian, D. G., & Noble, M. (1993). Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 13(3), 981–989.
- von Holstein-Rathlou, S., Petersen, N. C., & Nedergaard, M. (2018). Voluntary running enhances glymphatic influx in awake behaving,

- young mice. *Neuroscience Letters*, 662, 253–258. <https://doi.org/10.1016/j.neulet.2017.10.035>.
- Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., Iliff, J. J., Takano, T., Deane, R., & Nedergaard, M. (2013). Sleep drives Metabolite Clearance from the adult brain. *Science (New York N Y)*, 342(6156), <https://doi.org/10.1126/science.1241224>.
- Zhu, Y. C., Dufouil, C., Soumaré, A., Mazoyer, B., Chabriat, H., & Tzourio, C. (2010). High degree of dilated Virchow-Robin Spaces on MRI is Associated with increased risk of Dementia. *Journal of Alzheimer's Disease: JAD*, 22, 663–672. <https://doi.org/10.3233/JAD-2010-100378>.
- Zou, W., Pu, T., Feng, W., Lu, M., Zheng, Y., Du, R., Xiao, M., & Hu, G. (2019). Blocking meningeal lymphatic drainage aggravates Parkinson's disease-like pathology in mice overexpressing mutated α -synuclein. *Translational Neurodegeneration*, 8(1), 7. <https://doi.org/10.1186/s40035-019-0147-y>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Thalamic volume mediates associations between cardiorespiratory fitness (VO₂max) and cognition in Parkinson's disease

Andrew J. Petkus^{a,*}, Behnaz Jarrahi^b, Daniel P. Holschneider^{a,c}, Megan E. Gomez^d, J. Vincent Filoteo^{e,f}, Dawn M. Schiehser^{e,f}, Beth E. Fisher^g, John D. Van Horn^h, Michael W. Jakowec^a, Sarah C. McEwenⁱ, Giselle Petzinger^a

^a Department of Neurology, University of Southern California, 1520 San Pablo St., HCC-2, Suite 3000, Los Angeles, CA, 90033, USA

^b Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University School of Medicine, Palo Alto, CA, 94305, USA

^c Department of Psychiatry and the Behavioral Sciences, University of Southern California, 1333 San Pablo St., Los Angeles, CA, 90033, USA

^d Department of Psychology, Tibor Rubin Veterans Administration Medical Center, Long Beach, CA, 90822, USA

^e Psychology and Research Services, Veterans Administration San Diego Health Care System, San Diego, CA, 92161, USA

^f Departments of Psychiatry and Neurosciences, University of California San Diego, 9500 Gilman Dr., La Jolla, CA, 92093, USA

^g Division of Biokinesiology and Physical Therapy, University of Southern California, Los Angeles, CA, 90033, USA

^h Department of Psychology and School of Data Science, University of Virginia, Charlottesville, VA, 22904, USA

ⁱ Department of Translational Neurosciences and Neurotherapeutics, Saint John's Cancer Institute, Santa Monica, CA, 90404, USA

ARTICLE INFO

Keywords:

Thalamus
Cardiorespiratory fitness
Cognition
Structural magnetic resonance imaging
MRI
Imaging
Exercise

ABSTRACT

Introduction: Cognitive deficits occur in Parkinson's disease (PD). Cardiorespiratory fitness (CRF) is associated with better cognitive performance in aging especially in executive function (EF) and memory. The association between CRF and cognitive performance is understudied in people with PD. Brain structures underlying associations also remains unknown. This cross-sectional study examined the associations between CRF and cognitive performance in PD. We also examined associations between CRF and brain structures impacted in PD. Mediation analysis were conducted to examine whether brain structures impacted in PD mediate putative associations between CRF and cognitive performance.

Methods: Individuals with PD (N = 33) underwent magnetic resonance imaging (MRI), CRF evaluation (estimated VO₂max), and neuropsychological assessment. Composite cognitive scores of episodic memory, EF, attention, language, and visuospatial functioning were generated. Structural equation models were constructed to examine whether MRI volume estimates (thalamus and pallidum) mediated associations between CRF and cognitive performance (adjusting for age, education, PD disease duration, sex, MDS-UPDRS motor score, and total intracranial volume).

Results: Higher CRF was associated with better episodic memory (Standardized $\beta = 0.391$; $p = 0.008$), EF (Standardized $\beta = 0.324$; $p = 0.025$), and visuospatial performance (Standardized $\beta = 0.570$; $p = 0.005$). Higher CRF was associated with larger thalamic (Standardized $\beta = 0.722$; $p = 0.004$) and pallidum (Standardized $\beta = 0.635$; $p = 0.004$) volumes. Thalamic volume mediated the association between higher CRF and better EF (Indirect effect = 0.309) and episodic memory (Indirect effect = 0.209) performance ($p < 0.05$). The pallidum did not significantly mediate associations between CRF and cognitive outcomes.

Conclusion: The thalamus plays an important role in the association between CRF and both EF and episodic memory in PD.

1. Introduction

Motor deficits, such as gait and balance dysfunction, are the most frequently recognized characteristics of Parkinson's disease (PD);

however, non-motor features such as cognitive impairment are prominent and associated with poor quality of life. Mild cognitive impairment (MCI) is seen in 20–40% of patients in early PD and is associated with a high risk of developing dementia. Within eight years after diagnosis,

* Corresponding author. Department of Neurology, University of Southern California, 1520 San Pablo Street, Suite 3000, Los Angeles, CA, 90033-4606, USA.
E-mail address: Petkus@usc.edu (A.J. Petkus).

<https://doi.org/10.1016/j.parkreldis.2021.03.019>

Received 29 September 2020; Received in revised form 15 March 2021; Accepted 18 March 2021

Available online 29 March 2021

1353-8020/© 2021 Elsevier Ltd. All rights reserved.

78% of individuals with PD experience significant enough cognitive decline and disability to meet criteria for dementia [1,2]. Although Executive function (EF) is the most common cognitive domain affected by PD; other cognitive domains such as visuospatial function, episodic memory, attention and language are also commonly impacted. The pathophysiological basis for cognitive decline in PD includes alterations in dopamine, serotonin, and acetylcholine neurotransmission, as well as the neurodegeneration of a number of important cortical structures such as the prefrontal cortex (PFC) and cerebellum [3]. Hallmark neuropathology in PD is evidenced by changes in deep grey matter structures, in the basal ganglia (caudate, putamen, and pallidum), hippocampus, anterior cingulate, and thalamus [4,5]. Furthermore, in persons with PD, impairments in EF are associated with decreased volumetric size in the PFC, as well as the basal ganglia [4]. Increasing evidence supports the important role of the thalamus and its connections with the PFC and associated cognition, since thalamic lesions are concomitant with EF impairments observed in PFC neurodegeneration [5,6]. Functional and structural pathophysiological changes in the thalamus have also been associated with impaired EF, attention, memory, and visual spatial processing and overall cognitive functioning in PD. Currently there are no effective treatments for cognitive deficits in PD. Thus, identifying therapeutic interventions that may attenuate cognitive decline and the onset of dementia and slow pathophysiological changes in affected brain regions, such as deep grey matter structures, including the thalamus, may improve quality of life [7].

In PD, different forms of physical exercise, including aerobic exercise (e.g. treadmill training) and motor skill training (e.g. yoga), improve motor performance particularly gait and balance [8]. However, the impact of exercise on cognitive function in PD remains relatively understudied. A small number of aerobic exercise intervention studies using brisk walking, treadmill training, or recumbent bike-training have reported improvements in CRF along with moderate improvement in EF [9–13]. In healthy older adults, both aerobic exercise and CRF have been demonstrated to benefit EF and memory along with improvements in attention and visuospatial function [14–18]. In addition, investigations in cognitive aging have also revealed that cognitive benefits may be due in part to the association of CRF (defined as VO_{2max}) on grey matter volumetric (GMV) changes in the basal ganglia, PFC, hippocampus, and thalamus [19]. However, the role of aerobic exercise, and in particular the association of CRF and domain-specific cognitive performance such as attention, memory, visuospatial function, and language remain relatively unexplored in PD [9]. Furthermore, the underlying exercise- and fitness-related structural brain changes that may account for these cognitive benefits have yet to be elucidated in PD.

The aim of this cross-sectional study was to investigate the association of CRF (defined as estimated VO_{2max}) and performance in specific cognitive domains in people with PD. We also sought to examine the association of CRF and GMV in brain structures associated with cognitive deficits in PD. The specific cognitive domains examined included: EF, episodic memory, attention, visuospatial function, and language. Given reports of significant association in community dwelling older adults we hypothesized a significant association would be seen between CRF and EF, as well as CRF and episodic memory. Regarding brain structures of interest, we focused on deep grey matter structures (caudate nucleus and putamen, nucleus accumbens, globus pallidus, thalamus, anterior cingulate, and hippocampus), as well as the PFC, and cerebellum. These brain regions were selected based on at least one of the following: (i) associated neuropathological changes observed in PD; (ii) regions of exercise-induced neuroplasticity as reported in animal models; and (iii) association with increased CRF and GMV reported in cognitive aging populations. We employed a mediation analysis to investigate the extent to which putative associations between CRF and cognitive performance were mediated by the brain regions of interest implicated in PD.

2. Methods

2.1. Participants

The study sample consisted of 33 individuals with idiopathic PD who were enrolled in a cross-sectional study investigating associations between CRF, cognitive performance, and neuroanatomical volumetrics using magnetic resonance imaging (MRI). All participants had a diagnosis of PD using criteria used by the UK Brain Bank criterium [20]. Participants completed a structured interview and the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [21] with a movement disorder specialist (GP). Exclusion criteria included deep brain stimulation surgery, or a diagnosis of dementia based on MDS-PD dementia criteria [1]. Participants were recruited from the University of Southern California (USC) Movement Disorder Clinic, the Michael J. Fox trial finder, radio advertisements, and local PD patient support groups. All participants were optimally medicated during the completion of the study assessments. The USC Institutional Review Boards (IRB) approved study procedures and participants provided written informed consent.

2.2. Fitness assessment

CRF was quantified by estimating the maximum oxygen uptake volume (estimated VO_{2max}) as measured by the Ebbeling Single Stage Submaximal Treadmill Walking Fitness Test [22], a reliable and valid way to estimate VO_{2max} [23]. Briefly, participants first underwent a 4-min warm-up, walking at a brisk, but comfortable pace ranging from 2.0 to 4.5 mph, and 0% grade, eliciting a heart rate within 50–70% of age-predicted maximum, followed by a single testing stage of treadmill walking for 4 min at 5% grade. The steady state heart rate was determined from the average of the final 30 s at 5% grade. The equation to estimate VO_{2max} ($ml\ kg^{-1}\ min^{-1}$) from a single stage 4-min, 5% grade submaximal treadmill test is as follows: $VO_{2max} = 15.1 + 21.8 * SPEED\ (mph) - 0.327 * HEART\ RATE\ (bpm) - 0.263 * SPEED * AGE\ (yr) + 0.00504 * HEART\ RATE * AGE + 5.98 * GENDER\ (0 = female; 1 = male)$.

2.3. Neuropsychological assessment

The neuropsychological battery administered followed the Level-II guidelines from the PD-MCI MDS taskforce for assessing cognition [24]. The neuropsychological battery consisted of tests that measured cognitive performance across multiple domains including attention, language, visuospatial, EF, and episodic memory. To reduce the number of comparisons a composite score was created quantifying performance within each domain. Scores were transformed as needed so that for all tests higher scores represented better performance. Composite and raw scores on each test were z-score standardized based on the sample mean and standard deviation. Attention was measured by (i) the Delis Kaplan Executive Function System (D-KEFS), Color Word Interference Test (CWIT), with the number of seconds to complete the color naming and word reading subtests, (ii) the total score for the forward digit span condition of the Adaptive Digit Ordering Test (DOT-A), and (iii) the total raw score from the digit sequencing condition of the DOT-A. Language ability was measured by (i) the total raw score from the 4th edition of the Wechsler Adult Intelligence Scale (WAIS-IV) Similarities subtest, (ii) the total correct number of words produced on the D-KEFS Letter Fluency test, and (iii) the total correct number of words on the D-KEFS Category Fluency test. Episodic memory was measured by (i) the total number of correct words recalled across the five immediate recall trials of the 2nd Edition of the California Verbal Learning Test (CVLT-II), (ii) the total number of words recalled on the long-delayed free recall condition of the CVLT-II, (iii) the total points on the immediate recall subtest of the Wechsler Memory Scale-Third Edition Visual Reproduction test (WMS-III), and (iv) the total points on the delayed recall condition of the WMS-III Visual Reproduction test. Visuospatial ability was measured

by (i) total raw score on the Judgment of Line Orientation Test (JLO) and (ii) the total raw score on the Hooper Visual Organization Test (HVOT). EF was measured using the number of errors on the Wisconsin Card Sorting Test (WCST), (ii) the number of seconds to complete the color-word inhibition subtest of the D-KEFS CWIT, (iii) the number of seconds to complete the inhibition/switching subtest from the D-KEFS CWIT, (iv) the number of correct words produced on the verbal fluency switching subtest from the D-KEFS verbal fluency test, and (v) the total move score from the Tower of London-Drexel University test. For descriptive purposes participants were classified as meeting criteria for mild cognitive impairment (PD-MCI) by consensus (VF, AP, MG) based on Level II MDS Task Force Criteria.

2.4. Structural magnetic resonance imaging (MRI) acquisition

The structural MRI was completed on a 3.0 T MRI system (Siemens, Erlangen, Germany) fitted with a 12-channel receive-only phased array head coil at the UCLA Staglin IMHRO Center for Cognitive Neuroscience. A high-resolution whole-brain structural MRI scan was acquired using a T1-weighted 3-dimensional (3D) magnetization prepared rapid gradient echo (MPRAGE) protocol with the following parameters: TR = 2300 ms, TE = 2.98 ms, flip angle = 15°, 176 axial slices; 1 mm slice thickness, 256 × 256 matrix; 256 mm field of view. Participants were instructed to remain still as possible during the MRI acquisition to obtain good image quality. FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu/>; version v 5.1.0) was used for volumetric measurements. Briefly, preprocessing steps included conversion of MPRAGE images to FreeSurfer format, motion, non-uniformity and intensity correction, skull stripping using a hybrid watershed/surface deformation procedure, automated registration to a standard Talairach atlas space, and tissue segmentation (white matter, grey matter and cerebrospinal fluid). The white/gray matter boundary (i.e., white surface) and gray matter/cerebrospinal fluid boundary (i.e., pial surface) were identified and transformed into surfaces and the distance between the white and pial surfaces calculated at each point across the cortical mantle. Each participant's cortex was anatomically parcellated, labeled, and aligned to the FreeSurfer's standard averaged cortical surface template (FSaverage template) and smoothed on the level of the sphere using a 5-mm full-width half-maximum Gaussian spatial smoothing kernel. No obvious errors in parcellation and automatic labelling were observed for any participant upon inspection, and so no manual intervention was performed. Following automatic cortical and subcortical segmentation (i.e., recon-all function in FreeSurfer), volumetric measurements were collected for dorsolateral PFC, anterior cingulate, caudate nucleus, putamen, nucleus accumbens, pallidum, thalamus, cerebellum, and hippocampus based on the Desikan-Killiany atlas and were extracted from FreeSurfer for further statistical analysis. All brain volumes of interest were bilateral and scaled with respect to total intracranial volume (TICV) using the ICV-ANCOVA approach [25].

2.5. Statistical analysis

A multivariable linear regression analyses was used to first estimate associations between CRF and performance within each cognitive domain. The CRF was z-score standardized (mean = 0; standard deviation = 1) based on the sample mean and standard deviation and used for all statistical analysis. Associations between CRF and performance within each cognitive domain estimates were adjusted for covariates including age, sex, years since PD diagnosis, UPDRS Motor Score, and years of education. Multivariable linear regression analyses were conducted to examine the association between CRF and brain volumes of interest (including PFC, anterior cingulate, caudate nucleus, putamen, nucleus accumbens, pallidum, thalamus, cerebellum, and hippocampus). Extracted brain volumes of interest were corrected for intracranial volume and z-score standardized using the sample mean and standard deviation. The effect of CRF on brain volumes of interest were adjusted

study covariates. To decrease the false-discovery rate a Benjamini-Hochberg (B-H) p-value adjustment was applied.

Structural equation models (SEMs) were constructed to estimate whether structural MRI brain volumes of interest mediated any putative associations between CRF and cognitive performance. The SEM tested mediation using standard approaches by estimating three parameter effects of CRF on cognitive performance termed the Direct, Indirect, and total effects. The Direct effect (denoted by the pathway “c” in Figs. 1 and 2) is defined as the effect of CRF on cognitive performance that is independent of brain volume of interest and covariates. The Indirect effect of CRF on cognitive performance is defined as effect of CRF on cognitive performance mediated by the structural MRI brain volumes of interest. The Indirect effect is a product of the effect of CRF on structural MRI brain volumes of interest (represented by Figs. 1 and 2 path “a”) and MRI brain volumes of interest on cognitive performance (Figs. 1 and 2 path “b”). The total effect of CRF on individual cognitive performance is calculated by adding the Direct and Indirect effects. All parameter effect estimates were adjusted for covariates. The 95% confidence interval of the Indirect effect was estimated using bootstrapping sampling (with 5000 samples). Analyses were run with the SEM modeling software MPLUS in the R environment using the MPLUS Automation package.

3. Results

Participants were on average 64.6 (SD = 9.6) years old, 70% male, diagnosed with PD approximately five years (SD = 3.7) prior to the study and had an MDS-UPDRS motor score of 24.15 (SD = 9.8) (Table 1). Fifty two percent met criteria for PD-MCI. The age-based normative performance on individual cognitive tests within each cognitive domain are shown on Supplement Table S1. Multivariable linear regression analyses revealed that higher CRF was associated with better performance on tests of visuospatial function (standardized β = 0.570; 95% confidence interval (CI) = 0.238, 0.901; Benjamini-Hochberg adjusted [B-H] p = 0.005), episodic memory (standardized β = 0.391; 95% CI = 0.129, 0.653; B-H p = 0.008) and EF (standardized β = 0.324; 95% CI = 0.064, 0.585; B-H p = 0.025), (Table 2A). Associations were all adjusted for covariates. CRF was not significantly associated with performance on tests of attention or language ability. Regarding brain structures of interest, CRF was positively associated with larger volumes of the thalamus (standardized β = 0.722; 95% CI = 0.430, 1.015; B-H p = 0.004) and pallidum (standardized β = 0.635; 95% CI = 0.354, 0.916; B-H p = 0.004) (Table 2B). CRF was not significantly associated with volumes of the caudate nucleus, putamen, PFC, nucleus accumbens, anterior cingulate, hippocampus, or cerebellum.

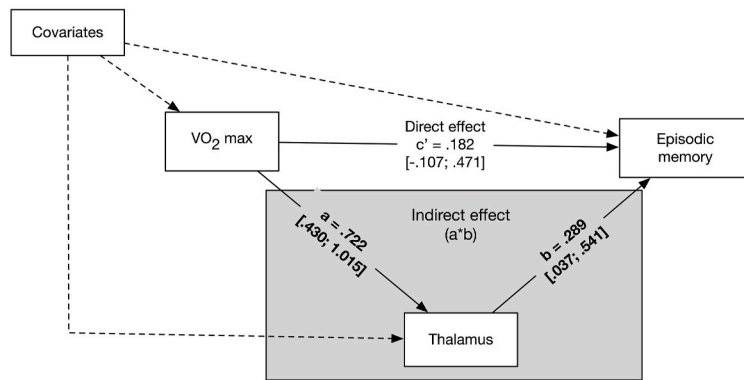
Mediation analyses were conducted to examine whether thalamic volume mediated putative associations between CRF and performance on tests of episodic memory (Fig. 1, Panel A), EF (Fig. 1, Panel B), and visuospatial function (Fig. 1, Panel C). The Indirect effect of CRF on episodic memory was statistically significant demonstrating that the thalamus partially mediated observed associations between higher CRF and better episodic memory. In addition, the Indirect effect of CRF on EF was statistically significant, demonstrating that the thalamus partially mediated observed associations between higher CRF and better EF. The Indirect effect of CRF on visuospatial function mediated by the thalamus was a trend to significance as indicated by the 95% confidence interval.

In contrast to the thalamus, the pallidum was not significantly associated with performance on either EF, episodic memory, or visuospatial function (Fig. 2, Panels ABC). In addition, the resulting Indirect effect of CRF on performance across the three domains mediated by the pallidum was not statistically significant.

4. Discussion

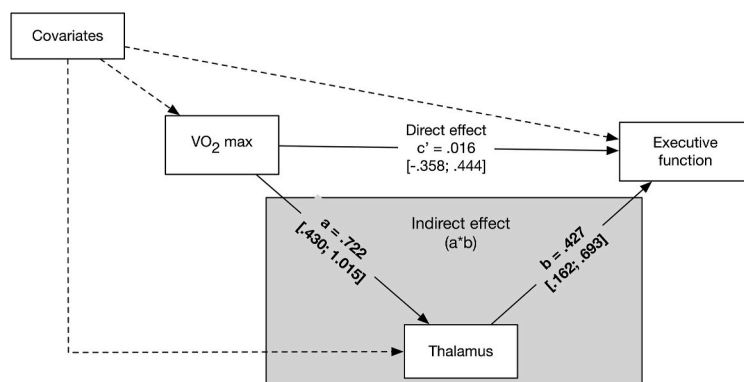
The relationship between CRF and brain structure has been extensively studied in the context of cognitive aging, through its contribution in neurodegenerative disorders such as PD has been relatively under-

Panel A



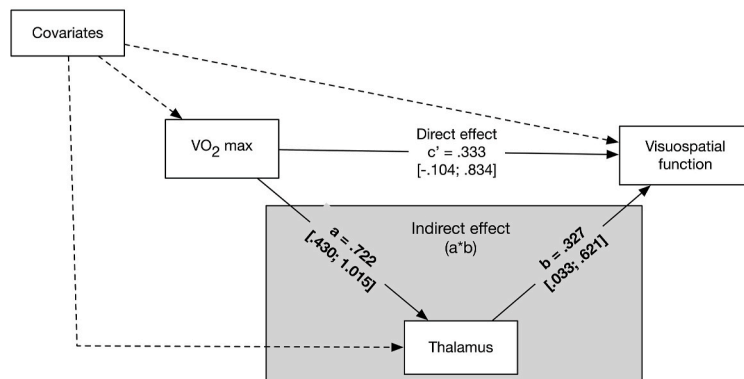
Effect	Estimate	95% CI
Direct effect of VO ₂ max on episodic memory (independent of the thalamus)	.182	[-.107; .471]
Indirect effect of VO ₂ max on episodic memory mediated by volume of the thalamus (.722*.289)	.209	 [.028; .390]
Total effect of VO ₂ max on episodic memory (Direct effect + Indirect effect)	.391	 [.120; .661]

Panel B



Effect	Estimate	95% CI
Direct effect of VO ₂ max on executive function (independent of the thalamus)	.016	[-.358; .444]
Indirect effect of VO ₂ max on executive function mediated by volume of the thalamus (.722 * .427)	.308	 [.023; .790]
Total effect of VO ₂ max on executive function (Direct effect + Indirect effect)	.324	 [.005; .803]

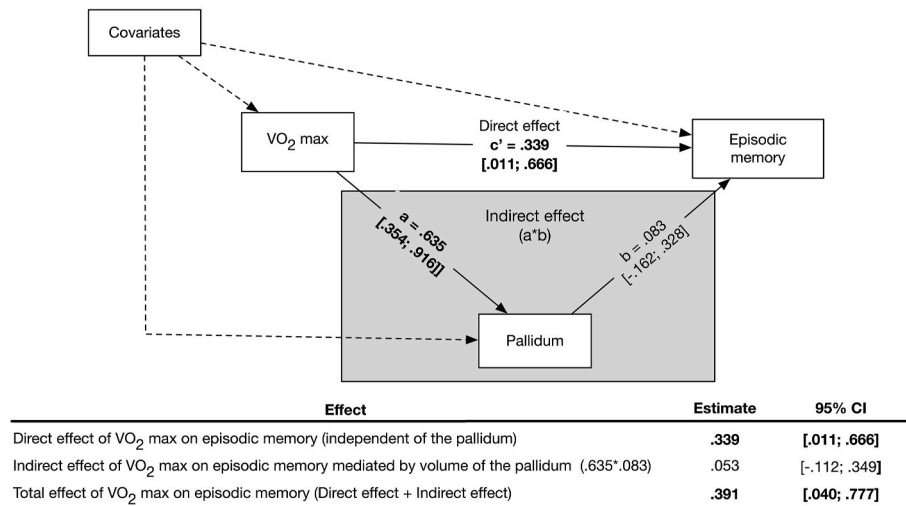
Panel C



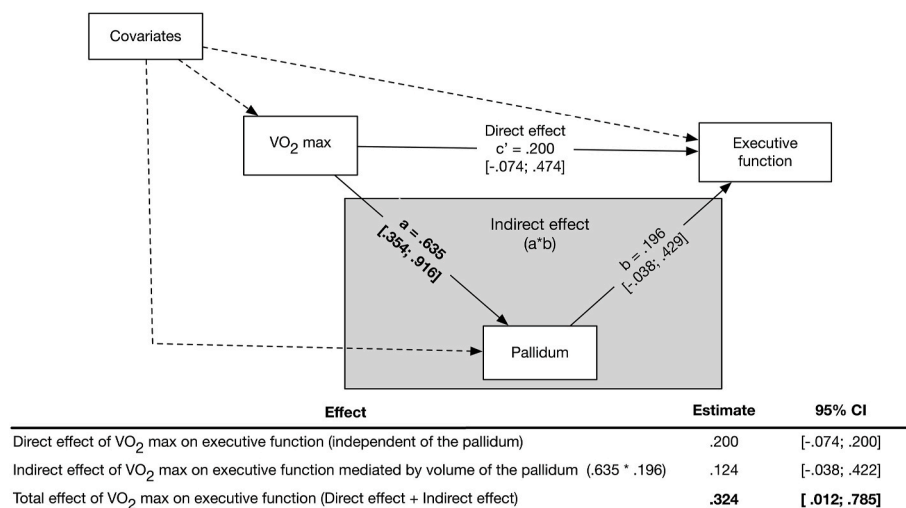
Effect	Estimate	95% CI
Direct effect of VO ₂ max on visuospatial function (independent of the thalamus)	.333	[-.104; .834]
Indirect effect of VO ₂ max on visuospatial function mediated by volume of the thalamus (.722 * .327)	.236	 [-.011; .704]
Total effect of VO ₂ max on visuospatial function (Direct effect + Indirect effect)	.570	 [.197; 1.050]

Fig. 1. Thalamic Volume Mediates the Association Between VO₂max and Episodic Memory and Executive Function. Panel A illustrates the structural equation mediation model with parameter effects, estimates, and 95% confidence intervals (CI). Thalamic volume is a significant mediator of the association between VO₂max and episodic memory, as demonstrated by the significant Indirect effect (grey box) of the thalamus on this association. The Indirect effect is the product of the effect of VO₂max on the thalamic volume (Path a) and the effect of thalamic volume on episodic memory (Path b). The Direct effect (Path c') is not significant and is defined as the effect of VO₂max on episodic memory while adjusting for the effect of thalamic volume and covariates. The total effect of VO₂max on episodic memory is significant and is calculated by adding the Direct and Indirect effects. Episodic memory is a composite of the total number of correct words recalled on the immediate and long-delay free recall trials from the California Verbal Learning Test-II, and visual reproduction one and two scores from the Wechsler Memory Scales. Panel B illustrates a similar structural equation mediation model as in Panel A and demonstrates that thalamic volume is a significant mediator (Indirect effect, grey box) of VO₂max on executive function. The total effect of VO₂max on executive function is also significant. Executive function is a composite of the number of seconds to complete the inhibition and inhibition-switching subtests of the D-KEFS Color-Word Interference test, the number of errors on the Wisconsin Card Sorting Test, total number of correct words on the switching fluency subtest from the D-KEFS Verbal Fluency test, and the total move score on the Tower of London-Drexel University test. Panel C illustrates a similar structural equation mediation model as in Panel A and demonstrates that thalamic volume is not a significant mediator (Indirect effect, grey box) of the association between VO₂max on visuospatial function. The total effect of VO₂max on visuospatial function is significant. Visuospatial function is a composite score of visuospatial ability consisting of the Judgement of Line Orientation and Hooper Visual Organization Test. All parameter effect estimates were adjusted for covariates. Dashed pathways represent effects of covariates. Bolded estimates are significant at $p < 0.05$. The 95% confidence intervals for parameter estimates are presented in brackets.

Panel A



Panel B



Panel C

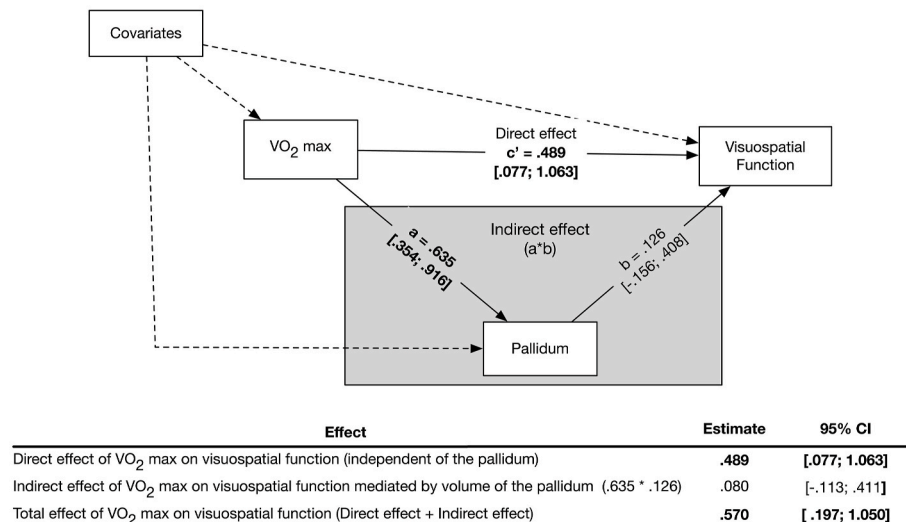


Fig. 2. Pallidum Volume Does Not Mediate the Association Between VO₂max and Cognitive Performance. Panel A illustrates the structural equation mediation model with parameter Effects, Estimates, and 95% Confidence Intervals (CI). Pallidum volume does not significantly mediate the association between VO₂max and episodic memory, as demonstrated by the non-significant Indirect effect (grey box) of the pallidum on this association. The Indirect effect is the product of the effect of VO₂max on the pallidum volume (Path a) and the effect of pallidum volume on episodic memory (Path b). The Direct effect (Path c) is significant and is defined as the effect of VO₂max on episodic memory while adjusting for the effect of pallidum volume and covariates. The total effect of VO₂max on episodic memory is significant and is calculated by adding the Direct and Indirect effects. Episodic memory is a composite of the total number of correct words recalled on the immediate and long-delay free recall trials from the California Verbal Learning Test-II, and visual reproduction one and two scores from the Wechsler Memory Scales. Panel B illustrates a similar structural equation mediation model as in Panel A and demonstrates that pallidum volume is not a significant mediator (Indirect effect, grey box) of VO₂max on executive function. The total effect of VO₂max on executive function is significant. Executive function is a composite of the number of seconds to complete the inhibition and inhibition-switching subtests of the D-KEFS Color-Word Interference test, the number of errors on the Wisconsin Card Sorting Test, total number of correct words on the switching fluency subtest from the D-KEFS Verbal Fluency test, and the total move score on the Tower of London-Drexel University test. Panel C illustrates a similar structural equation mediation model as in Panel A and demonstrates that pallidum volume is not a significant mediator (Indirect effect, grey box) of the association between VO₂max on visuospatial function. The Direct effect of VO₂max on visuospatial function is significant. The total effect of VO₂max on visuospatial function is significant. Visuospatial function is a composite score of visuospatial ability consisting of the Judgement of Line Orientation and Hooper Visual Organization Test. All parameter effect estimates were adjusted for covariates. Dashed pathways represent effects of covariates bolded estimates are significant at $p < 0.05$. The 95% confidence intervals for parameter estimates are presented in brackets.

Table 1

Demographics and Sample Characteristics. Total number of study participants was 33.

Table 1. Sample characteristics (N = 33)

	Mean	SD		
	or	or		
	N	%	Min	Max
Demographics				
Age (years)	64.61	9.55	49	88
Sex N (%)				
Male	23	70%		
Female	10	30%		
Years of education	16.88	1.95	13	20
Clinical characteristics				
Years since PD diagnosis	4.98	3.69	1	15
Hoehn and Yahr stage N (%)				
Two	29	88%		
Three	4	12%		
Part 3 of MDS-UPDRS	24.15	9.88	4	45
Dopamine equivalence	10.12	7.73	1	23
Meets MDS criteria for PD-MCI N (%)	17	52%		
Cardiorespiratory fitness				
Maximum VO ₂ (ml/min/kg)	30.35	4.51	22.90	40.83

Bolded estimates denote $p < 0.05$.

UPDRS=Unified Parkinson's Disease Rating Scale.

Table 2

Associations between maximum VO₂^a and domain-specific cognitive performance^b are presented in panel A while association between maximum VO₂ and structural magnetic resonance imaging derived brain volumes are presented in panel B (N = 33).

Panel A: Associations between max VO ₂ and domain-specific cognitive performance	95% Confidence Interval			B-H p ^d
	Est ^c	Lower	Upper	
Executive function ^d	.324	.064	.585	.025
Episodic memory ^c	.391	.129	.653	.008
Visuospatial ^f	.570	.238	.901	.005
Attention ^g	.081	-.241	.403	.623
Language ^h	.243	-.164	.651	.302
Panel B: Associations between max VO ₂ and structural magnetic resonance imaging derived brain volumes ^e	Est ^c	95% Confidence Interval		B-H p ^d
		lower	upper	
Thalamus	.722	.430	1.015	.004
Pallidum	.635	.354	.916	.004
Caudate nucleus	.155	-.260	.571	.464
Putamen	-.248	-.585	.088	.395
Dorsolateral prefrontal cortex	.095	-.326	.516	.813
Nucleus accumbens	.119	-.242	.479	.813
Anterior cingulate	-.113	-.409	.183	.813
Hippocampus	-.081	-.511	.348	.813
Cerebellum	.024	-.245	.294	.859

Bolded estimates denote $p < 0.05$.

^a Maximum VO₂ was estimated by the Ebbeling Single State Submaximal Treadmill Walking Test and z-score standardized (mean = 0; standard deviation = 1) based on the sample mean and standard deviation.

^b All cognitive outcomes were z-score standardized (mean = 0; standard deviation = 1).

^c Parameter estimates were adjusted for covariates.

^d p value represents the Benjamini-Hochberg adjusted p value to correct for multiple comparisons.

^e Brain volumes were derived from structural magnetic resonance imaging, were corrected for intracranial volume and were z-score standardized using the sample mean and standard deviation.

investigated. Findings from our study showed that greater CRF is associated with better performance on tests of EF, episodic memory, and visuospatial function in individuals with PD. Although the associations between CRF with EF and episodic memory are well established in aging

[18], our findings are the first to link CRF with specific cognitive domains impacted in PD, including EF, memory, and visuospatial function. Higher CRF was also associated with greater GMV of the thalamus, consistent with studies in cognitive aging [26], and in the pallidum as reported in children [27]. Unlike findings in older adults without PD, we did not observe an association between CRF and hippocampal or PFC volumes, which could be an artefact of the relative sparing of the hippocampus in the pathogenesis of PD, compared to subcortical regions [28].

We also found that thalamic volume mediated the association of higher CRF and EF and episodic memory, but not visuospatial function in PD. The thalamus and its connections to the PFC and the hippocampus are well known to play an important role in cognition [29]. In PD and its animal models, thalamic volume loss has been associated with behavioral deficits, including cognitive impairment [5,30]. Published studies in animal models demonstrating that aerobic exercise can elicit functional reorganization of several brain regions including the thalamus, may provide insight to the mechanisms by which CRF may influence thalamic volume [31,32]. Studies by Holschneider and colleagues [33] have demonstrated that exercised rats compared to their sedentary counterparts show increased functional activation during motor challenge in the mediodorsal thalamus (MD). The MD demonstrates dense connections to frontal cortical regions, including prefrontal, anterior cingulate and premotor cortex, and plays a central role in behavioral flexibility, memory, and working memory [29]. In addition, exercise increases functional activation in the midline thalamus (inter-mediodorsal, retrorhinal, rhomboid nuclei) and intralaminar thalamus (central medial, central lateral, paracentral, parafascicular), whose nuclei have strong connections to the medial PFC, striatum, and hippocampus, and play a role in spatial working memory, the temporal organization of memory, and executive functions [34–36]. Exercise also elicits functional adaptations in motor thalamic nuclei (ventrolateral, ventromedial, central medial) [33] which may contribute to the association of CRF with thalamic volume. Adaptations of the motor thalamus have been proposed to reflect neuroplasticity in the cerebellar-thalamocortical circuit, with changes noted especially at the level of the ventrolateral nucleus (VL), which constitutes a target for efferent fibers from the cerebellum [37,38]. Similar functional adaptations to exercise in these motor nuclei of the thalamus are also observed in the 6-OHDA rat model of Parkinsonism [39], with evidence for exercise-related increased functional connectivity between the motor thalamic nuclei and their connections to primary motor cortex, basal ganglia and cerebellum [32]. The current study undertook volumetric measurements on whole thalamus. Future studies should pursue parcellation of this structure and examine the effects exercise has on cognition and on individual thalamic nuclei. The latter could be undertaken using both volumetric assessments, as well as diffusion tractography and functional connectivity focused on the prefrontal-mediodorsal network, the medial prefrontal-midline thalamo-hippocampal system, as well as the cerebellar-thalamo-cortical circuit. Prior research from Greenough and colleagues has demonstrated increased cerebral angiogenesis in healthy rats undergoing aerobic exercise [40]. Although the precise role of angiogenesis in neuroplasticity and structural change remains to be fully elucidated, exercise-induced changes in regional blood flow may reflect localized molecular changes in enhanced neuronal synaptic connectivity including dendritic spine morphology, metabolism, as well as non-neuronal alterations in astrocyte structure and function.

While there was an association between CRF and the pallidum, there was no significant association between the pallidum with EF, memory, or visuospatial function. Thus, there was no mediating effect of the pallidum on CRF and its association with cognitive performance. Differences between the mediating effects observed with thalamus but not pallidum volume may indicate that pathophysiological changes of the pallidum contribute more to PD motor deficits than to cognitive deficits [41]. There was also no association between CRF and volumes of the

hippocampus and PFC. This is in contrast to findings in healthy aging studies involving principally aerobic exercise interventions where increases in CRF and both hippocampal volume and PFC were reported [42]. In this case, the cross-sectional nature of our study design may be one limitation to our findings, and support the need for larger, long-term aerobic exercise studies. Alternatively, differences between studies may be related to the level of cognitive function as over half of our participants were experiencing some decline in cognitive function and met diagnostic criteria for PD-MCI. However, our novel findings could potentially highlight focal specificity of CRF effects in PD patients but warrants replication in a larger PD sample.

The present study suffers from certain limitations which include: (i) cross-sectional aspect of the study which limits the ability to make any inferences of causality, (ii) we used an automated ROI approach to analyzing sMRI data which may not be sensitive to examining subtle effects compared to high-dimensional neuroimaging approaches, and (iii) participants were mostly Caucasian and well-educated potentially limiting the generalizability of our results, thereby limited the generalizability of our findings to other cultures, ethnicities, and socioeconomic groups.

In summary, we demonstrate that higher CRF is associated with better cognitive performance in multiple domains, including memory, visuospatial function, and EF in individuals with PD. While the underlying mechanisms by which CRF impacts the thalamus are unknown, individuals with PD who had higher CRF demonstrated better cognitive performance and larger GMVs of the thalamus and pallidum. The thalamus partially mediated the observed associations between CRF and both episodic memory and EF. These findings support the role of CRF and the critical importance of increasing physical aerobic exercise in promoting neurocognitive health in PD to maintain quality of life and daily functioning.

Funding sources

Don Roberto Gonzalez Family Foundation (GP); Parkinson's Foundation (GP); US Army Medical Research and Materiel Command Parkinson Research Program, Grant/Award Numbers: W81XWH18-0665 (GP), W81XWH18-00443 (MJ), W81XWH18-1-0666 (DPH), NIMH Mentored Research Scientist Career Development Award K01 MH099431 (SM); VA RR&D MERIT Award 1I01RX001691-01A1 (DMS).

Authors contributions

GMP, SCM, BJ, MWJ, DPH contributed to the conception and design of the study; AJP, BJ, SCM, GMP, MEG contributed to data acquisition; GMP, BJ, VDV, DMS, BEF, AJP were responsible for data analysis; AJP was responsible for statistical analyses; AJP, GMP, MWJ, SCM, DPH, DMS, JVH, BJ were responsible for writing and editing the manuscript; GMP, SCM, MWJ were responsible for acquisition of funding support.

Data availability statement

All data has been de-identified and stored as a RedCap data base at the USC-CTSI. Any requests for data should be directed to the principal investigator (Giselle M. Petzinger petzinger@med.usc.edu).

Declaration of competing interest

The authors have no financial disclosures and no conflicts of interest concerning the research related to this manuscript.

Acknowledgments

The authors would like to thank all the study participants for their contributions, as well as the contributions of friends of the PD Research Program at USC. Special thanks to lab members for their helpful

discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parkreldis.2021.03.019>.

References

- [1] I. Litvan, D. Aarsland, C.H. Adler, J.G. Goldman, J. Kulisevsky, B. Mollenhauer, M. C. Rodriguez-Oroz, A.I. Troster, D. Weintraub, MDS Task Force on mild cognitive impairment in Parkinson's disease: critical review of PD-MCI, *Mov. Disord.* 26 (10) (2011) 1814–1824.
- [2] D. Aarsland, K. Andersen, J.P. Larsen, A. Lolk, P. Kragh-Sorensen, Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study, *Arch. Neurol.* 60 (3) (2003) 387–392.
- [3] G.M. Halliday, J.B. Leverenz, J.S. Schneider, C.H. Adler, The neurobiological basis of cognitive impairment in Parkinson's disease, *Mov. Disord.* 29 (5) (2014) 634–650.
- [4] Y. Gao, K. Nie, B. Huang, M. Mei, M. Guo, S. Xie, Z. Huang, L. Wang, J. Zhao, Y. Zhang, L. Wang, Changes of brain structure in Parkinson's disease patients with mild cognitive impairment analyzed via VBM technology, *Neurosci. Lett.* 658 (2017) 121–132.
- [5] M.G. Li, J.F. He, X.Y. Liu, Z.F. Wang, X. Lou, L. Ma, Structural and functional thalamic changes in Parkinson's disease with mild cognitive impairment, *J. Magn. Reson. Imag.* 52 (4) (2020) 1207–1215.
- [6] F.X. Chen, D.Z. Kang, F.Y. Chen, Y. Liu, G. Wu, X. Li, L.H. Yu, Y.X. Lin, Z.Y. Lin, Gray matter atrophy associated with mild cognitive impairment in Parkinson's disease, *Neurosci. Lett.* 617 (2016) 160–165.
- [7] A.E. Lang, A.J. Espay, Disease modification in Parkinson's disease: current approaches, challenges, and future considerations, *Mov. Disord.* 33 (5) (2018) 660–677.
- [8] X. Shen, I.S. Wong-Yu, M.K. Mak, Effects of exercise on falls, balance, and gait ability in Parkinson's disease: a meta-analysis, *Neurorehabilitation Neural Repair* 30 (6) (2016) 512–527.
- [9] E.Y. Uc, K.C. Doerschug, V. Magnotta, J.D. Dawson, T.R. Thomsen, J.N. Kline, M. Rizzo, S.R. Newman, S. Mehta, T.J. Grabowski, J. Bruss, D.R. Blanchette, S. W. Anderson, M.W. Voss, A.F. Kramer, W.G. Darling, Phase I/II randomized trial of aerobic exercise in Parkinson disease in a community setting, *Neurology* 83 (5) (2014) 413–425.
- [10] C. Duchesne, F. Gheysen, A. Bore, G. Albouy, A. Nadeau, M.E. Robillard, F. Bobeuf, A.L. Lafontaine, O. Lungu, L. Bherer, J. Doyon, Influence of aerobic exercise training on the neural correlates of motor learning in Parkinson's disease individuals, *NeuroImag. Clin.* 12 (2016) 559–569.
- [11] K. Tanaka, A.C. Quadros Jr., R.F. Santos, F. Stella, L.T. Gobbi, S. Gobbi, Benefits of physical exercise on executive functions in older people with Parkinson's disease, *Brain Cognit.* 69 (2) (2009) 435–441.
- [12] A. Picelli, V. Varalta, C. Melotti, V. Zatezalo, C. Fonte, S. Amato, L. Salsuati, A. Santamato, P. Fiore, N. Smania, Effects of treadmill training on cognitive and motor features of patients with mild to moderate Parkinson's disease: a pilot, single-blind, randomized controlled trial, *Funct. Neurol.* 31 (1) (2016) 25–31.
- [13] F.J. David, J.A. Robichaud, S.E. Leurgans, C. Poon, W.M. Kohrt, J.G. Goldman, C. L. Comella, D.E. Vaillancourt, D.M. Corcos, Exercise improves cognition in Parkinson's disease: the PRET-PD randomized, clinical trial, *Mov. Disord.* 30 (12) (2015) 1657–1663.
- [14] S. Colcombe, A.F. Kramer, Fitness effects on the cognitive function of older adults: a meta-analytic study, *Psychol. Sci.* 14 (2) (2003) 125–130.
- [15] K. Wittfeld, C. Jochem, M. Dörr, U. Schminke, S. Glaser, M. Bahls, M.R.P. Markus, S.B. Felix, M.F. Leitzmann, R. Ewert, R. Bülow, H. Völzke, D. Janowitz, S. E. Baumeister, H.J. Grabe, Cardiorespiratory fitness and gray matter volume in the temporal, frontal, and cerebellar regions in the general population, *Mayo Clin. Proc.* 95 (1) (2020) 44–56.
- [16] K.I. Erickson, R.L. Leckie, A.M. Weinstein, Physical activity, fitness, and gray matter volume, *Neurobiol. Aging* 35 (Suppl 2) (2014) S20–S28.
- [17] J.M. Northey, N. Cherbuin, K.L. Pumpa, D.J. Smee, B. Rattray, Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis, *Br. J. Sports Med.* 52 (3) (2018) 154–160.
- [18] A.F. Kramer, S. Colcombe, Fitness effects on the cognitive function of older adults: a meta-analytic study-revisited, perspectives on psychological science 13 (2) (2018) 213–217.
- [19] T.D. Verstynen, B. Lynch, D.L. Miller, M.W. Voss, R.S. Prakash, L. Chaddock, C. Basak, A. Szabo, E.A. Olson, T.R. Wojcicki, J. Fanning, N.P. Gothe, E. McAuley, A.F. Kramer, K.I. Erickson, Caudate nucleus volume mediates the link between cardiorespiratory fitness and cognitive flexibility in older adults, *J. Aging Res.* 2012 (2012) 939285.
- [20] A.J. Hughes, S.E. Daniel, L. Kilford, A.J. Lees, Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases, *J. Neurol. Neurosurg. Psychiatry* 55 (3) (1992) 181–184.
- [21] C.G. Goetz, B.C. Tilley, S.R. Shaftman, G.T. Stebbins, S. Fahn, P. Martinez-Martin, W. Poewe, C. Sampaio, M.B. Stern, R. Dodel, B. Dubois, R. Holloway, J. Jankovic, J. Kulisevsky, A.E. Lang, A. Lees, S. Leurgans, P.A. LeWitt, D. Nyenhuis, C. W. Olanow, O. Rascol, A. Schrag, J.A. Teresi, J.J. van Hilten, N. LaPelle, Movement disorder society-sponsored revision of the unified Parkinson's disease rating Scale

- (MDS-UPDRS): scale presentation and clinimetric testing results, *Mov. Disord.* 23 (15) (2008) 2129–2170.
- [22] C.B. Ebbeling, A. Ward, E.M. Puleo, J. Widrick, J.M. Rippe, Development of a single-stage submaximal treadmill walking test, *Med. Sci. Sports Exerc.* 23 (8) (1991) 966–973.
- [23] M. Nakagaichi, K. Tanaka, Development of a 12-min treadmill walk test at a self-selected pace for the evaluation of cardiorespiratory fitness in adult men, *Appl. Human Sci.* 17 (6) (1998) 281–288.
- [24] V. Litvak, A. Jha, A. Eusebio, R. Oostenveld, T. Foltynie, P. Limousin, L. Zrinzo, M. I. Hariz, K. Friston, P. Brown, Resting oscillatory cortico-subthalamic connectivity in patients with Parkinson's disease, *Brain* 134 (Pt 2) (2011) 359–374.
- [25] L.M. O'Brien, D.A. Ziegler, C.K. Deutsch, J.A. Frazier, M.R. Herbert, J.J. Locascio, Statistical adjustments for brain size in volumetric neuroimaging studies: some practical implications in methods, *Psychiatr. Res.* 193 (2) (2011) 113–122.
- [26] M.A. Fletcher, K.A. Low, R. Boyd, B. Zimmerman, B.A. Gordon, C.H. Tan, N. Schneider-Garces, B.P. Sutton, G. Gratton, M. Fabiani, Comparing aging and fitness effects on brain anatomy, *Front. Hum. Neurosci.* 10 (2016) 286.
- [27] L. Chaddock, K.I. Erickson, R.S. Prakash, M. Vanpatter, M.W. Voss, M.B. Pontifex, L.B. Raine, C.H. Hillman, A.F. Kramer, Basal ganglia volume is associated with aerobic fitness in preadolescent children, *Dev. Neurosci.* 32 (3) (2010) 249–256.
- [28] M.C. Wen, A. Ng, R.J. Chander, W.L. Au, L.C. Tan, N. Kandiah, Longitudinal brain volumetric changes and their predictive effects on cognition among cognitively asymptomatic patients with Parkinson's disease, *Park. Relat. Disord.* 21 (5) (2015) 483–488.
- [29] M.M. Halassa, S. Kastner, Thalamic functions in distributed cognitive control, *Nat. Neurosci.* 20 (12) (2017) 1669–1679.
- [30] G.M. Halliday, Thalamic changes in Parkinson's disease, *Park. Relat. Disord.* 15 (Suppl 3) (2009) S152–S155.
- [31] Y. Ding, J. Li, Q. Lai, S. Azam, J.A. Rafols, F.G. Diaz, Functional improvement after motor training is correlated with synaptic plasticity in rat thalamus, *Neurol. Res.* 24 (8) (2002) 829–836.
- [32] Z. Wang, Y. Guo, K.G. Myers, R. Heintz, Y.H. Peng, J.M. Maarek, D. P. Holschneider, Exercise alters resting-state functional connectivity of motor circuits in parkinsonian rats, *Neurobiol. Aging* 36 (1) (2015) 536–544.
- [33] D.P. Holschneider, J. Yang, Y. Guo, J.M. Maarek, Reorganization of functional brain maps after exercise training: importance of cerebellar-thalamic-cortical pathway, *Brain Res.* 1184 (2007) 96–107.
- [34] M.J. Dolleman-van der Weel, A.L. Griffin, H.T. Ito, M.L. Shapiro, M.P. Witter, R. P. Vertes, T.A. Allen, The nucleus reuniens of the thalamus sits at the nexus of a hippocampus and medial prefrontal cortex circuit enabling memory and behavior, *Learn. Mem.* 26 (7) (2019) 191–205.
- [35] T. Cholvin, V. Hok, L. Giorgi, F.A. Chaillan, B. Poucet, Ventral midline thalamus is necessary for hippocampal place field stability and cell firing modulation, *J. Neurosci.* 38 (1) (2018) 158–172.
- [36] C. Varela, Thalamic neuromodulation and its implications for executive networks, *Front. Neural Circ.* 8 (2014) 69.
- [37] T.D. Aumann, J.A. Rawson, D.I. Finkelstein, M.K. Horne, Projections from the lateral and interposed cerebellar nuclei to the thalamus of the rat: a light and electron microscopic study using single and double anterograde labelling, *J. Comp. Neurol.* 349 (2) (1994) 165–181.
- [38] A.J. Haroian, L.C. Massopust, P.A. Young, Cerebellothalamic projections in the rat: an autoradiographic and degeneration study, *J. Comp. Neurol.* 197 (2) (1981) 217–236.
- [39] Z. Wang, K.G. Myers, Y. Guo, M.A. Ocampo, R.D. Pang, M.W. Jakowec, D. P. Holschneider, Functional reorganization of motor and limbic circuits after exercise training in a rat model of bilateral parkinsonism, *PLoS One* 8 (11) (2013), e80058.
- [40] J.E. Black, K.R. Isaacs, B.J. Anderson, A.A. Alcantara, W.T. Greenough, Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats, *Proc. Natl. Acad. Sci. U. S. A.* 87 (14) (1990) 5568–5572.
- [41] F. Magrinelli, A. Picelli, P. Tocco, A. Federico, L. Roncari, N. Smania, G. Zanette, S. Tamburin, Pathophysiology of motor dysfunction in Parkinson's disease as the rationale for drug treatment and rehabilitation, *Parkinsons Dis* 2016 (2016) 9832839.
- [42] K.I. Erickson, M.W. Voss, R.S. Prakash, C. Basak, A. Szabo, L. Chaddock, J.S. Kim, S. Heo, H. Alves, S.M. White, T.R. Wojcicki, E. Mailey, V.J. Vieira, S.A. Martin, B. D. Pence, J.A. Woods, E. McAuley, A.F. Kramer, Exercise training increases size of hippocampus and improves memory, *Proc. Natl. Acad. Sci. U. S. A.* 108 (7) (2011) 3017–3022.

Research Report

The Effects of Cardiorespiratory and Motor Skill Fitness on Intrinsic Functional Connectivity of Neural Networks in Individuals with Parkinson's Disease

Behnaz Jarrahi^{a,1}, Sarah C. McEwen^{b,c,1}, Daniel P. Holschneider^{f,h}, Dawn M. Schiehser^{d,e}, Andrew J. Petkus^f, Megan E. Gomezⁱ, Jack D. Van Horn^g, Vincent Filoteo^{d,e}, Michael W. Jakowec^f and Giselle M. Petzinger^{f,*}

^a*Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University School of Medicine, Palo Alto, California, USA*

^b*Pacific Brain Health Center, Pacific Neuroscience Institute, Santa Monica, CA, USA*

^c*Department of Translational Neurosciences and Neurotherapeutics, Providence Saint John's Cancer Institute, Santa Monica, CA, USA*

^d*Research & Psychology Services, VA San Diego Healthcare System (VASDHS), San Diego, CA, USA*

^e*Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, CA, USA*

^f*Department of Neurology, University of Southern California, Los Angeles, CA, USA*

^g*Department of Psychology and School of Data Science, University of Virginia, Charlottesville, VA, USA*

^h*Department of Psychiatry and the Behavioral Sciences, University of Southern California, Los Angeles, CA, USA*

ⁱ*Department of Psychology, Tibor Rubin Veterans Administration Medical Center, Long Beach, CA, USA*

Accepted 5 January 2021

Pre-press 27 January 2021

Published 19 October 2021

Abstract.

Background: Studies in aging older adults have shown the positive association between cognition and exercise related fitness, particularly cardiorespiratory fitness. These reports have also demonstrated the association of high cardiorespiratory fitness, as well as other types of fitness, on the reversal of age-related decline in neural network connectivity, highlighting the potential role of fitness on age- and disease-related brain changes. While the clinical benefits of exercise are well-documented in Parkinson's disease (PD), the extent to which cardiorespiratory fitness (assessed by estimated $\text{VO}_{2\text{max}}$ testing) or motor skill fitness (assessed by the Physical Performance Test (PPT)) affects neural network connectivity in PD remains to be investigated. The purpose of this study was to explore the hypothesis that higher fitness level is associated with an increase in the intrinsic network connectivity of cognitive networks commonly affected in PD.

Methods: In this cross-sectional resting state fMRI, we used a multivariate statistical approach based on high-dimensional independent component analysis (ICA) to investigate the association between two independent fitness metrics (estimated $\text{VO}_{2\text{max}}$ and PPT) and resting state network connectivity.

Results: We found that increased estimated $\text{VO}_{2\text{max}}$ was associated with increased *within* network connectivity in cognitive networks known to be impaired in PD, including those sub-serving memory and executive function. There was a similar trend for high levels of PPT to be associated with increased *within* network connectivity in distinct resting state networks. The *between* functional network connectivity analysis revealed that cardiorespiratory fitness was associated with increased functional connectivity between somatosensory motor network and several cognitive networks sub-serving memory, attention, and executive function.

¹These authors contributed equally to this study.

*Correspondence to: Giselle M. Petzinger, MD., Department of Neurology, University of Southern California, 1333 San Pablo St.,

MCA-243, Los Angeles, CA, 90033, USA. Tel.: +1 323 442 3367; E-mail: petzinge@med.usc.edu.

Conclusion: This study provides important empirical data supporting the potential association between two forms of fitness and multiple resting state networks impacting PD cognition. Linking fitness to circuit specific modulation of resting state network connectivity will help establish a neural basis for the positive effects of fitness and specific exercise modalities and provide a foundation to identify underlying mechanisms to promote repair.

Keywords: Physical activity, exercise, motor skill, functional network connectivity, resting state functional MRI, imaging

LIST OF ABBREVIATIONS

BA	Brodmann's area
BG	basal ganglia
BOLD	blood oxygenation level-dependent
CAN	cognitive/attention network
CEN	central executive network
CRF	cardiorespiratory fitness
DA	dopamine
DAN	dorsal attention network
DLPFC	dorsolateral prefrontal cortex
DMN	default mode network
EPI	echo-planar imaging
FD	frame-wise displacement of head movement
fMRI	functional magnetic resonance imaging
FDR	false discovery rate
FNC	functional network connectivity
ICA	independent component analysis
IC	independent component
Iq	quality index
MSF	motor skill fitness
MCI	mild cognitive impairment
MNI	Montreal Neurological Institute
PD	Parkinson's disease
PPT	physical performance test
ROI	region of interest
rs-fMRI	resting state functional magnetic resonance imaging
SRN	self-referential network
RSN	resting state network
SCN	subcortical network
SMN	sensorimotor network
SN	salience network
VAN	ventral attention network
VO _{2max}	maximal oxygen uptake

INTRODUCTION

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder that is the result of dopaminergic cell death and dopamine (DA) depletion. Loss of DA in turn leads to decreased synaptic connectivity and dysfunction in a number of cortical

and subcortical networks resulting in motor impairment such as bradykinesia, rigidity, and gait and balance instability, as well as cognitive impairment. Cognitive impairment may include deficits in executive function, attention, visual spatial, and memory domains which often progresses to dementia and poor quality of life. Although the etiology for cognitive change in PD is not fully elucidated, studies in PD have used resting state functional magnetic resonance imaging (rs-fMRI) and either seed-based or independent component analysis (ICA) to explore functional connectivity. These approaches have generally demonstrated loss of functional connectivity *within* the cognitive networks, including the default mode network (DMN), central executive network (CEN), dorsal attention network (DAN), and the salience network (SN) [1, 2]. In addition, changes have been reported *between* these well-established cognitive networks and also the sensorimotor network (SMN) and the subcortical network (SCN) [3]. While DA therapy has generally been shown to be helpful for motor deficits in PD, currently there is no effective treatment for cognitive impairment. Identifying interventions that promote neuroplasticity and repair of cognitive networks may lead to new therapeutic strategies for improving cognitive function and slowing disease progression.

In the last decade, studies in aging older adults have supported the potential role that exercise may play in promoting neuroplasticity and improving cognitive performance [4]. These studies highlight the relationship between exercise related cardiorespiratory fitness (CRF) and attenuation of age-related brain atrophy as well as improved functional network connectivity in brain regions sub-serving cognitive performance [5, 6]. In particular, CRF has been shown to improve functional network connectivity of cortical networks associated with age-related impairment including the DMN and DAN. Taken together, studies in aging have supported the idea that CRF may be associated with improved connectivity of brain regions that are particularly vulnerable to the adverse effects of aging and/or areas at risk for neurological diseases [5, 7, 8]. The gold standard metric for measuring CRF is VO_{2max} and is defined as

the capacity of the heart and lungs to deliver oxygenated blood to working muscles and brain. Since individuals with PD may experience fatigue during a traditional maximal exercise fitness assessment, a sub-maximal exercise test can be used to predict an estimated VO_{2max} [9]. In addition to CRF, exercise programs, particularly those incorporating aspects of skill including gait and balance practice may lead to improved motor skill fitness (MSF). While less is known about the role of motor skill fitness and its impact on neuroplasticity, a few studies in aging older adults have also supported the association of high levels of motor skill fitness with increased cognitive network activation and neural efficiency in brain regions also affected in aging [7, 8]. MSF is defined as physical activity training that incorporates aspects of complex motor performance including balance, agility, coordination, power, and speed, and higher levels of cognitive processing [10]. The Physical Performance Test (PPT) is a standardized test used to assess motor skill fitness that incorporates aspects of complex, fine and gross motor function, including coordination, balance, agility, and speed [10]. Importantly, fitness level (both CRF and MSF) may represent a means to determine exercise benefits that occur as a consequence of a variety of exercise types that engage either high aerobic practice such as running and swimming, and/or motor skill practice such as Tai Chi and yoga.

While the benefits of cardiorespiratory fitness and motor skill fitness have been shown in age-related changes in functional connectivity within the brain, there remain significant gaps in knowledge in understanding the role that CRF, and other types of fitness including MSF, may play in enhancing cognitive and motor network connectivity known to be dysfunctional in PD. Pre-clinical exercise studies in rodent models of PD have supported that different forms of exercise (aerobic versus skill-based) can lead to changes in synaptic and network connectivity as displayed through increased cortical and subcortical synaptogenesis, hippocampal neurogenesis, and changes in regional cerebral blood flow [11–15]. In addition, a few studies that have examined executive function, a domain commonly affected in PD, support the potential benefits of aerobic exercise and related improvement in cardiorespiratory fitness on cognitive circuits impaired in PD [16, 17].

In this study, we sought to explore the hypothesis that higher fitness level is associated with an increase in the intrinsic network connectivity of cognitive and motor networks commonly affected

in PD. Using rs-fMRI and a cross-sectional study design, we examined the association between cardiorespiratory fitness (estimated by the VO_{2max} test) and motor skill fitness (assessed with the PPT) on both *within* network connectivity, measured by network's spatial map intensity, and *between* network connectivity, measured by functional network connectivity (FNC) [18] of intrinsic resting state networks (RSNs). Understanding the effects of fitness on intrinsic brain network connectivity is crucial to better elucidate effective interventional strategies important for brain repair in PD.

For this study we recruited PD individuals who were physically active and routinely exercising based on self-report. We used blood oxygenation level-dependent (BOLD) rs-fMRI and an ICA-based approach to extract a select number of cognitive and motor RSNs implicated in PD, [1–3, 19]. We used high-dimensional spatial ICA to obtain more refined neural networks, and applied a multivariate, data-driven method that takes into account the relationships between all voxels across the brain with appropriate dimension reduction of response variables to decrease risk of spurious findings [20], and also to provide better sensitivity to detect subtle differences between PD individuals [21, 22].

MATERIALS AND METHODS

PD individuals and clinical assessments

The PD individuals for this cross-sectional study included 24 physically active, non-demented and ambulatory individuals with PD (15 males/9 females, mean age 65.5 years, range 51 – 88 years) with Hoehn and Yahr equal to II and years of diagnosis 4.9 ± 3.3 . Current physical activity levels were determined through self-report using the Global Physical Activity Questionnaire (GPAQ) [23]. Subjects provided written informed consent to participate in the study, which was approved by the Institutional Review Boards at the University of Southern California, and the University of California at Los Angeles. All PD individuals met the UK Parkinson's Disease Brain Bank criteria for the diagnosis of idiopathic PD [24]. Inclusion and exclusion criteria were determined through chart review, participant interview, and physical and neurological screening performed by a board-certified neurologist with movement disorder expertise (GP). Inclusion criteria for participation included: (i) willing and able to provide informed

consent; (ii) confirmed diagnosis of idiopathic PD based on the UK Brain Bank criteria; (iii) age 30 to 85, (iv) medically eligible for MRI imaging; (v) Hoehn and Yahr scale score of less than III [25]; (vi) stable PD medications for 3 months; and (vii) English as their primary language. Exclusion criteria for participation included: (i) electrically, magnetically, or mechanically activated implant (such as cardiac pacemakers or intracerebral respiratory clip); (ii) metal in any part of the body including metal injury to the eye; (iii) dementia based on performance or the inability to perform instrumental activities of daily living; (iv) other neurological diseases such as severe head trauma or stroke; (v) symptomatic orthostatic hypotension at the screening visit; and (vi) taking anticholinesterase inhibitors or anticholinergic medication. All subject assessments for the study were performed while on dopaminergic replacement medication (e.g., levodopa and DA agonists) and during the “on-state” period [26].

Fitness measurements

Cardiorespiratory fitness was determined using the Ebbeling Single Stage Treadmill Walking Test, which is a validated submaximal fitness test that can be used to estimate VO_{2max} in adult subjects [27]. Briefly, subjects first underwent a 4-minute warm-up, walking at a brisk, but comfortable pace starting at 2.0 mph and increasing by 0.2 mph increments every 30 secs, and not to exceed 4.5 mph, and at 0% grade, eliciting a heart rate within 50 to 70% of age-predicted maximum. This was followed by a single testing stage of treadmill walking for 4 minutes at 5% grade. The steady state heart rate was determined from the average of the final 30 seconds at 5% grade and the tester was careful to avoid extremes of their heart rate range [28]. The equation to estimate VO_{2max} (ml $kg^{-1} min^{-1}$) from a single stage 4-minute, 5% grade submaximal treadmill test is as follows: estimated $VO_{2max} = 15.1 + 21.8 * SPEED$ (mph) $- 0.327 * HEART RATE$ (bpm) $- 0.263 * SPEED * AGE$ (yr) $+ 0.00504 * HEART RATE * AGE + 5.98 * SEX$ (0 = female; 1 = male) [27]. The estimated VO_{2max} values were adjusted for the effects of age and sex in order to avoid multicollinearity issues. Estimated VO_{2max} was expressed as oxygen capacity per kilogram of body weight over time (mL/kg/min) [29], and is a value at which oxygen uptake volume (VO_2) plateaus or increases minimally despite increased workload on the graded exercise test.

The Physical Performance Test (PPT) was used as an objective evaluation of an individual's level of motor skill fitness [10]. PPT is a standardized, functional test with nine items that include upper limb fine and coarse motor functions, balance, coordination, and endurance, that requires timed performance in most items [10]. The PPT also simulates activities of daily living of various degrees of difficulty (writing, dressing, eating, stair climbing, walking, turning, bending, and lifting), and has been found to correlate well with the degree of geriatric disability, loss of independence, nursing home placement, and early mortality [30, 31]. The total score for the nine-item PPT ranges from 0 to 36 with lower scores indicating poorer physical performance. The PPT scores were assessed at a single point in time and adjusted for the effects of age and sex in order to avoid multicollinearity issues.

MRI acquisition

Structural MRI and rs-fMRI data were acquired on a Siemens 3T Trio ($N=11$) and Prisma systems ($N=13$) (Siemens, Erlangen, Germany) using a 12-channel head coil at the UCLA Staglin IMHRO Center for Cognitive Neuroscience. The functional T2*-weighted images were obtained with echo-planar imaging (EPI) sequence (repetition time = 2000 ms, echo time = 30 ms, field of view = 220 mm, image matrix = 64×64 , flip angle = 77° , slice thickness = 4 mm, interslice gap = 0.5 mm, 30 axial slices acquired in sequential ascending order). Head motion was minimized using padding. PD individuals were asked to lay still, not to think about anything in particular, and passively look at the fixation cross during the 5.13 min (154 volumes) rs-fMRI scanning. The first four images were automatically discarded by the scanner to allow for the establishment of steady-state magnetization. High-resolution T1-weighted magnetization-prepared rapid-gradient echo (MP-RAGE) structural images were also acquired for functional image registration and normalization (repetition time = 2300 ms, echo time = 2.91 ms, inversion time = 0.9 ms, flip angle = 9° , number of excitations = 1, resolution = 256×256 , slice thickness = 1.2 mm, 176 slices).

Image quality assessment and preprocessing

Upon completion of a scan, the imaging data were anonymized, and transferred to an offline workstation. Image data quality was assessed using the MRI Quality Control (MRIQC) software package [32].

Several of the quantitative image quality metrics from the quality assessment protocol of Human Connectomes Project [33] were calculated to ensure quality and consistency of images obtained in this aged population. They included metrics for (i) artifacts (AFNI's outlier ratio and quality index, head motion-related framewise displacement [FD], and ghost to signal ratio), (ii) temporal quality (DVARs, global correlation, and temporal signal to noise ratio and, (iii) structural quality (Shannon's entropy focus criterion, foreground-background energy ratio, and static signal to noise ratio). Upon passing the quality control (see supplementary material), images were preprocessed using the Statistical Parametric Mapping (SPM) software package (Wellcome Department of Cognitive Neurology, London, UK, version 12b) running under the MATLAB environment (Mathworks Inc., Natick, MA, USA, release 16a). In the first step, EPI images were realigned to correct head motions using rigid body transformations. Subsequently, images were corrected for acquisition time delay between different slices by temporally realigning the time series of each voxel to acquisition of the first slice. We chose to perform spatial realignment first to minimize the effect of inter-slice movement [34]. Spatial realignment parameters were computed by estimating the translational displacements along X, Y, and Z axes as well as the rotational displacements of pitch, yaw, and roll. Subjects' spatial realignment parameters did not exceed 2 mm (translational) or 1.5° (rotational) in relation to the reference. However, considering the potential influence of even small degrees of head motions on the rs-fMRI connectivity analysis [35], we calculated FD, an index of volume-to-volume changes in head position [36] and used it as a nuisance covariate in our analysis. Next, EPI and structural scans were co-registered and normalized to the standard template in Montreal Neurological Institute (MNI) space using the linear (affine transformation) followed by nonlinear deformations defined by linear combinations of 3D discrete cosine transform basis functions implemented in SPM, resampled to $2 \times 2 \times 2 \text{ mm}^3$ isotropic voxels, and spatially smoothed using a Gaussian filter with a full-width half maximum smoothness [FWHM] of 4 mm [37]. A relatively small smoothing kernel size was used to allow detection of RSN especially those containing refined subcortical structures. To further improve the accuracy and test-retest reliability of ICA, data intensity was normalized by dividing each voxel time course by its average intensity and converting data to percent signal change units [20].

High-dimensional independent component analysis (ICA)

Following a validated ICA analysis framework for rs-fMRI [1], we applied a relatively high model order ICA to the preprocessed data using the Group ICA of fMRI Toolbox (GIFT)-toolbox (Medical Image Analysis Lab, University of New Mexico; version 4.0b). Specifically, we chose 75-IC model order because recent ICA rs-fMRI studies have shown that such high-order ICA models yield more refined and particularly robust decomposition of components [38, 39]. A two-step data reduction approach using principal component analysis was carried out prior to performing the group ICA [40]. First, subject-specific data reduction principal component analysis with a standard economy-size decomposition retained 100 principal components. Next, subject reduced data were concatenated across time, and group data reduction retained 75 principal components using the expectation-maximization algorithm. The choice of retaining more principal components at the first reduction step was based on a previous study that showed more principal components at the first reduction step stabilized subsequent back-reconstruction and could better account for greater subject-specific variance [41]. Spatial ICA was subsequently performed using the infomax algorithm [42]. To ensure stability of estimation, ICA algorithm was repeated 100 times in ICASSO and the agglomerative hierarchical clustering with average-linkage criterion was used for calculating the cluster quality index (Iq) of the estimated ICs [43]. In the last step, subject-specific spatial maps and their corresponding time courses were back-reconstructed using the dual regression analysis technique (GICA-3 algorithm in GIFT), and scaled to Z-scores [41].

Resting state network (RSN) identification and feature extraction

We used a methodical approach to identify RSNs from noise or physiological artifacts. First, Iq measures from 100 ICASSO runs were examined to validate the reliability and stability of IC decomposition. ICs with Iq value below 0.8 were excluded from further analysis as lower Iq indicates that the likelihood that the IC is randomly produced is higher [44]. In the second identification step, the spatial correlations of IC spatial maps with *a priori* probabilistic maps of cerebrospinal fluid, white matter, and gray matter in MNI space (using templates pro-

vided in SPM12b) were calculated. Components that showed low correlations with gray matter, or high correlations with cerebrospinal fluid or white matter were excluded from analysis. Artifactual components were further identified based on (i) their motion-related spatial maps (e.g., rings near the borders of the brain), (ii) special confinement of their peak activation to cerebrospinal fluid, proximity to known cerebral blood vessels, or to regions susceptible to physiological pulsations with mixed high frequency power [45]. Identification of remaining ICs was carried out by regression-based spatial sorting to reference standard network templates from Allen and colleagues [20] as well as knowledge from established brain networks described in previous studies [20, 46–50]. Components averaged across all subjects were calculated with a one-sample *t*-test in SPM12b and thresholded at family-wise error rate (FWE-corrected $p < 0.05$). Component anatomical extents in MNI space, and corresponding Brodmann's areas (BA) and peak activations were determined using the cytoarchitectonic probability maps as implemented in the SPM Anatomy Toolbox [51, 52].

Following the methodological approach explained above, we identified a subset of 18 surviving components as RSNs compatible with those obtained in previous studies that used high-dimensional ICA [20, 38, 39, 46, 48, 50, 53, 54]. These 18 RSNs fell into one of the following four standard rs-fMRI network categories:

- Sensorimotor Network (SMN) consisting of IC 19 (bilateral ventral somatosensory), IC 46 (right dorsal somatosensory), IC 48 (left dorsal somatosensory), IC 54 (bilateral motor), and IC 62 (supramarginal gyrus).
- Subcortical Network (SCN) consisting of IC 24 (hippocampus) and IC 35 (basal ganglia; BG, and also included thalamus and amygdala).
- Default-Mode Network (DMN) consisting of IC 49 (posterior DMN), and IC 63 (containing both anterior and posterior DMN).
- Cognitive and Attention Network (CAN) consisting of IC 2 (self-referential network (SRN) comprising ventromedial prefrontal cortex), IC 17 (bilateral DLPFC part of the Central Executive Network (CEN)), IC 40 (right hemisphere of CEN), IC 45 (left hemisphere of CEN), IC 47 (dorsal attention network (DAN)), IC 32 (ventral attention network (VAN)), IC 50 (salience network (SN) including bilateral insula), IC 53 (second SN comprising the frontoinsula

regions), and IC 75 (third SN with dorsal anterior cingulate and insular cortices).

Spatial maps of these networks are shown in Fig. 1. Detailed information of component spatial maps including the rs-fMRI defined networks, anatomical regions, *t*-value of maxima, and MNI coordinates are listed in Table 1.

Multivariate statistical analysis

We used multivariate analysis of covariance (MANCOVA), which is a multivariate analytical method comparable to a standard ANOVA F-test, followed by a *post hoc* univariate analysis. The MANCOVAN toolbox in GIFT software package was used to discover which spatial map voxels (related to the connectivity *within* a network) and FNC correlations (related to the connectivity *between* networks) were uniquely associated with each of our two variables of interest (i.e., MSF measured by PPT and CRF measured by estimated VO_{2max}), while statistically controlling for the effects of covariates including age, sex and years of education. Head movement estimates (i.e., FD) were included as covariates of no interest (nuisance predictors). To minimize the potential disproportionate influence of covariates on the MANCOVA model fit, log transformations were applied to continuous covariates as recommended in [20]. To optimize for the large dimensions of the voxelwise ICA outcome measures (spatial maps and FNC correlations) and reduce the number of statistical tests performed, backward multivariate stepwise regressions (implemented as mStepwise in GIFT MANCOVAN toolbox) were performed for each response matrix separately. For more details on the multivariate analysis please refer to [20]. This approach first utilizes MANCOVA to determine contributions of each fitness parameter (CRF and MSF levels) on the ICs. Then, univariate tests corrected for multiple comparisons are carried out on a reduced design matrix, which reduces the number of statistical tests performed, to highlight the direction and strength of the relationship between fitness levels and ICA measures (spatial map intensity and FNC correlations).

Spatial map intensity statistical analysis

Following the multivariate analysis, univariate tests were performed on the reduced model applied to the original (not-dimension reduced) feature matrix to identify the effects of predictors (i.e. PPT and estimated VO_{2max}) on RSN spatial map intensities as

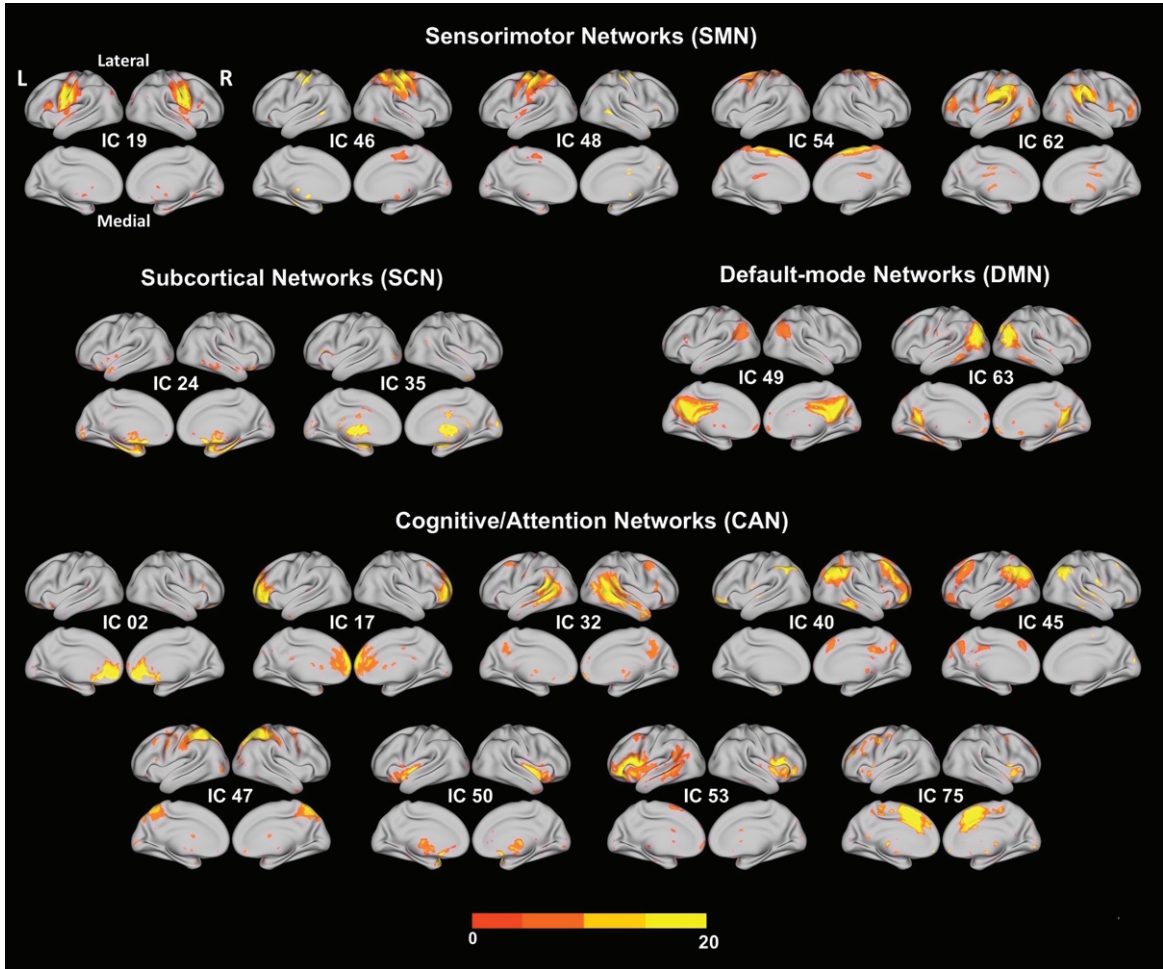


Fig. 1. Spatial maps of eighteen RSNs that were entered into the MANCOVA analysis. RSNs are grouped by their anatomical and functional properties into the sensorimotor networks (SMN; ICs 19, 46, 48, 54, and 62), the subcortical networks (SCN; ICs 24, and 35), the default-mode networks (DMN; ICs 49, and 63), and the cognitive and attention networks (CAN; ICs 2, 17, 32, 40, 45, 47, 50, 53, and 75). Spatial maps are plotted as t -statistics, thresholded at FWE-corrected p -value of 0.05 and displayed in neurological convention (right is right). The scale bar, red-to-yellow, represents t -values of the strength of each voxel's connectivity to the overall component time series. See Table 1 for MNI coordinates of maxima and corresponding t -values for each RSN. L, left; R, right.

detailed in [20]. In brief, partial correlation coefficients for each predictor were calculated from linear regressions with the feature matrix as a dependent variable while covarying out the effects of the other predictors (the remaining regressors in the model). Results from the univariate tests for RSN spatial map intensities were corrected for multiple comparisons using the false discovery rate (FDR) [55].

Functional network connectivity statistical analysis

In addition to the network spatial features, we also considered network temporal features, namely FNC.

FNC serves as a correlational value that measures the functional connectivity (i.e., temporal dependencies) among RSN time courses [20]. FNC was computed as the Pearson's correlation coefficient between RSN time course pairs as detailed in [18]. In brief, subject-specific time courses were detrended and de-spiked, and a fifth-order Butterworth band-pass filter was used to filter time courses with cut-off frequencies at [0.01 – 0.15] Hz. FNC correlation values were normalized using Fisher r -to- z transformation, i.e., $z = \text{atanh}(r)$, where r is the pairwise correlation value between RSN time courses. Results from the univariate tests for FNC were corrected with a strict control of false positives using the Bonferroni correction threshold of 0.003 (0.05/18).

Table 1

Peak coordinates of 18 selected RSNs. The MNI (Montreal Neurological Institute) coordinates show the coordinates of the peak voxels with negative x coordinates refer to left hemisphere activations. Brodmann's area as indicated by the cytoarchitectonic maximum probability map using the SPM-Anatomy toolbox developed by Eickhoff, et al. (2005) [49]. The quality index (Iq) associated with each component is listed in parentheses next to the component number

IC	Brain region	Cluster size (voxels)	t _{max}	MNI coordinates			Cytoarchitectonic Brodmann area (Probability, if available)
				x	y	z	
Sensorimotor Networks (SMN)							
IC 19 (0.98) <i>Ventral Somatosensory Network</i>	Left Postcentral Gyrus	759	13.87	−60	−14	34	Area 1 (40%) Area 3b (40%)
	Left Precentral Gyrus	same cluster	11.84	−54	−6	34	Area 4p (50%) Area 6 (30%)
	Right Postcentral Gyrus	649	11.86	50	−12	38	Area 3b (60%) Area 4p (50%)
	Right Precentral Gyrus	same cluster	11.14	54	−6	38	Area 6 (40%) Area 4p (40%)
IC 46 (0.98) <i>Right Dorsal Somatosensory Network</i>	Right Postcentral Gyrus	2493	14.41	42	−20	50	Area 3b (90%) Area 4a (40%)
	Right Precentral Gyrus	same cluster	13.35	40	−24	62	Area 1 (40%) Area 6 (40%)
IC 48 (0.96) <i>Left Dorsal Somatosensory Network</i>	Left Postcentral Gyrus	2225	15.29	−36	−40	56	Area 2 (50%) Area SPL (7PC) (40%)
	Left Precentral Gyrus	same cluster	14.40	−32	−26	56	Area 4p (40%) Area 4a (40%)
IC 54 (0.98) <i>Motor Cortex</i>	Left SMA	4412	17.93	0	−12	62	Area 6 (90%) Area 4a (10%)
	Right SMA	same cluster	13.93	8	2	72	Area 6 (80%)
	Left Precentral Gyrus	same cluster	12.86	−36	0	58	
IC 62 (0.93) <i>Supramarginal Gyrus</i>	Left Supramarginal Gyrus	1145	13.28	−58	−28	34	IPC (PFt) (60%) IPC (PF) (40%)
	Right Supramarginal Gyrus	556	12.85	58	−34	32	IPC (PF) (80%) IPC (PFcm) (40%)
Subcortical Networks (SCN)							
IC 24 (0.98) <i>Hippocampal</i>	Left ParaHippocampal Gyrus	361	8.73	−18	−10	−22	Hipp (SUB) (60%) Hipp (HATA) (40%)
	Right Hippocampus	160	10.40	20	−10	−18	Hipp (CA) (40%) Hipp (SUB) (50%)
	Right ParaHippocampal Gyrus	same cluster	7.74	20	−8	−26	Amyg (SF) (50%) Hipp (CA) (50%)
IC 35 (0.98) <i>Basal Ganglia (BG)</i>	Right Putamen	2107	12.90	28	6	−2	Hipp (EC) (60%) Hipp (SUB) (40%) Hipp (CA) (30%)
	Left Amygdala	same cluster	12.46	−22	0	−16	Amyg (SF) (70%)
	Left Putamen	same cluster	12.34	−24	6	0	
	Left Thalamus	same cluster	12.00	−6	−20	6	
	Right Thalamus	same cluster	11.57	8	−20	4	
	Right Amygdala	same cluster	11.56	26	2	−16	Amyg (SF) (30%)
Default-Mode Networks (DMN)							
IC 49 (0.97) <i>Posterior Default Mode Network (DMN)</i>	Right Precuneus	3530	18.94	4	−52	24	
	Left Posterior Cingulate Cortex	same cluster	18.83	−2	−44	32	
	Left Angular Gyrus	366	11.24	−46	−62	26	IPC (PGp) (30%)
	Left Inferior Parietal Lobule	same cluster	8.44	−30	−56	40	hIP3 (50%)
	Right Angular Gyrus	346	12.53	44	−64	32	IPC (PGp) (70%)
IC 63 (0.96) <i>DMN</i>	Right Precuneus	541	14.00	2	−58	22	
	Left Precuneus	same cluster	12.31	−4	−54	14	
	Left Middle Occipital Gyrus	491	10.64	−42	−72	24	IPC (PGp) (60%)
	Left Middle Temporal Gyrus	same cluster	10.49	−44	−68	22	IPC (PGp) (50%)
	Right Middle Temporal Gyrus	396	10.04	46	−62	22	IPC (PGp) (50%)

(Continued)

Table 1
(Continued)

IC	Brain region	Cluster size (voxels)	t _{max}	MNI coordinates			Cytoarchitectonic Brodmann area (Probability, if available)
				x	y	z	
Cognitive and Attention Network (CAN)							
IC 2 (0.98)	Left Anterior Cingulate Cortex	1310	13.59	−4	44	−2	
Self-Referential Network (SRN)	Left Mid Orbital Gyrus	same cluster	13.39	−2	48	−6	
	Right Anterior Cingulate Cortex	same cluster	12.09	8	34	10	
IC 17 (0.98)	Right Anterior Cingulate Cortex	4850	13.39	6	46	8	
Central	Left Superior Medial Gyrus	same cluster	12.94	−2	54	10	
Executive Network (CEN)	Right Superior Frontal Gyrus	same cluster	12.06	24	58	14	
IC 32 (0.98)	Right Middle Temporal Gyrus	1933	14.72	52	−20	−12	
Ventral Attention Network (VAN)	Right Angular Gyrus	same cluster	12.55	56	−56	24	IPC (PGa) (70%)
	Left Middle Temporal Gyrus	987	13.48	−58	−38	−2	
	Left SupraMarginal Gyrus	same cluster	10.17	−52	−52	26	IPC (PGa) (40%) IPC (PF) (30%) SPL (7A) (40%) SPL (7P) (10%)
IC 40 (0.98)	Right Inferior Parietal Lobule	1395	12.44	46	−54	40	IPC (PGa) (40%) hIP1 (10%)
Right CEN	Right Inferior Frontal Gyrus (p. Triangularis)	277	11.70	44	28	28	
	Right Middle Frontal Gyrus	same cluster	10.15	46	36	22	
	Right Middle Frontal Gyrus	271	11.62	42	50	0	
	Right Inferior Frontal Gyrus (p. Orbitalis)	same cluster	10.48	38	40	−18	
	Right Superior Frontal Gyrus	same cluster	9.36	24	54	2	
IC 45 (0.97)	Left Inferior Parietal Lobule	1645	14.29	−32	−72	40	IPC (PGp) (20%) hIP1 (30%)
Left CEN	Left Angular Gyrus	same cluster	12.18	−36	−60	42	
	Left Middle Frontal Gyrus	188	11.30	−44	12	46	
	Right Inferior Parietal Lobule	98	10.16	46	−56	46	IPC (PGa) (40%) IPC (PFt) (40%) IPC (PFm) (30%) hIP3 (30%) SPL (7A) (20%)
IC 47 (0.97)	Right Inferior Parietal Lobule	5056	14.47	50	−36	50	
Dorsal Attention Network (DAN)	Right Superior Parietal Lobule	same cluster	14.41	28	−60	50	hIP3 (30%) SPL (7A) (20%) hIP3 (30%) hIP2 (30%) IPC (PF) (20%)
	Right Angular Gyrus	same cluster	12.88	30	−60	44	
	Right SupraMarginal Gyrus	same cluster	12.49	44	−36	42	
IC 50 (0.97)	Left Insula Lobe	252	8.22	−32	18	−14	
Salience Network (SN)	Right Insula Lobe	235	9.76	46	2	−4	OP 4 (10%) Area 44 (40%)
IC 53 (0.98)	Left Inferior Frontal Gyrus (p. Opercularis)	1874	13.61	−48	14	14	
SN	Left Insula Lobe	same cluster	10.81	−36	8	6	Area 44 (10%) Area 44 (40%) Area 45 (30%)
	Right Inferior Frontal Gyrus (p. Opercularis)	292	10.62	50	18	6	
	Right Insula Lobe	same cluster	8.23	40	6	0	
	Left Middle Temporal Gyrus	193	9.91	−42	−52	14	
	Left Superior Temporal Gyrus	same cluster	8.54	−56	−48	18	IPC (PGa) (20%)
IC 75 (0.96)	Left Anterior Cingulate Cortex	2316	14.67	−8	22	26	
SN	Left Insula Lobe	83	10.67	−46	10	−8	
	Right Insula Lobe	50	9.05	44	16	−8	

RESULTS

Demographic and clinical characteristics

PD individuals' demographic and clinical information is summarized in Table 2. Our PD cohort had an average age of 65.5 ± 9.6 years and were approximately 4 to 5 years on average from disease onset (average disease duration of 4.9 ± 3.3 years), the average UPDRS part III was 24.6 ± 9.0 , all 24 PD individuals were Hoehn and Yahr stage II. The average adjusted (for age and sex) motor skill fitness was $PPT = 27.88 \pm 3.24$ (in terms of total scores), and cardiorespiratory fitness was estimated $VO_{2max} = 32.18 \pm 3.08 \text{ ml kg}^{-1} \text{ min}^{-1}$.

Multivariate analysis

A multivariate model selection strategy [20] was applied to determine the association of fitness (CRF, MSF) on ICA outcome measures (RSN spatial map intensity and FNC). The associations of each fitness type on the variability of the spatial map intensities are shown in Supplementary Figure 1.

Univariate analysis

Association of fitness measurements (Cardiorespiratory and Motor Skill) with RSN spatial map intensities in PD

We found an association between higher fitness levels and a number of RSNs that included cognitive-attention (CAN), default-mode (DMN), sensorimotor (SMN), and subcortical (SCN) networks. The univariate analysis demonstrated a

Table 2
Demographic information and clinical characteristics of PD individuals

Variable	PD subjects
n	24
Age	65.5 (9.6)
Sex, M/F	15/9
Years of diagnosis	4.9 (3.3)
UPDRS Part III	24.58 (8.99)
Hoehn & Yahr	24 Stage II
Education, years	17.0 (2.1)
Handedness, R/L	23/1
Estimated VO_{2max}	32.18 (3.08)
PPT	27.88 (3.24)

Data are means (SD) unless noted. PD = Parkinson's disease, Estimated VO_{2max} = Estimated Maximal oxygen uptake, PPT = Physical Performance Test total scores.

Table 3

Summary of univariate test results for spatial map intensities. Peak coordinates of largest significant clusters from univariate tests on RSN spatial map intensities are listed here

IC RSN	Average t-value	Average <i>p</i> -value	Brain region of the largest cluster	Cluster size (voxels)	Cluster <i>t</i> _{max}	Cluster MNI coordinates*			Cytoarchitectonic Brodmann area (Probability, if available)
						x	y	z	
A. Estimated VO ₂ max									
IC 62	2.72	0.020	Left Supramarginal Gyrus	137 (+)	3.89	−64	−26	32	Area Pft (IPL) (46%)
	−2.37	0.031	Right Supramarginal Gyrus	10 (−)	1.88	48	−32	38	Area PPop (IPL) (32%)
									Area Pft (IPL) (45%)
									Area 2 (24%)
IC 35	2.80	0.018	Left Putamen	388 (+)	4.20	−28	4	0	Amygdala (AStr) (39%)
			Left Amygdala	same cluster	2.90	−28	−6	16	
									Amygdala (LB) (9%)
IC 63	2.94	0.017**	Right Precuneus	258 (+)	3.92	6	−56	22	
IC 50	2.86	0.019**	Right Insula Lobe	159 (+)	5.78	42	4	−10	
IC 53	2.69	0.022	Left IFG (p. Opercularis)	362 (+)	3.74	−52	14	8	Area 44 (58%)
	−2.37	0.031	Left Temporal Pole	7 (−)	1.93	−50	14	−6	Area 45 (14%)
									Area 45 (9%)
B. PPT									
IC 2	2.44	0.028	Left Anterior Cingulate Cortex	102 (+)	3.08	−2	46	2	
IC 47	2.52	0.026	Right Angular Gyrus	579 (+)	3.49	28	−52	44	Area hIP3 (IPS) (23%)
									Area hIP1 (IPS) (20%)

*The Cluster MNI (Montreal Neurological Institute) coordinates of the peak voxels with negative x coordinates refer to left hemisphere activations. Brodmann's area as indicated by the cytoarchitectonic maximum probability map using the SPM-Anatomy toolbox developed by Eickhoff et al. (2005) [51]. **Represents RSN spatial map clusters that survived multiple comparison corrections (Bonferroni corrected, $p \leq 0.003$). IC RSN, independent component resting state network; VO_{2max} , maximal oxygen uptake; PPT, Physical Performance Test total scores.

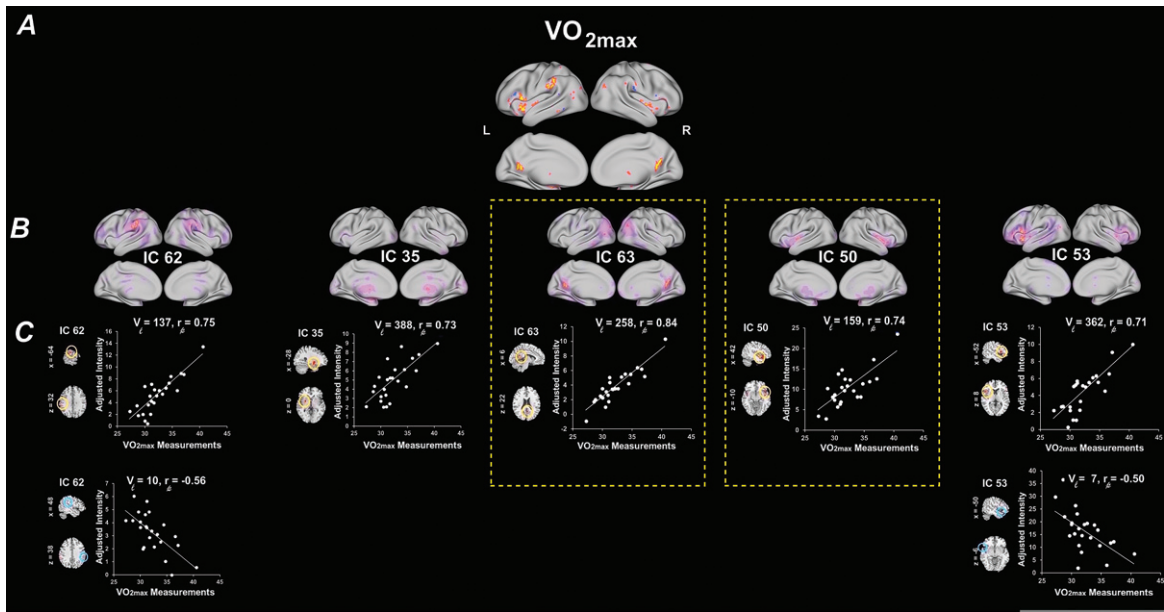


Fig. 2. Univariate results summarizing the effects of estimated VO_{2max} on RSN spatial map intensities. (A) 3D brain map depicting the composite renderings of significant effects of estimated VO_{2max} over all RSNs are displayed as the $-\text{sign}(t)\log_{10}(p)$ ($p < 0.05$, uncorrected). (B) Significant effects of estimated VO_{2max} are shown in individual RSNs overlaid on their corresponding RSN template (purple) and displayed as the $-\text{sign}(t)\log_{10}(p)$ ($p < 0.05$, uncorrected). (C) Scatter plots show the estimated VO_{2max} effects for the largest significant cluster in each affected RSN (indicated by yellow or blue circles on the RSN maps shown in representative orthogonal slices with corresponding MNI coordinates) with the number of contributing voxels (V_t), and the partial correlation coefficient (r_p). RSNs of significance are highlighted by yellow boxes with dashed lines. See Table 3 for the associated statistics, anatomical extent, the t -value of the maxima, and corresponding MNI coordinates for the largest significant clusters. Estimated VO_{2max} , maximal oxygen uptake; SMN, sensorimotor networks; SCN, subcortical networks; DMN, default-mode networks; CAN, cognitive and attention networks; L, left; R, right.

significant association between estimated VO_{2max} (CRF) and a trend with PPT (MSF) measures and distinct RSN spatial map intensities detailed below.

Regarding the role of CRF, we found that increased estimated VO_{2max} was significantly associated with several spatial map intensities of distinct RSNs (Table 3A). Specifically, estimated VO_{2max} was significantly associated with increased RSN spatial map intensities with IC 63 (DMN; Peak activation cluster: bilateral precuneus), and with IC 50 (CAN/SN; Peak: right insula). These associations were the only ones to survive the stringent FDR correction for multiple comparisons. The remaining associations show a trend for significance ($p < 0.03$, FDR corrected). Estimated VO_{2max} was positively associated with increased RSN spatial map intensities of IC 35 (SCN/BG; Peak: left putamen and left amygdala), IC 62 (SMN/supramarginal; Peak: left supramarginal gyrus), and IC 53 (CAN/SN; Peak: left inferior frontal gyrus). Negative associations were found with RSN spatial map intensities of IC 62 (SMN; Peak: right supramarginal gyrus), and IC

53 (CAN/SN; Peak: left temporal pole). Figure 2 illustrates the regions of RSN spatial map whose intensities were associated with estimated VO_{2max} . Associations were visualized by plotting the log of the p value with the sign of the corresponding t -statistic (i.e., $-\text{sign}(t)\log_{10}(p)$) to provide information on both the directionality and the statistical strength of the result.

There were no significant associations between PPT and RSN after stringent FDR multiple comparisons (Fig. 3). However, there was a trend for higher levels of PPT and increased RSN spatial map intensities with IC 2 (CAN/SRN; Peak: bilateral anterior cingulate), and IC 47 (CAN/DAN; Peak: right angular gyrus) ($p < .05$, FDR uncorrected) (Table 3B). Figure 3 illustrates the regions of RSN spatial map whose intensities were associated with PPT. Associations were visualized by plotting the log of the p value with the sign of the corresponding t -statistic (i.e., $-\text{sign}(t)\log_{10}(p)$) to provide information on both the directionality and the statistical strength of the result.

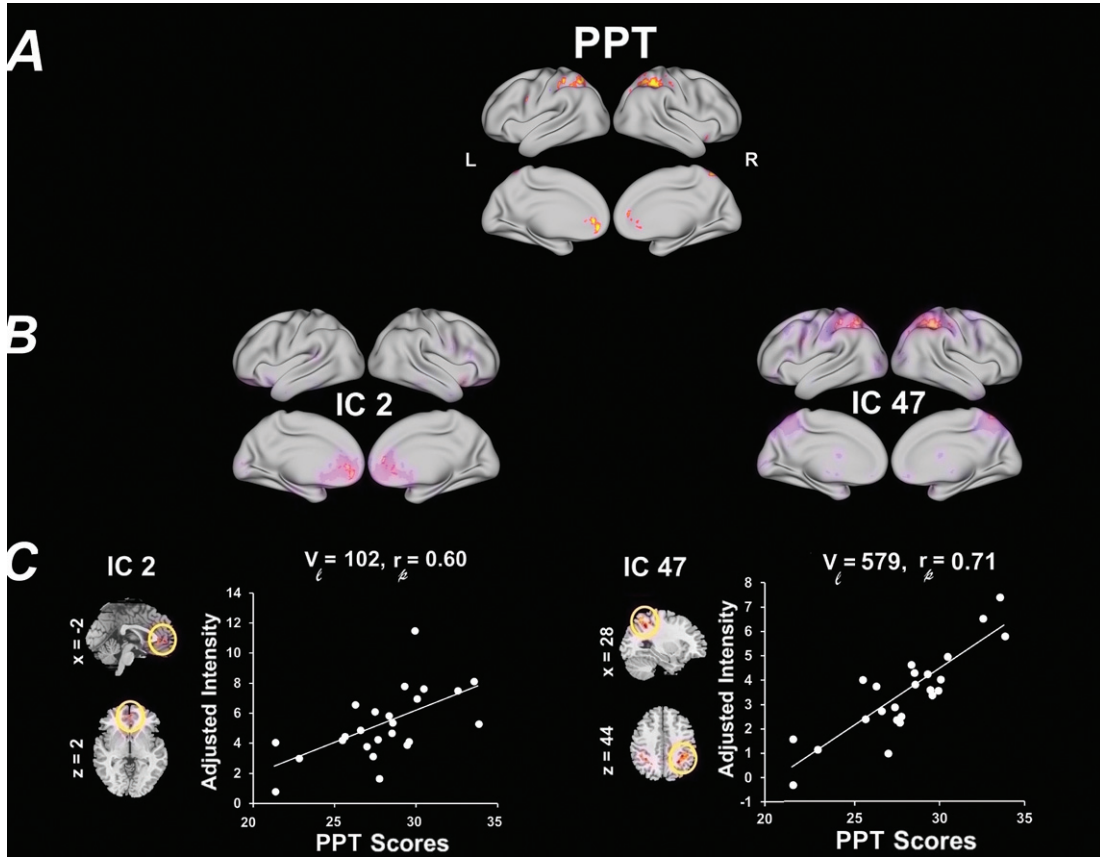


Fig. 3. Univariate results summarizing the effects of PPT on RSN spatial map intensities. (A) 3D brain map depicting the composite renderings of significant effects of PPT over all RSNs are displayed as the $-\text{sign}(t)\log_{10}(p)$ ($p < 0.05$, uncorrected). (B) Significant effects of PPT are shown in individual RSNs overlaid on their corresponding RSN template (purple) and displayed as the $-\text{sign}(t)\log_{10}(p)$ ($p < 0.05$, uncorrected). (C) Scatter plots show the PPT effects for the largest significant cluster in each affected RSN (indicated by yellow or blue circles on the RSN maps shown in representative orthogonal slices with corresponding MNI coordinates) with the number of contributing voxels (V_{ℓ}), and the partial correlation coefficient (r_p). See Table 3 for the associated statistics, anatomical extent, the t -value of the maxima, and corresponding MNI coordinates for the largest significant clusters. PPT, Physical Performance Test total scores; SMN, sensorimotor networks; SCN, subcortical networks; DMN, default-mode networks; CAN, cognitive and attention networks; L, left; R, right.

Functional network connectivity (FNC) analysis

Detailed information about the FNC pairs, and effect sizes are summarized in Table 4. Connectograms in Fig. 4 illustrate all of the pairwise FNC results for both fitness types measured ($p < 0.05$, Bonferroni uncorrected). The only FNC correlation that survived stringent multiple comparison correction ($p < 0.003$, Bonferroni corrected) was an association between estimated $\text{VO}_{2\text{max}}$ and an increase in FNC between IC 48 (SMN; Peak: left dorsal somatosensory) and IC 49 (DMN; $r_p = 0.71$, $p = 0.001$). The remaining correlations showed a trend towards significance. This included a trend between increased estimated $\text{VO}_{2\text{max}}$ and increased pairwise FNC between RSNs IC 48 (SMN; Peak: left dorsal somatosensory) and the following networks: IC 63 (DMN; $r_p = 0.59$, $p = 0.007$),

IC 40 (CAN/CEN; $r_p = 0.59$, $p = 0.008$), and IC 32 (CAN/VAN) ($r_p = 0.55$, $p = 0.015$). Estimated $\text{VO}_{2\text{max}}$ also showed a trend towards a positive association in pairwise FNC between IC 19 (SMN; Peak: bilateral ventral somatosensory) and the following networks: IC 47 (CAN/DAN; $r_p = 0.54$, $p = 0.018$), IC 40 (CAN/CEN; $r_p = 0.52$, $p = 0.022$) and IC 63 (DMN; $r_p = 0.49$, $p = 0.034$). Estimated $\text{VO}_{2\text{max}}$ was also associated with an increase in pairwise FNC between IC 53 (CAN/SN) and the following networks: IC 54 (SMN; Peak: motor cortex; $r_p = 0.56$, $p = 0.012$), IC 24 (SCN; Peak: hippocampus; $r_p = 0.47$, $p = 0.041$) and IC 46 (SMN; Peak: right dorsal somatosensory) ($r_p = 0.46$, $p = 0.050$). Finally, estimated $\text{VO}_{2\text{max}}$ was associated with an increase in FNC between IC 63 (DMN) and IC 45 (CAN/CEN) ($r_p = 0.49$, $p = 0.032$). Alterna-

Table 4

Summary of univariate test results for functional network connectivity (FNC). FNC components that were significantly affected by covariates of interests are listed here (p -value < 0.05 , uncorrected)

Measurement	Positive IC pairs	r_p	t -value	p -value	Negative IC pairs	r_p	t -value	p -value
PPT	IC 62 – IC 17	0.55	2.71	0.015	IC 24 – IC 35	–0.53	–2.61	0.018
	IC 47 – IC 53	0.56	2.79	0.012	IC 32 – IC 45	–0.48	–2.27	0.037
VO _{2max}	IC 46 – IC 53	0.46	2.11	0.050	IC 62 – IC 17	–0.50	–2.36	0.030
	IC 24 – IC 53	0.47	2.21	0.041	IC 62 – IC 63	–0.47	–2.21	0.041
	IC 19 – IC 63	0.49	2.31	0.034				
	IC 63 – IC 45	0.49	2.33	0.032				
	IC 19 – IC 40	0.52	2.52	0.022				
	IC 19 – IC 47	0.54	2.62	0.018				
	IC 48 – IC 32	0.55	2.70	0.015				
	IC 54 – IC 53	0.56	2.80	0.012				
	IC 48 – IC 40	0.59	2.98	0.008*				
	IC 48 – IC 63	0.59	3.04	0.007*				
	IC 48 – IC 49	0.71	4.23	0.001**				

Estimated VO_{2max}, maximal oxygen uptake; PPT, Physical Performance Test total scores; r_p , partial correlation coefficient. **Represents FNC correlations that are significant (Bonferroni corrected, $p \leq 0.003$). *Represents FNC correlations that show trend to significance (Bonferroni corrected, $p \leq 0.009$).

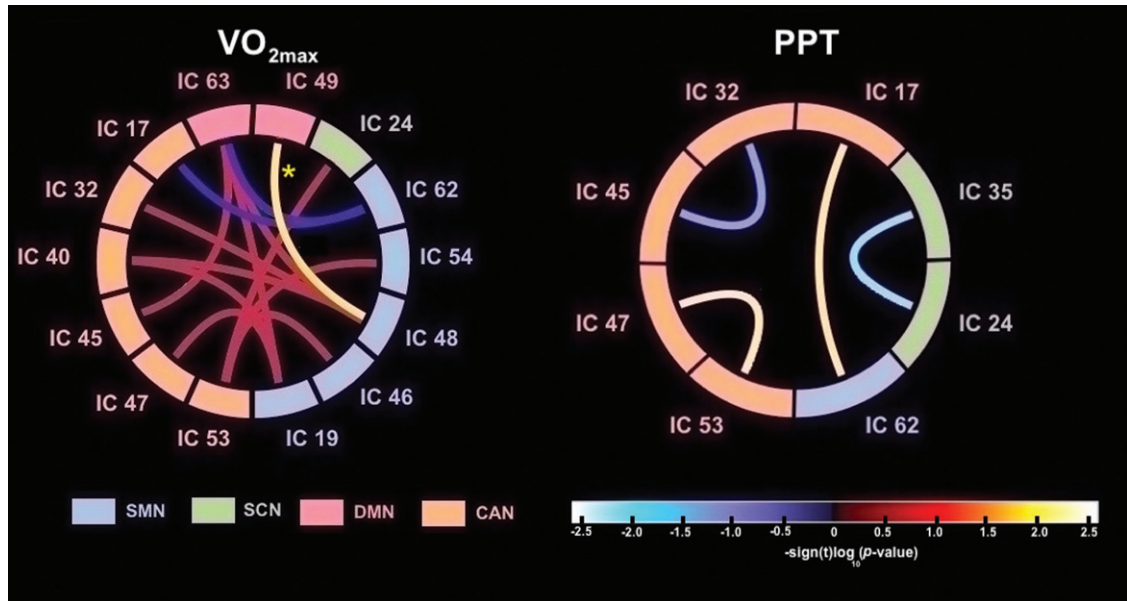


Fig. 4. Differences in FNC correlations related to fitness measures. In each connectogram, FNC correlations are shown using bezier curves. RSN within the same categories are shown in the same color. FNC, functional network connectivity; PPT, Physical Performance Test total scores; estimated VO_{2max}, maximal oxygen uptake; SMN, sensorimotor networks; SCN, subcortical networks; DMN, default-mode networks; CAN, cognitive and attention networks. The “*” represents a statistically significant FNC after FDR corrections for multiple comparisons.

tively, increased estimated VO_{2max} was negatively associated with pairwise FNC between IC 62 (SMN/supramarginal) and the following networks: IC 63 (DMN; $r_p = -0.50$, $p = 0.030$), and IC 17 (CAN/CEN; $r_p = -0.47$, $p = 0.041$).

No FNC correlation with PPT survived stringent multiple comparison corrections ($p < 0.003$, Bonferroni corrected). A trend towards significance was seen between increased PPT and a positive pairwise FNC

between IC 62 (SMN; Peak: supramarginal gyrus) and IC 17 (CAN/CEN) ($r_p = 0.55$, $p = 0.015$) and between IC 47 (CAN/DAN) and IC 53 (CAN/SN) ($r_p = 0.56$, $p = 0.012$). Alternatively, increased PPT was associated with reduced pairwise FNC between IC 24 (SCN; Peak: hippocampus) and IC 35 (SCN; Peak: basal ganglia) ($r_p = -0.53$, $p = 0.018$), and between IC 32 (CAN/VAN) and IC 45 (CAN/CEN) ($r_p = -0.48$, $p = 0.037$).

DISCUSSION

Impact of fitness on resting state networks

Cardiorespiratory (CRF) and motor skill fitness (MSF) have both been shown to be directly associated with improved cognitive structure and function in brain regions adversely affected by aging [5, 7]. These findings support the hypothesis that fitness may have its greatest impact on regions of the brain impacted in aging and neurological disease and that fitness level may serve as a valuable exercise parameter to predict behavioral benefit. There are a number of cognitive networks known to be affected in PD as shown by fMRI studies including central executive (CEN), default mode network (DMN), dorsal attentional network (DAN) and salience network (SN) [2, 56]. While exercise has been shown to be beneficial in PD, the relationship between fitness and functional brain connectivity in these PD affected networks remains to be elucidated. In this cross-sectional rs-fMRI study we examined the relationship between two types of fitness parameters (CRF and MSF) and *within* network and *between* network functional network connectivity (FNC) of established networks subserving cognitive function and impacted in PD. Our main significant findings were that increased CRF (estimated $\dot{V}O_{2\max}$) was associated with increased *within* network connectivity in cognitive networks subserving memory and executive function in the posterior DMN and salience network (SN), respectively, which are known to be impaired in PD. We also found trends for an association between MSF (assessed by the PPT) and increased within network connectivity in cognitive networks subserving executive function and attention in the self-referential network (SRN) and dorsal attention network (DAN), respectively. Taken together, fitness, particularly CRF is associated with cognitive networks impacted in PD.

Impact of cardiorespiratory fitness (CRF) on resting state networks

Specifically, we observed a significant association between increased CRF and increased *within* network spatial map intensity of the posterior DMN, localized in the bilateral precuneus, and between increased CRF and increased *within* network spatial map intensity of the SN, with peak activation localized in the right insula. Cross-sectional studies in older adults have also demonstrated a significant association between CRF and increased network connectivity

within the DMN and SN [5, 57]. Both the DMN and SN have been shown to play an important role in memory, and to be disrupted in older adults and in PD [58, 59]. The DMN comprises the hippocampal formation, medial temporal cortices, posterior cingulate cortex (PCC) and adjacent midline parietal areas, the precuneus, and the medial prefrontal cortex (mPFC) [60]. The DMN is implicated in memory consolidation, self-referential thought, mind-wandering, and autobiographical memory [61]. Aberrant functional connectivity of the DMN has been implicated in a number of neurodegenerative disorders including Alzheimer's disease (AD), frontotemporal dementia, Huntington's disease, and PD [62–66]. In PD, the DMN has emerged as a key functional substrate for cognitive deficits [67, 68]. Recent meta-analysis studies of the resting state functional connectivity in PD similarly found alterations in regions connected to the DMN [63]. The decreased functional connectivity of DMN during resting state in PD compared with healthy controls is reported to be significantly correlated with cognitive parameters [64, 69, 70] but not with disease duration, motor impairment, or levodopa therapy [70]. The SN has been widely studied in rs-fMRI data since it has been found to be a large scale network that mediates switching between the DMN and CEN, and to segregate the most relevant among internal and extra-personal stimuli in order to guide behavior [71]. Within the SN, the anterior insula, anterior to mid-cingulate cortex, middle frontal gyrus, and inferior parietal lobule support episodic memory encoding and retrieval by directing attention to relevant material, engaging working memory, organizing (“controlling”) available information strategy, and adjusting motivation. High functional connectivity in the SN has also been associated with high memory performance in “super-agers”, defined as older individuals who achieve a youthful performance on a memory task [72]. Taken together our results identify the potential role of CRF for promoting increased connectivity within RSNs which are known to sub-serve executive function and memory in PD.

Impact of motor skill fitness (MSF) on resting state networks

With respect to MSF, we observed a trend towards a significant association ($p < .05$, uncorrected) between increased PPT and increased *within* network spatial map intensity of the DAN, specifically within the inferior and superior regions of the parietal lobe. We

also observed a trend towards a significant association between increased PPT and increased *within* network spatial map intensity of the SRN, including the bilateral anterior cingulate cortex (ACC) and the orbitofrontal cortex. The DAN is disrupted early in PD and is integral for orchestrating top-down attentional control, including participating in successful goal-directed activities [73]. The SRN, particularly the ACC, is important for self-focused attention and internally generated information processing of one's current mental and physical state, conflict resolution, memories, emotions, and mood [74, 75]. In PD, alterations in the ACC are associated with early executive dysfunction as well as mood disorders, including depression, commonly observed in this disease [76]. While the association between higher levels of cardiorespiratory fitness and activation of top-down CEN and attention networks have been previously reported in older healthy adults [77, 78], our study suggests the potential association between MSF on cognitive networks sub-serving executive function and attentional control in PD and supports the need for future studies to explore the different fitness types on cognitive network function and connectivity.

Impact of cardiorespiratory fitness (CRF) on functional network connectivity (FNC)

Functional network connectivity (FNC) analysis demonstrated that CRF is significantly associated with increased network connectivity *between* the left sensorimotor network (SMN) and the DMN. Although not significant, there was a trend for an association with CRF and FNC between SMN and other cognitive networks including the CEN, DAN, and VAN. While there was a trend for association with MSF and FNC between SMN and CEN nothing survived multiple comparison correction. Overall, these findings suggest a potential association between high CRF and FNC between motor and cognitive networks.

Specifically, we found that high CRF was associated with FNC between the SMN (specifically the left dorsolateral somatosensory network) and the posterior DMN. There were also trends for increased CRF and increased FNC between the SMN (specifically the left dorsolateral and bilateral ventral somatosensory network) and the attentional networks (VAN and DAN), and between the SMN and the right CEN (right frontal parietal lobe). Studies of rsfMRI in PD have reported a loss of connectivity within regions of the SMN, such as the SMA, and that DA replacement

therapy in general is associated with increased SMN connectivity [3]. Therefore, CRF could augment this in individuals with PD. While others have reported the association of increased fitness and increased brain connectivity within the SMN in healthy young adults, our study is the first to report a potential association of CRF with the SMN and its connectivity to associated cognitive networks sub-serving memory, attention, and executive function in PD.

Mechanisms of exercise on plasticity and brain health impacting connectivity

Studies in animal models of PD and aging are useful for elucidating potential mechanisms underlying exercise-induced neuroplasticity and repair. Specifically, exercise studies in young and aging rodents have reported increased expression of angiogenesis and neurotrophic factors, especially brain-derived neurotrophic factors (BDNF), as well as an increase in neurogenesis and synaptogenesis [13, 14, 79, 80]. Furthermore, studies in our lab and others have also shown exercise induced neuroplasticity in animal models of neurodegeneration including synaptogenesis and changes in dopamine and glutamate neurotransmission, as well as changes in cellular metabolism [12, 80–82]. Studies in animal models are also elucidating the contribution of different forms of exercise, such as skill-based versus aerobic, on aspects of neuroplasticity, including differences in the distribution and degree of synaptogenesis and angiogenesis. For example, Greenough and colleagues reported that aerobic exercise that promotes cardiorespiratory fitness enhances angiogenesis in the cerebral cortex of rodents, while activities that target motor skill fitness, such as acrobatic activities (beam walking and ladders in the home cage), induce synaptogenesis [83]. Relevant to the differential contribution of motor skill fitness on the CEN and SMN are findings previously reported by our group in the 6-hydroxydopamine rodent model of PD. Rodents trained for 4 weeks in a skilled motor task (running in a wheel with irregularly spaced rungs) exhibited greater regional cerebral blood flow in both medial prefrontal cortex and sensorimotor cortices when imaged at rest than animals trained in a non-skilled motor task (running in a wheel with smooth surface) [15]. This finding was accentuated when animals were imaged during a locomotor challenge, with concomitant skill-related increases in functional connectivity between medial prefrontal cortex and sensorimotor cortices. Taken together, these studies

support that while exercise effects on brain circuitry may involve a number of different mechanisms, the predominance by any one of these physiological processes may be dictated in part by the type of exercise.

Limitations of these studies

Our findings should be interpreted in light of several potential limitations. First, our analysis was based on a cross-sectional analysis of a modest number of individuals with PD. Thus, these neuroimaging findings deserve replication with a larger sample size and over a longer period of time. This study did not include healthy age-matched controls, but age effects were controlled for in the analysis. The PD individuals were assessed after having received their usual anti-parkinsonian medication (“best on” state) and dopaminergic medication may have influenced some of the connectivity patterns as previously shown [84]. Furthermore, while we measured PPT and the estimated VO_{2max} , we did not directly assess the duration, intensity or type of exercise individuals engaged in. Future studies with “off” medication PD individuals, as well as detailed activity logs will provide more accurate information about the different dimensions of fitness (e.g., motor skill and cardiorespiratory) on the resting state networks without the potential confound of medication masking some symptoms. This study did not include cognitive behavior analysis; however, a strength is that it did examine a wide spectrum of cognitive networks thus providing rationale for future studies to explore the interactions of fitness with parameters of cognitive behavior. There is also a possibility of false negatives due to low thresholding, and lack of multiple comparisons because of small power, and small sample size. However, as a strong point of this study, we emphasize that we used ICA as a model free approach with no bias in selecting the brain regions. To add to the fidelity of our results, we particularly followed the ICA procedure that is suggested from the literature to yield more robust and reliable components (e.g., 75-IC decomposition, GICA-3 dual-regression algorithm [41], intensity normalization in preprocessing, and 100 re-runs of ICA algorithm with random initiation in ICASSO [43] and strict image quality control with MRIQC. Another strength of our study was the observational approach to evaluating exercise in PD individuals within the framework of their daily lives. This naturalistic approach suggests that our findings may be more broadly applicable, and that activity levels described are within the scope of what a person

could practically implement within his or her daily schedule.

CONCLUSIONS

Our study demonstrates the potential neuromodulatory role of cardiorespiratory fitness (estimated VO_{2max} test) and motor skill fitness (PPT) on intrinsic resting state networks (RSNs) impacting cognition in individuals with PD. This study provides important new empirical data to examine the association between the different types of fitness and multiple RSNs using a multivariate analysis approach. Considering the effect of PD on the intrinsic architecture of the brain, linking exercise related fitness parameters to RSN connectivity will help provide a means to monitor the effects of exercise on brain plasticity and to better understand the underlying neuronal mechanisms of plasticity to promote repair and treat cognitive and motor dysfunctions in PD.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <https://dx.doi.org/10.3233/BPL-200115>.

FUNDING

This research was supported by NIDA Mentored Quantitative Research Career Development Award K25 (DA048179) (BJ), NIDA T32 (DA035165) (BJ), NIMH Mentored Research Scientist Career Development Award K01 (MH099431) (SM), and by grants from the US Department of Defense (U.S. Army, CDMRP) #W81XWH18-1-0666 (DPH), #W81XWH19-1-0443 (MWJ), #W81XWH18-1-0665 (GP). VA Merit Award CX12004 (JVF), VA Merit Award 1101 RX001691-01A1 (DS), the Parkinson’s Foundation (GP), the Don Roberto Gonzalez Family Foundation (GP), and Anthony McClaren and the Climb Above Parkinson Foundation (GP, MJ).

ACKNOWLEDGMENTS

We would like to thank the individuals with Parkinson’s disease who participated in this work. We would like to thank Vincent Calhoun (Georgia Tech) and his lab for helpful discussions. This paper is dedicated to the memory of Andrew Whittaker, a

young man whose spirit showed us why we need a cure.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- [1] Filippi M, Elisabetta S, Piramide N, Agosta F. Functional MRI in Idiopathic Parkinson's Disease. *Int Rev Neurobiol*. 2018;141:439-67.
- [2] Tessitore A, Cirillo M, De Micco R. Functional Connectivity Signatures of Parkinson's Disease. *Journal of Parkinson's disease*. 2019;9(4):637-52.
- [3] Esposito F, Tessitore A, Giordano A, De Micco R, Paccone A, Conforti R, et al. Rhythm-specific modulation of the sensorimotor network in drug-naïve patients with Parkinson's disease by levodopa. *Brain*. 2013;136(Pt 3):710-25.
- [4] Voss MW, Nagamatsu LS, Liu-Ambrose T, Kramer AF. Exercise, brain, and cognition across the life span. *Journal of Applied Physiology*. 2011;111(5):1505-13.
- [5] Voss MW, Weng TB, Burzynska AZ, Wong CN, Cooke GE, Clark R, et al. Fitness, but not physical activity, is related to functional integrity of brain networks associated with aging. *Neuroimage*. 2016;131:113-25.
- [6] Smith JC, Nielson KA, Antuono P, Lyons JA, Hanson RJ, Butts AM, et al. Semantic memory functional MRI and cognitive function after exercise intervention in mild cognitive impairment. *J Alzheimers Dis*. 2013;37(1):197-215.
- [7] Voelcker-Rehage C, Godde B, Staudinger UM. Cardiovascular and coordination training differentially improve cognitive performance and neural processing in older adults. *Front Hum Neurosci*. 2011;5:26.
- [8] Voelcker-Rehage C, Niemann C. Structural and functional brain changes related to different types of physical activity across the life span. *Neurosci Biobehav Rev*. 2013;37(9 Part B):2268-95.
- [9] Noonan V, Dean E. Submaximal exercise testing: clinical application and interpretation. *Phys Ther*. 2000;80(8):782-807.
- [10] Reuben DB, Siu AL. An objective measure of physical function of elderly outpatients. The Physical Performance Test. *J Am Geriatr Soc*. 1990;38(10):1105-12.
- [11] Garcia PC, Real CC, Ferreira AF, Alouche SR, Britto LR, Pires RS. Different protocols of physical exercise produce different effects on synaptic and structural proteins in motor areas of the rat brain. *Brain Res*. 2012;1456:36-48.
- [12] Halliday MR, Abeydeera D, Lundquist AJ, Petzinger GM, Jakowec MW. Intensive treadmill exercise increases expression of hypoxia-inducible factor 1alpha and its downstream transcript targets: a potential role in neuroplasticity. *Neuroreport*. 2019;30(9):619-27.
- [13] Toy WA, Petzinger GM, Leyshon BJ, Akopian GK, Walsh JP, Hoffman MV, et al. Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiol Dis*. 2014;63:201-9.
- [14] van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A*. 1999;96(23):13427-31.
- [15] Wang Z, Guo Y, Myers KG, Heintz R, Peng YH, Maarek JM, et al. Exercise alters resting-state functional connectivity of motor circuits in parkinsonian rats. *Neurobiol Aging*. 2015;36(1):536-44.
- [16] Duchesne C, Lungu O, Nadeau A, Robillard ME, Bore A, Bobeuf F, et al. Enhancing both motor and cognitive functioning in Parkinson's disease: Aerobic exercise as a rehabilitative intervention. *Brain Cogn*. 2015;99:68-77.
- [17] Uc EY, Doerschug KC, Magnotta V, Dawson JD, Thomsen TR, Kline JN, et al. Phase I/II randomized trial of aerobic exercise in Parkinson disease in a community setting. *Neurology*. 2014;83(5):413-25.
- [18] Jafri MJ, Pearlson GD, Stevens M, Calhoun VD. A method for functional network connectivity among spatially independent resting-state components in schizophrenia. *Neuroimage*. 2008;39(4):1666-81.
- [19] McKeown MJ, Sejnowski TJ. Independent component analysis of fMRI data: examining the assumptions. *Hum Brain Mapp*. 1998;6(5-6):368-72.
- [20] Allen EA, Erhardt EB, Damaraju E, Gruner W, Segall JM, Silva RF, et al. A baseline for the multivariate comparison of resting-state networks. *Front Syst Neurosci*. 2011;5:2.
- [21] Koch W, Teipel S, Mueller S, Buerger K, Bokde AL, Hampel H, et al. Effects of aging on default mode network activity in resting state fMRI: does the method of analysis matter? *Neuroimage*. 2010;51(1):280-7.
- [22] Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. *Arch Neurol*. 2001;58(12):1985-92.
- [23] Keating XD, Zhou K, Liu X, Hodges M, Liu J, Guan J, et al. Reliability and Concurrent Validity of Global Physical Activity Questionnaire (GPAQ): A Systematic Review. *Int J Environ Res Public Health*. 2019;16(21).
- [24] Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1988;51(6):745-52.
- [25] Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology*. 1967;17(5):427-42.
- [26] Langston JW, Widner H, Goetz CG, Brooks D, Fahn S, Freeman T, et al. Core assessment program for intracerebral transplantations (CAPIT). *Mov Disord*. 1992;7(1):2-13.
- [27] Ebbeling CB, Ward A, Puleo EM, Widrick J, Rippe JM. Development of a single-stage submaximal treadmill walking test. *Med Sci Sports Exerc*. 1991;23(8):966-73.
- [28] Waddoups L, Wagner D, Fallon J, Heath E. Validation of a single-stage submaximal treadmill walking test. *J Sports Sci*. 2008;26(5):491-7.
- [29] Thompson PD, Arena R, Riebe D, Pescatello LS. ACSM's new preparticipation health screening recommendations from ACSM's guidelines for exercise testing and prescription, ninth edition. *Curr Sports Med Rep*. 2013;12(4):215-7.
- [30] Reuben DB, Siu AL, Kimpau S. The predictive validity of self-report and performance-based measures of function and health. *J Gerontol*. 1992;47(4):M106-10.
- [31] Terret C, Albrand G, Moncenix G, Droz JP. Karnofsky Performance Scale (KPS) or Physical Performance Test (PPT)? That is the question. *Crit Rev Oncol Hematol*. 2011;77(2):142-7.
- [32] Esteban O, Birman D, Schaer M, Koyejo OO, Poldrack RA, Gorgolewski KJ. MRIQC: Advancing the automatic prediction of image quality in MRI from unseen sites. *PLoS One*. 2017;12(9):e0184661.

- [33] Shehzad Z, Kelly C, Reiss PT, Cameron Craddock R, Emerson JW, McMahon K, et al. A multivariate distance-based analytic framework for connectome-wide association studies. *Neuroimage*. 2014;93 Pt 1(0 1):74-94.
- [34] Sladky R, Friston KJ, Tröstl J, Cunningham R, Moser E, Windischberger C. Slice-timing effects and their correction in functional MRI. *Neuroimage*. 2011;58(2):588-94.
- [35] Van Dijk KR, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage*. 2012;59(1):431-8.
- [36] Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage*. 2012;59(3):2142-54.
- [37] Ashburner J, Friston KJ. Nonlinear spatial normalization using basis functions. *Hum Brain Mapp*. 1999;7(4):254-66.
- [38] Abou Elseoud A, Littow H, Remes J, Starck T, Nikkinen J, Nissilä J, et al. Group-ICA Model Order Highlights Patterns of Functional Brain Connectivity. *Front Syst Neurosci*. 2011;5:37.
- [39] Kiviniemi V, Starck T, Remes J, Long X, Nikkinen J, Haapea M, et al. Functional segmentation of the brain cortex using high model order group PICA. *Hum Brain Mapp*. 2009;30(12):3865-86.
- [40] Calhoun VD, Adali T, Pekar JJ. A method for comparing group fMRI data using independent component analysis: application to visual, motor and visuomotor tasks. *Magn Reson Imaging*. 2004;22(9):1181-91.
- [41] Erhardt EB, Rachakonda S, Bedrick EJ, Allen EA, Adali T, Calhoun VD. Comparison of multi-subject ICA methods for analysis of fMRI data. *Hum Brain Mapp*. 2011;32(12):2075-95.
- [42] Bell AJ, Sejnowski TJ. An information-maximization approach to blind separation and blind deconvolution. *Neural Comput*. 1995;7(6):1129-59.
- [43] Himberg J, Hyvärinen A, Esposito F. Validating the independent components of neuroimaging time series via clustering and visualization. *Neuroimage*. 2004;22(3):1214-22.
- [44] Li YO, Adali T, Calhoun VD. Estimating the number of independent components for functional magnetic resonance imaging data. *Hum Brain Mapp*. 2007;28(11):1251-66.
- [45] Duff EP, Johnston LA, Xiong J, Fox PT, Mareels I, Egan GF. The power of spectral density analysis for mapping endogenous BOLD signal fluctuations. *Hum Brain Mapp*. 2008;29(7):778-90.
- [46] Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain. *Nat Rev Neurosci*. 2002;3(3):201-15.
- [47] Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, et al. Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A*. 2006;103(37):13848-53.
- [48] D'Argembeau A, Collette F, Van der Linden M, Laureys S, Del Fiore G, Degueldre C, et al. Self-referential reflective activity and its relationship with rest: a PET study. *Neuroimage*. 2005;25(2):616-24.
- [49] Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U S A*. 2001;98(2):676-82.
- [50] Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci*. 2007;27(9):2349-56.
- [51] Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K, et al. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage*. 2005;25(4):1325-35.
- [52] Ballo AW, Nadim F, Bucher D. Dopamine modulation of Ih improves temporal fidelity of spike propagation in an unmyelinated axon. *J Neurosci*. 2012;32(15):5106-19.
- [53] Caspers S, Geyer S, Schleicher A, Mohlberg H, Amunts K, Zilles K. The human inferior parietal cortex: cytoarchitectonic parcellation and interindividual variability. *Neuroimage*. 2006;33(2):430-48.
- [54] Mantini D, Perrucci MG, Del Gratta C, Romani GL, Corbetta M. Electrophysiological signatures of resting state networks in the human brain. *Proc Natl Acad Sci U S A*. 2007;104(32):13170-5.
- [55] Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage*. 2002;15(4):870-8.
- [56] Baggio HC, Segura B, Junque C. Resting-state functional brain networks in Parkinson's disease. *CNS Neurosci Ther*. 2015;21(10):793-801.
- [57] Voss MW, Erickson KI, Prakash RS, Chaddock L, Malkowski E, Alves H, et al. Functional connectivity: a source of variance in the association between cardiorespiratory fitness and cognition? *Neuropsychologia*. 2010;48(5):1394-406.
- [58] Kim YK, Yoon HK. Common and distinct brain networks underlying panic and social anxiety disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;80(Pt B):115-22.
- [59] Putcha D, Ross RS, Cronin-Golomb A, Janes AC, Stern CE. Altered intrinsic functional coupling between core neurocognitive networks in Parkinson's disease. *NeuroImage Clinical*. 2015;7:449-55.
- [60] Raichle ME. The brain's default mode network. *Annu Rev Neurosci*. 2015;38:433-47.
- [61] Fransson P. How default is the default mode of brain function? Further evidence from intrinsic BOLD signal fluctuations. *Neuropsychologia*. 2006;44(14):2836-45.
- [62] Kobeleva X, Firbank M, Peraza L, Gallagher P, Thomas A, Burn DJ, et al. Divergent functional connectivity during attentional processing in Lewy body dementia and Alzheimer's disease. *Cortex*. 2017;92:8-18.
- [63] Krajcovicova L, Mikl M, Marecek R, Rektorova I. The default mode network integrity in patients with Parkinson's disease is levodopa equivalent dose-dependent. *Journal of neural transmission (Vienna, Austria : 1996)*. 2012;119(4):443-54.
- [64] Lucas-Jimenez O, Ojeda N, Pena J, Diez-Cirarda M, Cabrera-Zubizarreta A, Gomez-Esteban JC, et al. Altered functional connectivity in the default mode network is associated with cognitive impairment and brain anatomical changes in Parkinson's disease. *Parkinsonism Relat Disord*. 2016;33:58-64.
- [65] Sorg C, Riedl V, Mühlau M, Calhoun VD, Eichele T, Läer L, et al. Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2007;104(47):18760-5.
- [66] Wolf RC, Sambataro F, Vasic N, Wolf ND, Thomann PA, Saft C, et al. Default-mode network changes in preclinical Huntington's disease. *Exp Neurol*. 2012;237(1):191-8.
- [67] Amboni M, Tessitore A, Esposito F, Santangelo G, Picillo M, Vitale C, et al. Resting-state functional connectivity associated with mild cognitive impairment in Parkinson's disease. *J Neurol*. 2015;262(2):425-34.
- [68] Gorges M, Muller HP, Lule D, Pinkhardt EH, Ludolph AC, Kassubek J. To rise and to fall: functional connectivity in cognitively normal and cognitively impaired patients

- with Parkinson's disease. *Neurobiol Aging*. 2015;36(4):1727-35.
- [69] Disbrow EA, Carmichael O, He J, Lanni KE, Dressler EM, Zhang L, et al. Resting state functional connectivity is associated with cognitive dysfunction in non-demented people with Parkinson's disease. *Journal of Parkinson's Disease*. 2014;4(3):453-65.
- [70] Tessitore A, Esposito F, Vitale C, Santangelo G, Amboni M, Russo A, et al. Default-mode network connectivity in cognitively unimpaired patients with Parkinson disease. *Neurology*. 2012;79(23):2226-32.
- [71] Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct*. 2010;214(5-6):655-67.
- [72] Sun FW, Stepanovic MR, Andreano J, Barrett LF, Touroutoglou A, Dickerson BC. Youthful Brains in Older Adults: Preserved Neuroanatomy in the Default Mode and Salience Networks Contributes to Youthful Memory in Superaging. *J Neurosci*. 2016;36(37):9659-68.
- [73] Jacob Y, Rosenberg-Katz K, Gurevich T, Helmich RC, Bloem BR, Orr-Urtreger A, et al. Network abnormalities among non-manifesting Parkinson disease related LRRK2 mutation carriers. *Hum Brain Mapp*. 2019;40(8):2546-55.
- [74] Ingram RE. Self-focused attention in clinical disorders: review and a conceptual model. *Psychol Bull*. 1990;107(2):156-76.
- [75] Yoon EJ, Ismail Z, Hanganu A, Kibreab M, Hammer T, Cheetham J, et al. Mild behavioral impairment is linked to worse cognition and brain atrophy in Parkinson disease. *Neurology*. 2019;93(8):e766-e77.
- [76] Vogt BA. Cingulate cortex in Parkinson's disease. *Handb Clin Neurol*. 2019;166:253-66.
- [77] Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci*. 2003;14(2):125-30.
- [78] Prakash RS, Voss MW, Erickson KI, Lewis JM, Chaddock L, Malkowski E, et al. Cardiorespiratory fitness and attentional control in the aging brain. *Front Hum Neurosci*. 2011;4:229.
- [79] Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience*. 2005;133(3):853-61.
- [80] Petzinger GM, Walsh JP, Akopian G, Hogg E, Abernathy A, Arevalo P, et al. Effects of treadmill exercise on dopaminergic transmission in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. *J Neurosci*. 2007;27(20):5291-300.
- [81] Caldwell CC, Petzinger GM, Jakowec MW, Cadenas E. Treadmill exercise rescues mitochondrial function and motor behavior in the CAG140 knock-in mouse model of Huntington's disease. *Chem Biol Interact*. 2019;315:108907.
- [82] Kintz N, Petzinger GM, Akopian G, Ptasnik S, Williams C, Jakowec MW, et al. Exercise modifies alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor expression in striatopallidal neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse. *J Neurosci Res*. 2013;91(11):1492-507.
- [83] Black JE, Isaacs KR, Anderson BJ, Alcantara AA, Greenough WT. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc Natl Acad Sci U S A*. 1990;87(14):5568-72.
- [84] Delaveau P, Salgado-Pineda P, Fossati P, Witjas T, Azulay JP, Blin O. Dopaminergic modulation of the default mode network in Parkinson's disease. *Eur Neuropsychopharmacol*. 2010;20(11):784-92.

RESEARCH ARTICLE

Global and Regional Changes in Perivascular Space in Idiopathic and Familial Parkinson's Disease

Erin K. Donahue, BA,^{1,2†} Amjad Murdos, BS,^{3,4†} Michael W. Jakowec, PhD,^{1,2} Nasim Sheikh-Bahaei, MD, PhD,^{5,6} Arthur W. Toga, PhD,^{3,6} Giselle M. Petzinger, MD,^{1,2} and Farshid Sepehrband, PhD^{3,6*}

¹Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

²Neuroscience Graduate Program, University of Southern California, Los Angeles, California, USA

³Stevens Neuroimaging and Informatics Institute, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

⁴Keck School of Medicine, University of Southern California, Los Angeles, California, USA

⁵Department of Radiology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

⁶Alzheimer's Disease Research Center, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

ABSTRACT: Background: The glymphatic system, including the perivascular space (PVS), plays a critical role in brain homeostasis. Although mounting evidence from Alzheimer's disease has supported the potential role of PVS in neurodegenerative disorders, its contribution in Parkinson's disease (PD) has not been fully elucidated. Although idiopathic (IPD) and familial PD (FPD) share similar pathophysiology in terms of protein aggregation, the differential impact of PVS on PD subtypes remains unknown. Our objective was to examine the differences in PVS volume fraction in IPD and FPD compared to healthy controls (HCs) and nonmanifest carriers (NMCs).

Methods: A total of 470 individuals were analyzed from the Parkinson's Progression Markers Initiative database, including (1) IPD (n = 179), (2) FPD (LRRK2 [leucine-rich repeat kinase 2], glucocerebrosidase, or α -synuclein) (n = 67), (3) NMC (n = 101), and (4) HCs (n = 84). Total PVS volume fraction (%) was compared using parcellation and quantitation within greater white matter

volume at global and regional levels in all cortical and subcortical white matter.

Results: There was a significant increase in global and regional PVS volume fraction in PD versus non-PD, particularly in FPD versus NMC and LRRK2 FPD versus NMC. Regionally, FPD and NMC differed in the medial orbitofrontal region, as did LRRK2 FPD versus NMC. Non-PD and PD differed in the medial orbitofrontal region and the banks of the superior temporal regions. IPD and FPD differed in the cuneus and lateral occipital regions.

Conclusions: Our findings support the role of PVS in PD and highlight a potentially significant contribution of PVS to the pathophysiology of FPD, particularly LRRK2. © 2021 International Parkinson and Movement Disorder Society

Key Words: imaging; perivascular spaces; α -synuclein; leucine-rich repeat kinase 2; glucocerebrosidase

Perivascular spaces (PVSs), also known as Virchow–Robin spaces, are fluid-filled spaces surrounding the penetrating vessels that accommodate the flux of the cerebrospinal fluid into the brain parenchyma.¹

Normally functioning PVS of the central nervous system (CNS) composes a vital waste clearance network^{2–4} referred to as the “glymphatic system.”^{1–5} Dilated PVS (dPVS), considered the pathological signature of the

***Correspondence to:** Farshid Sepehrband, PhD. Assistant Professor. Stevens Neuroimaging and Informatics Institute, Keck School of Medicine, University of Southern California, 2025 Zonal Avenue, Los Angeles, CA 90089, USA; E-mail: farshid.sepehrband@loni.usc.edu

Relevant conflicts of interest/financial disclosure: The authors report no conflicts of interest or disclosures.

Funding agencies: This work was supported by 2P41EB015922-21 and 1P30AG066530 (AWT and FS) grants and by grants from the US Department of Defense (U.S. Army, CDMRP) #W81XWH18-1-0666, #W81XWH19-1-0443 (MWJ), #W81XWH18-1-0665 (GMP), the

Parkinson's Foundation (GMP), and the Don Roberto Gonzalez Family Foundation (GMP), the Michael J Fox foundation #MJFF-006340 (AWT), Parivash Yazdani Parkinson's Research Fund (AWT).

[†]E.K.D. and A.M. contributed equally to this work.

Received: 29 June 2020; **Revised:** 23 November 2020; **Accepted:** 14 December 2020

Published online 20 January 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28473

PVS,⁶⁻⁹ is observed in various neurodegenerative diseases, including Alzheimer's disease (AD).¹⁰

The proposed mechanisms of PVS dysfunction in neurodegenerative disease include poor waste clearance and disruption of the integrity of the blood-brain barrier (BBB), impacting the normal transport of proteins, metabolites, and potential toxins to the periphery. For example, in AD, changes in the BBB and the glymphatic system may contribute to insufficient clearance of amyloid- β and phospho- τ .¹¹ In fact, increased dPVS is associated with AD progression and predictive of cognitive decline.^{5,12-14} In particular, recent magnetic resonance imaging (MRI) studies have found significantly larger PVS in AD individuals compared to healthy controls (HCs) in the centrum semiovale of the white matter but not in the basal ganglia,^{15,16} and these PVS differences associated with cognitive impairment were independent of the degree of amyloid- β accumulation.¹⁶ However, the association between PVS and cognitive decline remains controversial.^{17,18} These findings highlight the importance of further studies examining the anatomical distribution of PVS changes and their relationship with clinical features and disease progression.

Similar to AD, Parkinson's disease (PD) is a progressive neurodegenerative disorder, featuring both motor and nonmotor symptoms, including cognitive impairment progressing to dementia. Pathophysiologically, PD is also characterized by the accumulation of protein aggregates, including α -synuclein (SNCA) containing Lewy bodies, and their widespread distribution throughout the brainstem, basal ganglia, and cortical and subcortical regions.¹⁹ Although these aberrant aggregates play an essential role in the development of PD, other mechanisms may contribute to pathophysiology. For example, analogous to AD, a dysfunctional glymphatic system and the increased presence of PVS could lead to compromised clearance of toxic moieties, including protein aggregates and emergence or exacerbation of PD.²⁰⁻²²

PD can be divided into either idiopathic (IPD) or familial (FPD) forms based on the current understanding of common associated genes,²³ including leucine-rich repeat kinase 2 (LRRK2), SNCA, and glucocerebrosidase (GBA). Similar to IPD, familial forms are characterized by widespread SNCA pathology, although the role of PVS alterations remains unclear.²⁰⁻²² Although carrying these alleles puts individuals at higher risk for developing PD, some individuals may remain asymptomatic for a long period of time (termed nonmanifest carriers [NMC]) before developing PD, if at all.^{24,25} Such individuals may serve as a valuable population for understanding early pathophysiological changes before PD diagnosis.

A recent study comparing PD individuals and HC showed increased PVS in the basal ganglia.⁵ In addition, this same study reported that basal ganglia PVS may be a predictor of cognitive decline.⁵ Despite this report, the level and presence of PVS in cortical and

subcortical white matter have not yet been examined. Knowledge of the anatomical distribution of PVS throughout the brain may allow us to better understand its role in the pathophysiology of PD.

The aim of this study was to examine the differences in the global and regional distribution of PVS in individuals with PD (including IPD and FPD) compared to non-PD individuals (including NMC and HC). Because advancements in imaging have begun to delineate cortical changes in PD, we conducted regional analyses to identify changes in PVS in different anatomical regions.²⁶ Furthermore, we examined the differences in global and regional PVS along genetic lines to determine whether IPD and FPD differ in the underlying pathophysiology. For this study, we focused on quantitating PVS changes in cortical and subcortical white matter. We utilized a recently developed method that specifically quantitates PVS volume fraction within the context of the greater white matter volume.²⁷

Patients and Methods

Study Data

Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). After exclusion for mild cognitive impairment and low-quality MRI, 431 individuals with normal cognition were analyzed. The subjects were grouped into the following: (1) IPD, defined as PD noncarriers, based on screening for known LRRK2, SNCA, and GBA mutations ($n = 179$); (2) FPD, defined as PD gene carriers based on positive screening for known LRRK2, SNCA, and GBA mutations ($n = 67$); (3) nonmanifest gene carriers (NMC), unaffected carriers of known LRRK2, SNCA, and GBA mutations ($n = 101$); and (4) HCs without LRRK2, SNCA, or GBA mutations or PD and without a first-degree blood relative with PD (HC) ($n = 84$). Due to low numbers of total FPD within individual mutation types, all FPD participants were initially pooled. Subsequent analysis was carried out for LRRK2 individuals due to sufficient sample size within this particular subgroup. Table 1 presents demographic information, including sex, age, and frequency of LRRK2, SNCA, and GBA mutations. No age or sex differences were observed between any groups, including PD/non-PD, FPD/NMC, or LRRK2 FPD/NMC. For some analyses, groups were pooled into PD (IPD + FPD, $n = 246$) and non-PD (HC + NMC, $n = 185$).

Magnetic Resonance Imaging

PPMI participants with T1-weighted (T1w) and fluid-attenuated inversion recovery (FLAIR) data were used. PVS was mapped from T1w images, and then white matter hyperintensities were excluded using FLAIR

**TABLE 1.** Demographic information for individuals included in analyses

Group	IPD	FPD	NMC	HC
N (female)	179 (65)	67 (36)	101 (56)	84 (33)
Age (SD)	61.31 (10.44)	61.91 (10.29)	62.36 (6.98)	59.77 (11.83)
N (LRRK2+)	—	54	53	—
N (SNCA+)	—	2	1	—
N (GBA+)	—	11	47	—

Abbreviations: FPD, familial Parkinson's disease; GBA, glucocerebrosidase; HC, healthy controls; IPD, idiopathic Parkinson's disease; LRRK2, leucine-rich repeat kinase 2; NMC, nonmanifest carriers; SD, standard deviation; SNCA, α -synuclein.

images. T1w preprocessing and parcellation was done using the *FreeSurfer* (v5.3.0) software package.²⁸ Data processing was conducted using the Laboratory of Neuro Imaging (LONI) pipeline system (<http://pipeline.loni.usc.edu>),²⁹⁻³² similar to previous studies.^{33,34} Brain volume and white matter mask were derived from the Desikan-Killiany atlas.³⁵ Parcellation was performed with the *recon-all* module of *FreeSurfer* using an atlas-based parcellation approach. Before parcellation, preprocessing steps included motion correction, non-uniform intensity normalization, Talairach transformation, intensity normalization, and skull stripping.³⁵⁻⁴⁸ FLAIR images of each participant were corrected for nonuniform field inhomogeneity using the N4ITK module⁴⁹ of Advanced Normalization Tools (ANTs).⁵⁰ FLAIR images were then co-registered to T1w images using the *antsIntermodalityIntrasubject* ANTs module. False-positive PVS voxels were reduced by removing voxels with hyperintense FLAIR value.

PVS Mapping

Mapping was focused on areas with the highest image resolution, which included principally all cortical and subcortical white matter. Deep brain regions, including the basal ganglia, were not included due to poor automated PVS quantification in this region.

PVS mapping was performed using a newly developed technique by Sepehrband et al.²⁷ T1w images were preprocessed using *FreeSurfer*²⁸ for subject motion and nonuniform intensity normalization. T1w images were then filtered using an adaptive nonlocal mean filtering technique.⁵¹ Filtering was applied only on high-frequency spatial noises using a filtering patch with a radius of 1 voxel, which removes the noise at a single-voxel level and preserves signal intensities that are spatially repeated.⁵¹ Subsequently, we applied a Frangi filter⁵² to T1w using the *Quantitative Imaging Toolkit*.⁵³ The Frangi filter estimates a vesselness measure for each voxel from eigenvectors of the Hessian matrix of the image. The default setting for Frangi filter implementation was used as recommended in Frangi et al.⁵² The parameter c was set to half the value of the maximum Hessian norm. Frangi filter estimated vesselness measures at different scales and provided the

maximum likeliness. The scale was set to a range of 0.1 to 5 voxels to maximize the vessel inclusion. The output of this step is a quantitative map of vesselness in the regions of interest and is taken to be the maximum across scales, as suggested in the original paper.⁵² Vesselness threshold of 0.0002 was used to obtain PVS mask. For each segmented voxel, the corresponding FLAIR voxels were checked, and if the FLAIR voxel value was a white matter hyperintensity (above 90 percentile), then the voxel was excluded from the final PVS mask. All FLAIR and T1w MRI images were manually checked in a randomized and blinded fashion for misalignment and postprocessing failure. All the extracted PVS maps were quality controlled, and 40 subjects who failed the manual inspection were excluded.

Statistical Analysis

To compare the differences in PVS at a cortical and subcortical white matter level, the PVS volume fraction (%) was first calculated as the total cortical and subcortical white matter PVS over the total cortical and subcortical white matter volume. The first analysis compared the total cortical and subcortical white matter PVS volume fraction (%) of all PD (FPD and IPD) with all non-PD (NMC and HC) individuals. The mean differences in the PVS volume fraction between groups and regions were estimated by calculating the unpaired Cohen's d effect size. To calculate the confidence interval of the effect size, 5000 bootstrap samples were taken, bias corrected, and accelerated.⁵⁴ A 2-sided P -value of the Mann-Whitney U test was reported. The second analysis compared the following 4 groups—(1) IPD and (2) FPD (for PD participants) and (3) NMC and (4) HC (non-PD participants)—using an analysis of covariance (ANCOVA) adjusting for age, sex, and brain size. Multiple comparison correction was performed using conservative Bonferroni correction, and raw 2-sided Mann-Whitney P -values were reported.

For regional PVS evaluation, the PVS volume fraction (%) was calculated as the regional white matter PVS over the total regional white matter volume for all cortical and subcortical white matter regions of the Desikan-Killiany atlas.³⁵ The left and right hemispheres

averaged for 34 subregions. The first analysis compared the regional cortical and subcortical white matter PVS volume fraction (%) of all PD (FPD and IPD) with all non-PD (NMC and HC) individuals. The mean differences in the PVS volume fraction between groups and regions were estimated by calculating the unpaired Cohen's *d* effect size. To calculate the confidence interval of the effect size, 5000 bootstrap samples were taken, bias corrected, and accelerated.⁵⁴ A 2-sided *P*-value of the Mann-Whitney *U* test was reported. The second analysis used a pairwise comparison between the following groups: (1) IPD and FPD, (2) FPD and NMC, (3) IPD and HC, and (4) NMC and HC. This analysis was conducted using an ANCOVA adjusting for age, sex, and brain size. Multiple comparison correction was performed using conservative Bonferroni correction, and raw 2-sided Mann-Whitney *P*-values were reported.

Results

Global Cortical and Subcortical PVS Comparisons in PD and Non-PD

The initial comparison of PVS was made between PD (pooled IPD and FPD) and non-PD (pooled non-PD and NMC). The global PVS volume fraction (%) of the cortical and subcortical white matter was significantly higher in PD (0.74 ± 0.04) than non-PD individuals (0.61 ± 0.04) (Fig. 1a). This difference remained significant after controlling for age, sex, and total brain volume ($F(1418) = 5.4$, $P = 0.021$).

Global Cortical and Subcortical PVS Comparisons in Subgroups of PD and Non-PD

All participants were delineated into 4 groups, including those with PD subdivided into (1) IPD and (2) FPD and non-PD participants divided into (3) NMC and (4) HC. Using an ANCOVA analysis to compare the global PVS volume fraction (%) of the cortical and subcortical white matter between all 4 groups, a significant group difference remained significant after adjusting for age, sex, and total brain volume ($F(3424) = 2.9$, $P = 0.038$ [Fig. 1b]). Post hoc analysis showed a significant difference between NMC (0.57 ± 0.05) and IPD (0.73 ± 0.04) ($F(1269) = 5.9$, $P = 0.016$) and between NMC (0.57 ± 0.05) and FPD (0.77 ± 0.08) ($F(1158) = 7.7$, $P = 0.0006$). A sample difference in PVS distribution in cortical and subcortical white matter in NMC and in FPD is shown in Figure 2a–d. There were no significant differences in the following group comparisons: (1) NMC (0.57 ± 0.05) and HC (0.65 ± 0.06) ($P = 0.26$), (2) IPD and FPD ($P = 0.67$), (3) HC and FPD ($P = 0.24$), and (4) HC and IPD ($P = 0.33$). Among participants with LRRK2+, the PVS volume fraction of the PD was significantly higher than that of non-PD ($P = 0.021$). The difference remained statistically significant after correcting for age, sex, and brain volume ($F(1103) = 7.2$, $P = 0.008$).

Regional PVS Comparisons in PD and Non-PD

The initial comparison of PVS was made between PD (pooled IPD and FPD) and non-PD (pooled non-PD

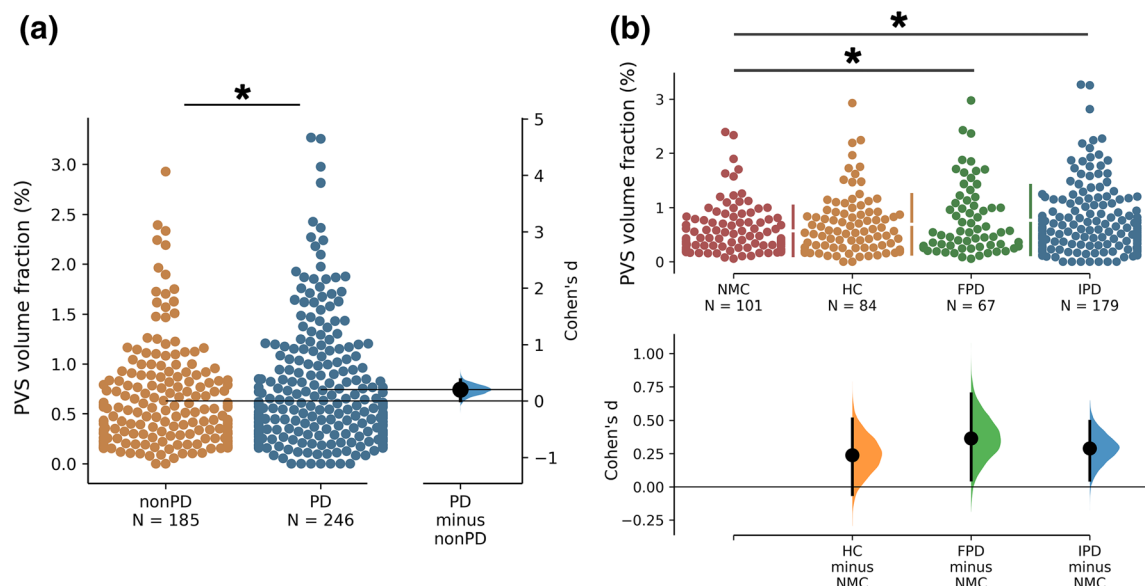


FIG. 1. Perivascular space (PVS) volume fraction (%) across global cortical and subcortical white matter in (a) PD (Parkinson's disease) versus (b) non-PD IPD (idiopathic PD), FPD (familial PD) individuals, NMCs (nonmanifest carriers), and HCs (healthy controls). Swarm plots of the present PVS data points and the second panels present the effect size as a bootstrap 95% confidence interval on an aligned axis, highlighting the effect size. Significance level: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P < 0.0001$ (****). [Color figure can be viewed at wileyonlinelibrary.com]

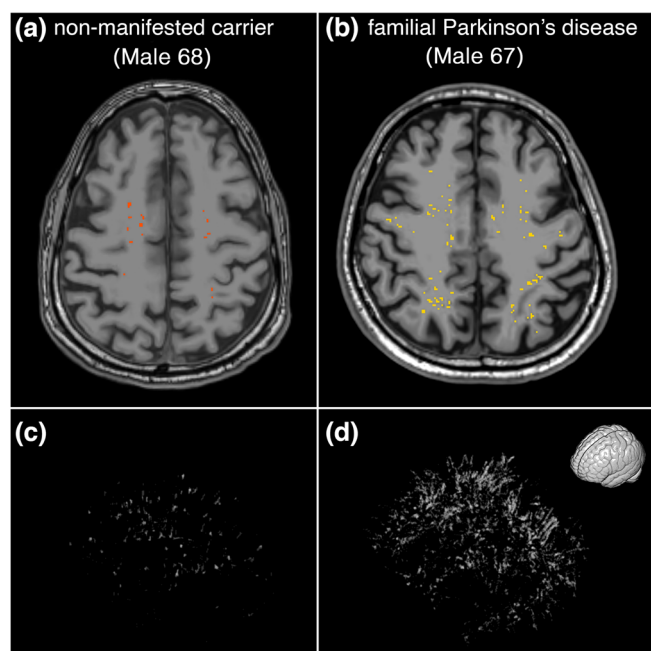


FIG. 2. Differences in perivascular space (PVS) distribution in PD (Parkinson's disease) (FPD [familial PD]) and non-PD (NMC [nonmanifest carrier]). (a, b) T1-weighted (T1w) axial image of white matter centrum semiovale in (a) non-PD (NMC) and (b) PD (FPD), with superimposed PVS regions illustrated in red and yellow, respectively. (c, d) 3D image of whole brain PVS for (c) non-PD (NMC) and (d) PD (FPD). Segmented PVSs are superimposed on an axial image of participant T1w images (a and b) and constructed into 3D image of the PVS across the entire white matter (c and d). The schematic 3D brain indicates the angle at which the PVS maps are illustrated. The varying PVS alteration from region to region illustrated created the interest in exploring the regional differences in PVS. [Color figure can be viewed at wileyonlinelibrary.com]

and NMC). The global PVS volume fraction (%) of the cortical and subcortical white matter was significantly higher. A multiple comparison analysis comparing all

34 regions showed 2 regions in which PVS volume fraction (%) was significantly higher in PD compared to non-PD: (1) the banks of the superior temporal region (PD = (0.86 ± 0.07) ; non-PD = (0.55 ± 0.06) ; $F(1418) = 9.6$, $P = 0.0021$; Fig. 3a) and (2) the medial orbitofrontal region (PD = (0.34 ± 0.03) ; non-PD = (0.23 ± 0.03) ; $F(1418) = 6.0$, $P = 0.014$; Fig. 3b). These differences remained significant after correcting for age, sex, and total brain volume.

Regional PVS Comparisons in Subgroups of PD and Non-PD

PD and non-PD participants were then delineated into 4 groups, with PD participants subdivided into (1) IPD and (2) FPD and non-PD participants divided into (3) NMC and (4) HC. The comparisons conducted in all 34 regions showed significant differences in 2 group comparisons: (1) IPD and FPD and (2) FPD and NMC. A significant PVS volume fraction difference between IPD and FPD was found in 2 regions: (1) the cuneus region (IPD = (0.32 ± 0.04) ; FPD = (0.12 ± 0.02) ; $F(1240) = 4.8$, $P = 0.029$; Fig. 4a) and (2) the lateral occipital region (IPD = (0.57 ± 0.05) ; FPD = (0.23 ± 0.03) ; $F(1240) = 12.43$, $P = 0.0005$; Fig. 4b). Both differences remained significant after correcting for age, sex, and total brain volume. A significant PVS difference between FPD and NMC was observed in the medial orbitofrontal region (FPD = (0.46 ± 0.10) ; NMC = (0.21 ± 0.03) ; $F(1158) = 7.53$, $P = 0.007$; Fig. 4c). These differences remained significant after correcting for age, sex, and total brain volume. Among participants with LRRK2, the medial orbitofrontal PVS volume fraction of PD was significantly ($P = 0.005$) higher than that of non-PD. The difference remained statistically significant after correcting for age, sex, and brain volume ($F(1103) = 6.8$, $P = 0.010$).

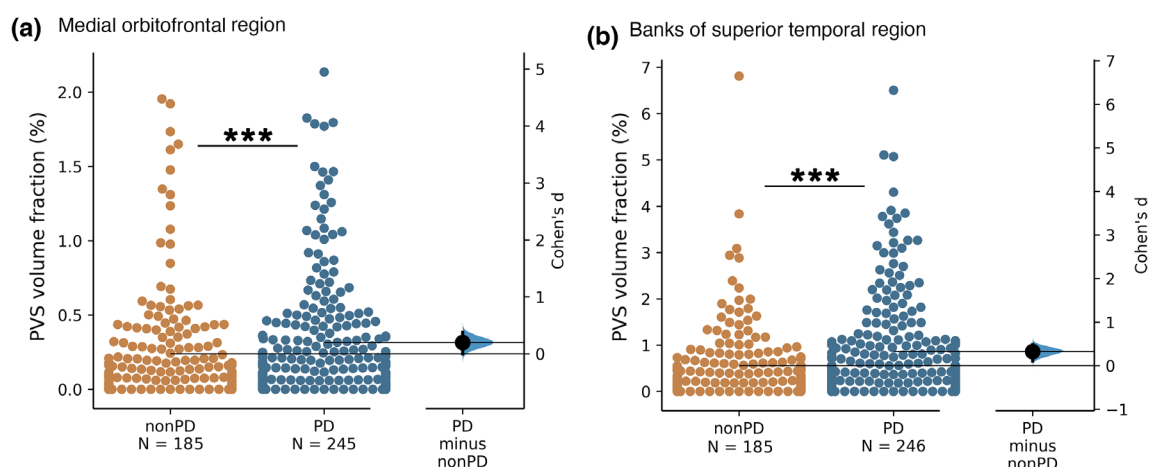


FIG. 3. Regional perivascular space (PVS) volume fraction (%) in PD (Parkinson's disease) and non-PD. Swarm plots of the present PVS data points and the second panels present the effect size as a bootstrap 95% confidence interval on an aligned axis, highlighting the effect size. Significance level: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P < 0.0001$ (****). (a) $P = 0.00063$, corrected $P = 0.0021$; (b) $P = 0.00097$, corrected $P = 0.014$. [Color figure can be viewed at wileyonlinelibrary.com]

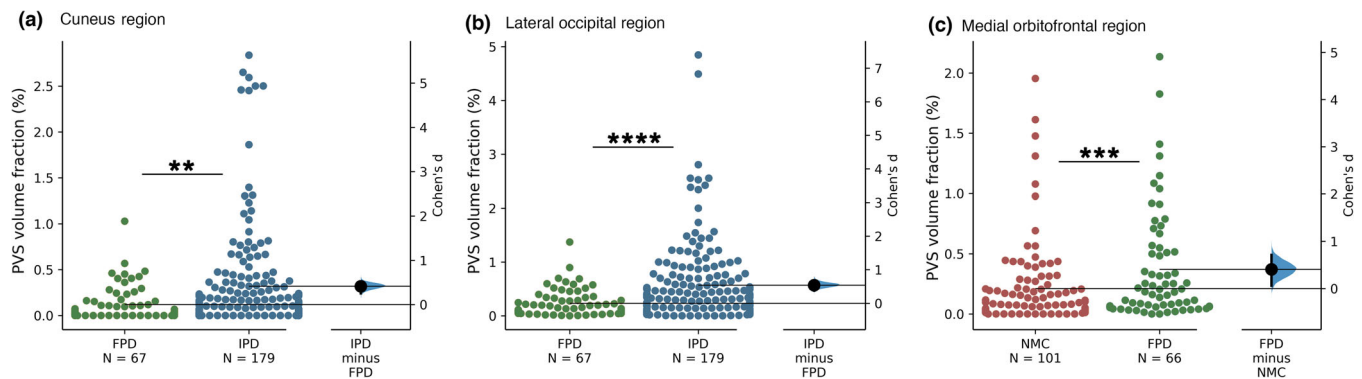


FIG. 4. Regional perivascular space (PVS) volume fraction (%) in (a, b) NMC (nonmanifest carrier) and FPD (familial Parkinson's disease) and (c) FPD and IPD (idiopathic PD). Swarm plots of the present PVS data points and the second panels present the effect size as a bootstrap 95% confidence interval on an aligned axis, highlighting the effect size. Significance level: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P < 0.0001$ (****). (a) $P = 0.0014$, corrected $P = 0.029$; (b) $P = 0.000006$, corrected $P = 0.0005$; (c) $P = 0.00096$, corrected $P = 0.007$. [Color figure can be viewed at wileyonlinelibrary.com]

No significant regional differences in PVS volume fraction (%) were found in any regional comparison between 2 groups (3) IPD and HC and (4) NMC and HC.

Discussion

The aim of this study was to investigate the global and regional differences in PVS volume fraction between PD and non-PD using MRI. We found a significant increase in the global amount of PVS volume fraction between PD (including both IPD and FPD) and non-PD (including both NMC and HC). This difference was primarily due to differences between FPD and NMC. Further analysis showed a significant increase in the global amount of PVS volume fraction between LRRK2 FPD and NMC. We did not observe any significant difference between IPD and HC. Our findings are different from those of a previous report by Park and colleagues, who found a significant increase in PVS in the basal ganglia in individuals with PD compared to age-matched HCs.⁵ The different results between the studies may be explained by varied methodological approaches, including different regional examination and delineation of the PD group into IPD and FPD, neither of which was noted in the study of Park and colleagues. In addition, we included regional analyses in the centrum semiovale and other cortical and subcortical white matter using a new approach developed by Sepehrband and colleagues.²⁷ Similar to our findings comparing FPD to NMC, studies in dementia and in aging have also reported increased global PVS in the centrum semiovale and other white matter regions compared to HCs.^{9,14,55,56} However, in these studies, the relationship between increased PVS and cognitive decline remains unclear.^{17,18}

Similar to global findings, we found significant regional differences between FPD and NMC in the medial orbitofrontal region. The medial orbitofrontal

region subserves executive function and has been shown to be disrupted early in PD.^{57,58} Other MRI imaging studies have also supported changes in the medial orbitofrontal region in PD, including cortical gray matter thinning.^{59,60} Further analysis of FPD versus NMC in LRRK2 FPD and NMC also showed a significant difference in PVS volume fraction within the medial orbitofrontal region. Interestingly, a recent study of LRRK2 individuals with PD has indicated an accumulation of LRRK2 aggregates in the prefrontal cortex compared to HCs.⁶¹

No region-specific changes were observed between IPD and HC, which may be explained in part by the large variability and small sample size in our HC group. The high variability in our HC group may be explained in part by age-related comorbidities, such as vascular changes known to be associated with dPVS. PVS is known to increase with age, differ along gender lines, and vary widely within the general population.⁶²

In the pooled group comparison, we observed a difference in the superior temporal region between PD and non-PD. However, there was no significant difference between FPD and NMC or between IPD and HC in this region. The superior temporal area is a region known to subserve memory, which is also commonly affected in PD.⁶³⁻⁶⁸ Volume loss in the superior temporal region has been reported to be associated with cognitive decline in PD.^{64,69-71} To the best of our knowledge, this is the first study to report global and regional significant changes in PVS in PD, particularly in FPD.

Interestingly, there was no significant global PVS volume fraction difference between IPD and FPD; however, there was a significantly higher regional PVS volume fraction in the white matter of the lateral occipital and cuneus regions in IPD compared to FPD. Both brain regions have been implicated in PD-related cognitive impairment through changes in perfusion and volume in the lateral occipital and cuneus regions, respectively.^{64,72}

Despite these observed differences, the clinical distinction between IPD and FPD remains unclear. Some reports indicate greater cognitive deficits and/or progression in familial forms such as SNCA and GBA,^{73,74} whereas others show the opposite⁷⁵⁻⁷⁷ or no clinical differences.^{78,79} Future studies are needed to better delineate any clinical differences between IPD and FPD.

Given the significant difference in PVS volume fraction between FPD and NMC, several possible hypotheses exist for the role of PVS in FPD. PVS may not be the primary physiological mechanism underlying FPD but rather a consequence of disease progression. Alternatively, PVS may be the result of a secondary insult contributing to the etiology of some familial forms of PD. Consistent with the localization of LRRK2 to the prefrontal cortex,⁶¹ PVS may also be serving as a marker for disease severity and warrants further investigation. Evidence from animal studies supports the interpretations of our findings. As part of the glymphatic system, one role of the PVS is the transport of toxic moieties, including misfolded and aggregated proteins.⁸⁰ Consequently, dysfunction of PVS may limit the clearance of toxins, such as protein aggregates, in PD.⁸¹ For example, surgical disruption of the glymphatic system in the SNCA transgenic rodent leads to increased PVS and increased SNCA protein aggregates.²² Aggregates, including SNCA, are thought to contribute to cognitive impairment in PD,^{82,83} although their exact role in PD pathophysiology is unclear. To date, the role of regional differences in SNCA accumulation across the brain and impairments in individual cognitive domains remains a gap in knowledge. PVS also plays a role in the regulation of water transport in the CNS through its interactions with aquaporin-4 (AQP4) that are primarily localized to astrocytic end feet.^{84,85} A number of studies in both PD and animal models of dopamine depletion have reported dysfunction of AQP4 in terms of its expression and localization.⁸⁶⁻⁹¹ Finally, the changes in PVS volume fraction may also reflect the changes in the integrity of the BBB. For example, an MRI study in PD showed increased BBB leakage in the midbrain.⁹² Animal studies with 6-hydroxydopamine lesioning have shown increased PVS and reduced tight junction protein expression, indicating reduced adhesion between endothelial cells.⁹³ Future studies are needed to elucidate the mechanisms underlying PVS in PD and may begin to illuminate its involvement in cognitive pathology and disease progression. The PVS may also serve as a potential therapeutic target, because dilation of PVS may be reversible.

There are several methodological limitations to our study. (1) PVS identification was suboptimal, given the relatively low resolution of the T1-weighted images and the absence of 3D T2-weighted images²⁷; and (2) subject motion and non-PVS white matter hyperintensities negatively impacted the certainty of the segmented PVS. To

address these limitations, we applied conventional preprocessing, including artifact correction and noise removal, and utilized FLAIR images to remove misclassified PVS. More specific acquisition strategies and higher-resolution scans are required to be sensitive to a larger number of PVS and not only visible large-caliber PVS.⁹⁴ Future studies of quantitative characteristics of PVS using noninvasive techniques, such as diffusion MRI, may be used to obtain more specificity.⁹⁵⁻⁹⁷

(3) Another limitation of our study is grouping of all forms of FPD in a single group, due to small sample size. Although we were able to perform follow-up analysis with LRRK2, both GBA and SNCA had group sizes too small to consider individually. We do not yet know if there are shared mechanisms among these different familial forms. Future studies should consider each genetic subtype of PD individually as they relate to PVS in both NMC and symptomatic patients with PD. However, a strength of this study is that even with grouping subtypes of FPD, we were able to detect significant differences between FPD and NMC, as well as between LRRK2 FPD and NMC, that may provide insight into the cause of the disease. (4) Finally, given that subjects in this study were not cognitively impaired, future studies should explore how cortical and subcortical white matter PVS correlates with disease progression, including cognitive function.

Conclusions

Our findings support a role of PVS in PD, particularly in FPD and PD individuals with LRRK2 mutations. Further studies will determine if changes in PVS play a role as a secondary insult or are a consequence of disease.

PPMI Investigators

Steering committee: Vanessa Arnedo, Adrienne Clark, Mark Fraiser, Catherine Kopil, Sohini Chowdhury, and Todd Sherer (The Michael J. Fox Foundation for Parkinson's Research, New York, NY, USA). Study core centers: Nichole Daegele, Christina Salerno (Institute for Neurodegenerative Disorders, New Haven, CT, USA); Cynthia Casaceli, Ray Dorsey, Renee Wilson, and Sugi Mahes (Clinical Trials Coordination Center, University of Rochester, Rochester, NY, USA); Karen Crawford (LONI, University of Southern California, Los Angeles, CA, USA); Paola Casalin and Giulia Malferrari (BioRep, Milan, Italy); Mali GaniWeisz, Avi Orr-Urtreger (Tel Aviv Medical Center, Tel Aviv, Israel); Thomas Montine (Stanford University, Stanford, CA, USA); and Chris Baglieri and Amanda Christini (Blackfynn, Philadelphia, PA, USA).

Site investigators: David Russell (Institute for Neurodegenerative Disorders, New Haven, CT, USA); Nabila Dahodwala (University of Pennsylvania, Philadelphia, PA, USA); Nir Giladi (Tel Aviv Medical Center, Tel Aviv, Israel); Stewart Factor (Emory University of Medicine Atlanta, GA, USA); Penelope Hogarth (Oregon Health and Science University, Portland, OR, USA); David Standaert (University of Alabama at Birmingham, Birmingham, AL, USA); Robert Hauser (University of South Florida, Tampa, FL, USA); Joseph Jankovic (Baylor College of Medicine, Houston, TX, USA); Marie Saint-Hilaire (Boston University, Boston, MA, USA); Irene Richard (University of Rochester, Rochester, NY, USA); David Shprecher (Banner Research Institute, Sun City, AZ, USA); Hubert Fernandez (Cleveland Clinic, Cleveland, OH, USA); Katrina Brockmann (University of Tübingen, Tübingen, Germany); Liana Rosenthal (Johns Hopkins University, Baltimore, MD, USA); Paolo Barone (University of Salerno, Salerno, Italy); Alberto Espay (University of Cincinnati, Cincinnati, OH, USA); Dominic Rowe (Macquarie University, Sydney, Australia); Karen Marder (Columbia University, New York, NY, USA); Anthony Santiago (The Parkinson's Institute, Sunnyvale, CA, USA); Shu-Ching Hu (University of Washington, Seattle, WA, USA); Stuart Isaacson (Parkinson's Disease and Movement Disorders Center of Boca Raton, Boca Raton, FL, USA); Jean-Christophe Corvol (Hospital Pitié-Salpêtrière, Paris, France); Javier Ruiz Martinez (Hospital Donostia, San Sebastian, Spain); Eduardo Tolosa (Hospital Clínic de Barcelona, Barcelona, Spain); Yen Tai (Imperial College London, London, UK); and Marios Politis (King's College London, London, UK).

Coordinators: Debra Smejdin and Linda Rees (Institute for Neurodegenerative Disorders, New Haven, CT, USA); Karen Williams (University of Iowa, Iowa City, IA, USA); Farah Kausar (University of California, San Francisco, CA, USA); Karen Williams (Northwestern University, Chicago, IL, USA); Whitney Richardson (University of Pennsylvania, Philadelphia, PA, USA); Diana Willeke (Paracelsus-Elena Klinik, Kassel, Germany); Shawnees Peacock (University of California, San Diego, CA, USA); Barbara Sommerfeld (Emory University of Medicine, Atlanta, GA, USA); Alison Freed (Oregon Health and Science University, Portland, OR, USA); Katrina Wakeman (University of Alabama at Birmingham, Birmingham, AL, USA); Courtney Blair (University of South Florida, Tampa, FL, USA); Stephanie Guthrie (Baylor College of Medicine, Houston, TX, USA); Leigh Harrell (University of South Florida, Tampa, FL, USA); Christine Hunter (Baylor College of Medicine, Houston, TX, USA); Cathi-Ann Thomas and Raymond James (Boston University, Boston, MA, USA); Grace Zimmerman (University of Rochester, Rochester, NY, USA); Victoria Brown

(Banner Research Institute, Sun City, AZ, USA); Jennifer Mule (Cleveland Clinic, Cleveland, OH, USA); Ella Hilt (University of Tübingen, Tübingen, Germany); Kori Ribb (Johns Hopkins University, Baltimore, MD, USA); Susan Ainscough (University of Salerno, Salerno, Italy); Misty Wethington (University of Cincinnati, Cincinnati, OH, USA); Madelaine Ranola (Macquarie University, Sydney, Australia); Helen Mejia Santana (Columbia University, New York, NY, USA); Juliana Moreno (University of Washington, Seattle, WA, USA); Deborah Raymond (Beth Israel Medical Center, New York, NY, USA); Krista Spekter (University of Washington, Seattle, WA, USA); Lisbeth Carvajal Parkinson's Disease and Movement Disorders Center of Boca Raton, Boca Raton, FL, USA); Stephanie Carvalo (Hospital Pitie-Salpetriere, Paris, France); Ioana Croitoru (Hospital Donostia, San Sebastian, Spain); Alicia Garrido (Hospital Clínic de Barcelona, Barcelona, Spain); and Laura Marie Payne (Imperial College London, London, UK).

Industry and Scientific Advisory Board: Veena Viswanth and Lawrence Severt (Allergan, Dublin, Ireland); Maurizio Facheris and Holly Soares (AbbVie, North Chicago, IL, USA); Mark A. Mintun (Avid Radiopharmaceuticals, Inc., Philadelphia, PA, USA); Jesse Cedarbaum (Biogen Idec, Cambridge, MA, USA); Peggy Taylor (BioLegend, Dedham, MA); Kevin Biglan (Eli Lilly and Company, Indianapolis, IN, USA); Emily Vandenbroucke and Zul qar Haider Sheikh (GE Healthcare, Princeton, NJ, USA); Baris Bingol (Genentech, San Francisco, CA, USA); Tanya Fischer, Pablo Sardi, and Remi Forrat (Genzyme Sano, Cambridge, MA, USA); Alastair Reith (GlaxoSmithKline, Brentford, UK); Jan Egebjerg and Gabrielle Ahlberg Hillert (H Lundbeck A/S, Copenhagen, Denmark); Barbara Saba (Institut de Recherches Internationales Servier, Neuilly-sur-Seine, France); Chris Min (Merck and Co., Kenilworth, NJ, USA); Robert Umek (Meso Scale Diagnostics, Rockville, MD, USA); Joe Mather (P zer Inc., Cambridge, MA, USA); Susan De Santi (Piramal Group, Mumbai, India); Anke Post, Frank Boess, and Kirsten Taylor (Hoffmann-La Roche Limited, Basel, Switzerland); Igor Grachev (Teva Pharmaceutical Industries, Petah Tikva, Israel); Andreja Avbersek and Pierandrea Muglia (UCB Pharma, Brussels, Belgium); Kaplana Merchant (TransThera Consulting, Portland, OR, USA); and Johannes Tauscher (Takeda, Osaka, Japan).

Acknowledgments: PPMI (a public-private partnership) is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including AbbVie, Allergan, Avid Radiopharmaceuticals, Biogen, BioLegend, Bristol-Myers Squibb, Celgene, Denali, GE Healthcare, Genentech, GlaxoSmithKline, Lilly, Lundbeck, Merck, Meso Scale Discovery, Pfizer, Piramal, Prevail Therapeutics, Roche, Sano Genzyme, Servier, Takeda, Teva, UCB, Verily, Voyager Therapeutics, and Golub Capital. Support provided by the Michael J Fox foundation, grant MJFF-006340, entitled "MJFF Bioinformatics: Infrastructure, data aggregation, distribution, and compliance activities in Parkinson's Progression Markers Initiative (PPMI) and complementary research efforts". We would also like to

acknowledge Bobby and Lisa Yazdani for establishing the Parivash Yazdani Parkinson's Research Fund, to speed up the process of discovery. Data used in the preparation of this article were obtained from the PPMI database (www.ppmi-info.org/data). Up-to-date information on the study is available at www.ppmi-info.org.

References

- Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. The Glymphatic system: a Beginner's guide. *Neurochem Res* 2015;40(12):2583–2599.
- Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A Paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Sci Transl Med* 2012;4(147):147ra111.
- Eide PK, Vatnehol SAS, Emblem KE, et al. Magnetic resonance imaging provides evidence of glymphatic drainage from human brain to cervical lymph nodes. *Sci Rep* 2018;8:7194. <https://doi.org/10.1038/s41598-018-25666-4>.
- Ringstad G, Valnes LM, Dale AM, Pripp AH, Vatnehol S-AS, Emblem KE, et al. Brain-wide glymphatic enhancement and clearance in humans assessed with MRI. *JCI Insight* [Internet]. 2018;3(13): <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6124518/>
- Park YW, Shin N-Y, Chung SJ, Kim J, Lim SM, Lee PH, et al. Magnetic resonance imaging-visible perivascular spaces in basal ganglia predict cognitive decline in Parkinson's disease. *Mov Disord* 2019;34(11):1672–1679.
- Rudie JD, Rauschecker AM, Nabavizadeh SA, Mohan S. Neuroimaging of dilated perivascular spaces: from benign and pathologic causes to mimics. *J Neuroimaging* 2018;28(2):139–149.
- Kwee RM, Kwee TC. Virchow-Robin spaces at MR imaging. *RadioGraphics* 2007;27(4):1071–1086.
- Taber KH, Shaw JB, Loveland KA, Pearson DA, Lane DM, Hayman LA. Accentuated Virchow-Robin spaces in the centrum semiovale in children with autistic disorder. *J Comput Assist Tomogr* 2004;28(2):263–268.
- Patankar TF, Mitra D, Varma A, Snowden J, Neary D, Jackson A. Dilatation of the Virchow-Robin space is a sensitive indicator of cerebral microvascular disease: study in elderly patients with dementia. *Am J Neuroradiol* 2005;26(6):1512–1520.
- Yao M, Hervé D, Jouvent E, Duering M, Reyes S, Godin O, et al. Dilated perivascular spaces in small-vessel disease: a study in CADASIL. *Cerebrovasc Dis Basel Switz* 2014;37(3):155–163.
- Booth HDE, Hirst WD, Wade-Martins R. The role of astrocyte dysfunction in Parkinson's disease pathogenesis. *Trends Neurosci* 2017;40(6):358–370.
- Ding J, Sigurdsson S, Jónsson PV, Eiriksdóttir G, Charidimou A, Lopez OL, et al. Large perivascular spaces visible on magnetic resonance imaging, cerebral small vessel disease progression, and risk of dementia: the age, gene/environment susceptibility–Reykjavik study. *JAMA Neurol* 2017;74(9):1105–1112.
- Debette S, Schilling S, Duperron M-G, Larsson SC, Markus HS. Clinical significance of magnetic resonance imaging markers of vascular brain injury: a systematic Review and meta-analysis. *JAMA Neurol* 2019;76(1):81–94.
- Zhu Y-C, Dufouil C, Soumaré A, Mazoyer B, Chabriat H, Tzourio C. High degree of dilated Virchow-Robin spaces on MRI is associated with increased risk of dementia. *J Alzheimers Dis* 2010;22:663–672.
- Ramirez J, Berezuk C, McNeely AA, Scott CJM, Gao F, Black SE. Visible Virchow-Robin spaces on magnetic resonance imaging of Alzheimer's disease patients and Normal elderly from the Sunnysbrook dementia study. *J Alzheimers Dis* 2015;43(2):415–424.
- Banerjee G, Kim HJ, Fox Z, Jäger HR, Wilson D, Charidimou A, et al. MRI-visible perivascular space location is associated with Alzheimer's disease independently of amyloid burden. *Brain J Neurol* 2017;140(4):1107–1116.
- Jeong EH, Lee YJ, Kim SJ, Lee J-H. Is the severity of dilated Virchow-Robin spaces associated with cognitive dysfunction? *Dement Neurocognitive Disord* 2015;14(3):114–119.
- Renske U, Marjolein H, Julie S, Annelien D, Ed G, Kroon Abraham A, et al. Subjective cognitive failures in patients with hypertension are related to cognitive performance and cerebral microbleeds. *Hypertension* 2014;64(3):653–657.
- Del Tredici K, Braak H. Review: Sporadic Parkinson's disease: development and distribution of α -synuclein pathology. *Neuropathol Appl Neurobiol* 2016;42(1):33–50.
- Tremblay M-E, Cookson MR, Civiero L. Glial phagocytic clearance in Parkinson's disease. *Mol Neurodegener* 2019;14(1):16.
- Henry AG, Aghamohammadzadeh S, Samaroo H, Chen Y, Mou K, Needle E, et al. Pathogenic LRRK2 mutations, through increased kinase activity, produce enlarged lysosomes with reduced degradative capacity and increase ATP13A2 expression. *Hum Mol Genet* 2015;24(21):6013–6028.
- Zou W, Pu T, Feng W, Lu M, Zheng Y, Du R, et al. Blocking meningeal lymphatic drainage aggravates Parkinson's disease-like pathology in mice overexpressing mutated α -synuclein. *Transl Neurodegener* 2019;8(1):7.
- Schiesling C, Kieper N, Seidel K, Krüger R. Review: familial Parkinson's disease – genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. *Neuropathol Appl Neurobiol* 2008;34(3):255–271.
- Anheim M, Elbaz A, Lesage S, Durr A, Condroyer C, Viallet F, et al. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. *Neurology* 2012;78(6):417–420.
- Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol* 2008;7(7):583–590.
- Wilson H, Niccolini F, Pellicano C, Politis M. Cortical thinning across Parkinson's disease stages and clinical correlates. *J Neurol Sci* 2019;398:31–38.
- Sepehrband F, Barisano G, Sheikh-Bahaei N, Cabeen RP, Choupan J, Law M, et al. Image processing approaches to enhance perivascular space visibility and quantification using MRI. *Sci Rep* 2019;26(9):1, 12351.
- Fischl B. FreeSurfer. *NeuroImage* 2012;62(2):774–781.
- Dinov I, Lozev K, Petrosyan P, Liu Z, Eggert P, Pierce J, et al. Neuroimaging study designs, computational analyses and data provenance using the LONI pipeline. *PLoS One* 2010;5(9):e13070. <https://doi.org/10.1371/journal.pone.0013070>
- Dinov ID, Van Horn JD, Lozev KM, Magsipoc R, Petrosyan P, Liu Z, et al. Efficient, distributed and interactive neuroimaging data analysis using the LONI pipeline. *Front Neuroinformatics* 2009;3:22.
- Moon SW, Dinov ID, Kim J, Zamanyan A, Hobel S, Thompson PM, et al. Structural neuroimaging genetics interactions in Alzheimer's disease. *J Alzheimers Dis* 2015;48(4):1051–1063.
- Torri F, Dinov ID, Zamanyan A, Hobel S, Genco A, Petrosyan P, et al. Next generation sequence analysis and computational genomics using graphical pipeline workflows. *Genes* 2012;3(3):545–575.
- Sta Cruz S, Dinov ID, Herting MM, González-Zacarias C, Kim H, Toga AW, et al. Imputation strategy for reliable regional MRI morphological measurements. *Neuroinformatics* 2020;18(1):59–70.
- Sepehrband F, Lynch KM, Cabeen RP, Gonzalez-Zacarias C, Zhao L, D'Arcy M, et al. Neuroanatomical morphometric characterization of sex differences in youth using statistical learning. *NeuroImage* 2018;172:217–227.
- Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* 2006;31(3):968–980.
- Fischl B, van der Kouwe A, Destrieux C, Halgren E, Ségonne F, Salat DH, et al. Automatically parcellating the human cerebral cortex. *Cereb Cortex N Y* 2004;14(1):11–22.
- Fischl B, Salat DH, van der Kouwe AJW, Makris N, Ségonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. *NeuroImage* 2004;23(Suppl 1):S69–S84.

38. Ségonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, et al. A hybrid approach to the skull stripping problem in MRI. *NeuroImage* 2004;22(3):1060–1075.
39. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage* 1999;9(2):179–194.
40. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II: inflation, flattening, and a surface-based coordinate system. *NeuroImage* 1999;9(2):195–207.
41. Ségonne F, Pacheco J, Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging* 2007;26(4):518–529.
42. Reuter M, Rosas HD, Fischl B. Highly accurate inverse consistent registration: a robust approach. *NeuroImage* 2010;53(4):1181–1196.
43. Reuter M, Fischl B. Avoiding asymmetry-induced bias in longitudinal image processing. *NeuroImage* 2011;57(1):19–21.
44. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. *NeuroImage* 2012;61(4):1402–1418.
45. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;33(3):341–355.
46. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* 2000;97(20):11050–11055.
47. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998;17(1):87–97.
48. Waters AB, Mace RA, Sawyer KS, Gansler DA. Identifying errors in Freesurfer automated skull stripping and the incremental utility of manual intervention. *Brain Imaging Behav* 2019;13(5):1281–1291.
49. Tustison NJ, Avants BB, Cook PA, Zheng Y, Egan A, Yushkevich PA, et al. N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging* 2010;29(6):1310–1320.
50. Avants B, Tustison N, Song G. Advanced normalization tools (ANTS). *Insight J* 2008;30:1–35.
51. Manjón JV, Coupé P, Martí-Bonmati L, Collins DL, Robles M. Adaptive non-local means denoising of MR images with spatially varying noise levels. *J Magn Reson Imaging* 2010;31(1):192–203.
52. Frangi AF, Niessen WJ, Vincken KL, Viergever MA. Multiscale vessel enhancement filtering. *International Conference on Medical Image Computing and Computer-Assisted Intervention*. In Springer-Verlag; 1998. p. 130–137.
53. Cabeen RP. Quantitative Imaging Toolkit: Software for Interactive 3D Visualization, Data Exploration, and Computational Analysis of Neuroimaging Datasets [Internet]. Ryan Cabeen. 2020. /about/publication/cabeen-2018-quantitative/. Accessed May 26, 2020
54. Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. Moving beyond P values: data analysis with estimation graphics. *Nat Methods* 2019;16(7):565–566.
55. Zong X, Lian C, Jimenez J, Yamashita K, Shen D, Lin W. Morphology of perivascular spaces and enclosed blood vessels in young to middle-aged healthy adults at 7T: dependences on age, brain region, and breathing gas. *NeuroImage* 2020;218:116978.
56. Beak HW, Choi HJ, Byun MS, Choe YM, Yi D, Han JY, et al. O1-08-03: prevalence of enlarged perivascular spaces in a memory clinic population. *Alzheimers Dement* 2015;11(7S_Part_3):P146.
57. Wen M-C, Xu Z, Lu Z, Chan LL, Tan EK, Tan LCS. Microstructural network alterations of olfactory dysfunction in newly diagnosed Parkinson's disease. *Sci Rep* 2017;2(7):1, 12559.
58. van der Vegt JPM, Hulme OJ, Zittel S, Madsen KH, Weiss MM, Buhmann C, et al. Attenuated neural response to gamble outcomes in drug-naïve patients with Parkinson's disease. *Brain J Neurol* 2013;136(Pt 4):1192–1203.
59. Radziunas A, Deltuva VP, Tamasauskas A, Gleizniene R, Pranckeviciene A, Petrikonis K, et al. Brain MRI morphometric analysis in Parkinson's disease patients with sleep disturbances. *BMC Neurol* 2018;18(1):88.
60. Kobayakawa M, Tsuruya N, Kawamura M. Decision-making performance in Parkinson's disease correlates with lateral orbitofrontal volume. *J Neurol Sci* 2017;372:232–238.
61. Taymans JM, Mutez E, Drouyer M, Sibrán W, Chartier-Harlin MC. LRRK2 detection in human biofluids: potential use as a Parkinson's disease biomarker? *Biochem Soc Trans* 2017;45(1):207–212.
62. Barisano G, Sheikh-Bahaei N, Law M, Toga AW, Sepehrband F. Body mass index, time of day, and genetics affect perivascular spaces in the white matter. *J Cereb Blood Flow Metab* November 2020. <https://doi.org/10.1177/0271678X20972856>.
63. Kim D, Kim JS, Jeong W, Shin M-S, Chung CK. Critical area for memory decline after mesial temporal resection in epilepsy patients. *J Neurosurg* 2020 Jan 3;1(aop):1–9.
64. Pagonabarraga J, Corcuera-Solano I, Vives-Gilabert Y, Llebaria G, García-Sánchez C, Pascual-Sedano B, et al. Pattern of regional cortical thinning associated with cognitive deterioration in Parkinson's disease. *PLoS ONE* 2013;8(1):e54980. <https://doi.org/10.1371/journal.pone.0054980>.
65. Mesulam MM. From sensation to cognition. *Brain J Neurol* 1998; 121(Pt 6):1013–1052.
66. Patterson K, Nestor PJ, Rogers TT. Where do you know what you know? The representation of semantic knowledge in the human brain. *Nat Rev Neurosci* 2007;8(12):976–987.
67. Kravitz DJ, Saleem KS, Baker CI, Mishkin M. A new neural framework for visuospatial processing. *Nat Rev Neurosci* 2011 Apr;12(4):217–230.
68. Song SK, Lee JE, Park H-J, Sohn YH, Lee JD, Lee PH. The pattern of cortical atrophy in patients with Parkinson's disease according to cognitive status. *Mov Disord* 2011;26(2):289–296.
69. Xu Y, Yang J, Hu X, Shang H. Voxel-based meta-analysis of gray matter volume reductions associated with cognitive impairment in Parkinson's disease. *J Neurol* 2016;263(6):1178–1187.
70. Valli M, Mihaescu A, Strafella AP. Imaging behavioural complications of Parkinson's disease. *Brain Imaging Behav* 2019;13(2):323–332.
71. Kim Y-D, Jeong HS, Song I-U, Chung Y-A, Namgung E, Kim Y-D. Brain perfusion alterations in depressed patients with Parkinson's disease. *Ann Nucl Med* 2016;30(10):731–737.
72. Prosser AMJ, Tossici-Bolt L, Kipps CM. Occipital lobe and posterior cingulate perfusion in the prediction of dementia with Lewy body pathology in a clinical sample. *Nucl Med Commun* 2017;38(12):1029–1035.
73. Koros C, Stamelou M, Simitsi A, Beratis I, Papadimitriou D, Papagiannakis N, et al. Selective cognitive impairment and hyposmia in p.A53T SNCA PD vs typical PD. *Neurology* 2018;90(10):e864–e869.
74. Thaler A, Gurevich T, Bar Shira A, Gana Weisz M, Ash E, Shiner T, et al. A “dose” effect of mutations in the GBA gene on Parkinson's disease phenotype. *Parkinsonism Relat Disord* 2017;36:47–51.
75. Somme JH, Molano Salazar A, Gonzalez A, Tijero B, Berganzo K, Lezcano E, et al. Cognitive and behavioral symptoms in Parkinson's disease patients with the G2019S and R1441G mutations of the LRRK2 gene. *Parkinsonism Relat Disord* 2015;21(5):494–499.
76. Saunders-Pullman R, Mirelman A, Alcalay RN, Wang C, Ortega RA, Raymond D, et al. Progression in the LRRK2-Associated Parkinson disease population. *JAMA Neurol* 2018;75(3):312–319.
77. Ben Romdhan S, Farhat N, Nasri A, Lesage S, Hdiji O, Ben Djebara M, et al. LRRK2 G2019S Parkinson's disease with more benign phenotype than idiopathic. *Acta Neurol Scand* 2018;138(5):425–431.
78. Trinh J, Amouri R, Duda JE, Morley JF, Read M, Donald A, et al. Comparative study of Parkinson's disease and leucine-rich repeat kinase 2 p. G2019S parkinsonism. *Neurobiol Aging* 2014;35(5):1125–1131.
79. Gunzler SA, Riley DE, Chen SG, Tatsuoka CM, Johnson WM, Mieyal JJ, et al. Motor and non-motor features of Parkinson's disease in LRRK2 G2019S carriers versus matched controls. *J Neurol Sci* 2018;388:203–207.

80. Boland B, Yu WH, Corti O, Mollereau B, Henriques A, Bezard E, et al. Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. *Nat Rev Drug Discov* 2018;17(9):660–688.
81. Jung JS, Bhat RV, Preston GM, Guggino WB, Baraban JM, Agre P. Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc Natl Acad Sci U S A* 1994;91(26):13052–13056.
82. Henderson MX, Trojanowski JQ, Lee VM. α -Synuclein pathology in Parkinson's disease and related α -synucleinopathies. *Neurosci Lett* 2019;709:134316. <https://doi.org/10.1016/j.neulet.2019.134316>
83. Irwin DJ, White MT, Toledo JB, et al. Neuropathologic substrates of Parkinson disease dementia. *Ann Neurol* 2012 Oct;72(4):587–598.
84. Ampawong S, Combes V, Hunt NH, Radford J, Chan-Ling T, Pongponratn E, et al. Quantitation of brain edema and localisation of aquaporin 4 expression in relation to susceptibility to experimental cerebral malaria. *Int J Clin Exp Pathol* 2011;4(6):566–574.
85. Asgari M, de Zélicourt D, Kurtcuoglu V. How astrocyte networks may contribute to cerebral metabolite clearance. *Sci Rep* 2015;5(1):15024.
86. Hoshi A, Tsunoda A, Tada M, Nishizawa M, Ugawa Y, Kakita A. Expression of aquaporin 1 and aquaporin 4 in the temporal Neocortex of patients with Parkinson's disease. *Brain Pathol Zurich Switz* 2017;27(2):160–168.
87. Thenral ST, Vanisree AJ. Peripheral assessment of the genes AQP4, PBP and TH in patients with Parkinson's disease. *Neurochem Res* 2012;37(3):512–515.
88. Xue X, Zhang W, Zhu J, Chen X, Zhou S, Xu Z, et al. Aquaporin-4 deficiency reduces TGF- β 1 in mouse midbrains and exacerbates pathology in experimental Parkinson's disease. *J Cell Mol Med* 2019;23(4):2568–2582.
89. Sun H, Liang R, Yang B, Zhou Y, Liu M, Fang F, et al. Aquaporin-4 mediates communication between astrocyte and microglia: implications of neuroinflammation in experimental Parkinson's disease. *Neuroscience* 2016;317:65–75.
90. Zhang J, Yang B, Sun H, Zhou Y, Liu M, Ding J, et al. Aquaporin-4 deficiency diminishes the differential degeneration of midbrain dopaminergic neurons in experimental Parkinson's disease. *Neurosci Lett* 2016;614:7–15.
91. Chi Y, Fan Y, He L, Liu W, Wen X, Zhou S, et al. Novel role of aquaporin-4 in CD4+ CD25+ T regulatory cell development and severity of Parkinson's disease. *Aging Cell* 2011;10(3):368–382.
92. Kortekaas R, Leenders KL, van Oostrom JCH, Vaalburg W, Bart J, Willemsen ATM, et al. Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. *Ann Neurol* 2005;57(2):176–179.
93. Wachter B, Schürger S, Schmid A, Gröger A, Sadler R, Speidel A, et al. 6-Hydroxydopamine leads to T2 hyperintensity, decreased claudin-3 immunoreactivity and altered aquaporin 4 expression in the striatum. *Behav Brain Res* 2012;232(1):148–158.
94. Zong X, Park SH, Shen D, Lin W. Visualization of perivascular spaces in the human brain at 7T: sequence optimization and morphology characterization. *NeuroImage* 2016;125:895–902.
95. Sepehrband F, Cabeen RP, Choupan J, Barisano G, Law M, Toga AW, et al. Perivascularspace fluid contributes to diffusion tensor imaging changes in white matter. *NeuroImage* 2019;197:243–254.
96. Sepehrband F, Cabeen RP, Barisano G, Sheikh-Bahaei N, Choupan J, Law M, et al. Nonparenchymal fluid is the source of increased mean diffusivity in preclinical Alzheimer's disease. *Alzheimers Dement Amst Neth* 2019;11:348–354.
97. Ballerini L, Booth T, Valdés Hernández MDC, Wiseman S, Lovreglio R, Muñoz Maniega S, et al. Computational quantification of brain perivascular space morphologies: associations with vascular risk factors and white matter hyperintensities. A study in the Lothian birth cohort 1936. *NeuroImage Clin* 2020;25:102120.

Cognition and motor learning in a Parkinson's disease cohort: importance of recall in episodic memory

Yu-Chen Chung^a, Beth E. Fisher^a, James M. Finley^a, Aram Kim^a, Andrew J. Petkus^b, Dawn M. Schiehser^c, Michael W. Jakowec^{b,*} and Giselle M. Petzinger^{b,*}

Impaired motor learning in individuals with Parkinson's disease is often attributed to deficits in executive function, which serves as an important cognitive process supporting motor learning. However, less is known about the role of other cognitive domains and its association with motor learning in Parkinson's disease. The objective of this study was to investigate the associations between motor learning and multiple domains of cognitive performance in individuals with Parkinson's disease. Twenty-nine participants with Parkinson's disease received comprehensive neuropsychological testing, followed by practice of a bimanual finger sequence task. A retention test of the finger sequence task was completed 24 h later. Hierarchical linear regressions were used to examine the associations between motor learning (acquisition rate and retention) and cognitive performance in five specific cognitive domains, while controlling for age, sex, and years of Parkinson's disease diagnosis. We found that a higher acquisition rate was associated with better episodic memory, specifically better recall in visual episodic memory, in individuals with Parkinson's disease.

No significant associations were observed between retention and cognitive performance in any domains. The association between motor acquisition and episodic memory indicates an increased dependency on episodic memory as a potential compensatory cognitive strategy used by individuals with Parkinson's disease during motor learning. *NeuroReport* 32: 1153–1160 Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.

NeuroReport 2021, 32:1153–1160

Keywords: cognitive performance, executive function, memory, motor skill acquisition, Parkinson's disease.

^aDivision of Biokinesiology and Physical Therapy, ^bDepartment of Neurology, University of Southern California, Los Angeles and ^cPsychology and Research Services, Veterans Administration San Diego Healthcare System (VASDHS), San Diego, California, USA

Correspondence to Michael W. Jakowec, PhD, Department of Neurology, University of Southern California, 1333 San Pablo St, MCA-241, Los Angeles, CA 90033, USA
Tel: + 323 442 3367; e-mail: mjakowec@surgery.usc.edu

*Michael W. Jakowec and Giselle M. Petzinger are co-senior authors.

Received 26 May 2021 Accepted 22 June 2021

Introduction

Parkinson's disease is a neurodegenerative disorder characterized by motor deficits, including progressive gait and balance dysfunction, leading to significant disability and poor quality of life. While motor deficits predominate, individuals with Parkinson's disease also manifest impairments in cognition [1]. Neurorehabilitation has emerged as a promising intervention in the treatment of Parkinson's disease. The effectiveness of neurorehabilitation, such as gait and balance training, depends on an individual's ability to both acquire and retain a motor skill, referred to as motor learning. In healthy individuals, multiple cognitive processes have been shown to contribute to motor learning [2]. Compared to age-matched healthy persons, individuals with Parkinson's disease demonstrate impaired motor learning, including a slower acquisition rate and poor retention [3–5], which may result from deficits in cognitive function, especially executive function.

Executive function impairment is common in Parkinson's disease [1]. In healthy individuals, executive function

plays an important role in motor learning, incorporating aspects of planning and information processing [2]. In Parkinson's disease, deficits in executive function have been proposed to underlie impairments in the skill acquisition phase of motor learning [6,7]. To compensate for impaired motor skill acquisition, evidence suggests that individuals with Parkinson's disease may implement explicit strategies including the recruitment of alternative cognitive domains to facilitate motor learning [8,9]. However, the alternative cognitive domains (attention, episodic memory, or visuospatial function) that may be recruited during motor skill acquisition in Parkinson's disease remains a gap in knowledge.

With respect to the consolidation phase of motor learning, few Parkinson's disease studies have examined the association between motor skill retention and cognitive performance in Parkinson's disease [10,11]. For example, Lin *et al.* (2007) reported no association with executive function, while Pendt *et al.* (2011) described that individuals with Parkinson's disease with low global cognitive performance, measured by Mini-Mental Status

Examination, had poor retention performance [10,11]. Taken together, the associations between the different phases of motor skill learning and cognitive performance in individuals with Parkinson's disease remains relatively unexplored.

We hypothesize that in individuals with Parkinson's disease, the early acquisition and retention of motor learning is associated with cognitive performance in multiple domains, including attention, visuospatial function, episodic memory, language and executive function. Elucidating these associations may provide insight towards potential compensatory cognitive processes used to facilitate motor learning, as well as optimal rehabilitation strategies to improve the overall function and quality of life in individuals with Parkinson's disease. In this study, we used the rate in which the skill was acquired (acquisition rate) and retention performance to quantify the two phases of motor skill learning, in individuals with Parkinson's disease who also underwent comprehensive neuropsychological testing.

Methods

Participants

Participants were 39 nondemented individuals with Parkinson's disease recruited from movement disorders clinics, the Michael J Fox trial finder, radio advertisements, and support group talks in the community. Inclusion criteria for eligible participants were (1) idiopathic Parkinson's disease according to the United Kingdom Parkinson's Disease Society Brain Bank criteria, (2) age 50 and above, (3) Hoehn and Yahr less than 3, (4) stable and optimized on medications longer than 60 days, and (5) able to provide consent. Exclusion criteria included (1) individuals who meet formal criteria of Parkinson's disease dementia [12], (2) moderate or severe depression using the Geriatric Depression scale [13], or (3) clinically significant medical or psychiatric illness. All participants signed an informed consent approved by the Institutional Review Board of the University of Southern California according to the Declaration of Helsinki.

Study design

After enrolment, participant demographics, including age, sex, education, and years since diagnosis were obtained. Participants completed the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) assessed by a board-certified neurologist (G.P.). A complete neuropsychological battery was completed followed by practice of a finger sequence task. On the subsequent day, participants completed a 24-h finger sequence retention test (Fig. 1).

Measures

Neuropsychological battery

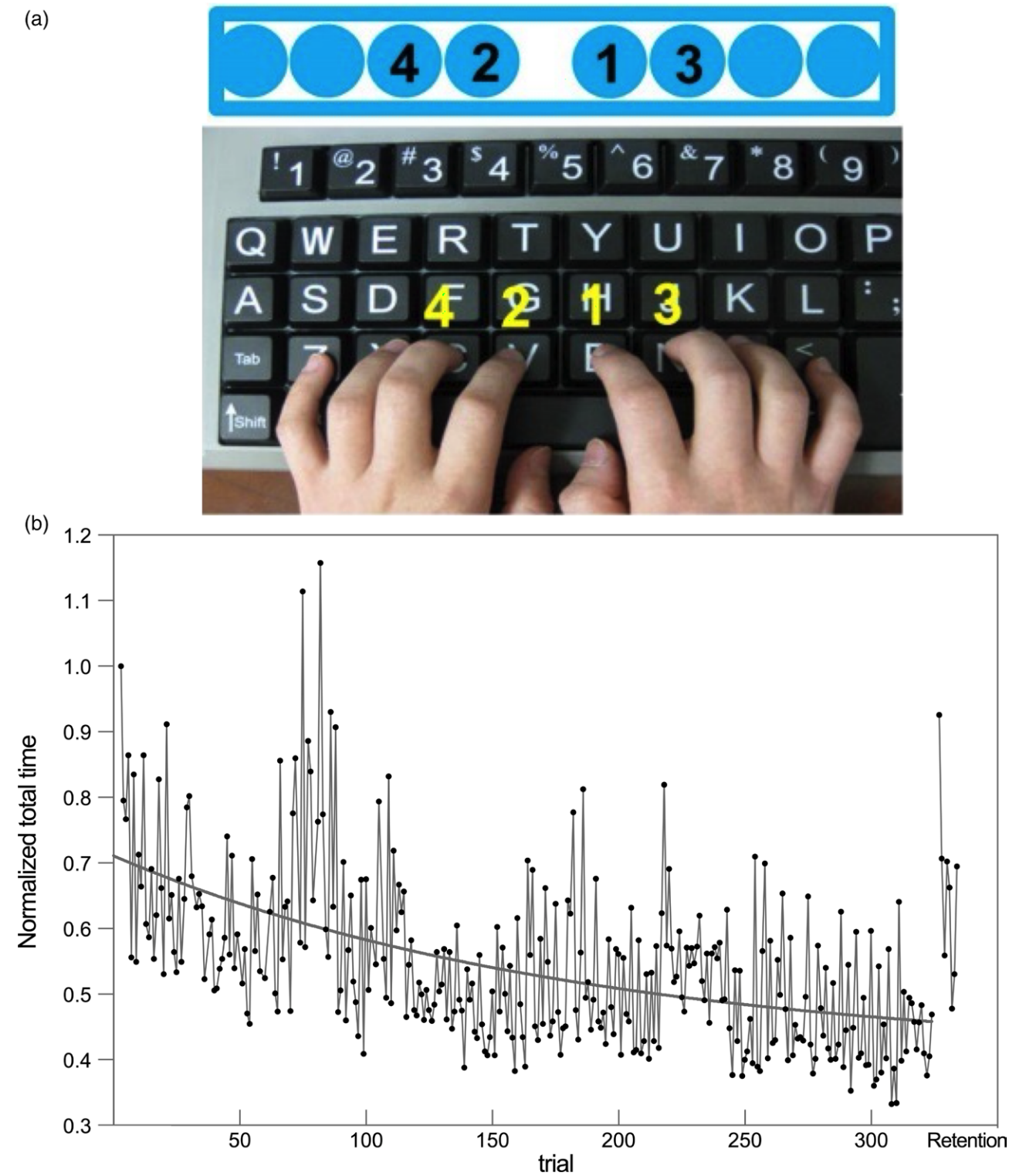
A validated neuropsychological battery was used to examine performance of cognitive specific domains including

executive function, attention, episodic memory (both learning and recall components), visuospatial function, and language [14]. Executive function was measured using (1) the Delis Kaplan Executive Function System (D-KEFS) [15], Color Word Interference Test (CWIT), with the number of seconds to complete the color-word inhibition subtest, (2) the number of seconds to complete the inhibition/switching subtest from the D-KEFS CWIT, and (3) the number of perseverative errors on the Wisconsin Card Sorting Test. Attention was measured by (1) the number of seconds to complete the color naming and word reading subtests of the D-KEFS CWIT and (2) the total score for the digit sequencing condition of the Adaptive Digit Ordering Test [16]. The learning component of episodic memory was assessed by (1) word learning measured using the total number of correct words recalled across the five learning trials of the California Verbal Learning Test – Second Edition (CVLT-II) [17], (2) the verbal learning slope defined as the difference in total number of correct words recalled between the first and fifth learning trial of CVLT-II, and (iii) the total points on the immediate recall subtest of the Wechsler Memory Scale – Third Edition Visual Reproduction test (WMS-III) [18]. The recall component of episodic memory was assessed by (1) the total number of words recalled on the long-delayed free recall condition of the CVLT-II, and (2) the total of points on the delayed recall condition of the WMS-III Visual Reproduction test. Visuospatial function was measured by (1) the total raw score on the Judgment of Line Orientation Test [19] and (2) the total raw score on the Hooper Visual Organization Test [20]. Language ability was measured by (1) the total correct number of words on the D-KEFS Category Fluency test, (2) the D-KEFS Letter Fluency test, and (3) the Similarities subtest from Wechsler Adult Intelligence Scale-4 [21]. Composite scores were created to capture performance within each domain. The raw scores of cognitive scales were first transformed to z scores based on the sample mean and SD with a greater z score representing better performance. The composite scores for each domain were calculated by averaging the z scores of individual cognitive scales included in each domain.

Finger sequence task

A finger sequence task was presented to each participant on a computer screen which they were required to replicate on a regular keyboard. Motor learning was measured with a bilateral finger sequence task consisting of three four-element number sequences using the index and middle fingers of both hands [22] (Fig. 1). Practice for each participant consisted of a total of 324 trials (i.e. 108 trials per sequence). The order of the three sequences were presented in a pseudo-random fashion such that different sequences were presented in adjacent trials. Participants were instructed to press the keys as accurately and quickly as possible. For each trial, the complete sequence was presented at one time. The sequence

Fig. 1



(a) Study procedure and finger sequence task, including the retention test; (b) the exponential decay equation result using normalized total time in a representative participant.

then disappeared from the computer screen upon the first key pressed. Feedback was provided after each trial and consisted of (1) confirmation of whether the response was correct or not and (2) total time to complete the sequence.

To examine retention performance, participants returned 24h later and performed a retention test consisting of nine trials. For each trial, a single sequence was displayed and then disappeared upon the first key pressed, but no feedback was provided after completion of each trial. Each sequence was presented in a pseudo-random fashion up to three times.

Motor learning parameters

The total time required for each trial was calculated as the time from the initial presentation of the number sequence to the final key press. Only accurate trials, defined as trials when the four keys were pressed in the correct sequence, were used for analysis. In this study, we aimed to investigate the associations between cognitive performance and motor learning in those individuals who were able to perform the finger sequence task successfully and achieved motor skill acquisition by showing improvement during practice. Thus, participants with low performance accuracy (<70%) throughout practice were considered not being able to perform the task with sufficient accuracy and were excluded [23]. For each participant, the total time of each trial was normalized to each individual's baseline performance (i.e. the total time of the first accurate trial) to account for individual differences in movement time. Accuracy was not included in calculating motor learning parameters, because (1) the included participants had high averaged accuracy across practice trials (90%) and (2) the group mean accuracy plateaued early, by trial 15, based on the observation of trial-by-trial data.

Two motor learning parameters were measured including (1) acquisition rate, defined as the speed of improvement during the practice of a motor skill and (2) retention, defined as the amount of improvement of a motor skill that persists after a period of time from the completion of practice [24].

Acquisition rate

Acquisition rate was used to characterize the time course over which participants reduced the time required to perform the finger sequences. Acquisition rate was estimated by fitting an exponential decay function (Equation 1) to the total time data using fmincon function in Matlab [25,26]:

$$y = A + Be^{-\alpha N} \quad (1)$$

In this equation, A is the estimated total time for executing each sequence upon reaching a plateau in performance, B is the overall improvement in total time across practice trials, α is the acquisition rate, and N is trial number. Acquisition rate quantifies the number of trials

Table 1 Participant demographics and clinical characteristics

Mean (SD)	Participants (n=29)
Demographics	
Age	63.8 (8.4)
Sex (male: female)	18:11
Education (years)	17.0 (1.9)
Years since diagnosis	5.4 (3.6)
Levodopa equivalent dose	625.5 (370.0)
MDS-UPDRS III ^a	58.1 (9.0)
Hoehn and Yahr stage (2:3) ^a	26:2
Cognitive performance	
Executive function	
D-KEFS color word test color-word inhibition (↓)	62.7 (14.8)
D-KEFS color word test color-word inhibition/switch (↓)	69.3 (26.4)
WCST perseverative errors (↓)	7.0 (10.2)
Attention	
Adaptive digit ordering test digit sequencing condition (↑)	6.8 (2.4)
D-KEFS color word test color naming and word reading (↓)	33.2 (5.1)
Learning in episodic memory	
CVLT-II total number of correct words (trials 1–5) (↑)	43.4 (12.4)
CVLT-II learning slope (change between trials 1 and 5) (↑)	5.9 (2.4)
WMS-III immediate recall subtest (↑)	76.0 (21.4)
Recall in episodic memory	
CVLT-II long delayed free recall condition (↑)	9.1 (3.5)
WMS-III delayed free recall condition (↑)	53.4 (23.0)
Visuospatial function	
Judgment of line orientation (↑)	24.2 (4.0)
Hooper visual organization (↑)	23.4 (3.4)
Language	
D-KEFS category fluency test (↑)	40.4 (9.5)
D-KEFS letter fluency test (↑)	42.0 (11.5)
WAIS-IV similarities subtest (↑)	29.1 (4.4)
Motor learning parameters	
Baseline performance (total time of first accurate trial, ms)	3450.8 (1450.9)
Acquisition rate/curve fitting parameters	
α (Exponential acquisition rate)	0.057 (0.068)
B	0.431 (0.157)
A	0.559 (0.158)
R^2	0.294 (0.178)
Retention performance	−0.232 (0.205)

Value is reported as mean (SD).

CVLT-II, California Verbal Learning Test – Second Edition; D-KEFS, Delis Kaplan Executive Function System; MCI, mild cognitive impairment; MDS-UPDRS III, Movement Disorder Society Unified Parkinson's Disease Rating Scale-III motor examination; WAIS-IV, Wechsler Adult Intelligence Scale-4; WCST, Wisconsin Card Sorting Test; WMS-III, Wechsler Memory Scale – Third Edition Visual Reproduction test. (↓)=lower better; (↑)=higher better.

^aMDS-UPDRS III and Hoehn and Yahr stage data were missing in one participant.

Table 2 Standardized beta estimates of the association between motor learning and cognitive composite z scores

Cognitive composite z score	Ln (α)		Retention performance	
	Standardized coefficient β	P value	Standardized coefficient β	P value
Executive function	0.15	0.52	0.03	0.89
Attention	0.27	0.21	0.22	0.31
Learning in episodic memory	0.36	0.12	0.25	0.27
Recall in episodic memory	0.50*	0.04	0.07	0.78
Visuospatial function	0.18	0.37	0.22	0.26
Language	0.30	0.14	0.17	0.40

Ln (α), log-transformed exponential acquisition rate; All regression coefficients were adjusted for age, sex and years since diagnosis. The composite score was the average of z score for each cognitive domain. The z score was calculated based on the sample mean and SD of 39 participants.

*Significant correlations.

required for participants to achieve approximately 63% of the performance improvement that would be observed once learning has plateaued. The 95% confidence interval (CI) for acquisition rate α was calculated using `lsqcurvefit` and `nlparci` functions in Matlab. Individuals whose 95% CI overlapped with zero were excluded from further analysis since this indicated that they did not exhibit a meaningful amount of learning.

Retention

Retention was defined as the difference in total time between the average of the retention trials (TT_{ret}) and the average of the last nine practice trials (TT_{end}^{ret}), normalized by the average of the last nine practice trials (Equation 2).

$$Ret = \frac{TT_{ret} - TT_{end}^{ret}}{TT_{end}^{ret}} \quad (2)$$

Statistical analysis

Demographic data, cognitive scores, and motor learning parameters were reported as averages and SDs. Participants whose acquisition rate or retention performance was greater or less than the group mean by 3 SDs were considered outliers and excluded from analysis. All 39 participants completed the practice and retention sessions for the finger sequence task. Ten participants were excluded from the final analysis due to poor accuracy ($<70\%$; $n=3$), little or no change in total time throughout practice resulting in a nonsignificant fit from exponential decay function ($n=6$), or outliers for retention performance with respect to the group mean below group mean by more than 3 SDs ($n=1$). Difference in demographics

and cognitive performance between the participants included and excluded in the analysis were compared using independent t -test and Mann-Whitney U test.

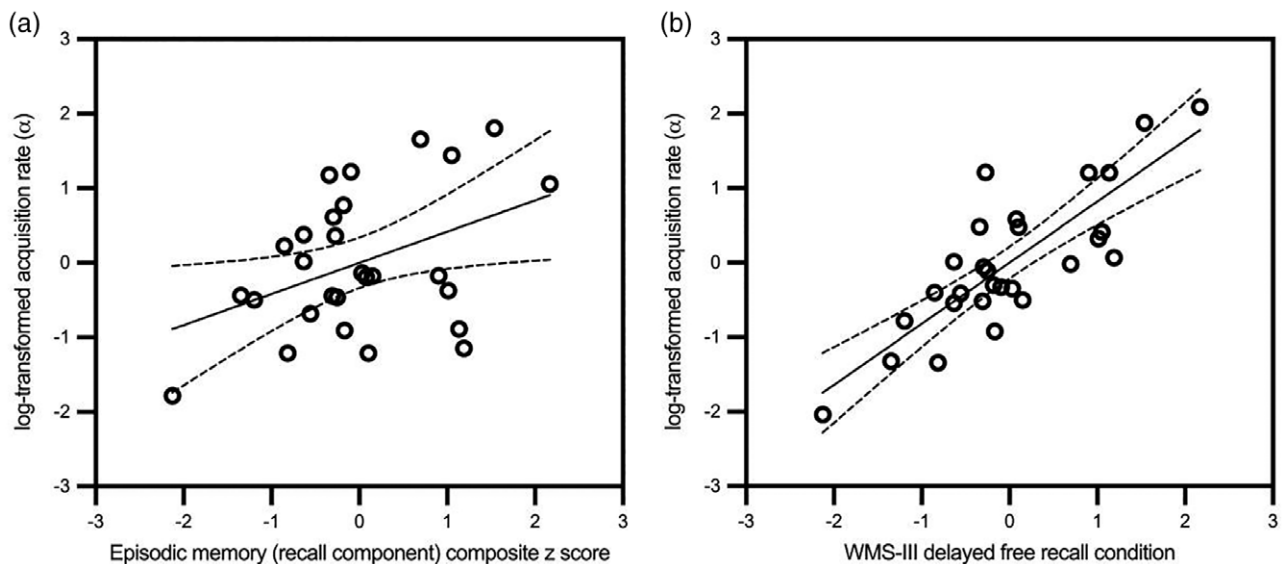
For the 29 Parkinson's disease participants included in the final analysis, Spearman's correlation was used to investigate the bivariate association between acquisition rate and retention performance. Since the acquisition rate was not normally distributed, a log transformed version of the acquisition rate was used as the dependent variable in the following regression analyses. Hierarchical linear regressions were used to examine the association (1) between the log-transformed acquisition rate and domain-specific cognitive performance using the composite scores for each cognitive domain, including executive function, attention, episodic memory (learning and recall components), visuospatial function, and language, and (2) between retention performance and domain-specific cognitive performance, while controlling for age, sex, and years of Parkinson's disease diagnosis. As an exploratory analysis, we examined the associations between the log-transformed acquisition rate, as well as retention performance and raw scores of individual domain-specific cognitive scales, while controlling for age, sex, and years of Parkinson's disease diagnosis. All statistical analyses were performed using SPSS Version 26 (SPSS Inc., Chicago, Illinois, USA), and alpha level was set at 0.05.

Results

Demographics

The twenty-nine Parkinson's disease participants who were included in the final analysis were on average 63.8

Fig. 2



Partial regression plots illustrating the relationships between (a) log-transformed acquisition rate and recall in episodic memory composite score and (b) log-transformed acquisition rate and recall in visual episodic memory, while controlling for age, sex, and years since diagnosis. The dash line represents the 95% confidence intervals of the regression estimates.

Table 3 Standardized beta estimates of the association between motor learning and cognitive scales

Cognitive scale	Ln (α)		Retention performance	
	Standardized coefficient β	P value	Standardized coefficient β	P value
Executive function				
DKEFS color word test color-word inhibition (↓)	-0.15	0.49	0.00	1.00
DKEFS color word test color-word inhibition/switch (↓)	-0.00	0.99	-0.01	0.96
WCST perseverative errors (↓)	-0.11	0.62	-0.03	0.89
Attention				
Adaptive digit ordering test	0.05	0.83	0.16	0.47
forward digit sequencing condition (↑)				
D-KEFS color word test color naming and word reading (↓)	-0.39+	0.05	-0.21	0.30
Learning in episodic memory				
CVLT-II total number of correct words (trials 1–5) (↑)	0.38	0.11	0.01	0.98
CVLT-II learning slope (change between trials 1 and 5) (↑)	0.24	0.26	0.22	0.28
WMS-III immediate recall subtest (↑)	0.18	0.41	0.28	0.18
Recall in episodic memory				
CVLT-II long delayed free recall condition (↑)	0.19	0.43	-0.24	0.32
WMS-III delayed free recall condition (↑)	0.55*	0.01	0.31	0.17
Visuospatial function				
Judgment of line orientation (↑)	0.12	0.56	0.31	0.13
Hooper visual organization (↑)	0.18	0.37	0.07	0.73
Language				
D-KEFS category fluency test (↑)	0.27	0.21	0.22	0.31
D-KEFS letter fluency test (↑)	0.35	0.08	0.15	0.47
WAIS-IV similarities subtest (↑)	0.09	0.66	0.05	0.80

Ln (α), log-transformed exponential acquisition rate; All regression coefficients were adjusted for age, sex and years since diagnosis. (↓)=lower better; (↑)=higher better.

CVLT-II, California Verbal Learning Test – Second Edition; D-KEFS, Delis Kaplan Executive Function System; WAIS-IV, Wechsler Adult Intelligence Scale-4; WCST, Wisconsin Card Sorting Test; WMS-III, Wechsler Memory Scale – Third Edition.

*Significant correlations.

+Trends towards significant correlations.

(SD=8.4) years old, with 17.0 (SD=1.9) years of education, predominantly male (62 %), and 5.4 (SD=3.6) years since diagnosis of Parkinson's disease. Participants had an average 625.5 (SD=370.0) levodopa equivalent dose, Hoehn and Yahr stages 2–3, and an UPDRS-III score of 58.1 (SD=9.0) (Table 1).

When compared to the 10 participants excluded from the analysis, we observed a trend towards significance that the participants who were included had better attentional performance than those who were excluded, as revealed by a higher score in the digit sequencing condition of the Adaptive Digit Ordering Test (6.8 ± 2.4 vs. 5.0 ± 2.6 ; $t(37) = -1.9$, $P = 0.05$).

Motor learning parameters (finger sequence task)

The average R^2 for the exponential fit to the total time measurement during acquisition was 0.294 (SD=0.178), with a range of 0.068–0.735. The mean acquisition rate α

was 0.057 (SD=0.068), with a range of 0.005–0.296, and the mean retention performance was -0.23 (SD=0.21), with a range of -0.63 to 0.13 (Table 1). There was no significant association observed between the acquisition rate α and retention performance ($P = 0.15$, $P = 0.44$).

Motor learning and cognitive performance

The hierarchical regression analysis showed that a higher acquisition rate was significantly associated with a higher composite score of recall in episodic memory (standardized coefficient $\beta = 0.50$; 95% CI, (0.03–0.97); $P = 0.04$, Table 2 and Fig. 2a). There were no significant associations observed with composite scores of any other cognitive domains, including executive function, attention, learning in episodic memory, visuospatial function, and language (all P values > 0.12). For motor retention, there were no significant associations observed with any cognitive domains (all P values > 0.26). All regression estimates were adjusted for age, sex, and years since diagnosis.

An exploratory analysis was run using the raw scores of individual scales of the five cognitive domains. The hierarchical regression analysis showed that a higher acquisition rate was specifically associated with better recall in visual episodic memory, as measured through the delayed recall condition of the WMS-III Visual Reproduction II test [standardized coefficient $\beta = 0.55$; 95% CI, (0.12–0.96); $P = 0.01$ (Table 3 and Fig. 2b)]. There was a trend towards significance ($P = 0.05$) between a higher acquisition rate and a higher attention, measured by the D-KEFS color word test color naming and word reading subtests. No significant associations were observed between acquisition rate and any other cognitive scales in executive function, visuospatial function, learning in episodic memory, and language. For retention performance, no significant associations were observed between retention performance and any cognitive scales in all domains.

Discussion

The aim of this study was to examine the associations between two phases of motor learning (acquisition and consolidation) and cognitive performance in individuals with Parkinson's disease. We found that a faster acquisition rate was significantly associated with better episodic memory in individuals with Parkinson's disease. Further exploratory analysis showed that this association was specifically driven by better recall in visual episodic memory. A higher acquisition rate was also associated with better attention. With regards to retention, no significant associations with any cognitive domains were found.

Motor skill acquisition consists of multiple learning processes [27]. In healthy adults, both executive function and episodic memory are engaged in the acquisition phase of motor learning [2]. Hippocampus-based episodic memory is involved in the fast learning process [28], in which performance improves fast, but decays quickly, and may

rely on single-cue strategies, in which individuals focus on one piece of information for accurate responses [9]. In contrast, frontostriatal circuit-based executive function is involved in the slow learning process of motor acquisition in which performance improves slowly, also decays slowly, and is better retained. This slow process may also utilize multi-cue strategies, in which multiple sources of information are integrated and used for accurate responses [9]. In individuals with Parkinson's disease, we found a stronger association between motor acquisition and episodic memory, compared to executive function. In support of our findings, Barzgari *et al.* [29] reported that an overall improvement of motor skill acquisition was associated with episodic memory in Parkinson's disease. In addition, Gamble *et al.* (2014) [8] using an implicit sequence learning task, showed that compared to healthy older adults, individuals with Parkinson's disease demonstrated less learning only in the latter part of practice when frontostriatal-based slow learning is proportionally more involved. Taken together, our findings suggest that in contrast to healthy adults, individuals with Parkinson's disease may demonstrate increased dependency on episodic memory for motor learning acquisition, potentially representing a compensatory cognitive strategy. Future studies including aged-matched participants with normal cognition as a control group will help delineate whether the association between episodic memory and motor skill acquisition is specific to or strengthened in individuals with Parkinson's disease. One alternative explanation of our findings is that increased dependency on episodic memory, specifically visuospatial, may be due to the nature of the bimanual finger sequence task used in this study, which requires the retainment of visual information prior to movement initiation. Further studies using different motor learning tasks are needed to investigate whether the association between episodic memory and motor skill acquisition is task specific.

No significant associations were observed between motor retention and any cognitive domains in individuals with Parkinson's disease. There are few studies examining the association between motor retention and cognitive performance in Parkinson's disease. Similar to our result, one study reported no association between motor retention and cognitive function in Parkinson's disease [11]. Our findings and those of others are consistent with reports of procedural learning where motor skill acquisition is not associated with prefrontal cortical area involvement of executive function. In fact, recent studies have supported a potential role of the cerebellum in motor retention in both healthy adults and individuals with Parkinson's disease. For example, positron emission tomography and functional MRI studies in healthy young individuals have demonstrated that the retention phase of motor learning may engage dorsal premotor, posterior parietal cortical areas, and cerebellum [30,31]. In addition, studies using animal models of Parkinson's disease have reported that

increased cerebellar perfusion occurred during and at the end of skilled aerobic exercise [32]. While the cerebellum serves as a brain region storing memory of acquired motor skills, more recent studies suggested that the cerebellum, acting in conjunction with prefrontal cortical area, may also be involved in executive function-related cognitive processes needed while learning new motor tasks [33,34]. Further studies are needed to better understand the role of the cerebellum and associated cognitive brain regions during motor acquisition and retention in Parkinson's disease.

For those participants who were included in the primary analysis, better attentional performance was observed, compared to those who did not successfully improve during practice. This suggests that attentional processing may have served as a fundamental cognitive function necessary for improving performance during the acquisition phase.

There are several limitations to this study that may impact the interpretation and generalizability of the findings. First, our results may not generalize to more advanced individuals with Parkinson's disease and future studies are needed to include individuals with more advanced cognitive impairments, including dementia. Second, this study was conducted while participants were on dopamine replacement therapy, and it is possible that the removal of dopamine replacement during the time of the study assessment may impact motor learning and cognitive processing differently. However, for this study we chose to examine participants on medication such as those findings may be more directly applied to a clinical setting. Finally, while the addition of healthy age-matched adults may provide insight regarding the relationship between motor learning and cognitive function that is specific to Parkinson's disease, for this study we interpreted our findings based on reported literature on motor learning and cognition in healthy adults.

In conclusion, we found that motor skill acquisition rate was associated with episodic memory, but not executive function, highlighting the involvement of episodic memory as a potential compensatory mechanism in Parkinson's disease. Different from the findings in acquisition rate, retention performance was not associated with any cognitive domains. However, retention and its association with cerebellum related cognitive processes warrant further investigation. Understanding the contribution of domain-specific cognitive performance in motor learning may determine specific strategies for optimizing motor learning including amount, type of feedback, and complexity of practice in individuals with Parkinson's disease.

Acknowledgements

Parkinson's Foundation grant number UN-202, US Army Medical Research and Materiel Command Parkinson Research Program, Grant/Award Number W81XWH18-0665.

Conflicts of interest

There are no conflicts of interest.

References

- Verbaan D, Marinus J, Visser M, van Rooden SM, Stiggelbout AM, Middelkoop HA, van Hilten JJ. Cognitive impairment in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2007; **78**:1182–1187.
- Ren J, Wu YD, Chan JS, Yan JH. Cognitive aging affects motor performance and learning. *Geriatr Gerontol Int* 2013; **13**:19–27.
- Stephan MA, Meier B, Zaugg SW, Kaelin-Lang A. Motor sequence learning performance in Parkinson's disease patients depends on the stage of disease. *Brain Cogn* 2011; **75**:135–140.
- Krebs HI, Hogan N, Hening W, Adamovich SV, Poizner H. Procedural motor learning in Parkinson's disease. *Exp Brain Res* 2001; **141**:425–437.
- Nieuwboer A, Rochester L, Müncks L, Swinnen SP. Motor learning in Parkinson's disease: limitations and potential for rehabilitation. *Parkinsonism Relat Disord* 2009; **15**(Suppl 3):S53–S58.
- Price A, Shin JC. The impact of Parkinson's disease on sequence learning: perceptual pattern learning and executive function. *Brain Cogn* 2009; **69**:252–261.
- Deroost N, Kerckhofs E, Coene M, Wijnants G, Soetens E. Learning sequence movements in a homogenous sample of patients with Parkinson's disease. *Neuropsychologia* 2006; **44**:1653–1662.
- Gamble KR, Cummings TJ Jr, Lo SE, Ghosh PT, Howard JH Jr, Howard DV. Implicit sequence learning in people with Parkinson's disease. *Front Hum Neurosci* 2014; **8**:563.
- Kemény F, Demeter G, Racsmány M, Valálik I, Lukács Á. Impaired sequential and partially compensated probabilistic skill learning in Parkinson's disease. *J Neuropsychol* 2019; **13**:509–528.
- Pendt LK, Reuter I, Müller H. Motor skill learning, retention, and control deficits in Parkinson's disease. *PLoS One* 2011; **6**:e21669.
- Lin CH, Sullivan KJ, Wu AD, Kantak S, Winstein CJ. Effect of task practice order on motor skill learning in adults with Parkinson disease: a pilot study. *Phys Ther* 2007; **87**:1120–1131.
- Emre M, Aarsland D, Albanese A, Byrne EJ, Deuschl G, De Deyn PP, et al. Rivastigmine for dementia associated with Parkinson's disease. *N Engl J Med* 2004; **351**:2509–2518.
- Schrag A, Barone P, Brown RG, Leentjens AF, McDonald WM, Starkstein S, et al. Depression rating scales in Parkinson's disease: critique and recommendations. *Mov Disord* 2007; **22**:1077–1092.
- Petkus AJ, Filoteo JV, Schiehser DM, Gomez ME, Hui JS, Jarrahi B, et al. Mild cognitive impairment, psychiatric symptoms, and executive functioning in patients with Parkinson's disease. *Int J Geriatr Psychiatry* 2020; **35**:396–404.
- Delis D, Kaplan E, Kramer J. *Delis-Kaplan Executive Function System: Examiner's Manual*. San Antonio, TX: The Psychological Corporation; 2001.
- Werheid K, Hoppe C, Thöne A, Müller U, Müngersdorf M, von Cramon DY. The Adaptive Digit Ordering Test: clinical application, reliability, and validity of a verbal working memory test. *Arch Clin Neuropsychol* 2002; **17**:547–565.
- Delis D, Kramer J, Kaplan E, Ober B. *Manual for the California Verbal Learning Test (CVLT-II)*. San Antonio, TX: The Psychological Corporation; 2000.
- Wechsler D. *Wechsler Memory Scale – Third edition: Administration and scoring manual*. USA: The Psychological Corporation; 1981.
- Benton AL, Varney NR, Des Hamsher K. Visuospatial judgment: a clinical test. *Arch Neurol* 1978; **35**:364–367.
- Hooper H. *The Hooper Visual Organization Test manual*. Los Angeles: Western Psychological Services; 1958.
- Wechsler D. *Wechsler Adult Intelligence Scale*. 4th ed. San Antonio, TX: Pearson; 2008.
- Lee YY, Winstein CJ, Gordon J, Petzinger GM, Zelinski EM, Fisher BE. Context-dependent learning in people with Parkinson's disease. *J Mot Behav* 2016; **48**:240–248.
- Adkins TJ, Lee TG. Reward modulates cortical representations of action. *Neuroimage* 2021; **228**:117708.
- Kantak SS, Winstein CJ. Learning-performance distinction and memory processes for motor skills: a focused review and perspective. *Behav Brain Res* 2012; **228**:219–231.
- Olivier GN, Paul SS, Lohse KR, Walter CS, Schaefer SY, Dibble LE. Predicting motor sequence learning in individuals with Parkinson disease. *J Neurol Phys Ther* 2019; **43**:33–41.
- Wadden KP, Asis K, Mang CS, Neva JL, Peters S, Lakhani B, Boyd LA. Predicting motor sequence learning in individuals with chronic stroke. *Neurorehabil Neural Repair* 2017; **31**:95–104.
- Smith MA, Ghazizadeh A, Shadmehr R. Interacting adaptive processes with different timescales underlie short-term motor learning. *Plos Biol* 2006; **4**:e179.
- Trevartha KM, Garcia A, Wolpert DM, Flanagan JR. Fast but fleeting: adaptive motor learning processes associated with aging and cognitive decline. *J Neurosci* 2014; **34**:13411–13421.
- Barzgari A, Sojkova J, Maritza Dowling N, Pozorski V, Okonkwo OC, Starks EJ, et al. Arterial spin labeling reveals relationships between resting cerebral perfusion and motor learning in Parkinson's disease. *Brain Imaging Behav* 2019; **13**:577–587.
- Shadmehr R, Holcomb HH. Neural correlates of motor memory consolidation. *Science* 1997; **277**:821–825.
- Tzvi E, Stoldt A, Witt K, Krämer UM. Striatal-cerebellar networks mediate consolidation in a motor sequence learning task: an fMRI study using dynamic causal modelling. *Neuroimage* 2015; **122**:52–64.
- Wang Z, Guo Y, Myers KG, Heintz R, Holschneider DP. Recruitment of the prefrontal cortex and cerebellum in Parkinsonian rats following skilled aerobic exercise. *Neurobiol Dis* 2015; **77**:71–87.
- Hallett M, Grafman J. Executive function and motor skill learning. *Int Rev Neurobiol* 1997; **41**:297–323.
- Kozioł LF, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu H, et al. Consensus paper: the cerebellum's role in movement and cognition. *Cerebellum* 2014; **13**:151–177.