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Dopamine depletion impairs gait automaticity by altering cortico-striatal and cerebellar processing in Parkinson's disease

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Abstract

Impairments in motor automaticity cause patients with Parkinson's disease to rely on attentional resources during gait, resulting in greater motor variability and a higher risk of falls. Although dopaminergic circuitry is known to play an important role in motor automaticity, little evidence exists on the neural mechanisms underlying the breakdown of locomotor automaticity in Parkinson's disease. This impedes clinical management and is in great part due to mobility restrictions that accompany the neuroimaging of gait. This study therefore utilized a virtual reality gait paradigm in conjunction with functional MRI to investigate the role of dopaminergic medication on lower limb motor automaticity in 23 patients with Parkinson's disease that were measured both on and off dopaminergic medication. Participants either operated foot

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pedals to navigate a corridor (‘*walk’* condition) or watched the screen while a researcher operated the paradigm from outside the scanner (‘*watch’* condition), a setting that controlled for the non-motor aspects of the task. Step time variability during *walk* was used as a surrogate measure for motor automaticity (where higher variability equates to reduced automaticity), and patients demonstrated a predicted increase in step time variability during the dopaminergic “off” state. During the “off” state, subjects showed an increased blood oxygen level-dependent response in the bilateral orbitofrontal cortices (*walk>watch*). To estimate step time variability, a parametric modulator was designed that allowed for the examination of brain regions associated with periods of decreased automaticity. This analysis showed that patients on dopaminergic medication recruited the cerebellum during periods of increasing variability, whereas patients off medication instead relied upon cortical regions implicated in cognitive control. Finally, a task-based functional connectivity analysis was conducted to examine the manner in which dopamine modulates large-scale network interactions during gait. A main effect of medication was found for functional connectivity within an attentional motor network and a significant condition by medication interaction for functional connectivity was found within the striatum. Furthermore, functional connectivity within the striatum correlated strongly with increasing step time variability during *walk* in the off state ($r=0.616$, $p=0.002$), but not in the on state ($r=-0.233$, $p=0.284$). Post-hoc analyses revealed that functional connectivity in the dopamine depleted state within an orbitofrontal-striatal limbic circuit was correlated with worse step time variability ($r=0.653$, $p<0.001$). Overall, this study demonstrates that dopamine ameliorates gait automaticity in Parkinson’s disease by altering striatal, limbic and cerebellar processing, thereby informing future therapeutic avenues for gait and falls prevention.

Abbreviations

BOLD, Blood Oxygenation Level Dependent; CV, Coefficient of Variation; DC, Dorsal Caudate; DCP, Dorsal Caudal Putamen; DLPFC, Dorsolateral Prefrontal Cortex; DRP, Dorsal Rostral Putamen; FDR, False Discovery Rate; FSL, Footstep Latency; MNI, Montreal Neurological Institute and Hospital; PCP, Postcommissural putamen; PMd, Dorsal Premotor Cortex; PPC, Posterior Parietal Cortex; Pre-SMA, Pre-Supplementary Motor Area; ROIs, Regions of Interest; SD, Standard Deviation; SPM12, Statistical Parametric Mapping version 12; TFCE, Threshold-Free Cluster Enhancement; UPDRS-III, Motor section of the Unified Parkinson's Disease Rating Scale; VRP, Ventral Rostral Putamen; VSi, Inferior Ventral Striatum; VSs, Superior Ventral Striatum

Key words

Parkinson's disease, Automaticity, Dopamine, Basal Ganglia, fMRI

Introduction

Impairments in motor automaticity are a hallmark feature of Parkinson's disease that cause patients to increasingly demand cortical resources in order to execute basic motor operations via attentional processes (Bohnen and Jahn, 2013; Wu *et al.*, 2015). A reduction in motor automaticity is of particular concern during gait in Parkinson's disease, as the cortical resources that would be used for compensation are not optimized for the fast and parallel processing required during locomotion (Clark, 2015). Furthermore, the excessive attentional demand of walking in Parkinson's disease demands a high computational cost and interferes with gait control in conditions of high workload (Schneider and Chein, 2003; Lewis and Barker, 2009; Lewis and Shine, 2014; Clark, 2015; Wu *et al.*, 2015). As a result, patients with Parkinson's disease have a greater risk of adverse mobility outcomes and falls, especially during more complex everyday situations where a secondary task is performed in parallel with gait (Schaafsma *et al.*, 2003; Clark, 2015; Hausdorff, *et al.*, 2003a; Vandenbossche *et al.*, 2012; Lewis and Shine, 2014; Strouwen *et al.*, 2015; Wu *et al.*, 2015). Despite the clinical importance of gait in Parkinson's disease, the

precise neural mechanisms underlying impairments in locomotor automaticity remain poorly understood, thus impeding targeted management (Wu *et al.*, 2015).

Increased step time variability is a robust predictor for falls in Parkinson's disease (Hausdorff *et al.*, 1998) and has previously been suggested as a surrogate measure for reduced locomotor automaticity (Frenkel-Toledo *et al.*, 2005; Yogev *et al.*, 2005; Peterson and Horak, 2016). A recently proposed framework by Wu and colleagues (2015) outlines several features that indicate whether a motor deficit is directly linked to an underlying impairment in motor automaticity in Parkinson's disease, namely: i) the motor skill is performed automatically (and without behavioural interference) in healthy subjects; ii) dual task performance results in significant deterioration in the motor skill in patients with Parkinson's disease as compared to healthy subjects; and iii) external cueing (or attention) significantly improves the performance of this motor skill (Wu *et al.*, 2015). If this theoretical model is applied to the existing literature, it indeed becomes evident that step time variability fits these criteria as an index of locomotor automaticity: i) stride time variability is resistant to interference during dual-task walking in healthy subjects (Yogev *et al.*, 2005), suggesting that regulation of stride time variability is an automated motor skill in healthy subjects (Friedman *et al.*, 1982; Yogev *et al.*, 2005; Wu *et al.*, 2015); ii) in Parkinson's disease, walking while performing a cognitive dual task exacerbates stride time variability (Hausdorff, *et al.*, 2003b; Yogev *et al.*, 2005; Plotnik *et al.*, 2011) and patients experience higher dual task cost during walking as compared to healthy subjects (Yogev *et al.*, 2005); iii) stride time variability in Parkinson's disease has been shown to reduce in the presence of external auditory cues (Willems *et al.*, 2006; Hausdorff *et al.*, 2007; Rochester *et al.*, 2011). Therefore, this evidence suggests that step time variability,

the regulation of which is an automated process in the healthy population, is impaired in Parkinson's disease and reflective of reduced motor automaticity (Yogev *et al.*, 2005; Bohnen and Jahn, 2013; Lewis and Shine, 2014; Wu *et al.*, 2015; Peterson and Horak, 2016).

Motor automaticity is typically achieved through motor learning, followed by the timely initiation and maintenance of automated motor sequences, even during interference (Wu *et al.*, 2015). During motor learning, the posterior striatum (putamen) is thought to 'chunk' motor action sequences under the influence of dopamine (Graybiel, 1998). This process allows performance of well-learned motor patterns to be executed as a single unit of activity rather than multiple serial computations, enhancing neural efficiency and reducing motor variability (Schneider and Chein, 2003; Poldrack *et al.*, 2005; Wymbs *et al.*, 2012; Wu *et al.*, 2015). The initiation of an automatic sequence is thought to involve a shift from the anterior associative fronto-striatal circuit to the posterior sensorimotor striatum (Miyachi *et al.*, 1997; Lehéricy *et al.*, 2005; Wymbs *et al.*, 2012; Wu *et al.*, 2015), which frees up frontal attentional resources that can then be used to process concurrent secondary demands (Carbon and Marié, 2003; Monchi *et al.*, 2007; Lewis and Shine, 2014; Wu *et al.*, 2015). The most well described circuits involved with maintaining an automated motor sequence include the spinal cord, brainstem locomotor regions, posterior striatum, primary cortical motor regions and cerebellum (Figure 1) (Poldrack *et al.*, 2005; Bohnen and Jahn, 2013; Clark, 2015; Wu *et al.*, 2015). Doyon et al. (2002) further proposed that during motor learning, a transfer of experience-dependent changes from the cerebellar cortex to the deep cerebellar dentate nucleus

takes place, and then with extended practice, from a cerebellar-cortical to a striatal-cortical network (Doyon *et al.*, 2002). Evidently, a well functioning striatum is integral for effective locomotor automaticity (Wu *et al.*, 2015).

Ascending dopaminergic neurons in the midbrain provide rich innervation to the entire striatum, thereby exerting neuromodulatory control over information processing across the striatum and parallel cortico-striatal loops that underpin the execution of coordinated behaviours (Alexander *et al.*, 1986; Kelly *et al.*, 2009; Helmich *et al.*, 2010; Surmeier *et al.*, 2010; Hacker *et al.*, 2012; Sharman *et al.*, 2013; Bell *et al.*, 2014). In Parkinson's disease, the pathological degeneration of nigrostriatal dopaminergic neurons impacts on the communication across the striatum and parallel cortico-striatal circuits (Alexander *et al.*, 1986; Kelly *et al.*, 2009; Helmich *et al.*, 2010; Surmeier *et al.*, 2010; Hacker *et al.*, 2012; Sharman *et al.*, 2013; Bell *et al.*, 2014), which likely impairs motor learning and automaticity (Figure 1) (Vandenbossche *et al.*, 2012; Bohnen and Jahn, 2013; Lewis and Shine, 2014; Hamacher *et al.*, 2015; Wu *et al.*, 2015; Peterson and Horak, 2016). Indeed, the dopaminergic insult is most severe in the posterior striatum (i.e. sensorimotor putamen) that is involved with motor learning and automaticity (Brooks *et al.*, 1990; Poldrack *et al.*, 2005; Wu *et al.*, 2015). Furthermore, step time variability improves with dopaminergic replacement therapies in Parkinson's disease (Figure 1) (Hausdorff, *et al.*, 2003a; Schaafsma *et al.*, 2003; Bryant *et al.*, 2016). The dopamine depletion in the posterior putamen is also likely to impact on the anterior-to-posterior striatal shift (Ashby *et al.*, 2010; Everitt and Robbins, 2016), thus placing additional load on the frontal attentional resources that further prevents patients from effectively utilizing compensatory locomotor control strategies when automaticity is reduced

(Lewis and Barker, 2009; Helmich *et al.*, 2010; Vandenbossche *et al.*, 2012; Lewis and Shine, 2014; Wu *et al.*, 2015). Finally, it has often been suggested that Parkinson's patients utilize the cortico-cerebellar pathways involved during early motor learning in order to maintain motor functions following the cortico-striatal impairments (Hanakawa *et al.*, 1999; Wu and Hallett, 2013; Peterson and Horak, 2016). However, to date little evidence exists describing the neural mechanisms underlying deficits in locomotor automaticity and the role of compensatory strategies in Parkinson's disease due in great part to the mobility restrictions that accompany the neuroimaging of gait *per se* (Shine *et al.*, 2013a; Wu *et al.*, 2015). As such, we currently lack a precise understanding of how dopaminergic pathology impairs motor automaticity during gait (Vandenbossche *et al.*, 2012; Bohnen and Jahn, 2013; Lewis and Shine, 2014; Hamacher *et al.*, 2015; Wu *et al.*, 2015; Peterson and Horak, 2016). Furthermore, the role of dopaminergic medication in regulating locomotor automaticity in Parkinson's disease remains poorly understood. Finally, understanding compensatory mechanisms for overcoming locomotor automaticity impairments in Parkinson's disease remains an important unresolved clinical question (Nonnekes *et al.*, 2015; Wu *et al.*, 2015; Peterson and Horak, 2016).

Approximate Position Figure 1

In this study we set out to investigate the neural mechanisms of dopamine on repetitive lower limb movements in Parkinson's disease. Twenty-three patients with Parkinson's disease performed a virtual reality gait paradigm in conjunction with functional MRI both on and off their dopaminergic medication. The virtual reality task required patients to either operate foot pedals to navigate a virtual corridor (*walk*

condition) or to watch the screen while a researcher operated the paradigm from outside the scanner (*watch* condition). We hypothesized that in the off medication state, patients with Parkinson's disease would show increased variability in their step times as compared to the medicated state, indicative of reduced motor automaticity (Hausdorff, *et al.*, 2003b; Yogev *et al.*, 2005; Gilat *et al.*, 2013). We further hypothesized that this increase in variability would be associated with increased Blood Oxygenation Level Dependent (BOLD) in frontal and parietal cortical regions that are associated with the attentional control of movements (Ouchi *et al.*, 2001; Bohnen and Jahn, 2013; Wu *et al.*, 2011; 2015; Peterson and Horak, 2016). In addition, a recent resting state functional MRI study showed that the strength of connectivity across the striatum was significantly reduced in Parkinson patients off their dopaminergic medication, which even at rest impacted on large-scale sensorimotor network dynamics (Bell *et al.*, 2014). As such, we hypothesized that dopamine denervation would also have the strongest effect on the connectivity across the striatum during the virtual gait task (Bell *et al.*, 2014), as shown by a significant condition (*watch, walk*) by medication (off, on) interaction effect. Furthermore, we hypothesized that dopamine depleted connectivity changes across the striatum would correlate with increasing step time variability (Hausdorff, *et al.*, 2003a; Schaafsma *et al.*, 2003 Bryant *et al.*, 2016) and impact on the connectivity across large-scale motor automaticity and attentional motor control networks involved with gait in Parkinson's disease (Kelly *et al.*, 2009; Helmich *et al.*, 2010; Hacker *et al.*, 2012; Bohnen and Jahn, 2013; Bell *et al.*, 2014; Wu *et al.*, 2015).

Materials and Methods

Study protocol

Twenty-three patients with Parkinson's disease that satisfied the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria were recruited for this study from the Parkinson's Disease Research Clinic, Brain and Mind Centre, The University of Sydney. None of the patients were diagnosed with dementia according to the Movement Disorders Society guidelines (Goetz *et al.*, 2008) or major depression according to Diagnostic and Statistical Manual of Mental Disorders-IV guidelines of the American Psychiatric Association (*Diagnostic and statistical manual of mental disorders (4th ed., text rev.)*). None of the participants had any additional neurological comorbidities including no history of stroke or significant head injury. In addition, high-resolution T1-weighted images of each subject passed visual inspection by an experienced radiologist for absence of any pathological white or grey matter lesions. All patients received dopamine-replacement therapy as part of their daily clinical management. Specifically, twelve patients were on levodopa monotherapy; two patients were on dopamine agonist monotherapy; four patients were on levodopa plus a dopamine agonist; two patients were on levodopa plus a monoamine oxidase inhibitor; three patients were on levodopa plus a dopamine agonist plus a monoamine oxidase inhibitor. Patients were tested on two separate occasions, once whilst on their usual medications and once in the practically defined off state, having been withdrawn from their dopaminergic medication overnight for more than 12 hours before testing. The order of testing (ON/OFF) was randomized and counterbalanced between subjects with a minimum interval of three weeks between trials (mean: 7.4 $\pm$5 weeks).

In addition to PD participants, we also acquired imaging data in an independent group of twelve healthy age matched control participants (see Materials & Methods:

Regions of Interest) that were scanned on a single occasion. The acquisition of healthy control data enabled data-driven functional localization of regions involved in lower limb motor control during the virtual gait paradigm in an independent and matched cohort. Ethical approval for this study was obtained from the University of Sydney Human Research Ethics Committee and written informed consent was obtained from each subject in accordance with the Declaration of Helsinki.

Cognitive and neurological assessment

All subjects were assessed on the Mini Mental State Examination. In addition, the motor section of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (UPDRS-III) and the Hoehn and Yahr Stages (HY), both on and off medications were obtained from patients with Parkinson disease. Motor symptom severity was assessed per body side by calculating a sum score of the UPDRS-III items for the left and right body side separately (items 3.3-3.8 and 3.15-3.17). The body side with the highest sum score on these UPDRS-III items was defined as being most affected. Furthermore, item 3 of the Freezing of Gait Questionnaire (FOG-Q3) "*Do you feel that your feet get glued to the floor while walking, making a turn or when trying to initiate walking (freezing)?*" was obtained from each subject. Finally, dopamine dose equivalency scores were recorded. Cognitive and neurological measures were compared between medication states using a paired-samples t-test or non-parametric Wilcoxon Signed Rank Sum test (alpha = 0.05).

Virtual Reality Task

The virtual reality task was performed while subjects lay supine inside the MRI scanner. The duration of the task was 10 minutes. The task stimuli were presented on

a screen that could be clearly viewed via a mirror mounted onto the head coil. The virtual environment consisted of a straight corridor presented in the first person that contained no additional environmentally salient or cognitive triggers, which have previously been utilised to trigger freezing behaviour (motor arrest) (Figure 2A) (Shine, *et al.*, 2013a; Gilat *et al.*, 2013; 2015). Indeed very little behavioural freezing (less than 0.05% of the total paradigm) was observed in the current experiment. Progression through this corridor was accomplished by alternately depressing left and right foot pedals at least 30° below parallel in a physiological sequence (e.g. left-right-left-…). Out of sequence steps (left-left or right-right) did not result in forward progression and were disregarded from behavioural analyses. Subjects were instructed to tap the pedals in a comfortable rhythm as per previous work (Shine, *et al.*, 2013a; Gilat *et al.*, 2013; 2015). The paradigm was made up of alternating blocks that instructed the participants to perform either of two rules. *Walk* blocks were initiated with the word "WALK" being presented on screen instructing the participants to start operating the pedals. In addition, participants performed a control *watch* block. *Watch* blocks were initiated with the word "WATCH" being presented on screen instructing participants to refrain from operating the pedals and watch the screen while a researcher operated the task from outside the MRI scanner in a similar rhythm as the participants. Subjects were therefore presented with the same visual input and baseline cognitive and limbic processes as during *walk*, without the need for any motor activation of the legs. Each block lasted for approximately one minute depending on the subject's latency and was ended when the word "STOP" was presented on screen in the colour red. Six seconds after participants had stopped accordingly, the alternative of the previous instruction (either "WALK" or "WATCH") was presented. Each participant completed 4-5 alternating blocks of both

conditions with the first condition always being a *walk* block to allow the researcher to ensure that the patient had understood the instructions.

Behavioural measures

Footstep latency was calculated during *walk* by measuring the time between two consecutive foot pedal depressions. The first five steps following a "WALK" cue and any step following a "STOP" cue were excluded from the analyses to remove the effects of motor initiation and cessation (Georgiades *et al.*, 2016). The mean and standard deviation were then used to calculate the coefficient of variation, which is a measure of step time variability (Hausdorff, *et al.*, 2003a; Gilat *et al.*, 2013). A coefficient of variation in footstep latencies was also calculated for each foot separately. A repeated measures ANOVA was then used to investigate the interaction between medication (off, on) and symptom side (most affected, least affected) on the coefficient of variation for each leg separately. In addition, the coefficient of variation was compared between the most- and least affected side using a paired-sampled t-test for both medication states. These analyses were performed to assess whether any increase in variability may be confounded by asymmetry in symptom severity. No behavioural measures were obtained during *watch* blocks, however all participants responded adequately to the "WALK" cue that followed a *watch* block (<2 seconds initiation times), indicating that they were likely to be paying close attention to the task.

Neuroimaging

Image acquisition

A General Electric 3T MRI was used to obtain T_2*-weighted echo planar functional

images in sequential order with repetition time (TR)=3s, echo time=32ms, flip angle=90°, 32 axial slices covering the whole brain, field of view=220mm, interslice gap=0.4mm and raw voxel size=3.9mmx3.9mmx4mm thick. High-resolution 3D T1-weighted anatomical images with voxel size=0.4x0.4x0.9mm were obtained for co-registration with functional scans.

Image pre-processing

Image processing and analyses were performed using Statistical Parametric Mapping Software (SPM12, Wellcome Trust Centre for Neuroimaging, London, UK). Functional images were pre-processed using the standard pre-processing pipeline provided with SPM12. Functional scans were: (i) manually realigned along the anterior-posterior commissure; (ii) slice-time corrected to the median (17^{th}) slice in each scan; (iii) realigned to create a mean realigned image and measures of 6 degrees of rigid head movements were created for later use in the correction of minor head movements; (iv) unwarped to deal with residual movement related variance induced by the susceptibility-by-movement interaction effects; (v) spatially normalized using the T_1-weighted image to improve segmentation accuracy; (vi) co-registered and estimated; and (vii) smoothed using an 8-mm full-width at half maximum isotropic Gaussian kernel. Spatial normalization was then manually checked for quality assurance.

Head motion correction

Multiple precautions were taken to ensure head motion was fully accounted for: (i) all subjects were instructed to minimize head motion by only moving the ankles, while not raising the legs and restrict hip rotation; (ii) cushions were placed inside the head

coil to ensure optimal performance with the least amount of head motion; (iii) following data collection, trials with >3mm or 3° of scan-to-scan movement were considered *a-priori* exclusion criterion; (iv) six motion and nuisance regressors were added into the first level analysis per subject, controlling for minor movement artefacts in the three directions of translation and axes of rotation; (v) each trial was analysed using ArtRepair (Mazaika *et al.*, 2007) and trials with a large amount of global drift or scan-to-scan head movement >1.5mm were corrected using the interpolation method; and finally, (vi) mean frame-wise displacement was calculated per trial and compared across sessions, showing no significant differences between the on and off states (Parkinson patients off versus on: Z=-0.304, p=0.761) or between groups (Parkinson patients off versus controls: U=124, Z=-0.487, p=0.644; Parkinson patients on versus controls: U=126, Z=-0.417, p=0.694).

Event related Whole Brain analysis

Individual first-level spatial maps were created in SPM12 using a general linear model analysis within an epoch-related design in a fixed-effects analysis. A design matrix was created for each subject by entering two regressors for each trial: a regressor that modelled the specific onset times and associated temporal derivatives of *walk* blocks and a regressor that similarly modelled the *watch* blocks. Contrast images from the first-level analyses were then entered into a second-level random-effects dependent samples t-test design to test the effects of dopaminergic medication within the Parkinson's disease group on the condition of interest (*walk>watch*), whereas a one-sample t-test design was used for the healthy control group. To further investigate imaging-behaviour associations, 8mm ROIs were created around the peak voxel for each significant second-level cluster found when contrasting *walk>watch* between

medication states in Parkinson's disease.

In addition, a whole brain parametric modulation analysis was used to investigate which brain regions were involved in the modulation of step time variability. First, a normalized footstep variability value per TR was calculated for the *walk* blocks for each subject. The normalized footstep variability was calculated as an absolute Z-score of footstep latencies, which were then de-meaned and averaged per TR. This parametric modulator vector was then entered into a general linear model together with the six head motion regressors and time derivatives of the hemodynamic response function in the first level analysis of SPM12. Contrast images from this analysis were then entered into a second-level random-effects analysis: dependent samples t-test to investigate differences between dopaminergic states in the Parkinson's disease group; and one-sample t-test analysis for the healthy control group. Regions with a negative relationship to the parametric modulator were associated with maintaining low step time variability, whereas an increased BOLD response indicates that those regions were involved during period of increasing step time variability, and hence, worsened automaticity.

The whole brain voxel maps for both analyses were displayed using xjView (www.alivelearn.net/xjview) software ($p<0.005$, $k>20$) (Lieberman and Cunningham, 2009) and a threshold-free cluster enhancement (TFCE) for multiple corrections was performed on each second level contrast (Smith and Nichols, 2009). To explore the direction of pattern in the BOLD responses found in this study, significant clusters (See Supplementary Table 3 for MNI coordinates) from the within-Parkinson group second level T-maps were saved as images and raw Beta scores were then extracted

from these ROIs using the MarsBar toolbox (Brett *et al.*, 2002) in SPM12, the values of which were averaged for reporting purposes only.

Task-based functional Connectivity

In this analysis, we examined the effect of dopaminergic medication on large-scale network interactions within and between motor automaticity, attentional motor control and striatal networks. Task based functional connectivity in these networks was calculated using predefined regions of interest (ROI) as further described below. The Response Exploration for Neuroimaging datasets toolbox (Duff *et al.*, 2007) was used to extract time series data of each predefined ROI for each patient. Task-based functional connectivity was then calculated using the Multiplication of Temporal Derivatives statistical method (Shine *et al.*, 2015). This method allows greater temporal resolution of time-resolved connectivity in BOLD time series data when compared to the conventional sliding-window Pearson's correlation coefficient (Shine *et al.*, 2015; 2016). The code is freely available at http://github.com/macshine/coupling/ (Shine *et al.*, 2015; 2016). First, mean functional connectivity was calculated within and between each network of interest and entered into condition (*watch, walk*) by medication (on, off) repeated measures ANOVAs (alpha = 0.05, False Discovery Rate (FDR) correction 0.05). For significant interactions post-hoc correlations were performed to further examine whether the task-based functional connectivity was associated with worse step time variability. Furthermore, to examine whether striatal dopamine modulated the communication across large-scale networks, we further examined the effects of dopamine on internal network connectivity. A correlation analysis was performed on the amount of

functional connectivity during *walk* in the on state compared to the off state (on-off) for the connectivity across each network.

Regions of Interest

Regions of interest were defined using a combination of data-driven regions identified using the age-matched healthy control group during performance of the virtual gait task and pre-defined *a priori* regions shown to be involved in attentional motor control in Parkinson's disease and the striatum (see Supplementary Materials: Regions of Interest for full description). Defined regions of interest were then utilized for subsequent functional connectivity analyses (see Supplementary Table 2 for MNI coordinates). These regions composed three different networks: motor automaticity, attention motor and striatal networks.

Motor Automaticity Network

As it is currently unclear how lower limb motor automaticity is achieved, data from the twelve matched healthy control subjects was used to explore which brain regions would be involved with the performance of the virtual reality task (*walk>watch*), and specifically to identify which brain regions would be associated with maintaining low step time variability. Based on this data-driven approach we included the bilateral primary motor cortex area of the legs, thalamus, putamen, superior orbitofrontal gyrus, lateral cerebellum and anterior cingulate into the motor automaticity network. In addition, the cerebellar locomotor and bilateral mesencephalic locomotor regions were included as predefined *a-priori* regions of interest (See Supplementary Materials: Regions of Interest and Supplementary Table 1 and Table 2 for MNI coordinates).

Attention Motor Network

The low variability in step times seen in the healthy control participants did not allow for the investigation of the attention demanding cortical regions that are hypothesized to be associated with high step time variability in the Parkinson's disease group following dopamine depletion (Wu and Hallett, 2005; Yogev *et al.*, 2005; Bohnen and Jahn, 2013; Lewis and Shine, 2014; Clark, 2015; Wu *et al.*, 2015; Peterson and Horak, 2016). As such, predefined ROIs were created for regions that have been shown to be involved with the attentional control of movements in Parkinson's disease, including the dorsal lateral prefrontal cortex (DLPFC), pre-supplementary motor area (pre-SMA), dorsal premotor cortex (PMd) and posterior parietal cortex (PPC) (Cole and Schneider, 2007; Kurz *et al.*, 2012; Shine, *et al.*, 2013b; Wu *et al.*, 2011; 2015).

Striatal Network

The striatal network consisted of seven predefined regions of interest in the striatum of each hemisphere in the Parkinson's disease group, as described by Bell et al. (Bell *et al.*, 2014). These striatal regions correspond to dissociable functional systems (Di Martino *et al.*, 2008; Kelly *et al.*, 2009) and enable broad coverage of the striatum (Bell *et al.*, 2014). The ROIs included the bilateral: inferior ventral striatum (VSi), superior ventral striatum (VSs), dorsal caudate (DC), dorsal caudal putamen (DCP), dorsal rostral putamen (DRP), ventral rostral putamen (VRP) and postcommissural putamen (PCP) (Di Martino *et al.*, 2008; Kelly *et al.*, 2009; Bell *et al.*, 2014). Based on the study by Di Martino and colleagues (2008) we further defined the caudal putamen seeds (DCP and PCP) as being most involved with sensorimotor tasks, the

dorsal caudate (DC) with cognitive control and the inferior ventral striatum (VSi) seeds, which approximate the nucleus accumbens, with limbic processing (Di Martino *et al.*, 2008). These seeds were used to define striatal cognitive-motor, limbic-motor and limbic-cognitive connections during a post-hoc analysis, as further described below.

Results

Participant demographics and behavioural outcomes

Patients with Parkinson disease and healthy control subjects were matched across multiple demographics (see Supplementary Table 4). The Parkinson's disease patients had a mean levodopa equivalent daily dose of 861 (±525) and disease duration (in years) of 6 (± 2.9). Patients demonstrated clinical improvement following dopaminergic treatment, as indicated by a significantly lower UPDRS-III score and HY stages in the on state (Table 1). The current cohort consisted of patients with a range of freezing of gait severity, with eight subjects scoring a 0 ('Never'), six subjects scoring a 1 ('Very rarely') and nine subjects scoring >1 ('Rarely-Often-Always') on the FOG-Q3. Importantly, Parkinson's disease patients off medication had similar modal footstep latencies as compared to when medicated, which together with similar scores on UPDRS-III items 3.7 (toe tapping) indicate that any group differences found were unlikely to be driven by an overall difference in lower limb motor performance (e.g. rigidity) (Table 1). However as predicted, step time variability was significantly higher in Parkinson patients off their medication as compared to when on medication (Table 1), suggesting between group differences in motor automaticity. Indeed, no significant interaction effect was found between

medication (off, on) and symptom side severity (most affected, least affected) on the coefficient of variation of footstep latencies (F=0.992, p=0.330). This analysis also revealed no main effect of medication (F=2.698, p=0.115) or main effect of symptom side severity (F=0.598, p=0.448). Furthermore, a difference score in toe tapping ability between the most- and least affected side did not significantly correlate with the coefficient of variation in footstep latencies in either medication state (Spearman correlation OFF: ρ=0.318, p=0.139; ON: ρ=-0.311, p=0.149). Together, these results indicate that the change in step time variability seen between medication states did not reflect asymmetry in symptom severity.

Table 1: Demographic and Behavioural Statistics within the Parkinson's disease group (n=23) during different medication states (off/on).

Task	OFF	ON	Test-value	P-value
UPDRS-III	27.8 (14)	22.9 (14)	2.62	0.015 [a]
HY	2 (1-3)	2 (1-3)	-2.12	0.034 [b]
Toe-Tap Right	1 (0-3)	1 (0-4)	-0.428	0.669 [b]
Toe-Tap Left	1 (0-3)	1 (0-3)	-0.775	0.439 [b]
Modal FSL	0.48 (0.15)	0.48 (0.12)	-0.011	0.991 [a]
CV	29.6 (20)	16.4 (8.3)	3.50	0.002 [a]

NOTE: UPDRS-III = Movement Disorder Society Unified Parkinson's Disease Rating Scale motor section III, Toe-Tap = Scores on item 3.7 of the Movement Disorder Society Unified Parkinson's Disease Rating Scale, Modal FSL = Modal Footstep Latency during performance of *walk* in the virtual reality task, CV = Coefficient of Variation in footstep latencies during the performance of *walk* in the virtual reality task. [a]Paired-samples t-test used and Mean (SD) and t-value reported, [b]Non-parametric Wilcoxon Signed Ranks Test used and Median (Range) and Z-value reported for ordinal variables.

Neuroimaging results

Whole brain activation (walk>watch)

The dopamine-depleted state of Parkinson's disease was associated with a significantly positive BOLD response in the bilateral orbitofrontal cortex during *walk* compared to negative BOLD during *watch* (TFCE corrected, see Figure 2B and Supplementary Table 3 for peak voxel coordinates). In addition, within-group analysis revealed that in both medication states patients demonstrated activation of the primary motor cortex. However, when on medication patients also utilized the pre-supplementary motor area, visual cortex and cerebellum, whereas patients off medication utilized the right mid frontal gyrus and bilateral orbitofrontal cortex (see Supplementary Figure 2). For exploration (i.e. non-statistical) purposes only, post-hoc one-sampled and paired-sampled t-tests were performed on the mean beta values in the orbitofrontal clusters that were found to be significantly different between medication states on the condition of interest (walk>watch) (See Figure 2C). These results confirmed that in the off state, the beta values in the orbitofrontal clusters were significantly different from zero during both WALK and WATCH blocks (all $p<0.01$, Bonferonni corrected) and significantly different between WALK and WATCH blocks (Left orbitofrontal: $t=4.36$, $p<0.001$; Right orbitofrontal: $t=5.42$, $p<0.001$, Bonferonni corrected). No differences in beta values were found for both orbitofrontal clusters in the dopaminergic on state between WALK and WATCH blocks (Left orbitofrontal: $t=0.560$, $p=0.592$; Right orbitofrontal: $t=0.881$, $p=0.151$), which were also not significantly different from zero (all $p>0.1$).

Approximate Position Figure 2

Whole brain activation (Parametric Modulator)

The goal of this analysis was to identify regions that were consistently associated with greater step time variability. The BOLD responses associated with the parametric modulator in the Parkinson's disease group are presented in Figure 3. In the off state Parkinson's disease patients had significant negatively signed beta values across the right dorsal premotor cortex and left posterior parietal cortex in contrast to the slight positively signed beta values in these regions in the on state (Figure 3 C and 3 E). This suggests that a negative association exists between BOLD response in these regions and step time variability in the off state. Furthermore, patients on medication had significant positively signed beta values in the right lateral cerebellum compared to the off state (Figure 3 C and 3 E), indicating that the cerebellar regions were recruited during periods of increasing step time variability when medicated, although these findings did not survive TFCE correction for multiple comparisons. As tremor amplitude in Parkinson's disease has previously been associated with increased beta values in the cerebellum (Helmich *et al.*, 2012), a post-hoc Spearman correlation analysis was performed between the sum score of UPDRS-III tremor items 3.15-3.18 and the beta weights of the right cerebellum cluster found to be significantly associated with the parametric modulator. The results showed no significant correlations between tremor scores and cerebellar Beta weights for both medication states (OFF: ρ=0.199, p=0.362; ON: ρ=0.142, p=0.517), indicating that tremor was unlikely to explain the results found.

To explore whether Parkinson patients utilized a cortico-cerebellar network in order to operate movements as a compensation for cortico-striatal impairments (Rascol *et al.*, 1997; Doyon *et al.*, 2002), a post-hoc correlation analysis was performed between the loading of the parametric modulator regressor onto the right lateral cerebellum

that was found to be significant during the parametric modulator analysis contrast (OFF>ON, see Figure 3 C) and the functional connectivity within the attention motor control network. A positive correlation was found between the loading of the variability regressor onto the right lateral cerebellum and the total functional connectivity within the regions of the cognitive network for the Parkinson's disease group when off medication (r=0.452, p=0.030, uncorrected), whereas the negative correlation found when patients were medicated did not reach statistical significance (r=-0.246, p=0.259) (Figure 3 F).

Approximate Position Figure 3

Task-based functional connectivity

As predicted a significant main effect of condition was found for all network connections showing an increased functional connectivity during *walk* as compared to *watch* (see Supplementary Figure 3, FDR corrected). Furthermore, a significant main effect of medication was found within the attention motor network (FDR corrected, see Figure 4). A significant condition (*watch, walk*) by medication (off, on) interaction effect was found within the striatum (F(1,22)=5.022, p=0.035, uncorrected, see Figure 4). Post hoc simple effect analysis showed that patients on medication were able to increase internal functional connectivity within the striatal network during *walk* compared to *watch* (Mean difference=0.367, p=0.003, FDR corrected), whereas patients off medication were not (Mean difference=0.106, p=0.285).

To examine how striatal dopamine modulates the interactions across large-scale networks, we further examined the effects of dopamine on internal network

connectivity. Results showed that dopaminergic modulation of the striatal network significantly correlated with the degree of dopaminergic modulation of internal motor automaticity network (r=0.536, p=0.008, Bonferonni corrected) and internal attention-motor network connectivity (r=0.464, p=0.026, uncorrected). As expected, there was no relationship between dopaminergic modulation of the attention-motor network and motor automaticity networks (r=0.219, p=0.315), indicating that the dopaminergic innervation of the striatum may be a key driving factor in modulating dopamine mediated change in functional connectivity in other large-scale networks.

Approximate Position Figure 4

Imaging-Behaviour Associations

We further examined the relationship between the major imaging findings in this study and behaviour, in order to explain how breakdown in network communication perturbs locomotor automaticity in the dopamine depleted state. Following the significant condition by medication interaction effect within the striatum, a post-hoc correlation analysis was performed between the amount of functional connectivity within the striatum during *walk* and step time variability (Bonferonni corrected for multiple comparisons). Internal striatal functional connectivity during *walk* correlated strongly with increasing step time variability in the off state (r=0.616, p=0.002), whereas no such correlation was found in the on state (r=-0.233, p=0.284). To further explore these findings, a separate post-hoc analysis was performed to see which striatal circuit (e.g. cognitive-motor, limbic-motor or limbic-cognitive) was driving this correlation with step time variability during *walk* (Figure 5A-B). Interestingly, a significant correlation was found between step time variability and the functional

connectivity in the striatal limbic-motor (r=0.688, p<0.001) and limbic-cognitive (r=0.597, p=0.003) circuits, whereas the cognitive-motor (r=0.380, p=0.068) functional connectivity did not reach statistical significance (Figure 5A-B). No significant correlations were found in the on state (limbic-motor: r=-0.153, p=0.487; limbic-cognitive: r=-0.227, p=0.297; cognitive-motor: r=-0.142, p=0.519).

As aforementioned, the whole brain analysis comparing *walk>watch* between medication states revealed increased bilateral BOLD responses in the orbitofrontal cortex in the off state (see Figure 2). The orbitofrontal cortex is known to have connections with multiple areas of the striatum allowing it to adapt behaviour (Haber *et al.*, 1995; Schoenbaum *et al.*, 2009; Rolls, 2015). As such, to explore its influences in the current study a second post-hoc analysis was performed to investigate whether the functional connectivity between the orbitofrontal cortex (see supplementary table 3 for MNI coordinates) and intra-striatal circuits was correlated with step time variability (Figure 5C). The results showed that the functional connectivity between the bilateral orbitofrontal cortex and inferior ventral striatum (limbic, r=0.653, p<0.001) and dorsal putamen seeds (motor, r=0.643, p<0.001) were significantly correlated to step time variability in the off state (Bonferonni corrected for multiple comparisons), whereas no correlation was found between step time variability and the functional connectivity between the orbitofrontal cortex and dorsal caudate nucleus (cognitive, r=0.293, p=0.174) (Figure 5C). Again no significant correlations were found in the on state (motor: r=-0.323, p=0.132; cognitive: r=-0.167, p=0.447; limbic: r=-0.304, p=0.159).

Approximate Position Figure 5

Finally, it has been proposed that local depletion of dopamine levels within the striatum in Parkinson's disease can lead to a reduced ability to consecutively process information through complementary yet competing cortico-striatal neural pathways (e.g. motor, cognitive and limbic pathways), which may eventually result in the inhibition of brainstem locomotor centres (Lewis and Barker, 2009; Lewis and Shine, 2014). We therefore wanted to explore whether the increased striatal limbic functional connectivity in Parkinson's disease patients off medication could have been influenced by a reduced ability to integrate this information into the consecutive motor or cognitive cortico-striatal pathways. As such, a post-hoc analysis was performed by correlating the degree of functional connectivity within the striatal limbic circuits (e.g. limbic-motor and limbic-cognitive) with the amount of functional connectivity between the primary motor cortex and dorsal putamen (motor cortico-striatal loop) and between the dorsolateral prefrontal cortex and dorsal caudate (cognitive cortico-striatal loop) (Figure 6A). Results showed that during the on state, the functional connectivity in intra-striatal limbic circuits correlated strongly with the functional connectivity between regions of the cognitive cortico-striatal loop (striatal limbic-motor: $r=0.615$, $p=0.002$; striatal limbic-cognitive: $r=0.711$, $p<0.001$), but not the motor cortico-striatal loop (striatal limbic-motor: $r=-0.125$, $p=0.569$, limbic-cognitive: $r=0.204$, $p=0.351$) (Figure 6B-C). Interestingly, no significant correlations were found in the off medication state for either the cognitive cortico-striatal loop (striatal limbic-motor: $r=-0.189$, $p=0.387$; limbic-cognitive: $r=0.077$, $p=0.728$) or the motor cortico-striatal loop (striatal limbic-motor: $r=0.080$, $p=0.718$; limbic-cognitive: $r=0.074$, $p=0.738$).

Approximate Position Figure 6

Discussion

To our knowledge, this is the first study to investigate the role of dopamine on the neural mechanisms underlying lower limb motor automaticity impairments in Parkinson's disease. Twenty-three patients with Parkinson's disease performed an interactive virtual reality paradigm consisting of two conditions (*walk, watch*) in conjunction with functional MRI both on and off dopaminergic medication. The main results were: (i) Parkinson's disease patients had greater step time variability off dopaminergic medication and recruited the bilateral orbitofrontal cortex when performing lower limb movements in the virtual reality task, as compared to when appropriately medicated; (ii) in the dopamine-depleted "off" state, patients with Parkinson's disease demonstrated an over-reliance on regions associated with cognitive control, which is in contrast to the recruitment of the cerebellum to maintain low variability in the "on" state; (iii) dopamine had a demonstrable influence on intra-striatal functional connectivity during lower limb movements; (iv) in the dopamine depleted-state, functional connectivity in orbitofrontal-striatal limbic circuits was correlated with step time variability; (v) with the administration of dopamine, the aforementioned striatal limbic circuits became coupled with cognitive cortico-striatal pathways that are putatively used to integrate the limbic information in order to maintain effective motor performance.

As predicted, patients with Parkinson's disease demonstrated an increased step time variability during the virtual reality gait task in the dopamine depleted state compared to when medicated, indicative of a loss of motor automaticity (Hausdorff, 2003b;

Yogev *et al.*, 2005; Plotnik *et al.*, 2011; Kelly *et al.*, 2012; Gilat *et al.*, 2013). This finding is in accordance with a broad literature showing that dopaminergic medication improves step time variability during over ground walking in Parkinson's disease (Hausdorff, *et al.*, 2003b; Schaafsma *et al.*, 2003; Bryant *et al.*, 2016) and is thought to indicate a shift from an automated towards a more attention demanding cognitive strategy of motor control (Bohnen and Jahn, 2013; Clark, 2015; Peterson and Horak, 2016).

During the *walk* condition, patients with Parkinson's disease in the dopamine depleted state demonstrated significantly greater BOLD activation across the bilateral orbitofrontal cortex compared to the on state (TFCE corrected). The orbitofrontal cortex is involved in many functions including modulation of attention and goal-directed behaviour (Rolls and Grabenhorst, 2008; Lewis and Barker, 2009; Takahashi *et al.*, 2011; Hartikainen *et al.*, 2012; Marinelli *et al.*, 2015;). These findings are in accordance with Ouchi et al. (2001) who used dopamine transporter PET imaging (DAT and $^{[11C]}$CFT) to show that Parkinson's disease patients off their medication have significantly increased activation in dopaminergic neurons of the bilateral orbitofrontal cortices during continuous straight walking, whereas gait in healthy controls activated the dopaminergic neurons in the putamen (Ouchi *et al.*, 2001). Importantly, orbitofrontal $^{[11C]}$CFT uptake in Parkinson's disease was inversely correlated with cadence during gait (Ouchi *et al.*, 2001).

The novel parametric modulator analysis used in the current study showed that during periods of increasing variability Parkinson's disease patients on medication engaged the bilateral cerebellum, a key hub known to be important for automated feed-forward

control of motor timing and adaptation (Rand *et al.*, 1998; Lang and Bastian, 1999; Doya, 2000; Morton and Bastian, 2006; Wu and Hallett, 2013). Recruiting this region may have allowed patients on medication to appropriately adapt to sudden changes in step timing variability without the need for attentional control (Horak and Diener, 1994; Rand *et al.*, 1998; Doya, 2000; Morton and Bastian, 2006)Without dopaminergic medication however, the same patients became unable to recruit the cerebellum and instead relied on cortical regions associated with cognitive control. The slow and serial processing of these cognitive resources (Schneider and Chein, 2003) could have required a longer time for peripheral information to be integrated with the stepping pattern resulting in a higher step time variability (Lucas *et al.*, 2013; Shine, *et al.*, 2013a; Clark, 2015; Hamacher *et al.*, 2015). These results however did not survive TFCE correction for multiple comparisons, warranting cautious interpretation.

A post-hoc analysis further showed that the loading of the variability regressor onto the cerebellum correlated significantly with the amount of functional connectivity within the attentional motor network in the off state. This novel finding could reflect a compensatory increase in network level organization where the attentional motor network might be attempting to engage the cerebellum following a loss of motor automaticity in the striatum (Wu *et al.*, 2009; 2011), although seemingly failing to do so. As the cerebellum receives relatively minor dopaminergic innervation (localized mostly in the vermis) (Melchitzky and Lewis, 2000) but shares strong reciprocal connections to the basal ganglia (Morton and Bastian, 2004; Bostan *et al.*, 2013), it could be through functional coupling with the basal ganglia, that dopamine modulates cerebellar circuits. The failure to recruit the cerebellum in the off state of Parkinson's

disease could therefore be the result of impaired basal ganglia – cerebellar coupling (Morton and Bastian, 2004; Bostan *et al.*, 2013). This is in accordance with previous resting state functional MRI studies showing reduced functional coupling between the striatum and the cerebellum in the dopamine depleted state of Parkinson's disease (Hacker *et al.*, 2012; Jech *et al.*, 2013; Bell *et al.*, 2014).

Striatal dysfunction can lead to an over-activation of the output nuclei of the basal ganglia (e.g. subthalamic nucleus and globus pallidus internus) that send inhibitory GABAergic projections to the cerebellum (via the pontine nuclei), thus hampering cerebellar compensation abilities in Parkinson patients off medication (Lewis and Barker, 2009; Bostan *et al.*, 2013; Lewis and Shine, 2014). This is further evidenced by an increase in neuronal activation of deep cerebellar nuclei following high-frequency stimulation of the subthalamic nucleus in rats (Moers-Hornikx *et al.*, 2011) and normalized cerebellar activation in patients with Parkinson's disease that received subthalamic deep brain stimulation (Asanuma *et al.*, 2006; Grafton *et al.*, 2006; Hill *et al.*, 2013; Wu and Hallett, 2013). Future studies are encouraged to further investigate these important dopamine related compensatory and pathological alterations in striatal-cerebellar and cortico-cerebellar networks during gait in Parkinson's disease (Wu and Hallett, 2013). In addition, these results lend weight to investigating the potential therapeutic implications of non-invasive cerebellar stimulation techniques (e.g. transcranial magnetic stimulation) to improve gait in patients with Parkinson's disease (Koch *et al.*, 2008). The cerebellum also shares connections with the frontal and parietal cortices via the thalamus, which is also intimately involved in cortico-basal ganglia circuitry (Lewis and Barker, 2009; Bohnen and Jahn, 2013; Verlinden *et al.*, 2016). It is therefore somewhat surprising that the current study did not find any

significant thalamic influence on step time variability. For instance, higher white matter microstructure radiations between the thalamus and cortical and cerebellar regions have recently been shown to be associated with improved gait measures, including step width variability in healthy elderly (Verlinden *et al.*, 2016). Future studies specifically examining thalamic-cortical connectivity as a function of motor automaticity may be more sensitive to the effects of dopamine on thalamic circuitry. In addition, it remains to be determined whether white matter changes are associated with increased gait variability in Parkinson's disease. The negative association found in the off state between step time variability and BOLD responses of the right dorsal premotor cortex and left posterior parietal cortex indicate that Parkinson's disease patients are unable to bring these brain regions online, leading to increasing step time variability. Alternatively, patients may have utilized these cortical regions as a compensatory strategy to improve their stepping performance in the off state. Future studies are now needed to test these hypotheses, as causality could not be inferred from these results.

The functional connectivity analysis revealed a significant main effect of condition where each within and between network connection significantly increased its functional connectivity during *walk* compared to *watch*, highlighting the involvement of these predefined networks in lower limb motor performance in Parkinson's disease (Lewis and Barker, 2009; Bohnen and Jahn, 2013; Hamacher *et al.*, 2015; Wu *et al.*, 2015; Peterson and Horak, 2016). Furthermore, a significant main effect of medication was found within the attentional motor network, showing overall increased functional connectivity in the off state. This dopaminergic effect likely reflects the attentional compensatory strategy employed by Parkinson patients off

medication as a result of impaired striatal functioning (Bohnen and Jahn, 2013; Clark, 2015; Wu *et al.*, 2015). Indeed, the dopaminergic innervation of the striatum significantly correlated with the effects of dopamine within the other networks, reflecting the importance of striatal dopamine in large-scale network function during lower limb movements (Kelly *et al.*, 2009; Jech *et al.*, 2013; Bell *et al.*, 2014). Furthermore, a significant condition by medication interaction effect was found within the striatum. This finding advances previous resting state functional MRI studies by showing that impaired integration across striatal subdivisions in the dopamine depleted state of Parkinson's disease found during rest also affects lower limb motor performance (Helmich *et al.*, 2010; Surmeier *et al.*, 2010; Hacker *et al.*, 2012; Sharman *et al.*, 2013; Bell *et al.*, 2014).

To our knowledge, this study is the first to show that impaired crosstalk across the dopaminergically-depleted striatum of Parkinson's disease correlates with increased step time variability. Furthermore, our post-hoc analyses revealed that intra-striatal limbic circuits were driving this correlation with worse step time variability. In fact, the functional connectivity within the orbitofrontal-ventral striatum limbic circuit was strongly correlated with increased step time variability in the dopamine depleted state. The current study therefore provides novel pathophysiological evidence to suggest that activation in the orbitofrontal cortex during gait in Parkinson's disease is related to activity within a limbic cortico-striatal circuit that, in the context of reduced dopamine, interferes with the striatal control of lower limb motor function.

Our findings are supported by primate work showing that the terminals of the orbitofrontal cortex are extensive throughout the dopaminergic neurons, which

influence a wide area of the striatum, particularly the ventral striatum and core of the nucleus accumbens (Haber *et al.*, 1995). Furthermore, a resting state functional MRI study by Di Martino et al. (2008) showed that in healthy adults the spontaneous fluctuations in BOLD response in the inferior ventral striatum primarily correlated with the orbitofrontal cortex, indicating that strong functional connections exist between these regions in humans (Di Martino *et al.*, 2008). Yang et al. (2016) also recently showed that functional connectivity during rest was increased between the ventral striatum and orbitofrontal cortex in Parkinson's disease patients off dopaminergic medication as compared to when medication, indicating that dopamine has a profound influence on this orbitofrontal-striatal limbic circuitry (Yang *et al.*, 2016).

The striatal projections allow the orbitofrontal cortex to regulate motor actions under the influence of dopamine, for instance to adapt behaviour in the face of unexpected outcomes (Schoenbaum *et al.*, 2009; Takahashi *et al.*, 2009; Rolls, 2015). It has previously been proposed that error signals processed by the midbrain dopaminergic neurons that project to the striatum originate within regions of the orbitofrontal cortex that encode expected value and performance outcome, and which later connect to the ventral striatum (Takahashi *et al.*, 2011; Rolls, 2015). The orbitofrontal cortex indeed receives negative prediction error feedback (i.e. sensory evidence that did not match the predicted performance outcome) from every sensory system, making it an important hub for multisensory integration that enables planning and learning performance outcomes (Kringelbach, 2005; Schoenbaum *et al.*, 2009; Takahashi *et al.*, 2009; Goble *et al.*, 2011; Rolls, 2015; Chanes and Barrett, 2016). As Parkinson's disease patients have known sensorimotor impairments (Nolano *et al.*, 2008; Conte *et*

al., 2013; Ehgoetz Martens *et al.*, 2013; Lucas *et al.*, 2013), the increased BOLD responses found in the orbitofrontal cortex in the Parkinson group off medication could reflect an attempt to adapt behaviour following increased negative prediction error feedback (Kringelbach, 2005; Schoenbaum *et al.*, 2009; Takahashi *et al.*, 2009). Indeed, our results further showed that increased intra-striatal limbic functional connectivity was correlated with increased functional connectivity within regions of the cognitive cortico-striatal pathway selectively in the "on" state. This might indicate that with sufficient dopaminergic resources, the striatum is able to functionally integrate limbic information into the parallel cortico-striatal pathways, which can then provide top-down control over motor performance to resolve the prediction error and prevent motor deterioration (i.e. compensation) (Postuma and Dagher, 2006; Lewis and Barker, 2009; Rolls, 2015). However, dopamine depletion likely impairs such parallel cortico-striatal integration within the striatum, as evidenced by the lack of such correlations in "off" state (Lewis and Barker, 2009; Bell *et al.*, 2014; Wu *et al.*, 2015). The prediction error would therefore remain unresolved and hence, continue to induce an increased limbic drive within the striatal network. This could impair motor performance directly by innervating the inhibitory basal ganglia output structures that project to the brainstem locomotor centres (Lewis and Barker, 2009; Lewis and Shine, 2014). In addition, the increased limbic drive likely demands a proportion of the depleted dopaminergic resources within the striatum, further depriving the dorsal striatum from its ability to process automated motor sequences (Wu *et al.*, 2015). Therefore, based on the results of the current study, it can be postulated that although the ventral striatal-orbitofrontal circuit may usually be employed as a compensatory strategy to overcome negative error feedback, without sufficient dopaminergic

resources utilizing this network can actually deteriorate motor performance in Parkinson's disease patients.

This study now forms the basis for future work. The orbitofrontal cortex has previously been associated with the integration of emotional information into decision processes (Bechara *et al.*, 2000). In addition, anxiety and depression are associated with impaired gait performance in Parkinson's disease patients (Rochester *et al.*, 2004; 2005; Lord *et al.*, 2011; 2013; Ehgoetz Martens *et al.*, 2015;), and these deficits are amenable to dopaminergic medication (Ehgoetz Martens *et al.*, 2015). Previous authors have postulated that depression and anxiety impair goal-directed attentional processing (Rochester *et al.*, 2004; 2005), possibly by increasing computational load of the ventral striatum-orbitofrontal pathway resulting in response conflict in the dopamine depleted cortico-basal ganglia motor circuitry (Lewis and Barker, 2009; Ehgoetz Martens *et al.*, 2013; 2015). Future studies should therefore investigate the complex interaction between dopamine, mood disturbance and gait impairments in Parkinson's disease.

In addition, the current patient cohort consisted mostly of patients with moderate bilateral disease (1 subject scored HY=1; 18 subjects scored HY=2-3; 3 subjects scored HY=3 and no subjects scored HY>3). As such, future work is needed to investigate whether the neural basis underlying gait variability changes with disease severity. Furthermore, it is recommended that future studies account for laterality in brain pathology when analysing neuroimaging data in a cohort of Parkinson's disease patients with obvious unilateral symptom severities. It is also important to note that although dopamine evidently plays a key role, gait disturbances in Parkinson's

disease likely reflect a multisystem neurodegenerative disorder beyond the loss of dopaminergic neurons, especially as the disease progresses (Bohnen and Albin, 2011; Bohnen and Jahn, 2013; Bohnen *et al.*, 2013). For instance, prefrontal cholinergic neurons are key players in attentional control functions that may be put under increasing pressure following striatal and prefrontal deterioration in Parkinson's disease (Bohnen *et al.*, 2013; Gonzales and Smith, 2015). Gait speed has indeed been shown to be most affected in patients with both nigrostriatal dopaminergic and cortical cholinergic denervation (Bohnen *et al.*, 2013). Future studies using larger cohorts are now needed to investigate the role of dopaminergic, cholinergic and other neurotransmitter systems on gait automaticity impairments and freezing of gait in Parkinson's disease and Parkinson's disease subgroups, for example Parkinson's disease patients with and without freezing of gait (Bohnen and Albin, 2011; Bohnen *et al.*, 2014).

Furthermore, dual task interference provides an alternative avenue to study motor automaticity impairments (Yogev *et al.*, 2005; Kelly *et al.*, 2012; Wu *et al.*, 2015). Future studies are therefore encouraged to further evaluate dual task interference to confirm that the increased step time variability seen in the Parkinson's disease patients off medication reflects reduced motor automaticity (Poldrack *et al.*, 2005; Wu *et al.*, 2015). However, similar to walking and most daily behaviours, simple motor tasks such as foot tapping are usually performed automatically in healthy subjects (Wu *et al.*, 2015). Furthermore, once a motor task is automatic it becomes difficult to revert back to controlled behaviour (Schneider and Chein, 2003; Wu *et al.*, 2015). It can therefore be assumed that the performance in healthy control participants and low variability seen in Parkinson' s patients on medication indicated a more automatic

motor performance. In addition, a supplementary correlation analysis was performed between the functional connectivity values and another behavioural motor outcome of the virtual reality paradigm, namely modal footstep latency, showing no significant correlations (See Supplementary Table 4). This further indicates that the findings of the current study are indeed specific to step time variability and thus motor automaticity. In accordance with the findings in this study, previous behavioural research has shown that the administration of dopaminergic replacement therapy often improves step time variability in Parkinson's disease patients (Hausdorff, *et al.*, 2003a; Lord *et al.*, 2011; Rochester *et al.*, 2011; Bryant *et al.*, 2016). Furthermore, impairments in dual-task walking are also often improved by optimal dopaminergic replacement therapy (Camicioli *et al.*, 1998; Lord *et al.*, 2011; Elshehabi *et al.*, 2016). Together, this evidence suggests that dopaminergic therapy may influence gait-related neural computations through modulation of motor automaticity.

While multiple motor skills rely upon motor automaticity, in this study, step time variability was employed as a surrogate for motor automaticity in an attempt to model simple gait. Equally, however, dual task paradigms can also provide insights into the breakdown of automaticity in Parkinson's disease by loading additional attentional resources with overt task demands. Future studies are now needed to further investigate the interaction between dopamine and dual tasking ability during gait in patients with Parkinson's disease. Finally, a limitation inherent in the study of motor automaticity is that indirect surrogate measures are required. Therefore, the component processes by which dopaminergic replacement therapy improves motor skills cannot be precisely isolated. While it is hypothesized that dopamine replacement therapy exerts its major mechanism of action by improving motor

automaticity in subcortical structures leading to reduced step time variability, dopamine may also modulate other sub-processes including, coordination, balance, proprioception, affective processing and cognition that may affect step time variability and motor automaticity.

In conclusion, this study showed that dopamine depletion in Parkinson's disease impairs motor automaticity by reducing striatal functioning and cerebellar compensation strategies, which lead to increased attentional motor control and excessive orbitofrontal-striatal limbic interference.

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Figure Legends

Figure 1: Schematic representation of the locomotor automaticity processes in health and hypothesized neural mechanisms underlying automaticity impairments in Parkinson's disease with and without dopaminergic replacement therapy. Left image – hypothesized posterior motor network underlying locomotor automaticity in health; Middle image – dopaminergic pathology in posterior striatum in Parkinson's disease is thought to cause patients to utilize attention demanding cortical resources to operate gait; Right image – dopaminergic replacement therapy is thought to normalize locomotor automaticity impairments in Parkinson's disease. NOTE: "Off"=Dopamine depleted state; "On"=On dopaminergic replacement therapy.

Figure 2: Whole Brain analysis for *walk>watch.* A=visual representation of the virtual reality task; B=BOLD responses within the Parkinson's disease group (n=23) between medication states (off>on) on the condition of interest (*walk>watch*); $p<0.005$, $k>20$, TFCE corrected.

Figure 3: BOLD responses ($p<0.005$, $k>20$, uncorrected) associated with step time variability as per the parametric modulator analysis within the Parkinson's disease group (n=23), between medication states (off/on). A= Whole brain BOLD responses within the Parkinson's disease group off medication (PD OFF); B= Whole brain BOLD responses within the Parkinson's disease group on medication (PD ON); C= Whole brain BOLD responses within the Parkinson's disease group between medication states (PD OFF > PD ON); D=Parametric modulator data from one subjects in each group over the time course of the virtual reality task; E=Mean Beta values for the regions associated with the parametric modulator contrast OFF>ON, as

per figure C; F= Scatterplot and linear correlation between the loading of the parametric modulator (Beta values) onto the right lateral cerebellum cluster that was found to be significantly different on the contrast (OFF>ON) as per Figure 3C and the total functional connectivity within regions of the attention motor control network for Parkinson patients off medication (OFF) and on medication (ON). NOTE: ΔFSL=Absolute normalized and demeaned footstep latency per Repetition Time (3 seconds) over the course of the virtual reality task *walk* condition; HC= Healthy Controls; CBM=Cerebellum, PMd=dorsal premotor cortex, PPC=Posterior Parietal Cortex; *indicates significant correlation.

Figure 4: Results for the Condition (*watch, walk*) by Medication (off, on) Repeated Measures ANOVA on functional connectivity values within and between the three networks in Parkinson's disease patients (n=23). Top left - Main effect of Medication (off, on); Bottom left - Condition x Medication Interaction effect; Each matrix provides summary statistics from the Repeated measures ANOVA analysis for within- and between network functional connectivity. P-values are embedded within each cell of the matrix. The colour scale represents F-values. Top right - Mean MTD values within the attention motor control network showing the main effect of medication; Bottom right - Mean MTD values within the striatum network showing the significant condition x medication interaction effect. NOTE: Auto=Motor Automaticity Network; Attn=Attention Motor Network; Stri=Striatum Network; PD off = Parkinson's disease patients off dopaminergic replacement medication; PD on = Parkinson's disease patients on dopaminergic replacement medication; *$p<0.05$, **$p<0.05$ FDR corrected.

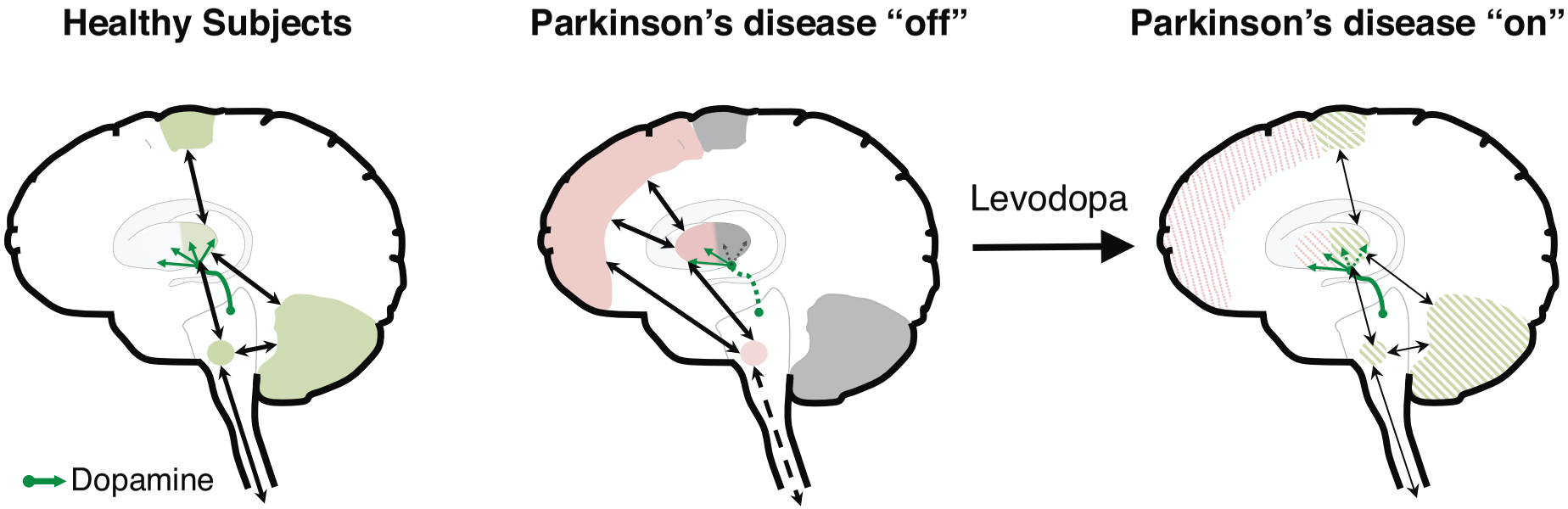
Figure 5: Post-hoc correlation analysis between functional connectivity in limbic circuits and step time variability during the *walk* condition of the virtual reality task in Parkinson's disease patients off their dopaminergic medication. A=Representation of the limbic intra-striatal and orbitofrontal-striatal functional connections; B=Scatter plots for the correlation analyses between functional connectivity in the intra-striatal limbic circuits and step time variability; C=Scatter plots for the correlation analyses between functional connectivity in the orbitofrontal-striatal limbic circuits and step time variability. NOTE: PD OFF=Parkinson's disease patients off their dopaminergic replacement medication (n=23); Step time variability measured as the coefficient of variation in footstep latencies; DC=Dorsal Caudate; DCP=Dorsal Caudal Putamen; VSi=Inferior Ventral Striatum; OFC=Orbitofrontal Cortex; CV=Coefficient of Variation; *Indicates a significant p-value ($p<0.05$) that survived Bonferonni correction for multiple comparisons.

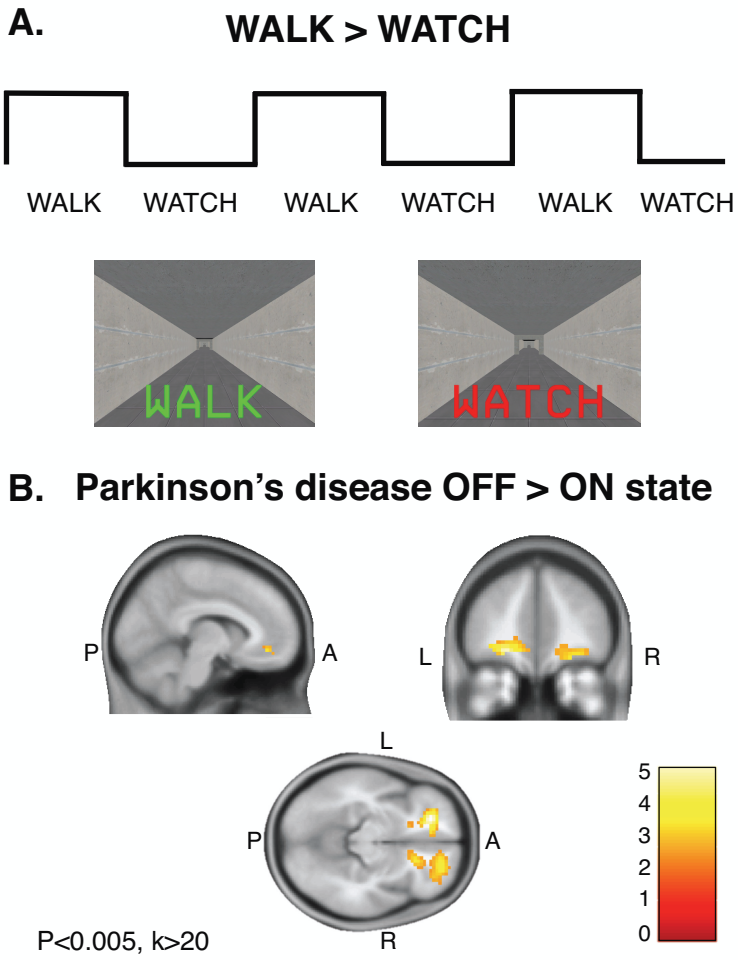
Figure 6: Post-hoc correlation analysis between the amount of functional connectivity in the cognitive and motor cortico-striatal loops and intra-striatal limbic pathways in Parkinson's disease patients on dopaminergic replacement medication (n=23). A=Representation of the cortico-striatal and intra-striatal limbic pathways used for the analysis; B=Scatter plots for the correlations between the amount of functional connectivity between the cognitive cortico-striatal loop and intra-striatal limbic pathways; C=Scatter plots for the correlations between the amount of functional connectivity between the motor cortico-striatal loop and intra-striatal limbic pathways. NOTE: PD ON = Parkinson's disease patients on dopaminergic replacement medication; DC=Dorsal Caudate; DCP=Dorsal Caudal Putamen; VSi=Inferior Ventral Striatum; DLPFC=Dorsolateral Prefrontal Cortex; M1=Primary

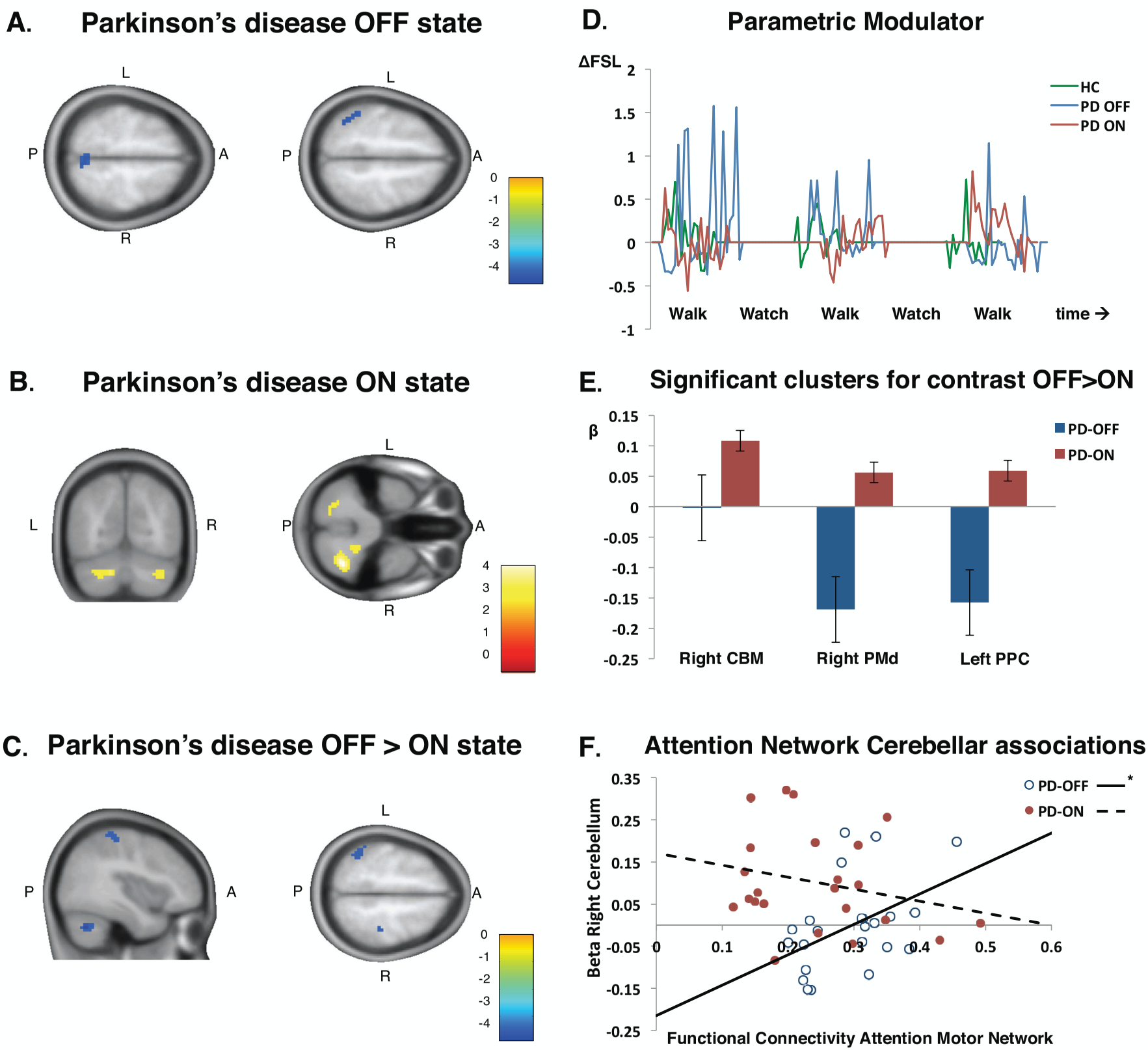
motor cortex of the leg area; *Indicates a significant p-value ($p<0.05$) that survived Bonferonni correction for multiple comparisons.

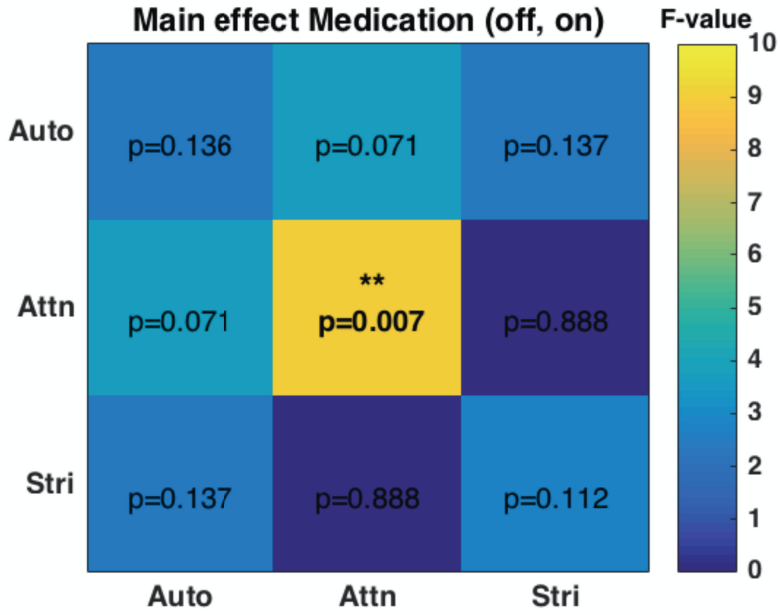
Highlights

- Parkinson's disease patients performed a virtual reality gait task during, fMRI
- The role of dopamine on gait automaticity impairments was investigated
- Limbic interference and poor striatal and cerebellar processing impair automaticity
- Dopamine ameliorates gait automaticity impairments in Parkinson's disease








Main effect Medication (off, on)
F-value
Auto
Attn
Stri
p=0.136
p=0.071
p=0.137
p=0.071
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p=0.007
p=0.888
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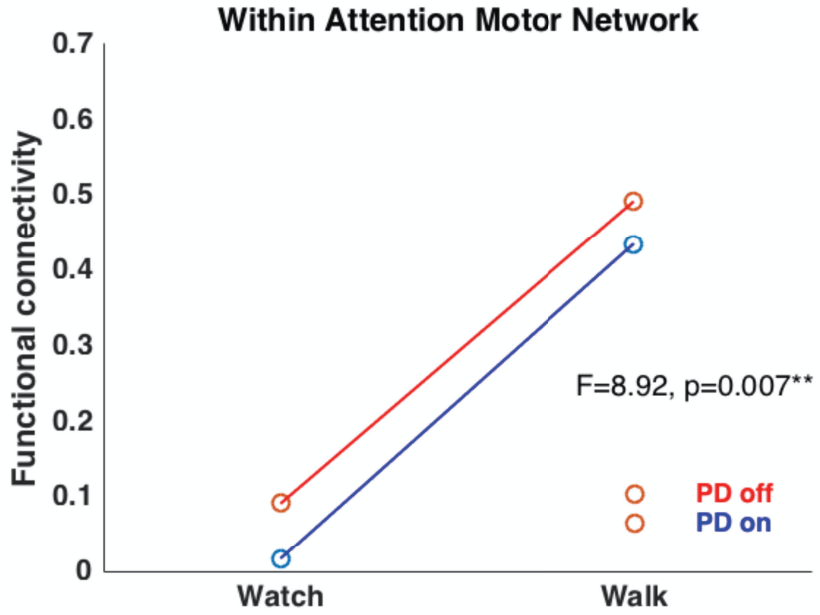
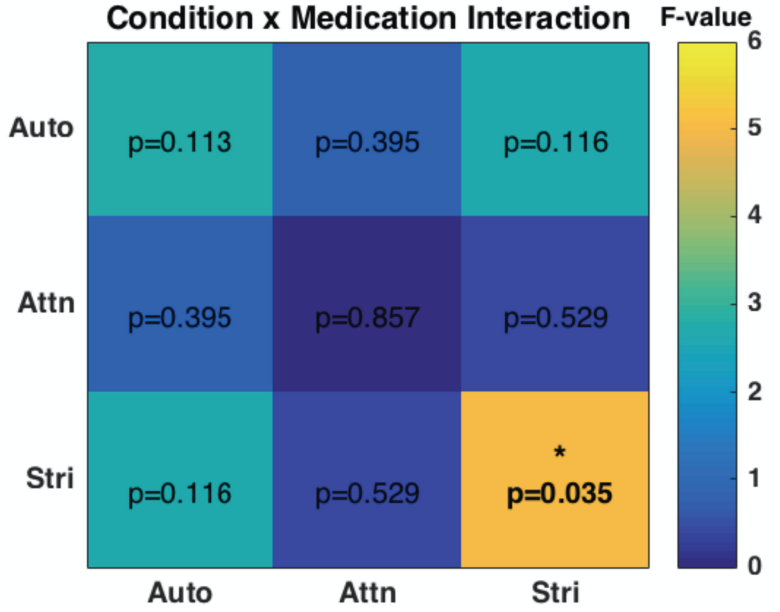

Within Attention Motor Network
Functional connectivity
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
F=8.92, p=0.007**
PD off
PD on
Watch
Walk


Condition x Medication Interaction
F-value
Auto
Attn
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p=0.113
p=0.395
p=0.116
p=0.395
p=0.857
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p=0.116
p=0.529
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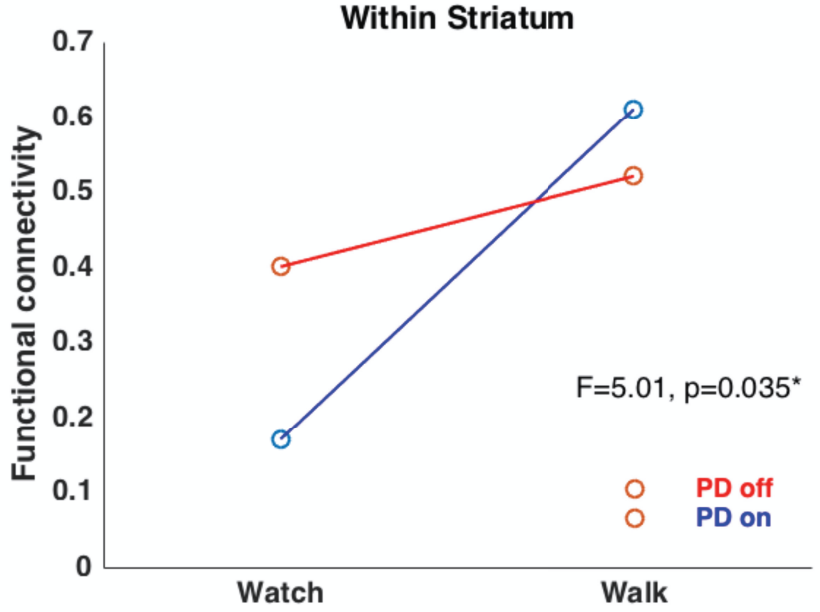
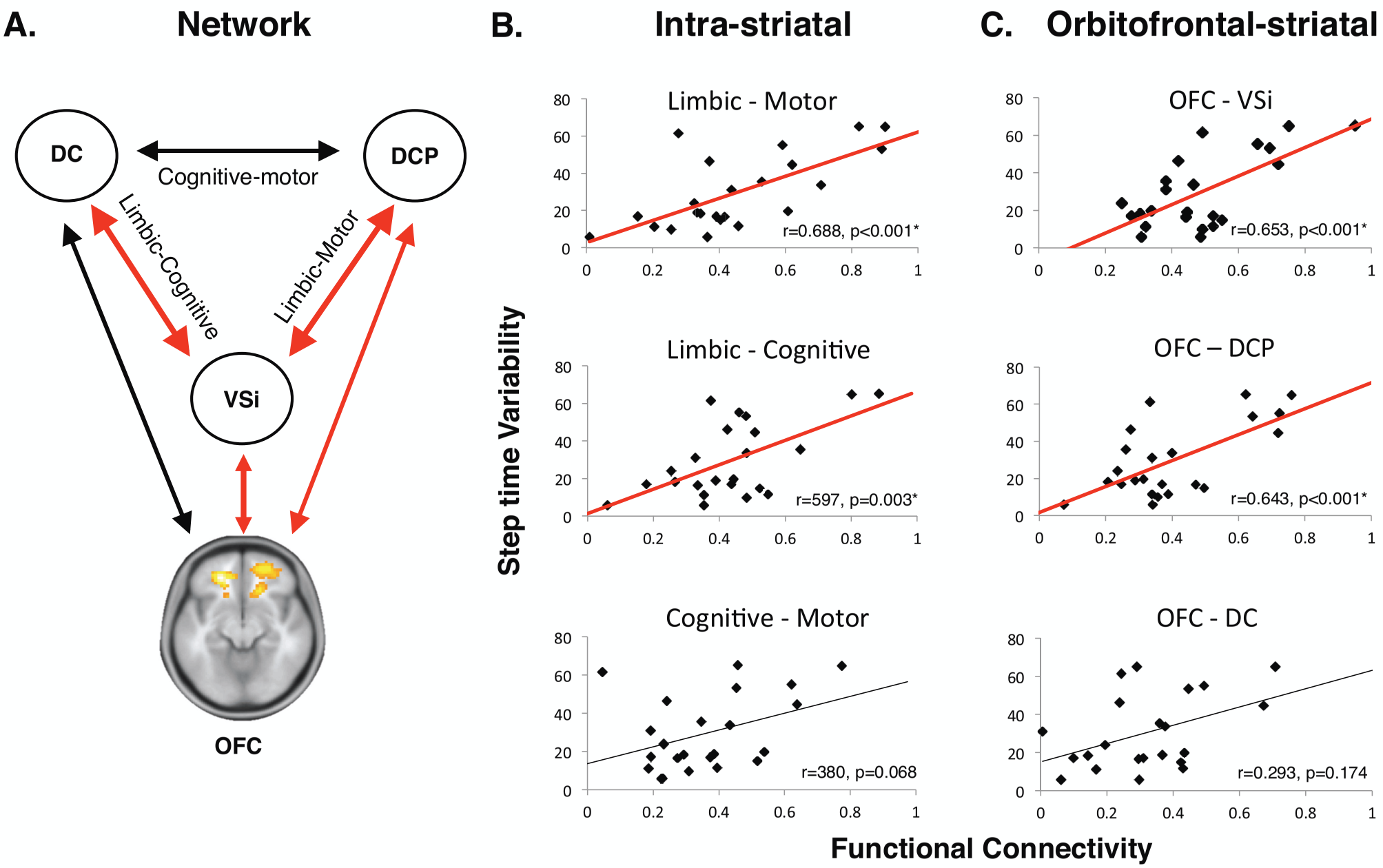
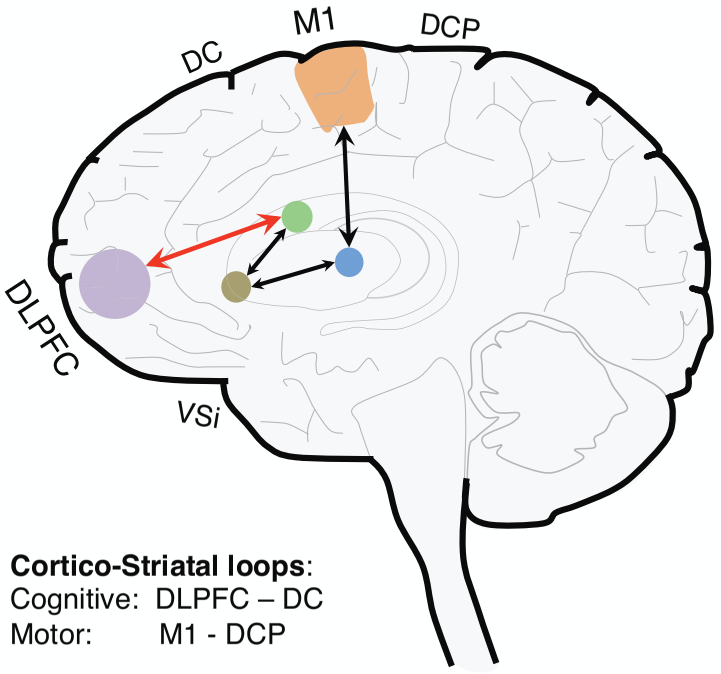

Within Striatum
Functional connectivity
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
F=5.01, p=0.035*
PD off
PD on
Watch
Walk


A.
Network
DC
DCP
Cognitive-motor
Limbic-Cognitive
Limbic-Motor
VSi
OFC
B.
Intra-striatal
C.
Orbitofrontal-striatal
Step time Variability
Limbic - Motor
r=0.688, p<0.001*
OFC - VSi
r=0.653, p<0.001*
Limbic - Cognitive
r=597, p=0.003*
OFC – DCP
r=0.643, p<0.001*
Cognitive - Motor
r=380, p=0.068
OFC - DC
r=0.293, p=0.174
Functional Connectivity

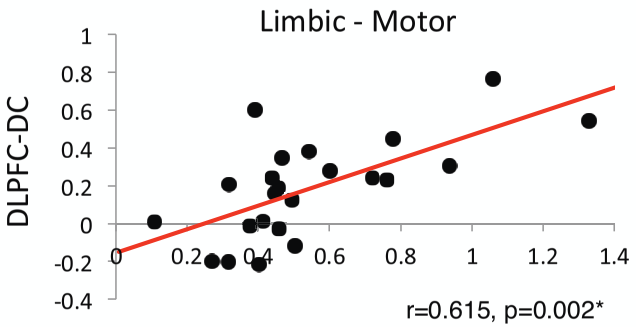
A. **Pathways**

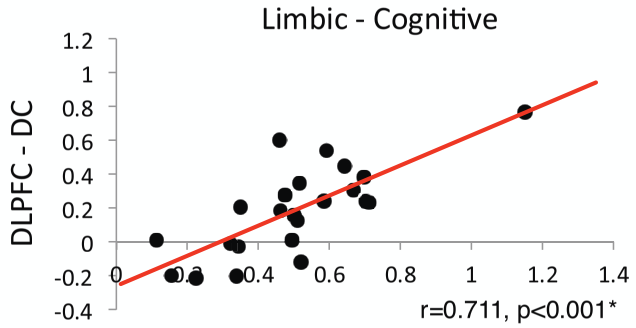


Cortico-Striatal loops:
Cognitive: DLPFC – DC
Motor: M1 - DCP

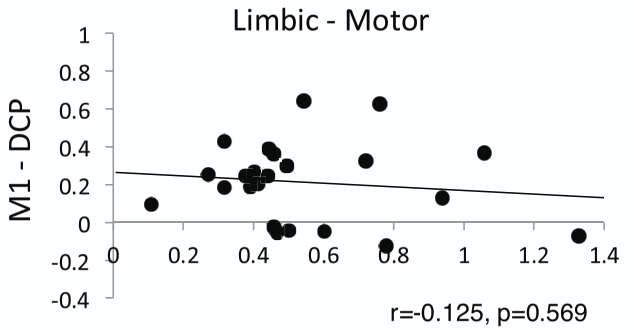
Intra-Striatal Limbic pathways:
Limbic – Motor: VSi – DCP
Limbic – Cognitive : VSi – DC

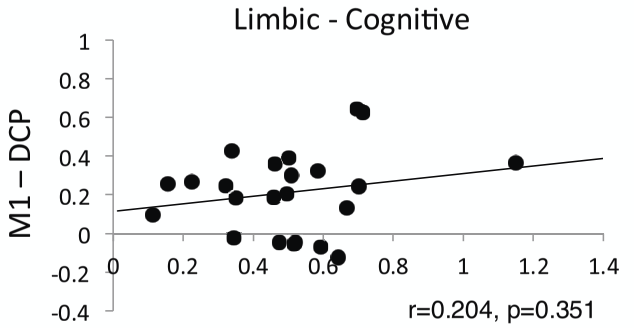
B. **Cognitive loop (DLPFC – DC)**





C. **Motor loop (M1– DCP)**





Functional Connectivity (PD-ON)