Prefrontal cortex activity and gait in Parkinson's disease with cholinergic and dopaminergic therapy

Samuel Stuart[1,2] *PhD*, Rosie Morris[1,2] *PhD*, Andrew Giritharan[1] *MD*, Joseph Quinn[1] *MD*, John G Nutt[1] *MD*, and Martina Mancini[1]* *PhD*

[1]Department of Neurology, Oregon Health and Science University, Portland, Oregon, USA

[2]Department of Sport, Exercise and Rehabilitation, Northumbria University, Newcastle upon Tyne, UK

*Corresponding author:

Martina Mancini, PhD

Assistant Professor

Balance Disorders Laboratory

Department of Neurology

Oregon Health and Science University

3181 SW Sam Jackson Road, OP-32

Portland

Oregon

97239

E-mail: mancinim@ohsu.edu

Tel: +1 503 418 2602

Words: abstract: 299

Manuscript: 3500

Tables: 2

Figures: 1

Supplementary Material: 1

References: 30

KEYWORDS: Prefrontal Cortex, Gait, Cognition, Parkinson’s disease

Abstract

Objectives

Degradation of striatal dopamine in Parkinson's disease (PD) may initially be supplemented by increased cognitive control mediated by cholinergic mechanisms. Shift to cognitive control of walking can be quantified by prefrontal cortex (PFC) activation. Levodopa improves certain aspects of gait and worsens others, and cholinergic augmentation influence on gait and PFC activity remains unclear. This study examined dopaminergic and cholinergic influence on gait and PFC activity while walking in PD.

Methods

A single-site, randomized, double-blind, cross-over trial examined effects of levodopa and donepezil in PD. 20 PD participants were randomized and 19 completed the trial. Participants were randomized to either levodopa+donepezil (5mg) or levodopa+placebo treatments, with two-weeks with treatment and a two-week washout. The primary outcome was change in PFC activity while walking, and secondary outcomes were change in gait, dual-task performance and attention.

Results

Levodopa decreased PFC activity compared to Off medication (effect size: -0.51), whereas the addition of donepezil reversed this decrease. Gait speed and stride length, under single and dual-task conditions, improved with combined donepezil and levodopa compared to Off medication (effect size: 1 for gait speed and 0.75 for stride length). Dual-task reaction time was quicker with levodopa compared to Off medication (effect size: -0.87), and accuracy improved with combined donepezil and levodopa (effect size: 0.47).

Conclusions

Cholinergic therapy, specifically donepezil 5mg/day for two-weeks, can alter PFC activity when walking, and improve secondary cognitive task accuracy and gait in PD. Further studies will investigate whether higher PFC activity while walking is associated with gait changes.

Introduction

A hallmark of healthy walking is automaticity, defined as the ability of the nervous system to successfully control movement with minimal use of executive-attentional resources. Recent evidence indicates that gait impairments in Parkinson's disease (PD) are often characterized by a shift in the locomotor control strategy from healthy automaticity, defined as the ability of the nervous system to successfully coordinate movement with minimal use of attention-demanding executive resources, to compensatory executive control[1]. This shift is potentially detrimental for gait as an executive control strategy is not optimized for locomotor control and it may place excessive demands on a limited pool of cognitive reserve. Gait impairments appear early in PD, progress throughout the course of the disease[2], often lead to falls[3], and reduced independence and quality of life[4]. Within PD, there is a combination of core motor impairments and prominence of cognitive deficits which also may occur early in the disease process[5], largely impacting executive function and attention. Cognitive function, particularly executive and attentional capabilities are associated to mobility, and particularly walking with previous studies linking cognitive ability to safe or effective walking in PD[5].

Treatment and management of gait impairments in PD can be complex, in fact, while levodopa improves certain aspects of gait, related to amplitude of movements, it worsens others, related to temporal aspects of gait and variability[6]. In general, as PD progresses, levodopa maybe less effective for mobility, likely because non-dopaminergic systems deteriorate in the disease course[7,8]. A considerable amount of research suggests that cholinergic dysfunction is also an important contributor to gait impairments in PD[7,8] implicating the cholinergic system as another target for therapeutic interventions[7].

Two main cholinergic projections are thought to influence mobility: 1) basal forebrain neurons, including the nucleus basalis of Meynert (nbM), providing the cholinergic projections to the cerebral cortex, including the prefrontal cortex (PFC); and 2) pedunculopontine nucleus-laterodorsal tegmental complex (PPN), a brainstem center, providing cholinergic inputs primarily to the thalamus, but also to the cerebellum, several brainstem nuclei, some striatal fibers, and the spinal cord[8]. Animal models demonstrate lesions within the nigrostriatal dopaminergic neurons and the PPN cholinergic neurons leads to gait impairments that cannot be corrected through dopaminergic therapies[9]. Furthermore, molecular imaging studies report that subjects with PD with

combined basal forebrain cholinergic and dopaminergic deficits showed more severe motor impairments, including slower gait, lower global cognitive performance, and more severe nigrostriatal denervation compared to subjects with PD with normal-range cortical cholinergic innervation[8,10]. Several recent studies have examined the impact of cholinergic augmentation on mobility in PD with controversial results[11]. In fact, one previous study found that cholinergic augmentation can improve gait in PD[12], whereas a separate study found no changes in gait[13].

According to the multisystem degeneration model of gait impairment[10], the degree of dopamine responsiveness of gait functions will depend on integrity of cholinergic system supporting mobility functions[10,14]. Additionally, degradation of striatal mechanisms of motor control may be initially supplemented by increasing cognitive control mediated by cortical cholinergic mechanisms[10] which could contribute to reduced automaticity of gait. Recently, cortical substrates of automaticity have been evaluated with functional near infrared spectroscopy (fNIRS). Mobile fNIRS devices are non-invasive headbands (or caps) that use near infrared light to measure hemodynamic changes in cerebral blood caused by local neuron firing (i.e. neurovascular coupling), which is similar to functional magnetic imaging (fMRI)[15]. fNIRS is increasingly being used to provide real-time imaging of cortical control of gait to evaluate activation in the supplementary motor area and PFC in healthy and neurological populations[16]. Although few studies have reported that PFC activity is elevated during steady state walking in PD compared to healthy elderly, to date, no study has examined PFC activity during walking in response to dopaminergic or cholinergic therapies.

An enhanced understanding of changes in gait automaticity due to common pharmacological therapies in PD will have implications to improve management of gait impairment in PD. If reduced gait automaticity is mainly due to lack of dopamine, then more aggressive dopaminergic strategies may be required. On the other hand, if reduced gait automaticity is due to decreased cholinergic activity, then cholinergic augmentation may be more effective for optimizing gait in PD. This study aimed to examine PFC activity while walking and spatio-temporal gait measures (both single and dual-task) in response to usual dopaminergic medication and cholinergic augmentation via donepezil. We selected gait characteristics (e.g. pace, variability, etc.) that are sensitive to PD pathology and have been implicated in poor mobility and falls risk [17]. We also examined attentional response through a computerized battery and used standard clinical assessments to investigate changes in disease severity (motor symptoms).

Methods

Participants and study design

This study was a randomized, double-blinded, cross-over trial in 20 people with PD who were >50 years old and able to walk independently; full methodology available at clinical trials.gov (NCT03599726). The study was approved by a Research Ethics Committee at Oregon Health and Science University (OHSU, eIRB #17805). Participants were eligible if they: 1) had a diagnosis of idiopathic PD with sensitivity to levodopa and off-medication Hoehn & Yahr scores of II-IV, 2) could stand unassisted for one minute and to walk continuously for 2 minutes without assistance or assistive devices, 3) currently taking Levodopa, and not already taking Donepezil, 4) were able to give informed consent to participate, and able to cooperate with the testing and be compliant with taking the experimental medications. Exclusion criteria were: 1) other factors affecting gait, such as musculoskeletal or orthopedic disorder, uncorrected vision or vestibular problem), 2) major depression, hallucinations or other psychiatric disturbances, 3) medical problems that might be worsened by donepezil, including tachycardia, bradycardia, arrhythmias, and peptic ulcer disease, 4) use of anticholinergics for Parkinsonism (e.g. cholinesterase inhibitors), bladder antispasmodics for urinary urgency or tricyclic antidepressants for depression.

All eligible participants that had completed a phone screening came into the laboratory for an informed consent process and initial screening. Participants completed three visits in total (see Figure 1), with the first visit involving screening, clinical, cognitive and mobility assessments while "Off" Parkinsonian medications (>12 hour withdrawal overnight). The second and third visits were conducted following the two-week period of medication intervention, and involved repeating some of the same clinical (MDS-UPDRS-III), cognitive (computerized attention battery) and mobility (2-minute walk under single and dual-task) assessments that were done in visit one. Prior to the first visit, participants were randomized in a 1:1 ratio to start with either levodopa+donepezil or a levodopa+placebo for a two-week intervention period. After two-weeks, participants came back to the laboratory for Visit 2, and prior to cross-over they had two-week washout, which was necessary as donepezil has a half-life of approximately 70hours and requires 14 days to reach a steady-state[18]. Donepezil was taken at 5mg per day for two weeks alongside usual levodopa medication, as this dosage is clinically efficacious and well tolerated. Participants

came back for laboratory testing in visits 2 and 3 while "On" their regular Parkinsonian medications.

The Research Pharmacy at OHSU was responsible for purchasing study medication and creating the blinded capsules, as well as maintaining and storing drugs, randomizing to maintain blinding, dispensing medication and checking compliance by returned capsule counts. Any adverse events were reported on a weekly basis and unexpected or serious adverse events were reported immediately to IRB, with referral to an independent neurologist (a safety monitoring officer).

Procedures

Clinical and Cognitive Assessment

Disease severity was measured using the Movement Disorder Society revised Unified PD Rating Scale motor sub-section (MDS-UPDRS-III), and freezing of gait status was recorded with the new freezing of gait questionnaire (NFOG-Q). Orthostatic hypotension symptoms were measured using the orthostatic hypotension symptom assessment (OHSA), fatigue was measured using the Multiple fatigue index (MFI), depression was measured with the geriatric depression scale (GDS-15), and retrospective falls were recorded from patient interview. Levodopa equivalent daily dosage (LED) scores were calculated according to standardized methods.

Four cognitive domains were examined. *Global Cognition* was measured using the Montreal Cognitive Assessment (MoCA). *Attention* was assessed using the North-East Visual Perceptual Battery, including simple reaction-time, choice reaction-time and digit vigilance[19]. *Executive Function* was measured with the frontal assessment battery, Royall's clock drawing (CLOX 1) and trail making test (TMT-A and TMT-B). *Visuo-spatial ability* was measured using clock copying (CLOX 2).

Gait assessment

PFC activity while walking was measured using an 8-channel mobile functional near infrared spectroscopy (fNIRS) (Octamon, Artinis, Netherlands), with two reference channels used to remove the components of superficial interference (peripheral blood flow changes in extra-cerebral layers of the head). This is a reliable technique for recording cortical activity during over-ground walking and the specific data collection and processing techniques have been reported in our previous studies[20,21]. Briefly, a digitizer (PATRIOT, Polhemus, VT, USA) was used to provide 3D

locations for fNIRS channels on the scalp over cortical regions of interest. Data from the digitizer was entered into the software package NIRS-statistical package metric mapping (NIRS-SPM, http://www.nitrc.org/projects/nirs_spm), which was implemented in MATLAB 2018a (Mathworks, MA, USA). NIRS-SPM registered fNIRS channel data onto Montreal Neurological Institute (MNI) standard brain space in order to locate the Broadmann areas (BA) that channels were recording from, with PFC recordings consisting of BA9 and BA10 for the participants. Our previously reported custom-made MATLAB algorithms analysed the fNIRS raw signals[20,21], which involved data filtering, baseline correction, reference channel correction, visual signal inspection and averaging across the fNIRS channels.

An instrumented mobility assessment was recorded by wireless, body-worn, inertial measurement units (IMUs) (Opals, APDM) that recorded tri-axial acceleration, angular velocity and magnetic field. IMUs were placed on both feet, shanks and wrists, as well as the sternum and sacrum via Velcro straps. Mobility data was wirelessly transmitted to a laptop that controlled the protocols, which stored and analyzed the raw data. At the beginning of each trial the IMUs were synchronized to the fNIRS system via a PortaSync device (Artinis). MobilityLab (version 2, APDM) was used to process and analyze the IMU data and provide the gait outcomes[22].

Gait was measured while participants walked for 2-minutes at a comfortable pace back and forth over a 10m distance, with a 180 degree turn at each end. Walking tasks were conducted under single and dual-task conditions. The dual-task involved the modified AX-CPT task that involved listening to a series of letters being read out and pressing a button (in the hand participants deemed they could react fastest with) when participants heard ‘A’ followed by the letter ‘I’, which reduced the risk of fNIRS signal noise and has been used in our previous work[21].

Outcome Measures

The primary outcome measure for this study was change in oxygenated hemoglobin (HbO_2) levels from baseline standing to walking within the PFC, which is a proxy for cortical activation. Secondary outcomes included change in de-oxygenated hemoglobin (HHb) and gait characteristics of gait speed, stride time variability, stride length, reaction time and accuracy of the dual-task.

Statistical Analysis

Data were analyzed using SPSS (v.25, IBM, Armonk, NY, USA) and assessed for normality using Shapiro-Wilks tests, meeting criteria for parametric analysis. Descriptive statistics (means and standard deviations (SD)) were calculated for continuous variables and Pearson Chi-square tests were used for frequency data comparisons. Statistical tests were two-tailed with a $p<0.05$ considered significant.

We hypothesized that the change in outcome between levodopa+donepezil and the baseline Off would differ from the change between levodopa+placebo and baseline Off. To estimate each of the "difference in difference" we used the standardized response mean (SRM) and a linear mixed effects models (LMEM). The SRM was calculated to compare changes from the Off medication and levodopa+placebo or levodopa+donepezil , which was interpreted as follows; trivial <0.2, small >0.2 <0.5, moderate >0.5 <0.8, and large >0.8[23]. The LMEM included an indicator of treatment and their interaction. This interaction term was used to determine whether the effect of levodopa+donepezil differs from levodopa+placebo. We also included terms to assess possible period effects and random effects for patients within treatments. LMEM were run with the Restricted Maximum Likelihood Estimation (REML) selection in MATLAB 2018a, which was selected over Maximum Likelihood (ML) to avoid bias due to our small cohort size[24]. Researchers were blinded to study intervention or placebo until following the further analysis of all outcomes.

Results

Table 1 shows the demographics, cognitive and clinical outcomes for the participants. One participant had adverse side effects (GI and worsening of depression) and did not complete the study, their data was removed from further analysis, so results are based upon 19 participants. Overall, participants had moderate disease severity (OFF medication MDS-UPRS-III score; 42), were not severely cognitively impaired (MoCA score; 26) and all participants were right handed.

Pre-frontal cortex activity while walking decreased with levodopa alone, but not with levodopa+donepezil

There was a moderate reduction in PFC activity when walking from Off medication baseline to levodopa+placebo (SRM=-0.51), but no change from Off medication to levodopa+donepezil (SRM=0.03), see Figure 2, Table 2. In addition, the delta from Off medication to

levodopa+donepezil was significantly different from the delta from Off medication to levodopa+placebo, confirmed with a significant difference in the LMEM (p=0.048, Supplementary Table 1).

Similar trends, although not significant, were observed for the change in PFC activity under dual-task conditions (Table 2 and Supplementary Table 1).

Accuracy of the concurrent DT improves with levodopa+donepezil, but not with levodopa alone

There was a moderate improvement in the accuracy of the concurrent dual-task when walking from Off medication baseline to levodopa+donepezil (SRM=0.47), but no change from Off medication to levodopa+placebo (SRM=0.16), see Figure 3, Table 2. On the contrary, the results on the reaction time of the concurrent dual-task were opposite, in fact, while there was a moderate reduction of reaction time when walking from Off medication baseline to levodopa+placebo (SRM=-0.87), there was a small increase from Off medication to levodopa+donepezil (SRM=0.01), see Figure 3, Table 2. However, only the delta for reaction time, and not accuracy, was significantly different among treatments (p=0.004, Supplementary Table 1).

Gait performance improved more with levodopa+donepezil than levodopa+placebo

There were significant improvements in secondary outcome measures of gait characteristics with levodopa+donepezil for single-task walking. Specifically, there was a moderate to large improvement in gait speed, stride length, and stride time variability from Off medication baseline to levodopa+donepezil (SRM>0.7), and only a small to moderate improvement in the same metrics from Off medication to levodopa+placebo (SRM>0.2), see Figure 2, Table 2. Similar, but attenuated trends were visible for dual-task walking. Only the delta for stride length was significantly different among treatments (p=0.002, Supplementary Table 1).

No change in motor or cognitive scores with levodopa+donepezil

Table 1 and Supplementary Table 1 show that there were no significant changes in the cognitive measures from Off medication baseline to levodopa+donepezil or levodopa+placebo. Effect size for the MDS-UPDRS III were moderate for both levodopa+donepezil or levodopa+placebo compared to Off levodopa (Table 2).

Discussion

This trial is the first to examine cortical, behavioral and cognitive response to levodopa and cholinergic augmentation in PD. We found that while PFC activity significantly decreased with levodopa alone compared to Off, that change was annulled with the combination of levodopa and donepezil. Interestingly, concurrent dual-task reaction time while walking was faster with levodopa alone, but concurrent dual-task accuracy while walking was slightly better with combined levodopa and donepezil. Results also suggest that gait improved more with combined levodopa and donepezil. Gait improvement supports previous reduction in fall rates and improvement in gait characteristics with Rivastigmine[12] or Donepezil[25], which may be underpinned by greater capacity for PFC activation with cholinergic therapies. However, our results also contrast with our previous work where balance and gait were not improved with cholinergic augmentation alone[13], which may indicate a need to combine cholinergic and dopaminergic therapies.

Gait impairments in PD lead to greater cognitive, particularly executive-attentional, burden to overcome deficits and maintain effective walking strategies[26]. Previous studies have highlighted that PFC activity when walking is greater in PD compared to older adult controls[20,21], but no studies determined the differences between Off and On levodopa. Within this cross-over design study with a relatively small cohort we found that levodopa can decrease PFC activity when walking compared to Off medication baseline, which may reflect reduced need for executive-attentional compensation for gait control with levodopa medication. Alternatively, the combination of levodopa and donepezil did not change PFC activity while walking compared to Off medication baseline in PD. Studying brain activity with fNIRS technology confined to the PFC limits us to only being able to determine activation and not neural projections or specific neurotransmitter activity[21]. However, gait also improved to a greater extent while on levodopa with donepezil compared to levodopa alone, which may be due to a cooperative relationship between dopamine and acetylcholine which is supported by the dopamine-acetylcholine synaptic balance theory of response[27]. Therefore, our results suggest that dopaminergic therapy may reduce executive-attentional burden of gait in PD, but with the addition of donepezil there may be more neural resource available to allow greater executive-attentional compensation to further improve secondary cognitive task accuracy and gait.

A previous review highlighted several associative studies that link cholinergic activity and gait[11]. For example, gait speed, step length and step time variability have been associated with cholinergic function. Within the current study, gait speed, stride length and stride time variability under single-task conditions were improved beyond levodopa+placebo with the addition of donepezil and there was a trend for the same result under dual-task conditions, which is likely due to greater cognitive compensation. Improved step length was significantly greater with levodopa+donepezil (Supplementary Table 1). Step length is thought to heavily rely upon cognitive function in PD[5,26], which is demonstrated by improvements in step length with cueing and reduction with cognitive decline in PD[5,21]. However, in contrast to previous research[12] we found only a moderate improvement in stride time variability, which is not totally unsurprising as two previous studies have found that this measure of gait is not related to cholinergic function, as measured by short latency afferent inhibition (SAI)[28].

Traditionally, gait and cognition relationships are studied in real-time using dual-task paradigms[26], with poorer gait performance compared to single-task indicating a lack of walking automaticity and a need for cognitive (cortical) compensation to control gait. Interestingly, in looking at the results of the concurrent dual-task while walking, we found that reaction time of the concurrent cognitive task improved more in the levodopa+placebo condition compared to the levodopa+donepezil. However, the results of the accuracy of the concurrent dual-task were opposite, in fact, accuracy of the concurrent dual-task improved for the levodopa+donepezil condition compared to baseline Off. These findings are in line with the PFC activity results, showing more PFC activity in the levodopa+donepezil compared to levodopa+placebo. Our preliminary findings support the role of attention under complex gait conditions and provides a means through which to intervene with cholinergic medication. Overall, our findings also suggest caution in interpreting high PFC activity during walking as reduced automaticity of walking, in fact, we showed better gait performance and better accuracy on the concurrent cognitive task with higher PFC activity while walking. Future study should examine if cognitive reserve plays a role in the ability to increase PFC activity while walking and improving gait performance.

None of the computerized attention battery measures improved beyond placebo with cholinergic intervention, but similar to our cognitive dual-task reaction time results there was a trend for choice reaction time becoming worse with the application of donepezil. Lack of change in computerized attentional tests is similar to our previous results with a different computerized attentional test[13].

The lack of improvement in cognitive outcomes may be due to the limited cohort size involved in the study and the limited battery used for follow-up testing. Whereas lack of clinical motor symptom changes may reflect the sensitivity of the scale that may be unable to detect subtle changes with current cholinergic therapies, which our inertial sensor technology is capable of quantifying. Current anti-cholinesterase inhibitors have limited brain uptake and therefore limited efficacy, which is also compacted by their burdensome side effects[12]. Therefore, there is a need to develop better treatment options to improve the cholinergic systems function within the brain, such as exercise, deep brain stimulation of the nucleus basalis of Meynert, and non-invasive nerve stimulation[11].

Study Strengths and Limitations

Our study differed from previous work to intervene in gait issues with cholinergic therapies in PD. The primary strength of our study is the use of cholinergic medication in addition to participants current dopaminergic therapy, which reflects medication use within clinical practice and may be important for a dopamine-acetylcholine cooperative response[27]. Our previous study found no improvement in gait or balance with donepezil (10mg/day for 6 weeks)[13], but we examined individuals while off dopaminergic medication which may have impacted results. This study suggests that combined dopaminergic and cholinergic therapies may be required for a beneficial effect on gait in PD.

Several limitations should be considered. While fNIRS provides important insight into cortical activity during gait, the depth of recording (~1-2cm into the brain) and low-resolution of signals (50Hz) do not allow comprehensive brain activity to be reported which may provide further understanding of treatment response[16]. We did not track falls rate during intervention periods and we included non-fallers within our cohort, which has been the focus of many previous studies of cholinergic therapies[17,25,29]. Similarly, due to the complexity of measuring cortical activity, cognition, clinical scores and gait, as well as intervening with medication, we were only able to capture data on a relatively small cohort of PD, which may increase the risk of a type II statistical error occurring. However, our calculation of SRMs point to moderate treatment effects within this cohort that appear to be clinically meaningful, but further work on a larger cohort is required to establish findings.

Conclusion

In conclusion, this double-blind, randomized cross-over design trial of dopaminergic therapy and additional cholinergic augmentation with donepezil at 5mg/day for 2 weeks showed that the addition of donepezil to usual levodopa increases PFC activity while walking and improves gait in PD compared to dopaminergic intervention alone.

Acknowledgements

We would like to thank the participants for the time they gave to participate in this study, Makena Strand (Research Assistant) for her help with recruitment and data collection, and Professor Fay Horak for helpful discussion. This study was funded by a Career Development Award 5R00HD078492-04 and a Medical Research Foundation of Oregon grant (PI Martina Mancini). Samuel Stuart is supported by a Parkinson's disease Foundation Post-doctoral Fellowship for Basic Scientists (PF-FBS-1898-18-01).

Conflicts of Interests

The Authors declare no competing interests.

Financial Disclosure Statement

Dr. Stuart received grant support from the Parkinson's Foundation (PF-FBS-1898-18-01) and Medical Research Foundation of Oregon.

Dr. Morris received grant support from the Medical Research Foundation of Oregon.

Dr. Giritharan has nothing to disclose.

Dr. Nutt received grants from Voyager Therapeutics, Inc.

Dr. Quinn received reimbursement for DSMB service from Retrophin and from VTV Pharmaceuticals and for the conduct of clinical trials from Sanofi, Abbvie, Alkahest, and EIP-Pharma. He also received grant funding from NIH and the Dept of Veterans' Affairs.

Dr. Mancini received grant support from the NIH (R00 078492, R44 AG056012-02) and the Medical Research Foundation of Oregon.

Data Sharing

All data contained within this study is available upon request from the corresponding author, and will be uploaded to clinical trials.gov (NCT03599726).

Author Contributions

SS and MM conceptualized and designed the study, recruited patients, contributed data, did statistical analysis, interpreted the results, and drafted and edited the manuscript. SS, MM, AG, JQ and JN recruited patients, contributed data, interpreted the results, and edited the manuscript. SS, RM, AG, JQ, JN, and MM contributed to the concept of the study, interpreted the results, and edited the manuscript.

References

1. Clark DJ. Automaticity of walking: functional significance, mechanisms, measurement and rehabilitation strategies. *Front Hum Neurosci.* 2015;9:246.
2. Pickering RM, Grimbergen YA, Rigney U, et al. A meta-analysis of six prospective studies of falling in Parkinson's disease. *Mov Disord.* 2007;22(13):1892-1900.
3. Fasano A, Canning CG, Hausdorff JM, Lord S, Rochester L. Falls in Parkinson's disease: A complex and evolving picture. *Movement disorders : official journal of the Movement Disorder Society.* 2017;32(11):1524-1536.
4. Muslimović D, Post B, Speelman JD, Schmand B, de Haan RJ, For the CSG. Determinants of disability and quality of life in mild to moderate Parkinson disease. *Neurology.* 2008;70(23):2241-2247.
5. Morris R, Lord S, Lawson RA, et al. Gait rather than cognition predicts decline in specific cognitive domains in early Parkinson’s disease. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences.* 2017;72(12):1656-1662.
6. Curtze C, Nutt JG, Carlson-Kuhta P, Mancini M, Horak FB. Levodopa Is a Double-Edged Sword for Balance and Gait in People With Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society.* 2015;30(10):1361-1370.
7. Bohnen NI, Albin RL, Müller ML, Chou K. Advances in therapeutic options for gait and balance in Parkinson's disease. *US neurology.* 2011;7(2):100.
8. Müller ML, Bohnen NI. Cholinergic dysfunction in Parkinson’s disease. *Current neurology and neuroscience reports.* 2013;13(9):377.

9. Grabli D, Karachi C, Folgoas E, et al. Gait disorders in parkinsonian monkeys with pedunculopontine nucleus lesions: a tale of two systems. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2013;33(29):11986-11993.
10. Bohnen NI, Frey KA, Studenski S, et al. Gait speed in Parkinson disease correlates with cholinergic degeneration. *Neurology.* 2013;81(18):1611-1616.
11. Morris R, Martini DN, Madhyastha T, et al. Overview of the cholinergic contribution to gait, balance and falls in Parkinson's disease. *Parkinsonism & related disorders.* 2019.
12. Henderson EJ, Lord SR, Brodie MA, et al. Rivastigmine for gait stability in patients with Parkinson's disease (ReSPonD): a randomised, double-blind, placebo-controlled, phase 2 trial. *The Lancet Neurology.* 2016;15(3):249-258.
13. Mancini M, Chung K, Zajack A, et al. Effects of augmenting cholinergic neurotransmission on balance in Parkinson's disease. *Parkinsonism & Related Disorders.* 2019;69:40-47.
14. Muller ML, Albin RL, Kotagal V, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson's disease. *Brain : a journal of neurology.* 2013;136(Pt 11):3282-3289.
15. Steinbrink J, Villringer A, Kempf F, Haux D, Boden S, Obrig H. Illuminating the BOLD signal: combined fMRI-fNIRS studies. *Magnetic resonance imaging.* 2006;24(4):495-505.
16. Stuart S, Vitorio R, Morris R, Martini DN, Fino PC, Mancini M. Cortical activity during walking and balance tasks in older adults and in people with Parkinson's disease: A structured review. *Maturitas.* 2018;113:53-72.
17. Hausdorff JM. Gait dynamics, fractals and falls: finding meaning in the stride-to-stride fluctuations of human walking. *Hum Mov Sci.* 2007;26(4):555-589.
18. Rogers SL, Friedhoff LT. Pharmacokinetic and pharmacodynamic profile of donepezil HCl following single oral doses. *Br J Clin Pharmacol.* 1998;46 Suppl 1(Suppl 1):1-6.
19. Elder GJ, Colloby SJ, Firbank MJ, McKeith IG, Taylor J-P. Consecutive sessions of transcranial direct current stimulation do not remediate visual hallucinations in Lewy body dementia: a randomised controlled trial. *Alzheimers Res Ther.* 2019;11(1):9-9.
20. Stuart S, Belluscio V, Quinn JF, Mancini M. Pre-frontal Cortical Activity During Walking and Turning Is Reliable and Differentiates Across Young, Older Adults and People With Parkinson's Disease. *Front Neurol.* 2019;10:536-536.
21. Stuart S, Mancini M. Prefrontal Cortical Activation With Open and Closed-Loop Tactile Cueing When Walking and Turning in Parkinson Disease: A Pilot Study. *J Neurol Phys Ther.* 2019.

22. Washabaugh EP, Kalyanaraman T, Adamczyk PG, Claflin ES, Krishnan C. Validity and repeatability of inertial measurement units for measuring gait parameters. *Gait Posture.* 2017;55:87-93.
23. Middel B, van Sonderen E. Statistical significant change versus relevant or important change in (quasi) experimental design: some conceptual and methodological problems in estimating magnitude of intervention-related change in health services research. *Int J Integr Care.* 2002;2:e15-e15.
24. McNeish D, Stapleton LM. Modeling Clustered Data with Very Few Clusters. *Multivariate Behavioral Research.* 2016;51(4):495-518.
25. Chung KA, Lobb BM, Nutt JG, Horak FB. Effects of a central cholinesterase inhibitor on reducing falls in Parkinson disease. *Neurology.* 2010;75(14):1263-1269.
26. Morris R, Lord S, Bunce J, Burn D, Rochester L. Gait and cognition: Mapping the global and discrete relationships in ageing and neurodegenerative disease. *Neuroscience and biobehavioral reviews.* 2016;64:326-345.
27. Calabresi P, Picconi B, Parnetti L, Di Filippo M. A convergent model for cognitive dysfunctions in Parkinson's disease: the critical dopamine-acetylcholine synaptic balance. *The Lancet Neurology.* 2006;5(11):974-983.
28. Rochester L, Yarnall AJ, Baker MR, et al. Cholinergic dysfunction contributes to gait disturbance in early Parkinson's disease. *Brain : a journal of neurology.* 2012;135(Pt 9):2779-2788.
29. Hiller A, Nutt J, Mancini M, Horak F, Kareus S. Are cholinesterase inhibitors effective in improving balance in Parkinson's disease. *J Neurol Disord S.* 2015;2(2).

Table 1. Demographics, cognitive and clinical outcomes

		Baseline Off medication Mean (SD)	Levodopa + Placebo	Levodopa + Donepezil
Demographic	Age (years)	72.68 (7.58)	-	-
	Sex	M (14) / F (6)	-	-
	Years of Education	17.00 (3.37)	-	-
	Height	175.82 (8.30)	-	-
	Weight (lbs)	190.21 (50.47)	-	-
	Depression (GDS-15)	7.11 (5.08)	-	-
	Falls status (n)	Faller 16 / Non-faller 3		
	Retrospective falls (no. in 12 months)	6.95 (22.59)	-	-
	MFI	57.47 (16.55)	-	-
	OHSA	2.84 (6.89)	-	-
	OHSDA	0.63 (2.52)	-	-
	Dominant side	0 L / 19 R	-	-
Cognition	MoCA	25.95 (2.99)	-	-
Attention	SRT - mean	364.45 (49.20)	387.35 (181.45)	359.27 (35.18)
	SRT - CV	25.24 (21.87)	29.87 (31.83)	18.44 (10.18)
	CRT - mean	498.07 (62.94)	505.58 (68.89)	538.33 (89.12)
	CRT - CV	16.80 (5.23)	17.62 (4.15)	19.96 (13.73)
	DV - mean	540.16 (59.82)	522.47 (69.53)	518.96 (67.82)
	DV - CV	21.20 (14.56)	19.84 (9.59)	17.65 (8.73)
Executive Function	FAB	14.70 (3.20)	16.00 (1.69)	16.1 (1.97)
	CLOX 1	12.21 (2.15)	13.10 (1.71)	12.58 (2.24)
	TMT-A (seconds)	36.36 (12.95)	36.36 (13.16)	34.12 (12.01)
	TMT-B (seconds)	103.70 (52.21)	130.03 (53.17)	124.57 (39.10)
Visuo-spatial ability	CLOX 2	13.90 (0.94)	13.70 (1.38)	13.74 (1.05)
Clinical	Hoehn and Yahr Stage (H&Y)	I (0) / II (13) / III (7)	I (0) / II (18) / III (1)	I (0) / II (18) / III (1)
	MDS-UPDRS-III	41.90 (10.54)	38.16 (11.01)	37.11 (12.13)
	Disease duration (years)	7.63 (4.78)	-	-
	FOGQ	5.32 (6.51)	-	-
	LED	717.95 (263.76)	-	-

[MFI = multiple fatigue index , OHSA = orthostatic hypotension symptom assessment, OHSDA = Orthostatic hypotension daily activity assessment, SRT = simple reaction time , CRT = choice reaction time, DV = digit vigilance, FAB = frontal assessment battery, CLOX1 and 2 = Royals clock drawing and copying, TMT = trail making test, UPDRS-III = unified Parkinson's disease rating scale, FOGQ = Freezing of gait questionnaire, LED = Levadopa equivalent daily dosage, Faller ≥ 1 fall in past 12 months]

Table 2. Summary of findings. Means and standard deviations (SDs) of primary and secondary gait measures, as well as clinical severity, at baseline and changes at two-weeks for complete cases in the two-arm crossover trial.

		Baseline Off medication	**Change after 2-weeks: $\Delta_{placebo}$ levodopa+placebo**	**SRM**	**Change after 2-weeks: $\Delta_{donepezil}$ levodopa+donepezil**	**SRM**
		Mean (SD)	**Mean (SD)**		**Mean (SD)**	
fNIRS (primary)						
HbO_2 (microM/l)	*Walk*	-0.031 (0.447)	-0.353 (0.687)	-0.51	0.007 (0.380)	0.03
	Walk - Dual-task	0.084 (0.496)	-0.303 (0.654)	-0.46	-0.040 (0.515)	-0.08
GAIT (secondary)						
Single-Task	*Gait Speed (m/s)*	0.87 (0.15)	0.04 (0.10)	0.40	0.07 (0.07)	1.00
	Stride length (m)	0.93 (0.13)	0.02 (0.09)	0.22	0.06 (0.08)	0.75
	Stride Time SD (s)	0.0358 (0.0112)	-0.0021 (0.0103)	0.20	-0.0042 (0.0096)	0.44
Dual-Task	*Gait Speed (m/s)*	0.83 (0.14)	0.06 (0.13)	0.46	0.08 (0.11)	0.73
	Stride length (m)	0.88 (0.13)	0.04 (0.12)	0.33	0.07 (0.12)	0.58
	Stride Time SD (s)	0.0384 (0.0107)	-0.0037 (0.0157)	0.23	-0.0032 (0.0116)	0.27
Cognitive Dual-Task	*Reaction Time (s)*	0.50 (0.08)	-0.05 (0.05)	-0.87	0.007 (0.07)	0.01
	Accuracy (-)	0.92 (0.07)	-0.03 (0.18)	0.16	0.03 (0.11)	0.47
CLINICAL						
Motor Symptoms	*MDS-UPDRS-III*	41.90 (10.54)	-3.74 (6.08)	-0.62	-4.79 (7.89)	-0.61

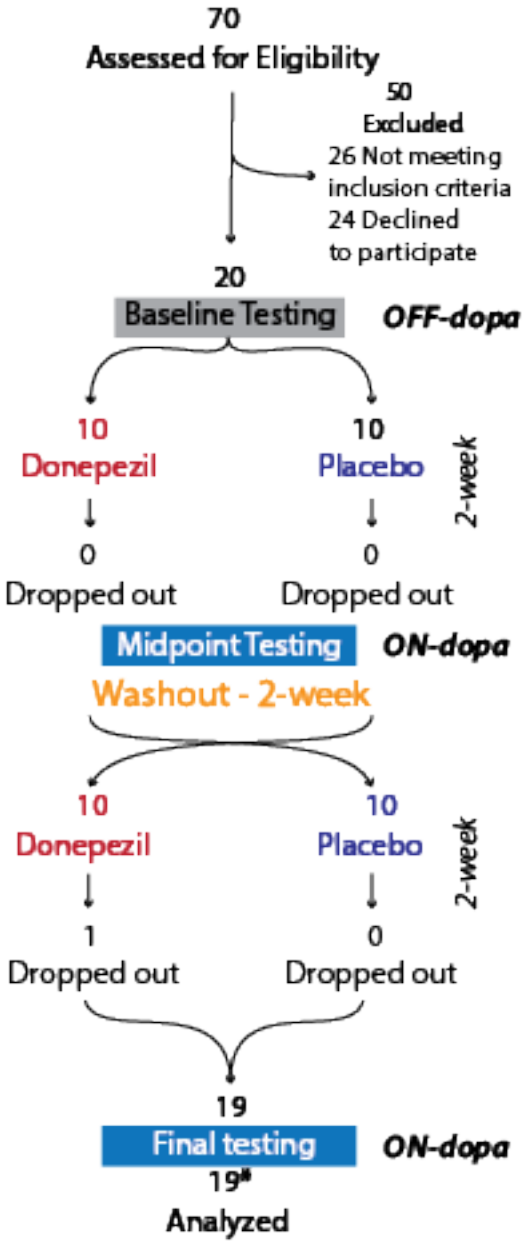
70
Assessed for Eligibility
50
Excluded
26 Not meeting inclusion criteria
24 Declined to participate
20
Baseline Testing
OFF-dopa
10
Donepezil
10
Placebo
2-week
0
Dropped out
0
Dropped out
Midpoint Testing
ON-dopa
Washout - 2-week
10
Donepezil
10
Placebo
2-week
1
Dropped out
0
Dropped out
19
Final testing
ON-dopa
19[a]
Analyzed

Figure 1

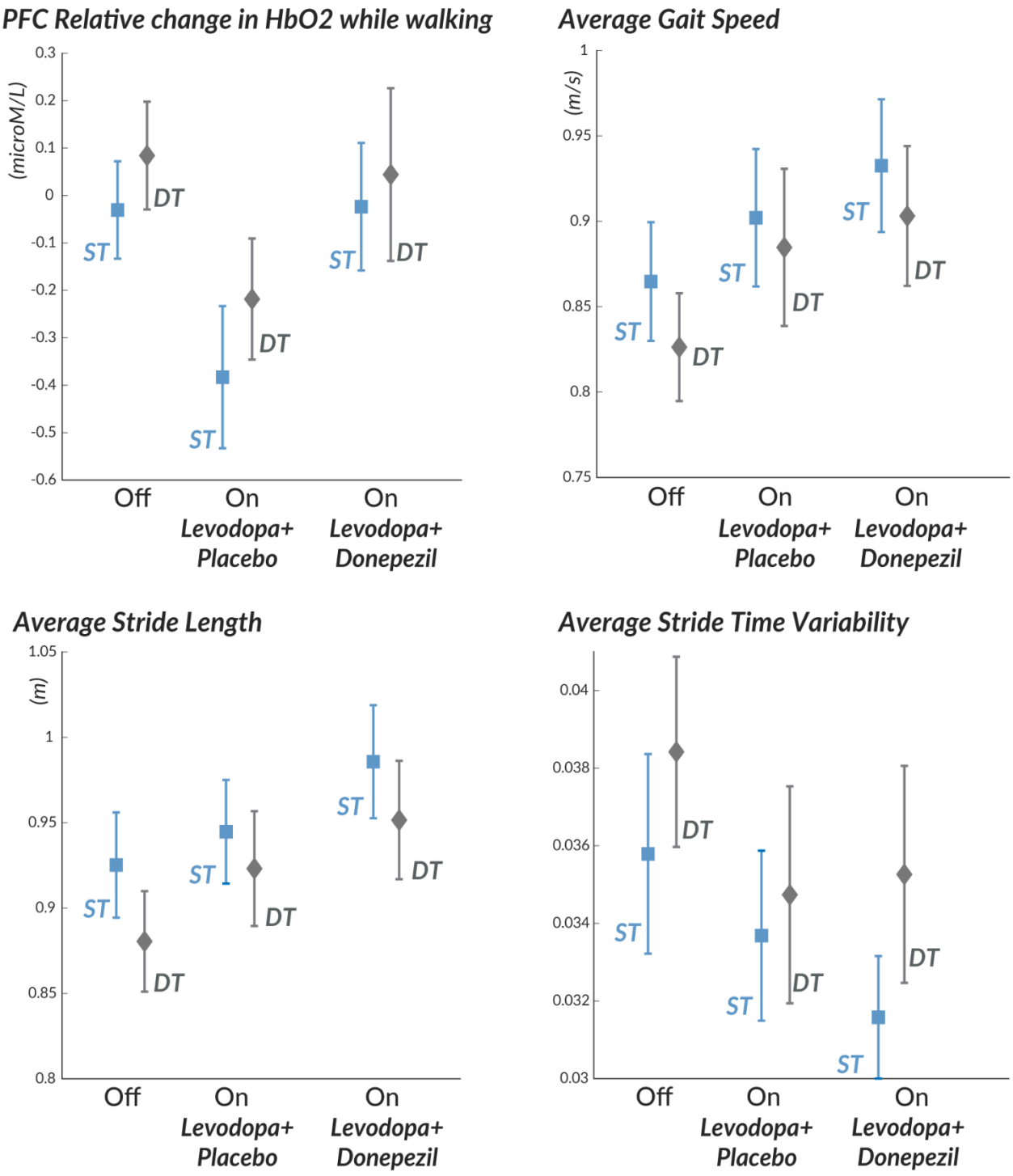

Figure 2 Mean and SEM of primary and secondary outcome measures of walking across the three time-points

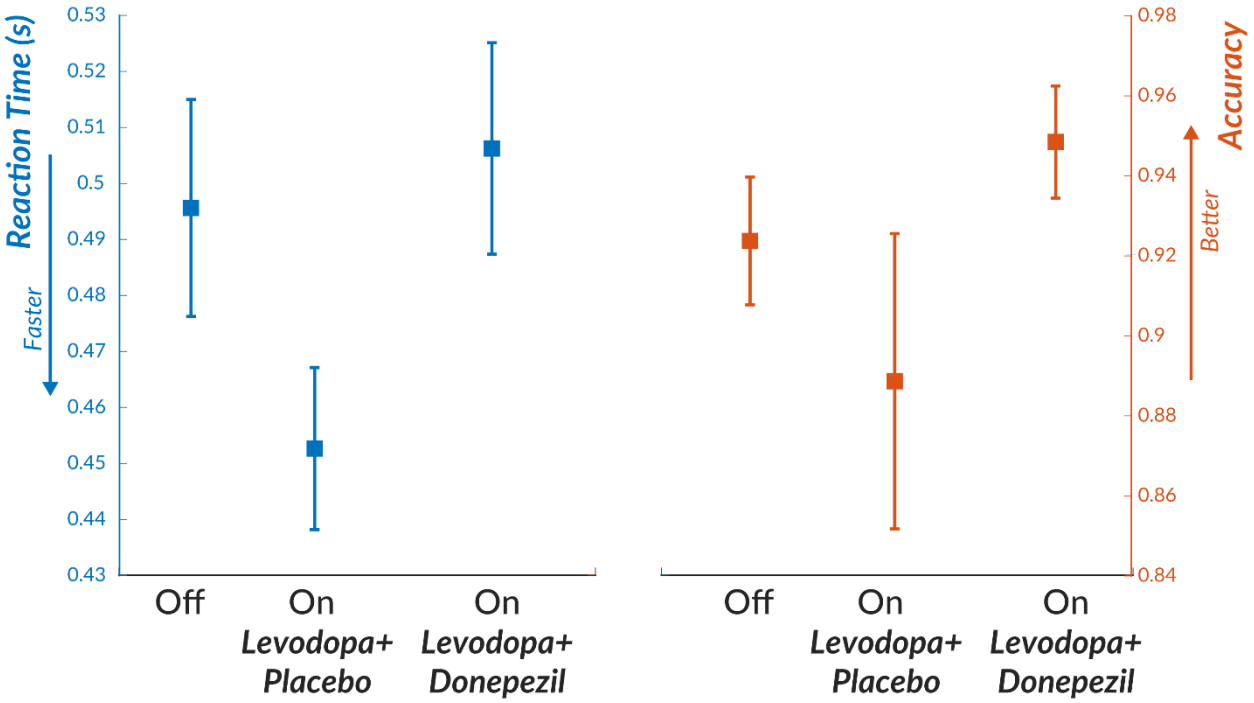

Figure 3 Mean and SEM of the reaction time and accuracy of the concurrent dual-task across the three timepoints

Supplementary Table 1 - Change in outcomes from baseline (Off Medication) with treatment and order

				Treatment				Treatment Order			
		Levodopa + placebo	**Levodopa + Donepezil**	**Estimate**	**SE**	**t**	**p**	**Estimate**	**SE**	**t**	**p**
		Mean (SD)	**Mean (SD)**								
fNIRS											
HbO_2	Walk	-0.35 (0.69)	0.01 (0.38)	0.37	0.18	2.05	**0.048***	-0.06	0.18	-0.31	0.762
	Walk - Dual-task	-0.30 (0.65)	-0.04 (0.52)	0.26	0.19	1.42	0.166	-0.35	0.19	-1.88	0.068
HHb	Walk	-0.02 (0.22)	0.14 (0.44)	0.16	0.11	1.45	0.156	0.03	0.12	0.22	0.826
	Walk - Dual-task	-0.08 (0.48)	0.07 (0.28)	0.15	0.10	1.62	0.115	-0.23	0.15	-1.55	0.130
GAIT											
Single-Task	Gait Speed	0.04 (0.10)	0.07 (0.07)	0.03	0.02	1.78	0.084	-0.01	0.04	-0.14	0.888
	Stride length	0.02 (0.09)	0.06 (0.08)	0.04	0.01	3.44	**0.002***	0.02	0.04	0.61	0.543
	Stride Time SD	-0.0021 (0.0103)	-0.0042 (0.0096)	0.00	0.00	-1.05	0.303	0.00	0.01	-0.28	0.783
Dual-Task	Gait Speed	0.06 (0.13)	0.08 (0.11)	0.02	0.02	0.79	0.437	0.02	0.05	0.33	0.745
	Stride length	0.04 (0.12)	0.07 (0.12)	0.03	0.01	1.93	0.062	0.04	0.05	0.66	0.514
	Stride Time SD	-0.0037 (0.0157)	-0.0032 (0.0116)	0.00	0.00	-0.37	0.714	0.00	0.01	0.06	0.955
Cognitive Dual-Task	Reaction Time	-0.05 (0.05)	0.0007 (0.07)	0.05	0.01	3.16	**0.004***	0.03	0.03	0.89	0.370
	Accuracy	-0.03 (0.18)	0.03 (0.11)	0.05	0.04	1.36	0.187	-0.05	0.08	-0.06	0.560
CLINICAL + ATTENTION											
Motor Symptoms	MDS-UPDRS-III	-3.74 (6.08)	-4.79 (7.89)	-1.05	1.30	-0.81	0.425	-0.81	5.31	-0.15	0.880
Attention	SRT - mean	22.90 (161.98)	-5.18 (35.39)	-28.09	38.49	-0.73	0.471	15.48	38.54	0.40	0.690
	SRT - CV	4.63 (40.71)	-6.81 (16.09)	-11.44	7.86	-1.46	0.155	6.51	12.09	0.54	0.594
	CRT - mean	7.51 (63.49)	40.27 (66.61)	32.75	16.69	1.96	0.058	-17.63	25.15	-0.70	0.488
	CRT - CV	0.82 (3.50)	3.17 (15.08)	2.35	3.34	0.70	0.486	0.83	3.34	0.25	0.805
	DV - mean	-17.69 (41.52)	-21.21 (59.31)	-3.51	11.56	-0.30	0.763	-34.12	19.38	-1.76	0.087
	DV - CV	-1.36 (17.71)	-3.55 (16.82)	-2.19	2.48	-0.88	0.384	-2.43	7.73	-0.31	0.756

[Early = first 40 seconds, Late = last 40 seconds, SD = standard deviation, CV = coefficient of variation, SRT = simple reaction time, CRT = choice reaction time, DV = digit vigilance, UPDRS-III = unified Parkinson's disease rating scale]