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Dysfunctional Limbic Circuitry Underlying Freezing Of Gait In Parkinson's Disease

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Abstract

Freezing of gait (FOG) is a poorly understood symptom affecting many patients with Parkinson's disease (PD). Despite growing evidence of a behavioural link between anxiety, attention and FOG in PD, no research to date has investigated the neural mechanisms that might explain this relationship. The present study therefore examined resting state MRI functional connectivity between the amygdala, striatum and frontoparietal attentional control network in PD patients with (freezers: n=19) and without FOG (non-freezers: n=21) in the dopaminergic 'off' state. Functional connectivity was subsequently correlated with an objective measure of FOG severity and a subjective scale of affective disorder within each group. Connectivity between the right amygdala and right putamen was significantly increased in freezers compared to non-freezers ($p<0.01$). Furthermore, freezers showed increased anticoupling between the frontoparietal network and left amygdala ($p=0.011$), but reduced anti-coupling between this network and the right putamen

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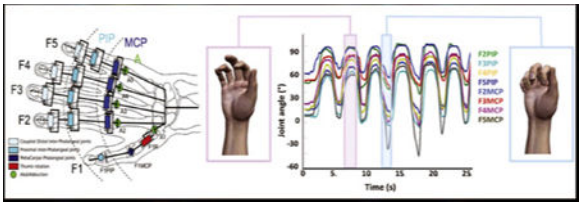
Declaration of interest

Dr Horak has significant financial interest in ADPM, a company that may have a commercial interest in the results of this research and technology. This conflict has been reviewed and managed by OHSU and the VA. All other authors declare that they have no conflicts of interest, financial or otherwise.

(p=0.027) as compared to non-freezers. Key functional connections between the amygdala, putamen and frontoparietal network were significantly associated with FOG severity and a fear of falling. This study provides the first evidence that dysfunctional fronto- striato-limbic processes may underpin the link between anxiety and FOG in PD. It is proposed that freezers have heightened striato-limbic load and reduced top-down attentional control at rest, which when further challenged by the parallel processing demands of walking may precipitate FOG.

Graphical abstract



Keywords

Parkinson’s disease; Gait disorders; Anxiety; Amygdala; Putamen; Functional MRI

Introduction

Freezing of Gait (FOG) is a devastating symptom of Parkinson’s disease (PD), which can be defined as a “brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk” (Nutt et al., 2011). FOG affects approximately 50–80% of patients with PD eventually, causing frequent falls and a decreased quality of life (Nutt et al., 2011; Auyeung et al., 2012; Walton et al., 2015b). Unfortunately, FOG is difficult to treat because it has a complex and poorly understood pathophysiology (Nieuwboer and Giladi, 2013; Lewis and Shine, 2014).

The pathological hallmark of PD is the degeneration of nigral-striatal dopaminergic neurons and it has, thus, been hypothesized that the dopamine-depleted striatum in PD becomes impaired in its ability to concurrently process information from segregated yet complimentary motor, cognitive and limbic cortico-striatal loops (Lewis and Barker, 2009). During certain, challenging situations the processing capacity of the striatum could become overwhelmed, resulting in an over-activation of the striatal output nuclei of the basal ganglia that send excessive GABAergic inhibitory projections to thalamic and brainstem locomotor regions causing a breakdown of gait and ultimately FOG (Lewis and Barker, 2009; Lewis and Shine, 2014). According to this model, any increase in processing demands in the striatum would increase the likelihood for FOG to occur in the absence of adequate dopaminergic resources.

In support of the model by Lewis and Barker (2009), certain motor (i.e. turning), cognitive (i.e. dual-tasking) and affective (i.e. anxiety) situations can all provoke FOG in PD, especially in the dopaminergic ‘off state’ (Schaafsma et al., 2003; Nutt et al., 2011; Ehgoetz Martens et al., 2014). Furthermore, important insights into the pathophysiology of FOG have been gained from studies that used functional MRI to investigate the motor and cognitive

network changes associated with freezing. For instance, task based functional MRI studies have shown reduced activation in cortico-striatal locomotor pathways and a decrease in functional connectivity between the frontoparietal attentional control network and the basal ganglia during freezing behaviour (Shine et al., 2013a; 2013b). Resting state functional MRI (rsfMRI) studies have most consistently revealed altered connectivity in the sensorimotor (Fling et al., 2014; Wang et al., 2016) and reduced connectivity in the frontoparietal and visual networks in freezers compared to non-freezers (Tessitore et al., 2012; Canu et al., 2015). However, no studies have yet investigated the limbic network differences associated with FOG in PD.

FOG often coincides with panic attacks in PD (Lieberman, 2006) and there has been increasing evidence of a relationship between limbic dysfunction (such as anxiety) and FOG (Giladi and Hausdorff, 2006; Burn et al., 2012; Ehgoetz Martens et al., 2014). For example, threatening situations (i.e. walking in the dark or crossing an elevated plank in virtual reality), which provoke greater anxiety, also elicit a greater amount of FOG in PD (Ehgoetz Martens et al., 2013; 2014). Visceral responses associated with anxiety, such as changes in heart rate and skin conductance, have also been shown to occur immediately prior to and during FOG episodes (Maidan et al., 2010; Mazilu et al., 2015).

The amygdala is a key neural structure of the limbic network that plays a critical role in emotional processing, particularly of stimuli that induce fear and anxiety (Mitchell et al., 2008; Marchand, 2010; Rohr et al., 2015; He et al., 2016; Vriend et al., 2016; Diederich et al., 2016; Sprooten et al., 2017). One of the primary functions of the amygdala is to rapidly detect internal and external sensory stimuli that pose a potential threat (Butler et al., 2007; Mitchell et al., 2008; Diederich et al., 2016). In addition, the amygdala has widespread efferent connections that allow it to quickly induce changes in both the body (rapid visceral and motor reactions) and brain (bias attentional resources towards the potential threat) during times of perceived threat (Bar-Haim et al., 2007; Mitchell et al., 2008; Marchand, 2010; Rohr et al., 2015). The motor reactions to threat often occur rapidly in a reflex-like manner and there is evidence to suggest that the amygdala projects to the striatum in order to achieve such feed-forward, emotional-motor responses (Butler et al., 2007; Marchand, 2010). Indeed, the amygdala directly innervates the striatum and has been shown to modulate ongoing dopamine signalling (Jackson and Moghaddam, 2001; Marchand, 2010).

The limbic input to the striatum may be further exacerbated in freezers due to a breakdown in the frontoparietal attentional control network (FPN). The FPN is often recruited to exert top-down control over the emotional responses from the threat system during instances of false alarm, or once the threat no longer poses a significant risk (Marchand, 2010; Sylvester et al., 2012; Ochsner et al., 2012; Rohr et al., 2015; De Witte and Mueller, 2016). However, many studies have demonstrated altered FPN network functioning in PD freezers (Tessitore et al., 2012; Shine et al., 2013b; Canu et al., 2015), accompanied by impaired executive functions that largely rely on overlapping brain networks (Amboni et al., 2008; Shine et al., 2013b; Brugger et al., 2015; Walton et al., 2015b). Furthermore, freezers are known to rely on attentional resources to operate their gait due to a loss of automaticity (Vandenbossche et al., 2012; Peterson et al., 2015; de Souza Fortaleza et al., 2017), which could make them particularly susceptible for limbic interference, since both limbic and motor processes

compete for attentional resources during gait. This competition could in turn lead to motor breakdown, especially as attentional resources are biased by the amygdala towards processing perceived threat (Eysenck et al., 2007; Bishop, 2007; Pessoa, 2009; Derakshan et al., 2009). Taken together, it is possible that a reduced ability of the FPN to exert top down control over the amygdala in PD may result in an amygdala demand on the striatum, which due to a lack of dopaminergic and attentional resources could increase the risk of FOG (Lewis and Barker, 2009; Ehgoetz Martens et al., 2014).

In this study, rsfMRI was used to investigate whether there are baseline differences in functional connectivity between the amygdala, striatum and the FPN between PD patients with and without FOG withdrawn overnight from their dopaminergic medication. It was hypothesized that PD patients with FOG would show increased resting state functional connectivity (rsFC) between the amygdala and striatum. Secondly, we hypothesized that PD patients with FOG would show reduced rsFC between the amygdala and FPN. Finally, we hypothesized that the degree of alteration in these limbic pathways at rest would be associated with an objective measure of FOG severity tested during a turning task outside the scanner and a subjective measure of affective, in particular anxiety.

Experimental Procedures

Participants

Data was collected from 46 patients that were diagnosed with idiopathic Parkinson's disease by a neurologist and movement disorder specialist and recruited through the Parkinson's Center of Oregon clinic at the Oregon Health & Science University (OHSU). Individuals were excluded from participation if they could not safely walk 20 feet without walking aids, or if they had a joint replacement, musculoskeletal or vestibular disorder, claustrophobia, severe tremor, or metal implants in their bodies. Data from six participants had to be excluded from the analyses due to violation of stringent movement thresholds during functional imaging acquisition as further described below. The remaining subjects (n=40) were subsequently divided into those with FOG (PD+FOG: n=19) as based on a score of >3 on the New Freezing of Gait Questionnaire (NFOG-Q) and those without FOG as based on a null score on this questionnaire (PD-FOG: n=21) (Nieuwboer et al., 2009). All patients were tested in the practically defined off state, having been withdrawn from their dopaminergic medication for a minimum of 12 hours prior to testing. Oregon Health & Science University Institutional Review Board approved this study and all patients gave their written informed consent prior to participation in accordance with the declaration of Helsinki.

Demographic variables

All patients were assessed on the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Hoehn and Yahr Staging (HY), Montreal Cognitive Assessment (MOCA) and Parkinson's Disease Questionnaire 39 (PDQ-39). The scores (ranging 0–4) on three items of the PDQ-39 were used as crude measures of affective disorder within the groups. Specifically, items 17 ("*During the last month have you felt depressed*?") and 21 ("*During the last month have you felt anxious?*") were used as general measures of affective disorder, whereas item 9 ("*During the last month have you felt*

frightened or worried about falling over in public?") was included as a subjective measure of anxiety during gait (i.e. fear of falling). For reporting purposes, a Postural Instability Gait Difficulty score (PIGD) was also calculated from the MDS-UPDRS-III by summing the items that reflect gait and balance difficulties (Stebbins et al., 2013). In addition, the sum of the MDS-UPDRS- III items for the left- and right sides of the body were used to determine the worst symptom side for each patient. Finally, each patient's mean levodopa equivalent daily dose (LEDD) was calculated (Tomlinson et al., 2010).

Similar to the wider PD population (Gallagher and Schrag, 2012), four patients in each group were taking a stable dose of medications for mood disorders for at least one month prior to their participation. Furthermore, three patients (2 PD+FOG and 1 PD-FOG) scored <21 points on the MOCA, implicating probable cognitive impairments (Dalrymple-Alford et al., 2010). However, because: i) cognitive decline is prevalent in the PD population (Aarsland et al., 2017); ii) the current study did not involve a cognitive task; and iii) the data from these participants passed quality assurance; it was decided to include the data from these participants in the analyses and minimize loss of statistical power. To support the robustness of our findings, supplementary analyses were performed following exclusion of these participants.

Objective FOG measure

In order to obtain a measure for FOG severity a freezing ratio (FOG-ratio) was calculated during a 2-minute long turning task in which participants made 360° turns on the spot, alternating between clockwise and anti-clockwise turns as fast as they could safely do, see Mancini et al. (2017). Turning on the spot is a highly provocative trigger for FOG, which is otherwise difficult to elicit in a laboratory setting (Snijders et al., 2012). Data was collected at 128Hz from 3 inertial sensors (Opals, by APDM Inc.) placed on the shins and at the lumbar level and stored for offline analysis using Matlab 2016b (Mathworks Inc.). Specifically, power spectral density from the anterior-posterior acceleration data was calculated for each trial and normalized for each subject to the area under the power spectral density curve (see Figure 1). The FOG-ratio was then calculated as the ratio between the square of the total power in the frequency band corresponding with freezing episodes (3–8 Hz), and the total power in the frequency band corresponding with locomotion (0.5–3 Hz) (Moore et al., 2008; Mancini et al., 2017). Higher FOG-ratio scores therefore indicate greater FOG severity (Mancini et al., 2017).

Image acquisition

Participants were scanned using a 3.0 Tesla Siemens Magnetom Trio scanner with a 12-channel head coil at the Oregon Health and Science University's Advanced Imaging Research Centre. High-resolution structural 3D T1- and T2-weigthed images were obtained for co-registration with functional images. Tl-weighted images were acquired using a sagittal magnetization prepared rapid gradient echo (MPRAGE) sequence (TR=2300 ms, TE=3.58ms, voxel size=1mm x 1mm x 1.1mm, slice s=160). T2-weighted images were acquired using the following parameters: TR=3200 ms, TE=497ms, voxel size=1mm^3, slices=160). Diffusion field maps were also acquired to correct for geometric distortions caused by susceptibility artefact. Resting state functional BOLD images were obtained using

a gradient-echo planar imaging (EPI) sequence (TR=2000ms, TE=30ms, field of view=240mm, flip angle=90°, voxel size=3.75×3.75×3.8mm). Whole brain coverage was achieved with 36 contiguous and interleaved axial slices acquired parallel to the plane transecting the anterior and posterior commissure. Steady-state magnetization was assumed after 5 frames (10s). Participants were instructed to relax but keep as still as possible with the eyes open while viewing a standard crosshair. All participants completed two rsfMRI scans consisting of 10 minutes (300 frames) each to maximize the number of volumes that could be retained following data quality assurance.

Image pre-processing

General pre-processing—Pre-processing was performed according to the HCP pipelines (Glasser *et al.,* 2013), which in turn utilize FSL (Smith et al., 2004; Woolrich et al., 2009; Jenkinson et al., 2012) and FreeSurfer (Dale et al., 1999; Fischl and Dale, 2000; Desikan et al., 2006) for different analysis steps. In short, T1 and T2-weighted volumes were corrected for gradient distortion and aligned to the MNI AC-PC axis prior to non-linear normalization to the MNI atlas. To improve alignment, the T1 and T2-weigthed volumes were then re-registered using boundary-based registration (Greve and Fischl, 2009). The recon-all tool from FreeSurfer was utilized for brain segmentation and the delineation of the cortical ribbon was subsequently improved by using the enhanced matter-pial surface contrast of the T2-weighted sequence. Distortions in the BOLD data were corrected using FSL's TOPUP tool and processed by a preliminary six degrees of freedom linear registration to the first frame. The average frame was then calculated and used as a final reference. In a single final step the BOLD data was registered to this final reference and the T1-weighted volume by concatenating all the individual registrations into a single registration.

Surface based registration—Surface registration of the BOLD data involved the definition of a high-resolution mesh of the cortical ribbon from the structural T1 and T2-weighted volumes. This cortical ribbon was subsequently used to quantify the partial contribution of each voxel in the BOLD data to the surface registration. Time series in the cortical mesh were calculated by a weighted average of the voxels neighbouring each vertex within the grid. The weights were derived from the number of voxels, wholly or partially within the cortical ribbon. Voxels with high coefficients of variation indicating poor tissue alignment or presence of large blood vessels were excluded. The resulting time series from the cortical mesh were then down sampled into a standard space of anchor points (i.e. grayordinates). The grayordinates were defined in the brain atlas and mapped uniquely to each patient's brain following 2-mm full-width-half-maximum Gaussian smoothing. The subcortical regions were treated and registered as volumes. Around two-thirds of the grayordinates were vertices in the cortical ribbon and the remaining grayordinates subcortical voxels.

Nuisance correction—Rigorous nuisance regression was performed by regressing out ventricle, grey matter and white matter average signals, the six motion and nuisance regressors in the three directions of translation and axes of rotation on the actual and previous TR as well as their squares corresponding to the Volterra series expansion of motion (Friston et al., 1996; Power et al., 2012; 2014). The regression coefficients, or beta

weights, were only calculated from frames with low movement while the regression was performed across all frames to preserve temporal order in the data to allow for filtering in the time domain. The time series were finally subjected to a first order Butterworth band pass filter (9–80 mHz).

Regions of interest—Pre-processed time series were extracted from the average bold signal within the bilateral amygdala and striatal regions of interest (ROIs) for each participant, after coregistration with the FreeSurfer subcortical atlas. The striatal ROIs consisted of the bilateral nucleus accumbens, caudate nucleus and putamen (Figure 2). For the size (i.e. number of voxels) of these ROIs see Appendix A. A data-driven parcellation schema proposed by Gordon was used to delineate 24 frontal and parietal ROIs that comprised a predefined frontoparietal attentional control network (FPN) (Figure 2) (Gordon et al., 2016). For the size (i.e. number of grayordinates) of each parcel see Appendix B.

Motion censoring—We excluded frames coming from volumes with a relative head displacement greater than 0.4 mm in any direction (Frame displacement) (Power et al., 2012). Only data from subjects with at least 5 minutes (150 frames) were included. Data from six participants were therefore excluded from further analysis while it assured good data quality for the remaining participants.

Functional connectivity

Functional connectivity was calculated by performing Pearson correlations between the pre-processed time series of the selected regions of interest after motion censoring. The functional connectivity values were then normalized for each participant using Fisher r-Z transformation for further statistical analysis.

Statistical analyses

Demographic statistics—Demographic statistics were compared between the groups using t-tests or Mann Whitney-U tests depending on data distribution. A χ-square test was applied for gender distribution and the laterality of motor signs.

Amygdala - Striatal connectivity—To investigate whether FOG in PD was characterized by increased amygdalar-striatal projections, the rsFC between the amygdala and striatal ROIs was compared between PD+FOG and PD-FOG using independent samples t-tests. Only connections within the same hemisphere were analysed to limit the number of comparisons and prevent alpha inflation while a False Discovery Rate (FDR, $\alpha<0.05$) correction for multiple comparisons was also applied. Based on the results of these analyses, only the putamen seeds were used for further processing. As it has been most well established that the amygdala projects to the ventral striatum in order to modulate behaviour (Mogenson et al., 1980; Marchand, 2010), a correlation analysis was also performed to investigate whether the connectivity between the amygdala and nucleus accumbens seeds (i.e. more ventral striatum) might be associated with the connectivity between the nucleus accumbens and putamen (i.e. more dorsal motor striatum). In addition, to examine result laterality, a post-hoc correlation analysis was performed to investigate whether the rsFC

between the amygdala and putamen on the left was associated with the rsFC between the amygdala and putamen on the right.

Subcortical - frontoparietal network connectivity—To investigate whether there was a difference in top-down attentional control over the amygdala across groups a functional connectivity score between the time series of the bilateral amygdalar seeds and an averaged time series of all 24 FPN ROIs was calculated. Differences in functional connectivity were compared between the groups using t-tests. The same analysis was also performed using the bilateral putamen instead of the bilateral amygdala to investigate whether the FPN also differentially influenced striatal processing across the groups. A post-hoc analysis was then performed to investigate which rsFC between these subcortical seeds and each of the 24 FPN ROIs was most significantly different between the groups. These rsFC values were compared between groups using t-tests and FDR correction was applied.

Connectivity - FOG associations—In addition to comparing the rsFC between PD +FOG and PD-FOG groups as defined by the NFOG-Q scores, permutation testing (Nichols and Holmes, 2002) was performed to investigate whether the rsFC between the amygdala and the putamen and FPN were also differentially associated to the objective measure of FOG severity across the groups. By performing such permutation testing insights could also be gained on the directionality of the limbic effects on FOG severity. First, a correlation analysis was performed between the objective FOG-ratio and the rsFC values of interest (i.e. between the bilateral amygdala and putamen and between the bilateral amygdala and the 24 ROIs of the FPN) for each group separately. These correlation values were then Fisher r-to-Z transformed and a difference in Z-scores between the predefined groups was calculated (ΔZ_{orig}). Matlab was then used to perform 10,000 permutations with full exchangeability on the group labels and to calculate a ΔZ for each of the randomly permuted groups (ΔZ_{perm}) (Nichols and Holmes, 2002). A twosided t-test was then performed with an alpha of 0.05 to investigate whether the ΔZ_{orig} was more extreme than the bottom 2.5^{th} or upper 97.5^{th} percentile of all 10,000 ΔZ_{perm} (e.g. permutation distribution) for each FOG-ratio and rsFC association. Each ΔZ_{orig} that was more extreme than the 2.5^{th} or 97.5^{th} percentile of ΔZ_{perm} was considered significantly different than based on chance levels according to a two-sided test with alpha=0.05 (Nichols and Holmes, 2002).

Connectivity - affective associations—A spearman rho correlation analysis was performed within each group to assess whether the scores on the PDQ-39 affective items were associated with the objective FOGratio as well as with the functional connectivity scores that were found to be significantly different between the PD+FOG and PD-FOG groups.

Results

Participant demographics

The groups were matched across all demographic variables (see Table 1), including gender ($\chi^2(1)=2.16$, p=0.141). The distribution of the dominant motor severity side was similar between the groups ($\chi^2(2)=0.087$, p=0.958), with most patients having higher MDS-

UPDRS-III scores for the left side of the body (PD+FOG (left/right/bilateral): 11/5/3; PD-FOG: 12/5/4). However, the actual sum of the MDS- UPDRS-III scores was not significantly different between the left and right side of the body for either group (PD+FOG: t(18)=1.36, p=0.190; PD-FOG: t(20)=0.667, p=0.512). Furthermore, no differences between the PD +FOG and PD-FOG groups were found on the general anxiety and depression items of the PDQ-39. However, a trend towards a significantly higher score on item 9 of the PDQ-39 (i.e. fear of falling) was found for PD+FOG compared to PD-FOG (Table 1). Importantly, removal of the participants that were taking medications for mood disorders nor the participants with low MOCA scores significantly altered the main results, indicating that these subjects were unlikely driving the findings of this study (see Appendix C).

Amygdala - Striatal connectivity

PD+FOG had significantly increased rsFC between the right amygdala and right putamen (t(38)=2.81, p=0.0076, FDR corrected) and a trend towards increased rsFC for the left amygdala to the left putamen (t(38)=1.94, p=0.060) compared to PD-FOG. No differences in rsFC were found between the amygdala and either the nucleus accumbens (left: p=0.624; right: p=0.143) or caudate (left: p=0.208, right: p=0.361), see Figure 3A.

RsFC of the left amygdala and left nucleus accumbens as well as the rsFC between the left nucleus accumbens and left putamen were positively correlated for both groups (PD+FOG: r=0.46, p=0.048; PD-FOG: r=0.45, p=0.041, uncorrected). However, only the PD+FOG group showed a similar positive association on the right, although this did not reach statistical significance (PD+FOG: r=0.40, p=0.090; PD- FOG: r=0.18, p=0.445). The post-hoc analysis revealed that the rsFC between the left amygdala to left putamen and the rsFC between the right amygdala to right putamen were strongly correlated in PD+FOG (r=0.73, p<0.001). A positive association was also found for PD-FOG (r=0.40, p=0.072), although seemingly less so than for PD+FOG as shown by a trend toward significance when comparing the independent correlation coefficients (Fisher r-Z=1.47, p=0.071). These results indicate that both hemispheres in PD+FOG were likely involved in processing limbic information between the amygdala and putamen.

Subcortical - frontoparietal network connectivity

An increased anti-correlation (i.e. anti-coupling or negative connectivity) was found between the left amygdala and FPN in PD+FOG compared to PD-FOG (t(38)=−2.66, p=0.011) (Figure 3B). The rsFC between the right amygdala and FPN showed no group difference (t(38)=−0.87, p=0.390). In contrast, an overall decreased anticoupling was found between the putamen and FPN (Right: t(38)=2.30, p=0.027; Left: t(38)=2.01, p=0.051) (Figure 3B). To investigate which connection between the subcortical seeds and the FPN were driving the group differences, a post-hoc analysis was performed on the rsFC between the left amygdala, bilateral putamen and all the 24 ROIs of the FPN. Appendix D shows the connections that had a significantly different rsFC between the groups. The most significant differences in rsFC were found between the left amygdala and the left lateral prefrontal cortex (p=0.0037), left amygdala and right inferior parietal cortex (p=0.0072), left amygdala and right lateral orbitofrontal cortex (p=0.0091) and right putamen and medial frontal cortex (p=0.0023). However, due to the many comparisons performed (3 subcortical seeds x 24

FPN ROIs in total) none of these results survived FDR correction, warranting cautious interpretation.

Connectivity - FOG associations

As expected, the PD+FOG group had significantly greater FOG-ratio scores compared to PD-FOG (PD+FOG Mean (SD)=3.21 (2.9); PD-FOG=1.04 (0.73), t(38)=3.12, p<0.01). Permutation testing revealed a positive association between the FOG-ratio and the rsFC between the left amygdala and left putamen in freezers, which was significantly different from the slightly negative association found in non-freezers (Z_{orig} PD+FOG=0.332; PD-FOG=−0.291; p=0.023). No significant associations with FOG-ratio were found for the rsFC between the right amygdala and right putamen in either group. As expected, a significantly negative association was found in freezers between FOG severity and the rsFC between the amygdala and regions of the FPN, that is, the greater the anti-coupling between these regions, the worse the freezing severity. Specifically, PD+FOG showed a negative association between FOG-ratio and rsFC between the left amygdala and the left lateral prefrontal (Parcel ID: 7) (Z_{orig} PD+FOG=−0.303; PD-FOG=0.442, p=0.0097) and right dorsolateral prefrontal cortex ROI (Parcel ID: 276) (Z_{orig} PD+FOG=−0.242; PD-FOG=0.439, p=0.019) as well as the rsFC between the right amygdala and right intraparietal ROI (Parcel ID: 261) of the FPN (Z_{orig} PD+FOG=−0.789; PD-FOG=0.548, p<0.001) (Gordon et al., 2016), compared to a positive association seen in non-freezers.

Connectivity - affective associations

No significant correlations were found between the scores on the general depression or anxiety items of the PDQ-39 and the functional connectivity scores for either group (all p>0.1). However, a significant negative association was found between the scores on item 9 of the PDQ-39 (i.e. fear of falling) and the functional connectivity between the FPN and both the left amygdala (rho=−0.544, p=0.016) and right amygdala (rho=− 0.497, p=0.030) within the PD+FOG group (i.e. greater anti-coupling relates to worse fear of falling), but not within the PD-FOG group (left: rho=0.395 p=0.080; right: rho=0.206, p=0.383). Furthermore, a trend towards a significant positive association was found within the PD +FOG group between the scores on item 9 of the PDQ-39 (i.e. fear of falling) and the increased functional connectivity between the left amygdala and left putamen (rho=0.424, p=0.070). No such correlation was found for the right side within the PD+FOG group (rho=0.074, p=0.764) or for either side in the PD-FOG group (left: rho=−0.110, p=0.645; right: rho=0.146, p=0.538). No significant correlation was found between the scores on PDQ-39 item 9 and the FOGratio, although the association trend was positive in the PD +FOG group as expected (PD+FOG: rho=0.271, p=0.262; PD-FOG: rho=−0.070, p=0.768). However, these findings should be interpreted with caution due to the ordinal level of measurement for the PDQ-39 items and the explorative nature of the correlations.

Discussion

The present study used rsfMRI to investigate whether baseline dysfunctional limbic circuitry is associated with FOG in PD. The results showed that in comparison to non-freezers, the freezer group had: i) increased rsFC between the right amygdala and putamen; ii) increased

anti-coupling between the left amygdala and the averaged frontoparietal attentional control network; iii) decreased anti-coupling between the putamen and this network (although the subcortical-FPN ROI connectivity results were uncorrected). Permutation testing revealed that increased objective FOG severity in the PD+FOG group was significantly associated with increased functional connectivity between the left amygdala to left putamen and increased anti-coupling between the left amygdala to left lateral prefrontal cortex, left amygdala to right dorsolateral prefrontal cortex, and right amygdala to right intra-parietal cortex. Finally, the scores on item 9 of the PDQ-39 (i.e. fear of falling) within the freezers group were negatively associated with the functional connectivity between both the left and right amygdala and the FPN and trending towards a positive association with the increased connectivity between the left amygdala to left putamen. Together, these results support our hypotheses that FOG is associated with increased baseline striato- limbic connectivity, in which FOG is likely exacerbated due to a lack of top-down control by the FPN over the amygdala.

The amygdala has been shown to play an integral role in the neural circuit controlling psychophysiological responses to fear in normal adults, such as changes in heart rate and skin conductance (Kuniecki et al., 2003; Williams et al., 2005). These psychophysiological responses also occur during FOG in PD (Maidan et al., 2010; Mazilu et al., 2015), suggesting that the amygdala could be involved in the manifestation of those freezing episodes. Furthermore, PD patients with a PIGD subtype are more prone to experiencing anxiety and FOG (Burn et al., 2012) as well as having greater amygdalar grey matter loss compared to tremor-dominant patients and age-matched healthy controls (Rosenberg-Katz et al., 2016). The PIGD group in that study also had greater putaminal grey matter loss compared to controls, and putamen grey matter loss was significantly correlated with subjective FOG severity (Rosenberg-Katz et al., 2016). Our study compliments these prior findings, and extends the current literature by demonstrating that functional connectivity between the amygdala and putamen at rest is also different in PD patients with FOG that have a more PIGD subtype, compared to patients without FOG, where increasing connectivity related to more severe freezing.

Increased resting state connectivity between the amygdala and dorsal striatum (i.e. putamen) was previously also demonstrated in patients with anxiety disorders (Liao et al., 2010) and in healthy subjects during experimentally-induced fear related to the anticipation of aversive electric shocks (Phelps et al., 2001; Butler et al., 2007). The authors of those studies postulated that even at rest subjects switched to evolutionary conserved subcortical processing during perceived danger in order to facilitate a state of motor readiness that can readily activate reflexive motor responses when necessary (Grillner et al., 2005; Butler et al., 2007; Liao et al., 2010). This further highlights that the basal ganglia serve as a critical interface for the translation of emotional information into behavioral responses (Butler et al., 2007; Lewis and Barker, 2009; Marchand, 2010), such as FOG.

In the current study, increased anti-coupling between the amygdala and FPN in PD+FOG was related to freezing severity and a subjective fear of falling. These findings are in accordance with vast literature showing that reduced connectivity between the amygdala and FPN is associated with increased anxiety in healthy subjects and patients with anxiety

disorders (Mitchell et al., 2008; Sylvester et al., 2012; Coombs et al., 2014; Dodhia et al., 2014; Rohr et al., 2015; De Witte and Mueller, 2016; He et al., 2016). The ability to provide top-down control over emotional responses has been linked to executive processes that rely on overlapping brain networks involving the FPN, amygdala and striatum (Pessoa, 2009; Ochsner et al., 2012; Sylvester et al., 2012; Rohr et al., 2015). Patients with anxiety disorder indeed have impairments in executive functions related to attentional set shifting and response inhibition, which are key features required for effective emotion regulation (Bar-Haim et al., 2007; Eysenck et al., 2007; Ansari et al., 2008; Rohr et al., 2015). Interestingly, PD patients with FOG have impairments in the same executive functions as those with anxiety (Naismith et al., 2010; Cohen et al., 2014; Walton et al., 2015b; Ehgoetz Martens et al., 2016), further complimenting the hypothesis that PD+FOG patients likely have reduced ability to exert top-down control over emotional responses.

During rest, the FPN in freezers is likely to have sufficient resources to process limbic information and prevent patients from feeling anxious, as partly indicated by the lack of a group difference on the general anxiety item 21 of the PDQ-39. However, because PD+FOG patients may rely on the same attentional resources to operate their stepping (Vandenbossche et al., 2012), the need to use attentional resources to process limbic information will likely impair gait performance. Indeed, anxiety in PD has been shown to affect gait performance in a similar manner to that of an attention demanding cognitive dual-task (Ehgoetz Martens et al., 2017), supporting the notion that anxiety consumes the attentional resources that PD +FOG rely on for gait control (Vandenbossche et al., 2012).

Furthermore, recent work has shown that PD patients off their dopaminergic medication have maladaptive hyper-activation of the ventro-posterior putamen during the consecutive performance of attention-demanding cognitive tasks and repetitive lower limb movements compared to healthy controls (Nieuwhof et al., 2017). This region of the putamen was not engaged in either task separately and its activation was related to worse task performance (Nieuwhof et al., 2017). Interestingly, this region of the putamen is functionally connected to the FPN (Choi et al., 2012). The authors suggested that this finding reflected an overspill of activation from neighbouring striatal regions causing functional blurring across cortico-striatal circuits (Nieuwhof et al., 2017). Therefore, the decreased anti-coupling between the putamen and FPN found in the current study may be linked to functional overlapping connections of motor and limbic cortico-striatal pathways. Given that the putaminal processing capacity is already impaired in PD+FOG (Bartels et al., 2006; Ouchi et al., 2001), having to share its connections with limbic processing could predispose patients for FOG when anxious (Lewis and Shine 2014).

Several connectivity findings of the present study were left lateralized. For instance, although PD+FOG showed positive rsFC between the amygdala and putamen bilaterally (Figure 1A), only the left amygdala to left putamen connectivity related to worse freezing severity in that group. Furthermore, only the left amygdala showed significant anti-coupling with the FPN, and only the left amygdala to left putamen connectivity was trending towards a significant association with subjective fear of falling in PD+FOG. These results are in line with previous work showing that anxiety in PD may be specifically related to left hemispheric involvement (Bogdanova and Cronin-Golomb 2012). It has further been

postulated that the right amygdala modulates the immediate fear response to aversive stimuli (e.g. a visual threat), whereas the left amygdala is involved in the expression of a fear response to previously encountered aversive events that do not exist in the immediate environment (i.e. a mental representation of fear of falling) (Phelps et al., 2001). This may explain why in the absence of an immediate aversive stimuli in the current study only the rsFC of the left amygdala to putamen correlated with the FOGratio. Indeed, the majority of patients in this study had higher MDS-UPDRS-III scores on the left side of the body, indicating that these results are unlikely explained by left lateralized neuropathology. Based on the positive associations between the rsFC of the amygdala to nucleus accumbens and rsFC of the nucleus accumbens and putamen in freezers, it can further be postulated that limbic information from the amygdala was processed by both the putamen and nucleus accumbens. However, future studies are needed to investigate the precise roles of the striatal sub-regions and emotional laterality in the limbic processing that occurs in PD patients with FOG.

Overall, this study provides novel insights into the neural mechanisms underlying FOG and the concomitant role of limbic circuit dysfunction in its manifestation. We hope that this will form the basis for much needed improvements in the clinical management of PD. Indeed, FOG affects the majority of the PD population eventually (Auyeung et al., 2012) and limbic disturbances (such as anxiety) that contribute to FOG are also known to aggravate other debilitating symptoms of PD, such as tremor and dyskinesia (Lieberman, 2006; Burn et al., 2012; Ehgoetz Martens et al., 2014; Chen and Marsh, 2014). However, even though reducing anxiety is likely to result in clinical benefits for multiple debilitating and poorly manageable symptoms of PD, a paucity of treatment data still exists (Chen and Marsh, 2014). Future clinical trials investigating the effects of anxiolytic agents on both anxiety and FOG are warranted (Ehgoetz Martens et al., 2014; Chen and Marsh, 2014), although careful consideration of their contra-indications is advised in this elderly population (Bakken et al., 2014). It would therefore also be compelling to investigate whether non-pharmacological strategies with known anxiolytic effects, such as cognitive behavioural therapy (Stanley et al., 2009; Chen and Marsh, 2014) and exercise interventions (Wipfli et al., 2008), would also be effective in treating anxiety in Parkinson’s disease and whether this would translate to reduced FOG.

The present study has several limitations. For instance, the enrolled participants did not undergo a thorough clinical assessment of mood disturbance. However, previous work has shown that freezers with similar anxiety levels as non-freezers still froze more during a ‘high threat’ gait task compared to a ‘low threat’ gait task (Ehgoetz Martens et al., 2014). This may explain why no group difference was found in the current study for the general anxiety item 21 of the PDQ-39, whereas PD+FOG showed a trend towards a significantly higher reported fear of falling on item 9 compared to PD-FOG. Together, these findings indicate that changes in limbic circuitry can interfere with motor performance in freezers without necessarily being associated with anxiety symptoms at baseline. Future studies investigating the pathophysiology underlying anxiety-induced FOG are encouraged to better assess baseline mood disturbances. In addition, although turning on the spot is a highly provocative trigger for FOG, which is otherwise difficult to elicit in a laboratory setting (Snijders et al., 2012), future studies should also employ a threatening gait task while obtaining

psychophysiological and self-reported outcome measures of anxiety to confirm that their findings were indeed related to the contribution of anxiety on FOG (Maidan et al., 2010; Ehgoetz Martens et al., 2014; Mazilu et al., 2015). Several findings of the present study also did not survive correction for multiple comparisons, and the use of uncorrected tests is a limitation of this study warranting careful interpretation and indicating that future studies should aim to test larger samples. Those studies should also consider including healthy age-matched control participants to further ensure that the limbic circuit dysfunctions found are specific to PD and FOG, and assess performance on executive tasks to confirm that attentional deficits indeed contribute to the limbic circuit dysfunctions found in the current study in PD+FOG.

It is important to note that even though the amygdala has consistently been shown to be associated with fear and emotion-induced motor responses, emotion regulation is operated by a much larger limbic network (Phelps et al., 2001; Butler et al., 2007; Marchand, 2010; Ochsner et al., 2012; Sylvester et al., 2012; Rohr et al., 2015; Sprooten et al., 2017; Thobois et al., 2017). Future studies are therefore also encouraged to employ a data driven approach to further explore the extent of the limbic circuitry involved in the manifestation of anxiety-induced FOG in PD, which has been restricted by the regional approach utilised here. For instance, studies could employ virtual reality in combination with functional MRI to simulate a threatening environment that would allow for the investigation of the neural mechanisms underlying real time fear-induced freezing behaviour (Shine et al., 2013 a; Ehgoetz Martens et al., 2014).

Finally, anxiety in PD is mediated by neurotransmitters besides dopamine, such as via the serotonergic raphe nuclei and noradrenergic locus coeruleus (Prediger et al., 2012). The noradrenergic locus coeruleus in particular may be implicated in anxiety and FOG given its strong ascending innervation of the amygdala and cortico-limbic regions and profound degeneration in PD (Prediger et al., 2012), the extend of which was recently shown to relate to subjective FOG severity (Ono et al., 2016).

In conclusion, the results of the current study suggest that FOG in PD may be related to baseline alterations in communication across fronto-striato-limbic regions involved with processing threat and emotionally-induced responses that could increase the risk for FOG, especially in the absence of sufficient dopaminergic and attentional resources.

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Appendix A

Size of the subcortical regions of interest used in the present study based on the Human Connectome Project pipelines using Freesurfer automated segmentation (for more information see: Glasser et al., 2014).

ROI	# Voxels
Left Nucleus Accumbens	135
Right Nucleus Accumbens	140
Left Amygdala	315
Right Amygdala	332
Left Caudate	728
Right Caudate	755
Left Putamen	1060
Right Putamen	1010

Note: Voxels = Number of voxels (2mm x 2mm x 2mm).

Appendix B

Size of the 24 parcels of the frontoparietal attentional control network as derived from Gordon et al., (2016).

Parcel ID	# Grayordinates
7	35
9	49
24	111
78	145
96	181
108	43
109	151
148	68
149	48
167	143
168	70
170	116
182	97

Parcel ID	# Grayordinates
240	104
260	52
261	37
272	99
273	167
276	48
277	104
319	89
320	44
327	171
328	77

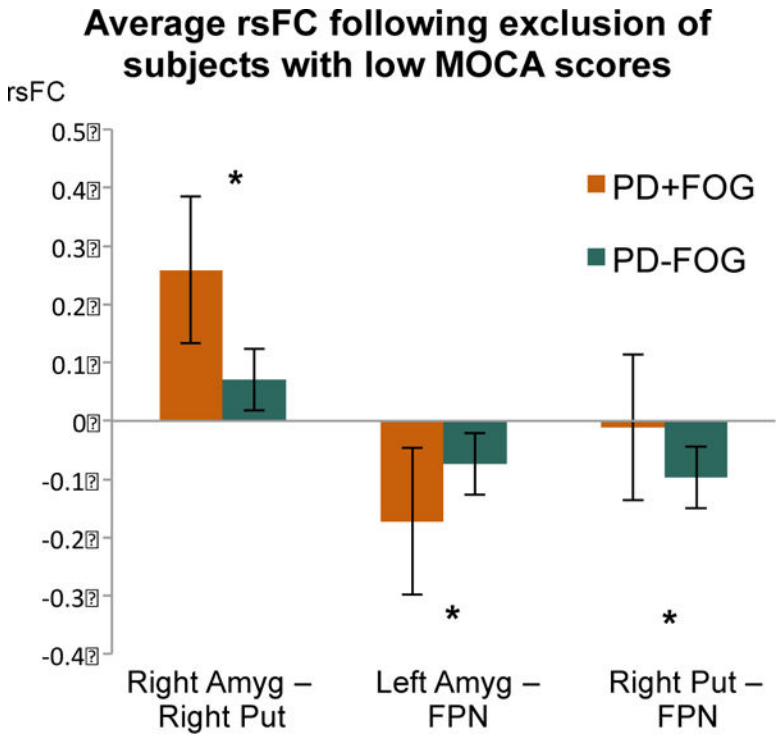
Note: Grayordinates=number of Grayordinates, with an average vertex spacing of 2mm on the cortical surface.

Appendix C – Supplementary Analyses

A: Overview of main findings of the t-tests following exclusion of patients with a MOCA scrore <21 (2 freezers, n=17;1 non-freezer, n=20)

Comparison	T-value	P-value
Right Amygdala - Right Putamen	2.56	0.015 *
Left Amygdala - averaged FPN	2.46	0.018 *
Right Putamen - averaged FPN	2.16	0.037 *

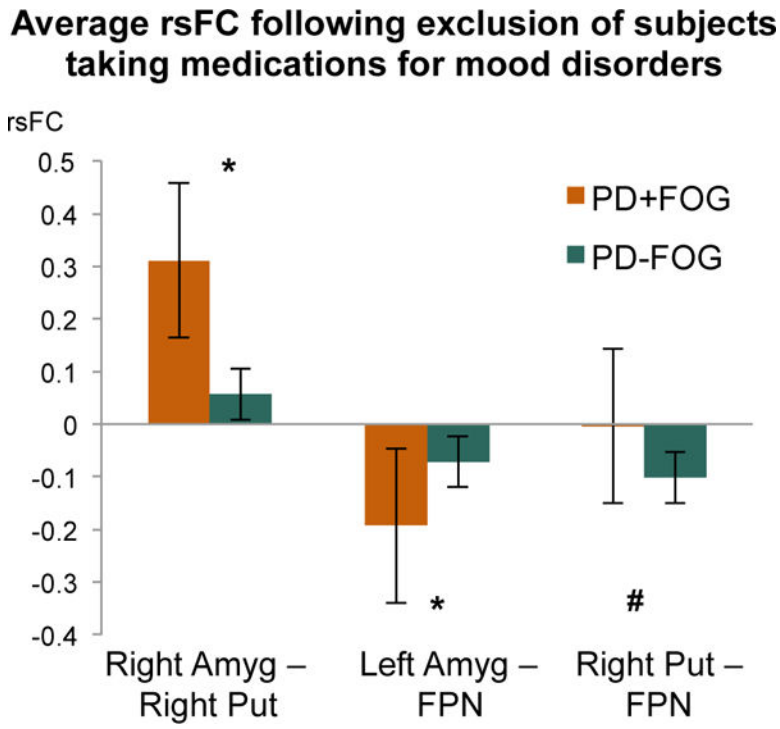
NOTE: FPN=averaged frontoparietal attentional control network, df=35,
* p<0.05, uncorrected.



B: Overview of main findings of the t-tests following exclusion of patients taking medications for mood disorders (4 freezers, n=15; 4 non-freezers, n=17).

Comparison	T-value	P-value
Right Amygdala - Right Putamen	2.64	0.012 *
Left Amygdala - averaged FPN	2.66	0.011 *
Right Putamen - averaged FPN	1.78	0.083 #

NOTE: FPN=averaged frontoparietal attentional control network, df=30,
* p<0.05, uncorrected.



Appendix D

Post-hoc results of the t-tests comparing rsFC between the amygdala, putamen and each parcel of the FPN between PD patients with and without FOG.

Parcel ID	Label	Left Amygdala	Left Putamen	Right Putmen
7	Left lateral prefrontal cortex	**0.0037** *		
9	Left inferior temporal cortex		0.0286	
24	Medial frontal cortex			**0.0023** *
96	Left Posterior Parietal Cortex	0.0426		
108	Left Dorsolateral Prefrontal Cortex		0.0400	0.0141
170	Right Medial Lateral Temporal Cortex			0.0244
240	Right Orbitofrontal Cortex		0.0321	0.0271
260	Right Inferior Parietal Cortex	**0.0072** *		
261	Right Intraparietal Cortex	0.0158		
272	Right Dorsolateral Prefrontal Cortex	0.0325		
277	Right Lateral Orbitofrontal Cortex	**0.0091** *		
319	Right Prefrontal Cortex		0.0380	0.0372
320	Right Prefrontal Cortex	0.0479		

NOTE: Independent samples t-test used (df=38). rsFC=resting state functional connectivity; FPN=frontoparietal attentional control network; PD+FOG=Parkinson's disease with freezing of gait; PD-FOG: Parkinson's disease patients without freezing of gait; Parcel ID=according to the Gordon parcellation scheme (Gordon et al., 2016). Only significant p-values are presented (p<0.05,

* indicates p<0.01, uncorrected). Grey shading indicates non-significance with p>0.05.

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Highlights

- FOG in PD is associated with increased resting state connectivity between the amygdala and putamen
- FOG in PD is associated with anti-coupling between the amygdala and frontoparietal network
- These limbic alterations relate to FOG severity and a subjective fear of falling

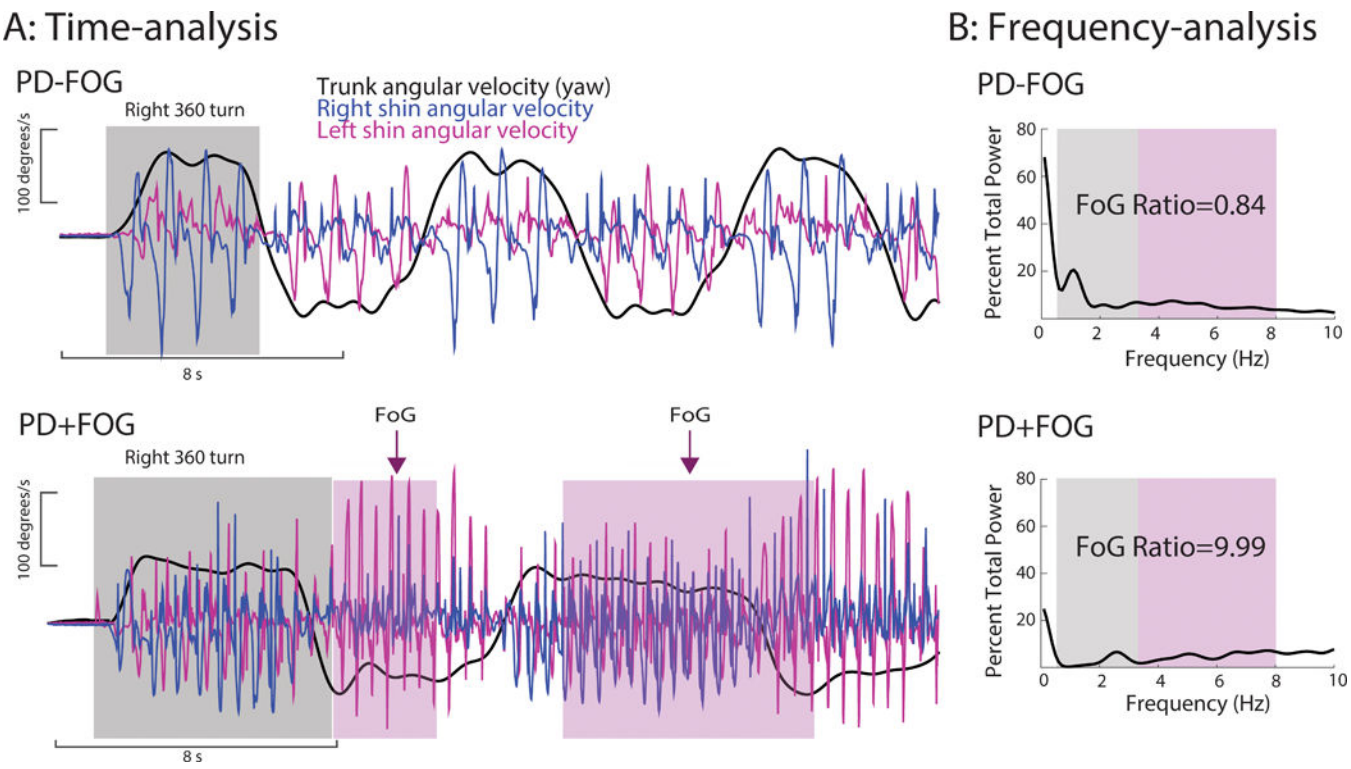

Figure 1: Representation of the objective FOG severity measure (FOG-ratio)

Representation of the time and frequency analysis of the objective FOG severity measure (FOG-ratio). A) Time series of trunk and bilateral shin angular velocities during the turning task. B) Frequency analysis of the shin anteroposterior acceleration power spectral densities during the turning task. NOTE: PD-FOG=representative data from a relatively mobile PD patient without FOG; PD+FOG=representative data from a PD patient with severe FOG; FoG=Freezing of Gait.

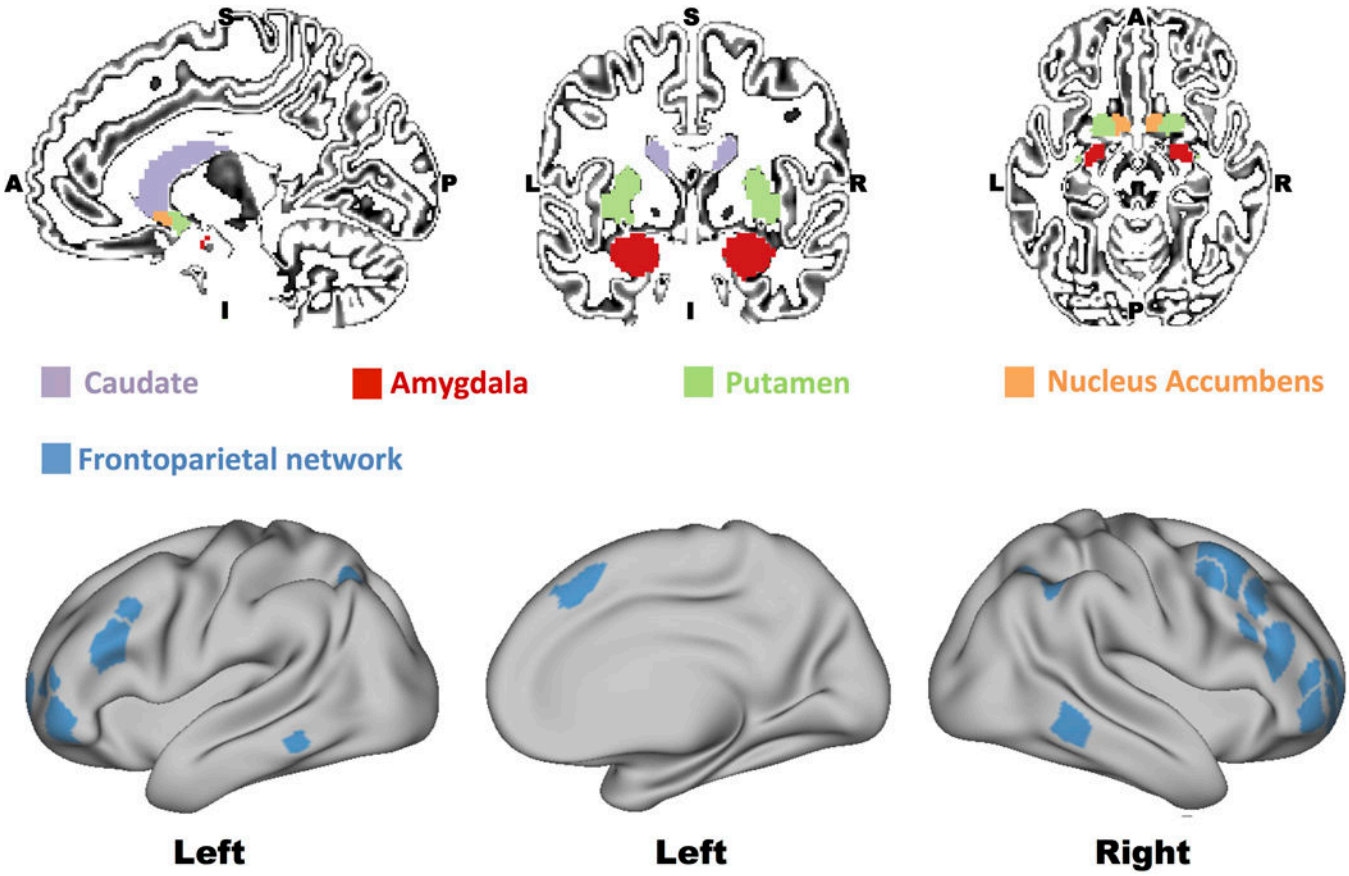

Figure 2: Regions of Interest (ROI)

Representation of the subcortical regions of interest (ROI) and the frontoparietal attention control network (Gordon et al., 2016) used in the current study.

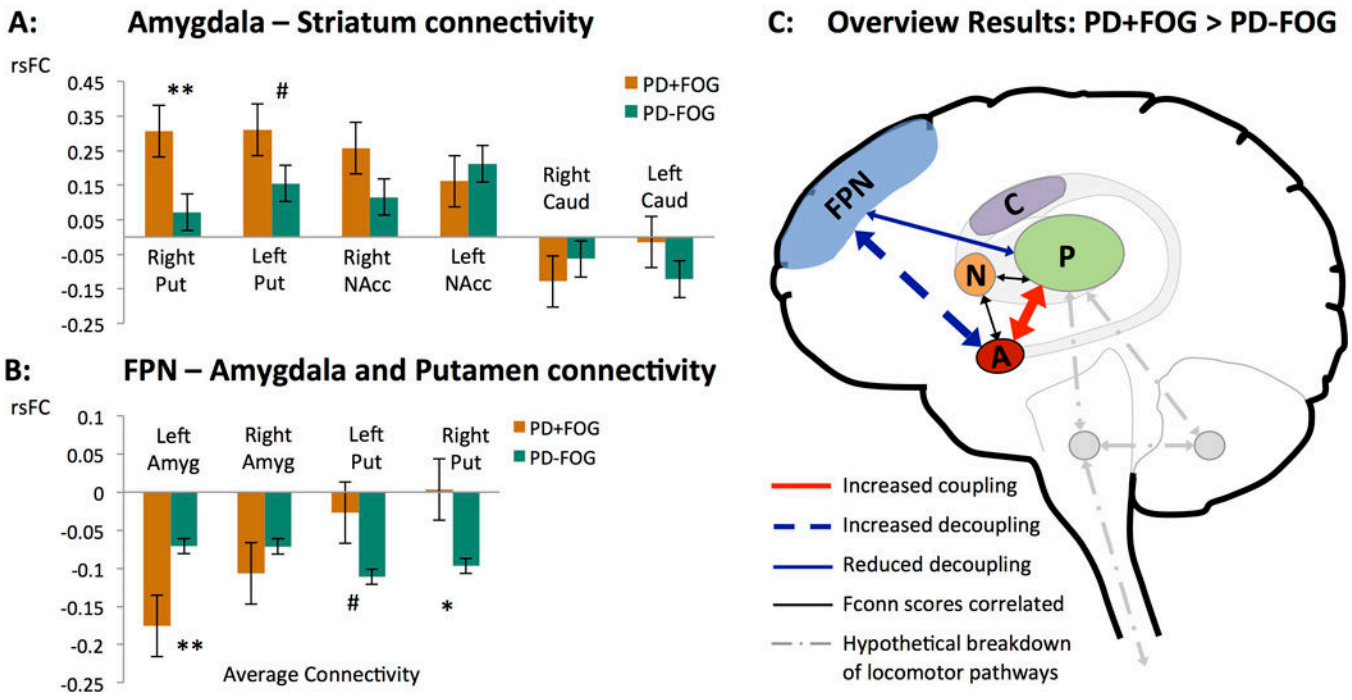

Figure 3: Representation of the rsFC differences found between PD+FOG and PD-FOG

Representation of the resting state functional connectivity (rsFC) differences found between Parkinson's disease patient with freezing of gait (PD+FOG) and Parkinson's disease patients without freezing of gait (PD-FOG). A) Amygdala - Striatum independent t-test results showing the average rsFC (Z-value) per group for the unilateral connections; B) Independent t-test results comparing the rsFC between the average time series of all 24 ROIs of the frontoparietal attentional control network (FPN) and the bilateral amygdala and putamen seeds showing the average rsFC (Z- value) per group; C) Visual representation of the results where coupling indicates increased correlations between BOLD time series while anti-coupling indicates increased negative correlations between BOLD time series. Put=Putamen (P); NAcc=Nucleus Accumbens (A); Caud=Caudate (C); Amyg=Amygdala (A). **Denotes statistical significance ($p<0.05$, FDR corrected); *Denotes statistical significance ($p<0.05$), but uncorrected; #Denotes a trend towards significance ($p<0.1$).

Table 1:

Demographic Statistics between groups

	PD+FOG n=19	PD-FOG n=21	Test-value	P-value
Age	68.4 (7.2)	66.9 (7.6)	t=0.645	0.523
MOCA	25.7 (4.5)	25.7 (3.0)	t=0.019	0.985
DD	9.31 (6.6)	6.01 (4.6)	t=1.85	0.073
SD	11.0 (6.6)	7.63 (6.3)	t=1.60	0.119
UPDRS total	71.1 (18)	61.7 (21)	t=1.50	0.143
UPDRS-III	42.7 (14)	36.1 (11)	t=1.63	0.111
PIGD	6 (2–15)	4(0–11)	Z=−2.39	0.017
HY	2 (2–4)	2 (2–3)	Z=−1.45	0.146[a]
LEDD	820 (300–2304)	460 (300–2464)	Z=−1.14	0.254[a]
PDQ item 17 ‘Depression’	1 (0–3)	1 (0–4)	Z=−0.451	0.652[a]
PDQ item 21 ‘Anxiety’	1 (0–2)	1 (0–3)	Z=−0.520	0.603[a]
PDQ item 9 ‘Fear of falling’	1 (0–2)	0 (0–2)	Z=−1.79	0.073[a]

NOTE: Independent t-test used (df=38) and Mean (SD) reported unless otherwise indicated.

[a]=Mann-Whitney U test used (n1=19, n2=21) and Median (range) reported. PD+FOG=Parkinson’s disease patients with freezing of gait; PD-FOG=Parkinson’s disease patients without freezing of gait; MOCA=Montreal Cognitive Assessment; DD=Disease duration in years; SD=Symptom duration in years; UPDRS=Movement Disorders Society Unified Parkinson’s Disease Rating Scale; UPDRS-III=Section 3 (Motor examination) of the UPDRS; PIGD: Postural Instability Gait Difficulty score of the UPDRS-III; HY=Hoehn and Yahr Stage; LEDD= Mean Levodopa Equivalent Daily Dose; PDQ=Parkinson’s Disease Questionnaire 39.