The functional network signature of heterogeneity in freezing of gait

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Freezing of gait is a complex, heterogeneous, and highly variable phenomenon whose pathophysiology and neural signature remains enigmatic. Evidence suggests that freezing is associated with impairments across cognitive, motor and affective domains; however, most research to date has focused on investigating one axis of freezing of gait in isolation. This has led to inconsistent findings and a range of different pathophysiological models of freezing of gait, due in large part to the tendency for studies to investigate freezing of gait as a homogeneous entity. To investigate the neural mechanisms of this heterogeneity, we used an established virtual reality paradigm to elicit freezing behaviour in 41 Parkinson's disease patients with freezing of gait and examined individual differences in the component processes (i.e. cognitive, motor and affective function) that underlie freezing of gait in conjunction with task-based functional MRI. First, we combined three unique components of the freezing phenotype: impaired set-shifting ability, step time variability, and self-reported anxiety and depression in a principal components analysis to estimate the severity of freezing behaviour with a multivariate approach. By combining these measures, we were then able to interrogate the pattern of task-based functional connectivity associated with freezing (compared to normal foot tapping) in a sub-cohort of 20 participants who experienced sufficient amounts of freezing during task functional MRI. Specifically, we used the first principal component from our behavioural analysis to classify patterns of functional connectivity into those that were associated with: (i) increased severity; (ii) increased compensation; or (iii) those that were independent of freezing severity. Coupling between the cognitive and limbic networks was associated with 'worse freezing severity', whereas anti-coupling between the putamen and the cognitive and limbic networks was related to 'increased compensation'. Additionally, anti-coupling between cognitive cortical regions and the caudate nucleus were 'independent of freezing severity' and thus may represent common neural underpinnings of freezing that are unaffected by heterogenous factors. Finally, we related these connectivity patterns to each of the individual components (cognitive, motor, affective) in turn, thus exposing latent heterogeneity in the freezing phenotype, while also identifying critical functional network signatures that may represent potential targets for novel therapeutic intervention. In conclusion, our findings provide confirmatory evidence for systems-level impairments in the pathophysiology of freezing of gait and further advance our understanding of the whole-brain deficits that mediate symptom expression in Parkinson's disease.

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Introduction

Freezing of gait is a complex and poorly understood symptom of Parkinson's disease that typically presents as a paroxysmal and transient episode in which patients report feeling as though their feet are 'glued' to the floor (Schaafsma et al., 2003). Management of the condition remains difficult, partly because of the heterogeneous nature of the disorder. For instance, freezing of gait can be provoked in a wide variety of situations (e.g. when distracted, turning or when feeling anxious) (Nutt et al., 2011). These common 'triggers' can be broadly classified into impairments within three behavioural categories: cognitive, motor and limbic.

Impairments in cognitive flexibility, namely attentional set-shifting deficits are a widely recognized 'cognitive signature' associated with freezing of gait (Amboni et al., 2008; Hallett, 2008; Shine et al., 2013d; Szeto et al., 2015). For instance, patients with freezing of gait typically perform significantly worse on part B of the Trail Making Test (TMT) (Hall et al., 2014, 2015; Beck et al., 2015), and intra-extra dimensional set shifting test (Stefanova et al., 2014). Additionally, patients with freezing of gait show greater dual-task interference (Peterson et al., 2015) and commit more errors (Pieruccini-Faria et al., 2014) when asked to perform a secondary cognitive task while walking. These behavioural deficits align with the observation that dividing attention, or switching motor programmes often provokes freezing of gait (Schaafsma et al., 2003; Nutt et al., 2011; Beck et al., 2015), whereas dual motor-cognitive interventions can improve clinical freezing (Killane et al., 2015).

The motor signature of freezing is generally characterized by impairments in gait automaticity, which is normally operationalized by an increase in step-to-step variability (Hausdorff et al., 2003), step length variability (Thevathasan et al., 2012; Barbe et al., 2014) and/or step time variability (Pieruccini-Faria et al., 2015; Weiss et al., 2015) during regular walking, even in the absence of freezing. Additionally, step time variability in patients with freezing of gait is also exacerbated during split belt treadmill walking (Nanhoe-Mahabier et al., 2013), while approaching narrow doorways (Almeida and Lebold, 2010; Ehgoetz Martens et al., 2013b; Silveira et al., 2015), walking in threatening situations (Ehgoetz Martens et al., 2013a, 2014b), during sharp turns (Bhatt et al., 2013), and while dual-tasking (Pieruccini-Faria et al., 2014; Beck et al., 2015). In fact, variability in stepping has even been shown to be worse in patients with freezing of gait while performing a stationary virtual reality tapping task (Gilat et al., 2013) and repetitive stepping in place task (Nantel et al., 2011).

Finally, emotional disturbances, such as anxiety, have been suggested to represent a key limbic signature of freezing (Giladi and Hausdorff, 2006). Panic attacks (Vazquez et al., 1993; Lieberman, 2006) and physiological heart rate changes (Maidan et al., 2011) have been temporally linked to the onset of freezing of gait episodes, and threatening or stressful situations (e.g. walking over an elevated virtual plank) can also reliably trigger freezing of gait (Bloem et al., 2004; Ehgoetz Martens et al., 2014b). Recent research has also shown that patients with freezing of gait have worse symptoms of anxiety and depression compared to those without freezing of gait (Burn et al., 2012; Ehgoetz Martens et al., 2016c), and greater anxiety is also associated with worse freezing severity (Ehgoetz Martens et al., 2016b).

Several models have been proposed to explain the pathophysiology of freezing (Nieuwboer and Giladi, 2013). However, most mechanistic explanations have focused on impairments in one component domain (e.g. cognitive or motor), and thus essentially underestimate the role of system-level impairments in freezing. In contrast, the 'cross-talk' model (Lewis and Barker, 2009) explicitly embraces the interactions between the component dimensions of freezing. Specifically, the model suggests that freezing of gait is triggered by episodic discordant 'cross-talk' between competing yet complimentary frontostriatal circuits that span motor, cognitive and limbic cortical areas. This 'cross-talk' is proposed to overload the information processing capacity within the dopamine-depleted striatum, thus producing momentary synchronous firing in the output nuclei of the basal ganglia, which in turn would lead to increased inhibition in brainstem locomotor areas, and consequently freezing of gait (Lewis and Shine, 2016). In this model, the corticostriatal architecture is critical for balancing integration and segregation across disparate motor, cognitive, and limbic circuits, which is required for optimal information processing and hence guided the regions of interest selected for this study. It can be proposed that the corticostriatal dysfunction seen in Parkinson's disease may lead to interference and over-integration between these normally segregated networks.

Functional imaging studies have provided broad empirical support for the cross-talk model. Specifically, patients with Parkinson's disease and freezing have been shown to demonstrate reduced frontal and parietal cortical activation but increased subcortical activation (mesencephalic locomotor region) during imagined walking (Snijders et al., 2011), while the opposite pattern (i.e. increased frontal and parietal activation but reduced subcortical activation) is observed during freezing episodes (Shine et al., 2013b). Others have looked beyond the role of individual regions, and examined the interplay between regions, as well as large-scale networks. For example, reduced functional coupling in fronto-parietal networks at rest was identified in patients with freezing of gait compared to those patients without (Tessitore et al., 2012b), and reduced coupling between the cognitive control network and the basal ganglia network was shown to be specifically associated with episodes of freezing behaviour elicited during functional MRI scanning (Shine et al., 2013c). These studies suggest that freezing of gait can be thought of as occurring due to dynamic dysfunctional interactions across normally coordinated neural networks (Fasano et al., 2015). However, recent reviews have

promoted the need to characterize the heterogeneity and complexity of freezing of gait, rather than to study contributors to freezing in isolation (Fasano *et al.*, 2015; Snijders *et al.*, 2016). Therefore, a crucial next step towards understanding the freezing phenomenon is to examine the neural signature of freezing across multiple levels of neural network organization.

Here, we used a task-based functional MRI approach in combination with a validated virtual reality task (Gilat *et al.*, 2013) to investigate the neural signature of the heterogeneity of freezing of gait in Parkinson's disease. We aimed to clarify: (i) how specific regions are coordinated during freezing compared to normal foot tapping (i.e. a proxy for normal walking); (ii) how the freezing signature varies as a function of the first principal component (which relates to 'freezing severity'); and (iii) whether different behavioural signatures of freezing (i.e. cognitive, motor, limbic) are associated with unique neural underpinnings. Based on previous research (Shine *et al.*, 2013c), we hypothesized that freezing epsiodes would be characterized by abnormal patterns of functional connectivity within and between anatomical cortico-striatal circuitry. This was thought to reflect a loss of specificity between the cortex and the striatum, and hence to increase the vulnerability of the system to 'overload'. Given the heterogeneity in freezing, we further hypothesized that the freezing network signature would be uniquely associated with individual differences in the cognitive, motor, limbic component processes of freezing.

Materials and methods

Participants

Forty-one patients with Parkinson's disease and freezing of gait were studied at the Brain and Mind Centre at the University of Sydney. Inclusion criteria included: (i) a diagnosis of Parkinson's disease in accordance with the United Kingdom Parkinson Disease Society Brain Bank Diagnostic Criteria; (ii) a self-reported score of $\geqslant 1$ on question 3 of the Freezing of Gait Questionnaire (FOGQ3): 'do you feel that your feet get glued to the floor while walking, making a turn or when trying to initiate walking (freezing)?'; (iii) clinically-evident freezing of gait, confirmed visually by a neurologist (S.J.G.L.); and (iv) the completion of the virtual gait paradigm in the MRI scanner in the 'OFF' state (i.e. after a minimum of 12 h withdrawal from their regular dopaminergic medication). Exclusion criteria included: (i) any additional neurological comorbidities (e.g. history or stroke or head injury); and (ii) any pathological lesions or abnormalities that were identified by an experienced radiologist from the participants' structural high-resolution T_1-weighted image. Because of the nature of our study (i.e. to investigate a clinical phenomenon that only occurs in a particular group of individuals), we did not include a traditional 'control' group. The current study received ethical approval from the University of Sydney Human Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Data collection and analysis

Clinical and neuropsychological assessment

Participants' motor symptom severity was assessed with Part III of the Movement Disorders Society Unified Parkinson's Disease Rating Scale. Global cognition was assessed with the Mini-Mental State Examination, and attentional set-shifting was assessed with the TMT, parts A and B. Finally, affective disturbance was assessed using the Hospital Anxiety and Depression Scale (HADS).

Virtual gait task

Participants lay supine inside the MRI scanner with a mirror that was mounted to the head coil, which enabled participants to have a clear view of the screen where the virtual environment was projected. As detailed in previous work (Shine *et al.*, 2013a), foot pedals were positioned under the participants' feet (Fig. 1A), and participants were instructed to flex and extend their ankle in order to 'tap' the pedals, which in turn allowed the participants to navigate forward through the virtual environment (Shine *et al.*, 2013a). Forward progression was only achieved when participants alternately depressed the pedals (i.e. left-right-left).

The virtual environment consisted of a straight corridor (presented in first-person view) that contained environmentally salient triggers, such as wide and narrow gaps and doorways. As in previous experiments, cognitive cues were also included to exacerbate set-shifting deficits. Both narrow doorways and cognitive cues have previously been shown to elicit freezing behaviour in susceptible individuals (Shine *et al.*, 2013a). Walking and stopping in the virtual environment was initiated by simple (e.g. WALK and STOP) and complex cue words (e.g. 'WALK' if the colour-word pair match and 'STOP' if the colour-word pair do not match) that were displayed on the screen (for further details see Shine *et al.*, 2013b). Performance on the task was titrated to acceptable levels prior to scanning. Patients were familiarized with the task prior to scanning to ensure acceptable performance. Sixteen participants completed the full 10-min protocol, and 25 participants completed a shorter 5-min protocol.

The timing of each participants' footsteps during the task was collected by recording the onset of each sequential pedal depression. From this output, the modal footstep latency (i.e. step time) for the duration of the protocol was calculated as the weighted average after removing all cognitive cues and freezing of gait episodes (Shine *et al.*, 2013b). Freezing of gait was defined by any footstep latency that was longer than twice the modal footstep latency. This definition is in line with past work and has been previously shown to correlate with the severity freezing of gait episodes experienced in the 'real' world (Gilat *et al.*, 2013; Matar *et al.*, 2013; Shine *et al.*, 2013a). The coefficient of variation of step time (i.e. step time variability) was calculated for the duration of the protocol for which freezing of gait episodes were also removed.

Functional MRI acquisition and preprocessing

A General Electric 3 T MRI was used to obtain T_2*-weighted echo planar functional images were acquired in sequential order with repetition time = 3000 ms, echo time = 40 ms, flip angle = 90°, 40 axial slices covering the whole brain, field of view = 220 mm, interslice gap = 0.4 mm and

Figure 1 Experimental design and protocol. (A) The *left* panel illustrates a participant lying in the MRI scanner with the foot pedals at their feet. The *right* panel shows an example of the virtual environment that the participant views while lying in the MRI scanner. As the participant performs the task, depressing the pedals (e.g. left-right-left-right), step time is recorded and periods of normal foot tapping and freezing are calculated. The relationship between the percentage of time spent frozen and the FCI (**B**: r = 0.40, P = 0.01); inverse of TMT- B (**C**: r = 0.35, P = 0.02); step time variability (**D**: r = 0.56, P < 0.001); and HADS (**E**: r = 0.14, P = 0.37) are also illustrated. PCA = principle component analysis.

raw voxel size = 3.9 mm × 3.9 mm × 4 mm thick. High-resolution 3D T_1-weighted anatomical images with voxel size = 0.4 × 0.4 × 0.9 mm were obtained for co-registration with functional scans.

Statistical Parametric Mapping Software (SPM12, Wellcome Trust Centre for Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.ak/spm/software) was used for image preprocessing. Functional scans were: (i) manually realigned along the anterior-posterior commissure; (ii) slice time corrected to the median (21st) slice in each repetition time; (iii) realigned to create a mean realigned image and measures of 6° of rigid head movements were calculated 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 (vii) smoothed using an 8-mm full-width at half-maximum isotropic Gaussian kernel.

Multiple precautions were taken to ensure strict control of the effects of head motion: (i) all participants were instructed to minimize head motion by keeping the legs and hips stationary and only moving the ankles to depress the foot pedals; (ii) cushions were placed inside the head coil to limit the physical possibility of head motion; (iii) ArtRepair was used to analyse each trail and applied the interpolation method to correct for large amounts of global drift or scan-to-scan head movement > 1.5 mm; and (iv) six motion and nuisance regressors were regressed out of each participants' extracted time series during statistical modeling.

The freezing component index

To estimate a low-dimensional signature of the factors that relate to freezing across subjects, we ran a principal components analysis on a set of three behavioural variables that were hypothesized a priori to be associated with the freezing phenotype: cognitive, TMT-B Z-score (inverted so that worse scores were positive); motor, step time variability; and limbic, HADS total. We labelled the first principal component (which explained 79.24% of the variance) the 'Freezing Component Index' (FCI). To confirm that the FCI related to freezing of gait severity, we correlated the principal component to the percentage of time spent frozen during the virtual reality task (any footstep latency that was longer than twice the modal footstep latency) using a Spearman's correlation (Fig. 1B).

Region of interest selection

To improve the specificity of our imaging analysis, we predefined a set of regions of interest. Based on previous work (Lewis and Barker, 2009), we chose cortical and subcortical regions that covered the motor, cognitive and limbic networks, along with striatal regions that are functionally related to each network. The cortical regions were chosen using the term-based meta-analyses tool NeuroSynth (http://www.neurosynth.org/). Specifically, we identified the top regions associated with the terms: 'cognitive control network', 'motor network' and 'anxiety'. It should be noted that anxiety and depression identified similar regions during the NeuroSynth search; however, we selected anxiety as the search term since it has been more robustly linked to freezing. The peak MNI coordinates for each region (Table 1) were then used to construct 8 mm region of interest spheres (Fig. 2). Based on previous research (Di Martino et al., 2008; Bell et al., 2015), we also defined a set of 2 mm region of interest spheres to parcellate the striatum into seven distinct regions.

Functional connectivity analysis

To conduct a task-based functional connectivity analysis, time series data were extracted from the first level general linear model for each of the 31 regions of interest (17 cortical, 14 striatal) using the MarsBaR toolbox (http://marsbar.sourceforge.net/). Further preprocessing steps included the normalization of each region to its own mean and standard deviation (SD), low pass filtering (f $\leqslant$ 0.125 Hz), and removal of the mean signal across all regions at each time point. SPM12 software was then used to model freezing and normal foot tapping (plus their temporal and spatial derivatives) using an epoch design, after which the regressors were convolved with a canonical haemodynamic response function. Normal foot tapping epochs were defined as five consecutive footsteps that were not interrupted by an environmentally salient cue (such as a doorway) or by a cognitive cue. The criterion of a minimum of five freezing events was selected to permit effective modelling of freezing events (Poldrack et al., 2011). Thus, participants who experienced less than five freezing events during functional MRI scanning were removed from all of the subsequent imaging analyses. Our cohort of freezers was made up of participants from two separate studies, which varied the task length during functional MRI. Thus, our final sample included 20 participants: 13 participants who completed a 10-min protocol and seven who completed a 5-min protocol.

To estimate the time-resolved functional connectivity between each of the 31 regions of interest over the course of the task, we used the multiplication of temporal derivatives approach (Shine et al., 2015) with a window size of 10 repetition times (i.e. 30 s). Briefly, this technique affords a windowed estimate of functional connectivity as it evolves over time (code is freely available at http://github.com/macshine/coupling/). To determine whether functional connectivity between regions changed with respect to freezing behaviour, the pairwise connectivity estimates were fitted to a general linear model comparing epochs of freezing with those of normal foot tapping. To control for multiple comparisons, we performed a non-linear permutation testing by creating a null distribution of 5000 randomly permuted connectivity matrices (Nichols and Holmes, 2002). Edges were deemed significant if the connectivity strength was either stronger or weaker than the 97.5th (or 2.5th) percentile of the null distribution, respectively. To further minimize type I errors, we also thresholded results using a large effect size (Cohen's $d \geqslant 0.8$; Supplementary Table 1).
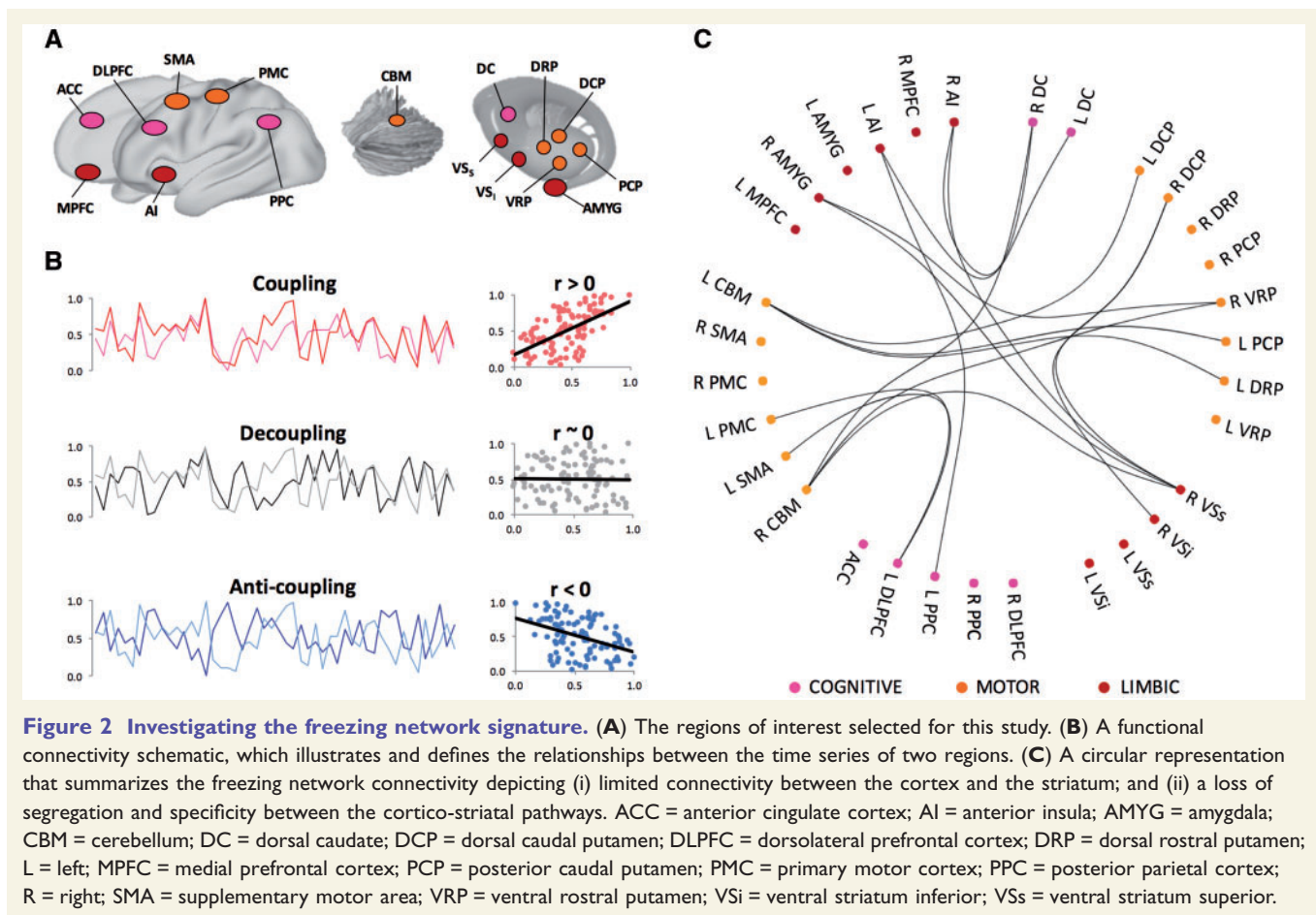
To determine the relationship between abnormal functional connectivity and freezing severity and heterogeneity, we seperately correlated the freezing > normal foot tapping functional connectivity matrix with the FCI and each individual component, respectively (Supplementary Table 2). A similar non-parametric permutation approach as detailed above was used to correct for multiple comparisons. Specifically, we randomly re-sorted the FCI vector across participants and then estimated the strength of correlation between the reordered FCI and each pairwise functional connection 5000 times per edge—edges < 2.5th percentile ($P < 0.05$) were retained for further interpretation. Only the significant edges that both survived permutation testing and were also significantly correlated with the FCI were reported.
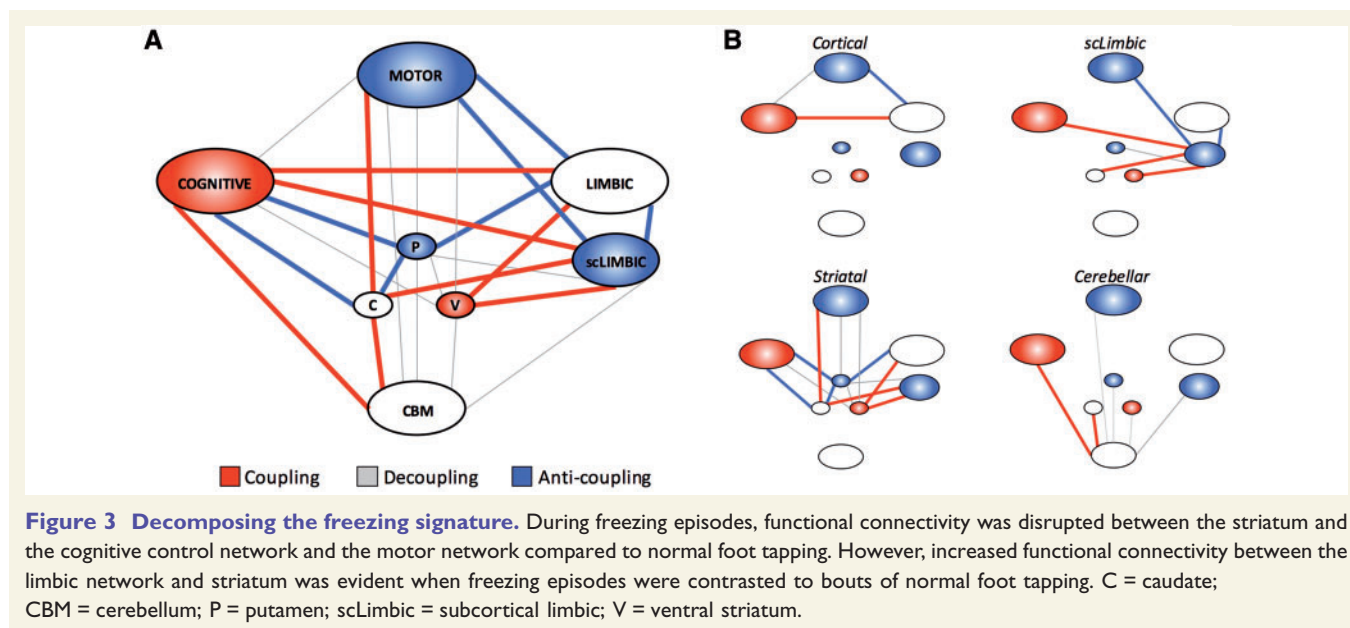
To aid in the interpretation of our results, edges were qualitatively sorted according to their relationship with the FCI. We reasoned that edges that were similarly correlated with the FCI (e.g. an edge with increased coupling during freezing was positively correlated the FCI or an edge with decreased coupling during freezing was inversely correlated with the FCI) may represent an index of freezing 'severity' (i.e. greater coupling/anti-coupling was associated with a greater 'freezing severity') (Fig. 3). In contrast, if the FCI was correlated in the opposite direction to the mean pattern observed in an edge (e.g. an edge with increased coupling was associated with a lower 'severity' score on the FCI), then the edge may be 'compensatory'. Finally, if an edge was found to be independent of the severity of freezing of gait correlates, it was labelled as 'independent', suggesting that there was a significant pattern of abnormal connectivity that was consistently

Table 1 Cortical regions of interest identified using *NeuroSynth*

Region of interest		x	y	z	Z-score
Motor					
PMC	R	38	−2	58	10.06
	L	−24	−28	58	10.09
SMA	R	4	−2	60	8.05
	L	−8	−4	56	8.62
CBM	R	32	−64	−58	7.71
	L	−16	−66	−58	7.76
Cognitive					
ACC	M	0	−26	32	5.81
DLPFC	R	44	18	32	6.43
	L	−46	18	36	6.8
PPC	R	48	−46	40	5.61
	L	−42	−62	40	4.23
Limbic					
MPFC	R	18	36	32	4.36
	L	−14	46	26	5.51
AI	R	44	6	−10	6.02
	L	−38	−2	−10	5.62
Amygdala	R	22	−4	−18	7.38
	L	−24	−2	−24	9.21

ACC = anterior cingulate cortex; AI = anterior insula; CBM = cerebellum; DLPFC = dorsolateral prefrontal cortex; L = left; M = middle; MPFC = medial prefrontal cortex; PMC = primary motor cortex; PPC = posterior parietal cortex; R = right; SMA = supplementary motor area.

Figure 2 Investigating the freezing network signature. (**A**) The regions of interest selected for this study. (**B**) A functional connectivity schematic, which illustrates and defines the relationships between the time series of two regions. (**C**) A circular representation that summarizes the freezing network connectivity depicting (i) limited connectivity between the cortex and the striatum; and (ii) a loss of segregation and specificity between the cortico-striatal pathways. ACC = anterior cingulate cortex; AI = anterior insula; AMYG = amygdala; CBM = cerebellum; DC = dorsal caudate; DCP = dorsal caudal putamen; DLPFC = dorsolateral prefrontal cortex; DRP = dorsal rostral putamen; L = left; MPFC = medial prefrontal cortex; PCP = posterior caudal putamen; PMC = primary motor cortex; PPC = posterior parietal cortex; R = right; SMA = supplementary motor area; VRP = ventral rostral putamen; VSi = ventral striatum inferior; VSs = ventral striatum superior.

Figure 3 Decomposing the freezing signature. During freezing episodes, functional connectivity was disrupted between the striatum and the cognitive control network and the motor network compared to normal foot tapping. However, increased functional connectivity between the limbic network and striatum was evident when freezing episodes were contrasted to bouts of normal foot tapping. C = caudate; CBM = cerebellum; P = putamen; scLimbic = subcortical limbic; V = ventral striatum.

present in the cohort of patients with freezing of gait irrespective of freezing severity. A similar analysis was then conducted using the individual component scores (i.e. cognitive, motor, limbic); however, 'independent' edges were ignored due to overlap across the three component domains.

Results

Executive summary

In this study, we made an explicit choice to expose the detailed heterogeneity of the freezing of gait phenotype. To clarify our results, we would like to first emphasize the overarching framework that we applied to investigate the multivariate nature of freezing. First, by contrasting connectivity during freezing episodes to normal foot tapping we constructed a 'Freezing Network Signature' (Figs 2C and 3). Second, we related the network signature of freezing to the FCI (which is significantly correlated with per cent time spent frozen during the task) in order to assess which aspects might be associated with 'severity', 'compensation', or 'independent' altogether (Fig. 4). Third, by decomposing the FCI into its cognitive, motor and limbic components, we examined the relationship between individual behavioural components and the freezing network signature further (Fig. 5). Characteristics of participants both included and excluded from the functional MRI analysis are presented in Table 2. Notably the two groups (MRI+ and MRI−) were matched in age, symptom severity, cognitive status, FOGQ3, anxiety, total HADS, processing speed, set-shifting ability and step time variability. There were a few group differences such that the cohort included in the functional MRI analysis (MRI+) had a significantly higher dopamine dose equivalence ($P = 0.04$), greater depression score (although not clinical significant)

($P = 0.03$), faster foot step latency ($P < 0.01$), and greater amount of freezing ($P < 0.01$).

Freezing network signature

Figure 3 illustrates a summary of the overall network as a function of freezing of gait (i.e. freezing > normal foot tapping). During freezing episodes, the functional connectivity was disrupted between the striatum and the cognitive control network and the motor network compared to normal foot tapping. However, increased functional connectivity between the limbic network and striatum was evident when freezing episodes were contrasted to bouts of normal foot tapping. Further details are provided below.

Cognitive network

During episodes of freezing, anti-coupling was observed between the cognitive control network and striatum, whilst increased coupling was observed within the cognitive control network when compared to normal foot tapping. The cerebellum was also significantly more coupled to the caudate and cognitive control network.

Motor network

There was substantial disruption to the motor network during freezing compared to normal foot tapping. Greater anti-coupling was found both within the cortical motor network, and within the putamen. Furthermore, the motor network was also decoupled from the putamen and and limbic structures and instead coupled to the dorsal caudate nucleus.

Limbic network

Increased coupling between the limbic network (cortical and subcortical) and the ventral striatum was found
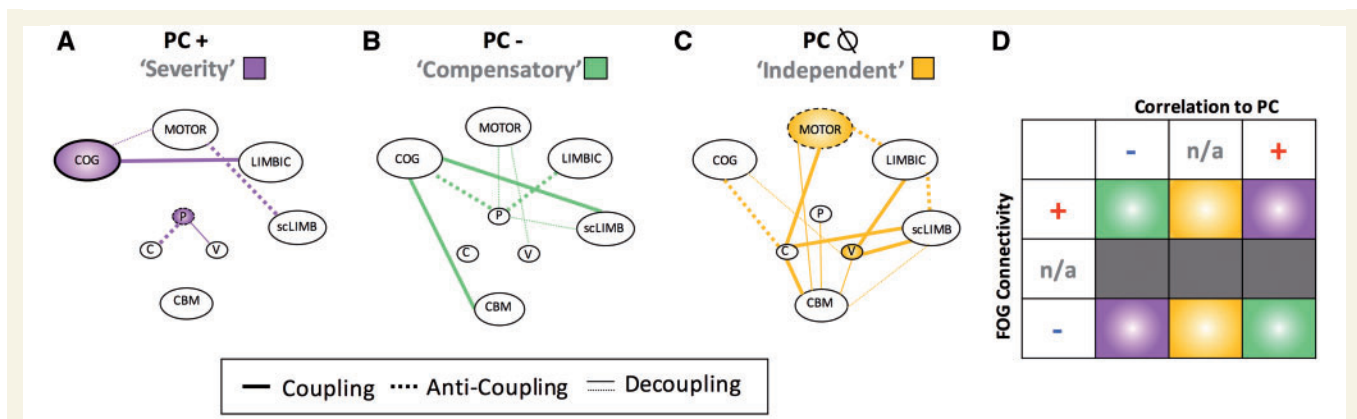
Figure 4 Associations between the freezing network signature and the FCI. Functional connections identified in the Freezing Network Signature, which were associated with a worse FCI are depicted in (**A**), whereas the connections that were associated with a better FCI are depicted in (**B**). The remaining functional connections that were not significantly correlated to the FCI are depicted in **C**, and (**D**) demonstrates a summary key that describes how the classification was determined. C = caudate; CBM = cerebellum; P = putamen; PC = principal component; scLimbic = subcortical limbic; V = ventral striatum.
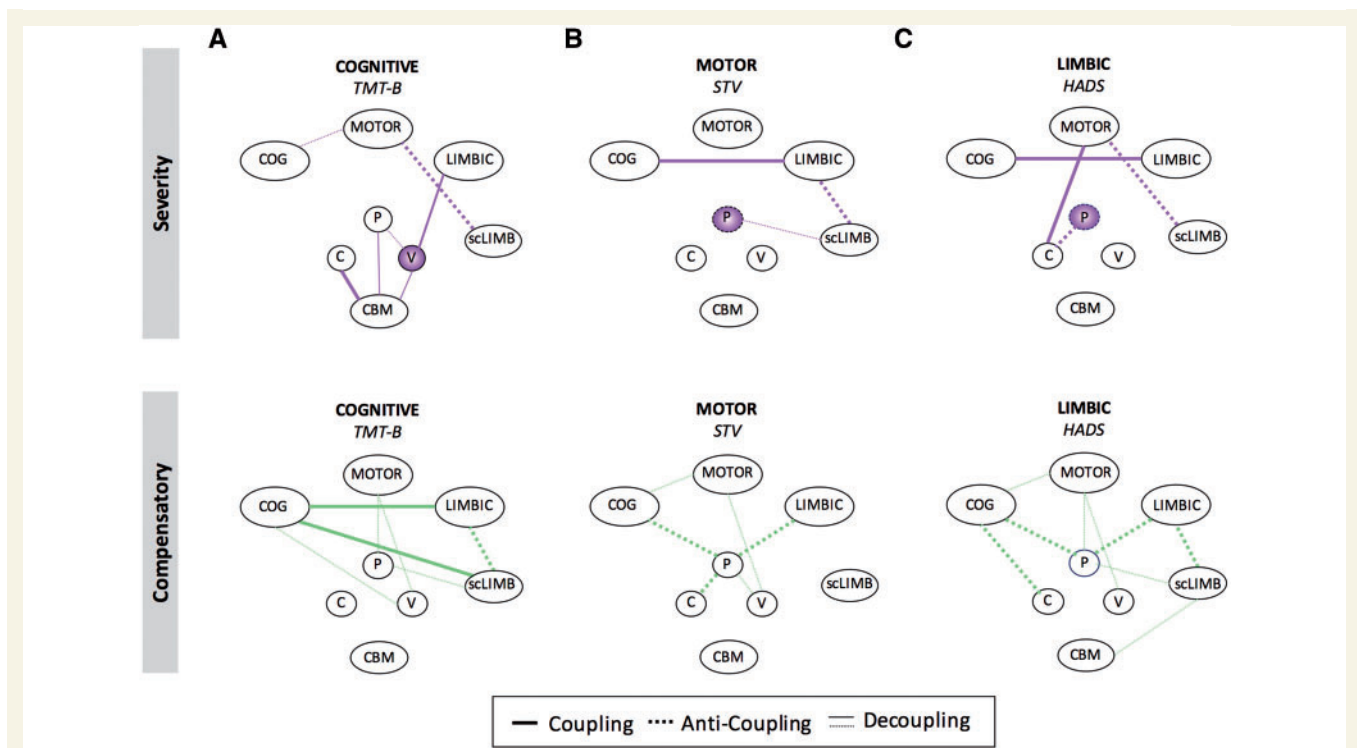
Figure 5 Unique network signatures for individual components of freezing. Functional connections identified in the freezing network signature that were associated with more severe components are illustrated in the *top* panel (**A**, cognitive; **B**, motor; **C**, limbic). Functional connections identified in the freezing network signature that were associated with reduced component scores (i.e. compensatory) are illustrated in the *bottom* panel. C = caudate; CBM = cerebellum; COG = cognitive; P = putamen; scLimbic = subcortical limbic; STV = step time variability; V = ventral striatum.

during freezing compared to normal foot tapping. Furthermore, increased coupling between the limbic network (cortical and subcortical) and the cognitive control network was found. In contrast, anti-coupling between the cortical and subcortical limbic network and within the subcortical network was marked during freezing compared to normal foot tapping. Notably, the subcortical limbic regions were tightly coupled to the dorsal caudate, despite that the cognitive control network was anti-coupled from the dorsal caudate nucleus.

Table 2 Demographic, clinical and neuropsychological and virtual reality characteristics

	Total participants (n = 41)		MRI + (n = 20)		MRI − (n = 21)	
	Mean	SD	Mean	SD	Mean	SD
Gender, n	9 F (22%)		3 F (15%)		6 F (29%)	
Age	67.54	6.40	66.45	5.34	68.57	7.26
DDE	877.9	579.9	1069.5*	687.6	686.3*	374.4
MDS UPDRS-III	33.89	14.0	34.15	12.19	33.62	15.84
MMSE	27.90	2.07	27.65	2.16	28.14	2.01
FOGQ3	2.5	1.01	2.53	1.02	2.48	1.03
HADS-Anxiety	5.78	3.63	6.3	3.25	5.29	3.98
HADS-Depression	5.37	2.77	6.35*	2.83	4.43*	2.42
HADS Total	11.15	5.9	12.65	5.79	9.71	5.77
TMT-A (Z-score)	−0.34	1.14	−0.26	0.97	−0.42	1.31
TMT-B (Z-score)	−0.84	1.68	−0.80	1.40	−0.88	1.95
Step time variability	23.08	7.86	24.99	7.04	21.27	8.32
Modal foot step latency, s	0.52	0.27	0.38*	0.17	0.64*	0.29
Time spent frozen, %	0.10	0.09	0.14*	0.08	0.07*	0.14
Protocol completed (5-min:10-min)	25:16		7:13		18:3	

*Significant group difference, $P < 0.05$.
DDE = dopamine dose equivalence; FOGQ3 = question 3 of the Freezing of Gait Questionnaire; MDS UPDRS-III = Movement Disorders Society Unified Parkinson's Disease Rating Scale motor subsection; MMSE = Mini-Mental State Examination; MRI + = participants included in the functional MRI analysis; MRI− = participants removed from the functional MRI analysis.

Relating the freezing network signature to the freezing component index

To assess which aspects of the freezing network signature were associated with severity, compensation, or were independent of severity, we first verified there was a significant relationship between the percentage of time spent frozen during the task and the FCI (r = 0.40, P = 0.01; Fig. 1B). Furthermore, the FCI was also positively correlated with FOGQ3 (r = 0.32, P = 0.045). Figure 4 summarizes the relationship between functional connectivity and the FCI, wherein the FCI was correlated with each significant connection from the freezing signature (freezing > normal foot tapping; Fig. 3).

Severity versus compensation

Overall, a worse FCI (a proxy for freezing severity) was associated with: (i) greater coupling within the cognitive control network and also between the cognitive control network and the limbic cortex; (ii) anti-coupling within the putamen, and between the putamen and caudate nucleus, as well as a loss of anti-coupling between the putamen and ventral striatum; and (iii) anti-coupling between the motor cortex and the limbic subcortex, and cognitive control network.

In contrast, reduced freezing severity (i.e. 'Compensation') was associated with: (i) anti-coupling between the putamen and cortical networks (cognitive control, limbic and motor network); (ii) anti-coupling between the ventral striatum and motor network; and (iii) increased coupling between the cognitive control network and the cerebellum and subcortical limbic network.

Freezing component signatures

To understand the heterogeneous network signature associated with freezing, we decomposed the FCI into its cognitive, motor and limbic parts, and further examined the relationship between individual behavioural components and the freezing network signature. Figure 5 depicts the edges associated with better (green) or worse (purple) scores for each of the three component domains (cognitive, motor and limbic).

Cognitive signature (TMT-B)

Worse set-shifting ability was associated with decoupling between cognitive control network and the motor network, as well as decoupling between the putamen and the ventral striatum. Increased coupling within the ventral striatum was observed, as well as between the ventral striatum and the cortical limbic network, and between the cerebellum and caudate nucleus was also associated with worse set-shifting ability. Anti-coupling between the motor network and the subcortical limbic network was related to worse set-shifting ability. In contrast, better set-shifting ability was associated with coupling between the cognitive control network and the limbic network (cortex and subcortex), and anti-coupling between the cortical and subcortical limbic network. Decoupling between the cortex and striatum was also associated with greater cognitive flexibility.

Motor signature (step time variability)

Worse step time variability was primarily associated with limbic edges. Greater coupling between cortical limbic and cognitive control networks, and greater anti-coupling between cortical and subcortical limbic networks as well as

within the putamen. Decoupling between the putamen and subcortical limbic regions was also associated with greater step time variability.

In contrast, reduced step time variability was primarily associated with reduced coupling between the cortex and striatum and across the striatum. More specifically, anti-coupling between the putamen and the cognitive control network and cortical limbic network, as well as anti-coupling between the dorsal caudate and the putamen were related to less variable step timing. Additionally, decoupling between ventral striatum and both the motor network and putamen, as well as decoupling between the motor network and cognitive control network was also associated with reduced step time variability.

Limbic signature (HADS)

Worse anxiety and depression was associated with increased coupling between the cortical limbic and cognitive control network, as well as between the motor network and the dorsal caudate nucleus. Furthermore, anti-coupling between the subcortical limbic network and the motor network, within the putamen, and between the putamen and the dorsal caudate was also related to worse affective disturbance.

Similar to the step time variability, less affective disturbance was associated primarily with anti-coupling between the cortex and the striatum. Specifically, anti-coupling was found between the putamen and the cognitive control network and cortical limbic network, as well as between the dorsal caudate and the cognitive control network. Additionally, anti-coupling between the cortical and subcortical limbic network was found. Decoupling between the putamen and the subcortical limbic network and motor network as well as decoupling between the motor network and the various other cortical and striatal regions (i.e. the cognitive control network, subcortical limbic network, putamen, and the ventral striatum) was also related to less severe anxiety and depression symptoms.

Discussion

In this study, we used a task-based connectivity analysis to identify latent heterogeneity within the neural signature underlying freezing of gait in Parkinson's disease. When compared to periods of normal foot tapping (i.e. a proxy for normal walking), freezing episodes were characterized by an overall loss of synchrony between the cortex and the striatum, as well as a loss of segregation and specificity between the cortico-striatal pathways (Figs 2B and 3). Subsequent group-level behavioural interrogation allowed us to dissociate the freezing signature into three distinct categories (Fig. 4): a set of intracortical and intrastriatal connections that were related to freezing severity; a group of cortico-striatal connections that were putatively related to compensation; and a network of cortico-striatal and striato-cerebellar connections that were independent of

freezing severity. Finally, we related these deficits to individual differences in the behavioural factors (cognitive, motor, limbic) that predispose individuals towards freezing episodes (Fig. 5). Together, these results provide confirmatory evidence for systems-level impairments in the pathophysiology of freezing of gait in Parkinson's disease.

Severity versus compensation

In contrast to previous studies, the approach used in this study was able to decipher the patterns within the freezing of gait phenotype that were related to individual differences in freezing severity. Specifically, worsened scores on the first principal component (i.e. inferring worse behavioural freezing) was associated with increased connectivity within the cognitive control network and between the cognitive control network and other cortical networks (i.e. motor and limbic; Fig. 4). That is, greater cortical 'cross-talk' was associated with worse freezing, and less cross-talk between the cortex and striatum was associated with less freezing of gait. It is plausible that cortical areas may become too integrated due to striatal dysfunction or periods of increased limbic drive. Alternatively, actively reducing the 'cross-talk' between the striatum and cortex (e.g. via maximizing temporal separation of regional activity) may reduce the severity of freezing of gait and reflect a compensatory strategy to overcome freezing episodes. There is growing evidence that dopaminergic replacement therapy may restore the appropriate corticostriatal connectivity (Esposito *et al.*, 2013; Tahmasian *et al.*, 2015; Gilat *et al.*, 2017), and normalize the disrupted network topology (Berman *et al.*, 2016; Kim *et al.*, 2017), thus potentially minimizing 'crosstalk'. Further research is needed to determine whether dopaminergic replacement therapy restores network segregation (Nieuwhof and Helmich, 2017), and whether network segregation versus integration might be protective against freezing of gait. The current findings also converge with previous suggestions that freezing represents dysfunction within a distributed network of widespread frontal and parietal cortical regions (Shine *et al.*, 2013c), and supplements studies that have shown a reduction in frontoparietal blood oxygen level-dependent activation (Snijders *et al.*, 2011; Shine *et al.*, 2013b), grey matter atrophy in frontal and parietal cortices (Kostic *et al.*, 2012; Tessitore *et al.*, 2012a; Herman *et al.*, 2014), and reduced perfusion rate in frontal networks (Imamura *et al.*, 2012) in association with freezing severity. Interestingly, freezing severity has been associated with reduced functional connectivity within the 'executive-attention' neural network in the 'resting' state (Tessitore *et al.*, 2012b), whereas here we demonstrate the opposite pattern and relationship during a task-based protocol. These differing results could emphasize that freezing is likely related to altered cortical control of gait, or perhaps via failed compensatory strategies that facilitate too much cortical integration. These findings also indirectly support predictions of the cross-talk model, which hypothesizes that an

overflow of communication between the cortex and the striatum, paired with a loss of segregation (demonstrated here by pathological cortical coupling) among competing yet complimentary brain networks may overwhelm the striatum, thus generating excessive inhibitory output from the globus pallidus to the brainstem structures controlling gait, ultimately manifesting freezing behaviour (Lewis and Barker, 2009).

Similar to recent work, the current study also identified patterns of abnormal cerebellar connectivity that were associated with freezing of gait. In previous work, Fasano et al. (2017) used a novel lesion network mapping technique to show that lesions in the dorsal midline cerebellum were implicated in generating freezing symptoms; however, it was inconclusive whether the cerebellum's role in lesion-induced freezing was relevant for parkinsonian freezing of gait. Several studies have shown abnormalities in cerebellar locomotor regions' structural and functional connectivity in Parkinson's disease patients with freezing (Schweder et al., 2010; Fling et al., 2014; Vercruysse et al., 2015; Myers et al., 2017), yet a recent study investigating cerebellar theta burst stimulation found that it did not improve freezing of gait in patients with Parkinson's disease (Janssen et al., 2017). In the current study, the connectivity between the cerebellum and the striatum was a marked feature of the network signature of freezing independent of severity, whereas coupling between the cerebellum and cognitive control network was found to be compensatory (i.e. associated with less freezing). Therefore, we provide further evidence that the cerebellum plays an important role in the underlying pathophysiology of freezing of gait; however, future research is needed to understand its compensatory relationship in order to help guide the development of effective new therapies.

Recent work by Nieuwhof et al. (2017) has also shown a loss of segregation within cortico-striatal loops during dual-task performance while foot-tapping in Parkinson's disease (Nieuwhof et al., 2017). Here, we advanced these findings by mapping patterns of abnormal connectivity during freezing, which were further related to individual differences in predispositional components (Fig. 3). More specifically, we used a low dimensional behavioural signature to infer patterns of functional connectivity associated with increased severity; with increased compensation; or those that were independent of severity. Based on Nieuwhof's predisposition hypothesis, it may have been expected that a loss of cortico-striatal segregation (i.e. greater striatal overlap) might be independent of freezing severity, and thus represent trait-like factors common in all patients with freezing of gait. Indeed, rather than the typical cortico-striatal circuitries being functionally coupled (e.g. cognitive control network – dorsal caudate; motor network – dorsal putamen; limbic network – ventral striatum), we observed in the current study little specificity (i.e. greater overlap) in the functional connectivity between the cortical networks and the striatum during freezing compared to normal foot tapping. Moreover, all positive edges between the cortex and striatum (e.g. motor network – caudate, subcortical limbic – caudate) were independent of freezing severity. Furthermore, increased limbic connectivity with the striatum, as well as disrupted communication within the motor network and between the cognitive control network and the striatum were also independent of freezing severity. Therefore, one interpretation could be that these abnormalities in communication were consistently present across all patients with freezing of gait during episodes of freezing, and thus may represent the true neural underpinnings of freezing of gait that are unchanged by heterogeneous trait-like factors.

In contrast, the first principal component (a proxy for freezing severity) was associated with altered connectivity across the striatal nuclei (Fig. 4). More specifically, worse freezing behaviour was associated with a loss of coupling within the putamen and between the dorsal caudate and putamen, and accompanied by greater coupling between the ventral striatum and putamen. These findings are in keeping with previous work which has demonstrated decreased blood oxygen level-dependent signal in subcortical areas (such as the caudate, globus pallidus internus, thalamus and mesencephalic locomotor region) during freezing (Shine et al., 2013b). Furthermore, these results may reflect the dopaminergic aetiology of freezing of gait, since dopamine degeneration progresses posterior-dorsal to anterior-ventral within the striatum (Poldrack, 2005; Wu and Hallett, 2005), thus affecting the putamen and dorsal caudate more so than the ventral striatum. This degenerative process could lead to recruitment of the dopamine-preserved ventral striatum as a compensatory strategy to aid with cortico-striatal processing, which may also contribute to greater overlap and loss of segregation within cortico-striatal pathways. This notion is consistent with research suggesting that a bottleneck of processing might occur when motor and cognitive inputs are funnelled into the relatively spared ventro-anterior putamen due to the gradient dopamine depletion in the dorsoposterior putamen (Lewis and Barker, 2009). Hypothetically, in instances where the ventral striatum may be needed for information processing within its own segregated cortico-striatal limbic loop, this over-recruitment may lead to vulnerability across other systems that rely on compensatory ventral striatum processing. In this way, limbic input may have a greater capacity to interfere with motor output such as walking, which might offer a further explanation for the emerging relationship between anxiety and freezing of gait.

Component signatures of freezing

Several of the regions that have been associated with freezing of gait are also known to be intimately involved with motor, cognitive and limbic functions, which in turn may explain the well-known clinical relationship between freezing and impairment across multiple behavioural domains (Schaafsma et al., 2003; Giladi and Hausdorff, 2006; Naismith et al., 2010; Nutt et al., 2011; Ehgoetz Martens et al., 2014b; Hall et al., 2015). Indeed, this very feature of

freezing forms an important basis of the 'cross-talk' model, which describes freezing as a transient overload of information processing capacity of within the broader network of the brain (i.e. independent of the domain that catalyses overload), ultimately leading to a breakdown in motor function and freezing (Lewis and Barker, 2009; Lewis and Shine, 2016). Given that the precise circuitry that provokes freezing likely differs depending on the specific trigger that will cause an episode (cognitive, motor, limbic), this study aimed to explore how functional connectivity correlated to particular cognitive, motor, and limbic components of freezing (i.e. set-shifting ability, step time variability, anxiety and depression severity), in an effort to better understand their unique contributions and neural underpinnings.

Cognitive signature

Set-shifting impairments are well known in Parkinson's disease, and have been linked with dopaminergic depletion in the caudate nucleus as well as reduced structural and functional connectivity to frontal areas (Bartels *et al.*, 2006; Shine *et al.*, 2013d). In the current study, worse set-shifting ability was associated with greater coupling between the cerebellum and the caudate as well as between the ventral striatum and cortical limbic regions. In contrast, greater cognitive flexibility was associated with coupling between the cognitive control network and the limbic network and less connectivity between the cortex and the striatum. In patients with Parkinson's disease, including patients with freezing of gait, a relationship between affective disturbance and performance on the TMT has been established, such that anxiety and depression can interfere with set-shifting abilities (Ehgoetz Martens *et al.*, 2016d). Our findings may fit with this notion, since greater synchronization within the limbic cortico-striatal pathway relates to worsened TMT-B performance, whereas greater 'top-down' control of the limbic network is associated with more cognitive flexibility, which confirms the dependent nature of cognitive and affective disturbance in patients with freezing of gait.

Motor signature

Increased step time variability has also been robustly linked with freezing of gait (Pieruccini-Faria *et al.*, 2015; Weiss *et al.*, 2015) and is thought to reflect a loss in motor automaticity due to dopaminergic denervation predominantly in the posterior dorsal putamen (Wu and Hallett, 2005). This area of the putamen is thought be responsible for chunking motor sequences to improve efficiency and free attentional resources (Graybiel, 1998). Given that dopaminergic depletion affects the posterior-dorsal putamen early on, compensatory attentional and cognitive control networks have been postulated to compensate by being brought online to control and coordinate movements. Recent neuroimaging work has indeed shown that greater activation in frontal areas (Wu and Hallett, 2005) and increased

intrastriatal functional connectivity was associated with greater movement variability in patients with Parkinson's disease (Gilat *et al.*, 2017). In patients with freezing of gait, it might be expected that this loss of automaticity (represented by increased step time variability) places an even greater demand on limited attentional resources and will be similarly related to dysfunctional striatal connectivity. Here, we found that worsened step time variability was indeed related to disrupted connectivity within the striatum, predominately between the putaminal nuclei. Furthermore, less variability was associated with anti-coupling between the putamen and the cortex, as well as reduced connectivity between intra-striatal nuclei and between the cognitive control network and motor network. Overall these findings are in support of previous work suggesting a loss of dopamine in the dorsal striatum is related to greater step time variability. Likewise, the pattern of results in this study could also reflect a compensatory shift in processing, such that the ventral-anterior striatum may be recruited more to aid with cortico-striatal processing to control movement since it may have more preserved dopaminergic neurons. Furthermore, individuals with less step time variability also had reduced connectivity between the cognitive control network and motor network to employ top-down control of movement, which is sensible given that cognitive control of gait typically enhances variability and reduces rhythmicity.

Limbic signature

Although affective disturbance and freezing of gait are closely related, little research has examined the neural correlates of anxiety or depression in Parkinson's disease, let alone the integrity and functional connectivity of the limbic cortico-striatal pathway in patients with freezing of gait. Here, we demonstrate for the first time that limbic-striatal connectivity is a key component of the freezing neural signature. Moreover, increased input from the limbic network may contribute to interference in processing at the level of the basal ganglia via 'limbic load', which has been previously suggested based on behavioural results as a potential mechanism underpinning freezing of gait (Ehgoetz Martens *et al.*, 2014b). Considering the loss of segregation across the striatum, the ventral striatum may become increasingly utilized for motor-related processing and movement control, which could be especially problematic in anxious patients, who have an 'overactive' limbic network (and threat detection system) to begin with. Surprisingly, however, we found that greater affective disturbance in patients with freezing of gait was associated with predominately motor network connectivity rather than limbic *per se*.

Similar to variability and set-shifting, less affective disturbance was associated with less functional connectivity between the cortex and the striatum. This might suggest that anxiety in Parkinson's disease, or perhaps that which is associated with freezing of gait is intimately related to movement control deficits. A common discussion point is

whether anxiety is a response to movement impairments or a driving cause. It is well known that uncertainty in sensory information provides the basis for anxious processes. Thus, perhaps sensory impairments that underlie movement symptoms could also provoke anxiety over time in Parkinson's disease. Previous work has demonstrated that sensory deficits are linked to freezing as well (Ehgoetz Martens *et al.*, 2013*b*, 2014*a*, 2016*a*), such that freezing was exacerbated when asked to walk toward a doorway in the dark compared to a lit frame, and also suggest that the threat in an environment compounds freezing behaviours since freezing was also greater when walking toward a doorway in the dark compared to a dark open space (Ehgoetz Martens *et al.*, 2013*a*). Further research is needed to test whether anxiety is related to sensory impairments in Parkinson's disease early on in the disease course (Ehgoetz Martens *et al.*, 2016*b*), and also to further disentangle whether anxiety is the chicken or egg to freezing.

Clinical implications

Overall, these findings suggest that unique neural signatures exist and relate to different behavioural correlates of freezing. The suggestion that freezing of gait subtypes may exist has been previously put forward (Fasano *et al.*, 2015); however, to date, limited evidence has been presented. This is the first study to examine multiple components of freezing, and demonstrate that unique neural signatures are present during freezing of gait episodes. Based on our results, one could speculate that different subtypes of freezing of gait arise due to individual vulnerabilities within the diverse systems required for dynamic control of adaptive behaviour and locomotion. For instance, if one is highly anxious, input from the limbic system to the striatum could 'tip the scale', causing freezing episodes to occur when the processing capacity of the system exceeds its limit. Similarly, if someone has significant impairments to motor automaticity and hence, relies more heavily on cognitive control resources for normally automatic gait, then instances that perturb or divide these necessary resources may in turn provoke freezing of gait. However, these relationships are not always straightforward and remain opaque, for example in situations where kinesia paradoxa enable freezers to execute movement in highly stressful situations, cross-talk between circuitries might channel adequate compensation rather than overload the system (Nieuwhof and Helmich, 2017). Thus, further research is certainly needed to illuminate whether segregation versus integration is detrimental or compensatory across various situations specific to freezing.

It should be noted that these findings do not necessarily dispute the involvement of a common downstream pathway of freezing (Lewis and Shine, 2016), but rather exposes the fact that there are potentially distinct upstream causes that may represent dissociable targets for treatment. For example, treating anxiety may have beneficial effects for specific individuals with freezing of gait (Ehgoetz Martens *et al.*, 2016*c*), whereas cognitive training might be more effective for others (Walton *et al.*, 2014; Leung *et al.*, 2015). Alternatively, an individual with either a motor or anxious phenotype may benefit from sensory-based training programmes that work on improving the use of sensory information and restoring gait automaticity (Sage and Almeida, 2009; Lefaivre and Almeida, 2015). However, much research is still needed to clarify this point and further research is also needed to characterize these subtypes, and determine whether in a data-driven approach there is any evidence of these different types, and whether they lend support to different cortical mechanisms and thus different 'cortically' geared therapies.

Limitations and considerations

It is important to point out the shortcomings and limitations of this work. First, we did not include any 'ON' state patients with freezing of gait in this cohort. Although this is a rare subtype of freezing of gait, it is currently unclear whether the findings presented here would generalize to this subgroup. Second, although the virtual gait task is able to capture much of the coordination involved in gait, it also has many limitations. For example, the constraints imposed by the functional MRI scanner are incompatible with analyses that interrogate the delicate control of upright stance and dynamic balance required while walking. Additionally, the freezing events detected could be confounded by fatigue or a loss in concentration. Another caveat in this study was that the participants included in the imaging cohort had a faster modal footstep latency on average than the cohort who were excluded. Although a slower foot tapping speed may set the threshold to detect freezing higher than those with a faster foot step latency, we did not observe an association between modal foot step latency and percentage of time spent frozen ($P > 0.1$). Thus, it is unlikely that foot step latency confounds the neuroimaging results reported in this study. Finally, given that our focus was on investigating the particular episodic characteristics of freezing, we did not include a control group in this study. However, future research should consider examining differences in continuous gait patterns between Parkinson's disease patients with and without freezing to further understand the role of the motor, cognitive and limbic networks and their contribution to gait variability and gait disturbances.

Conclusion

Overall, these findings provide confirmatory evidence for systems-level impairments in the pathophysiology of freezing of gait. Importantly, the findings of this study show how functional connectivity during freezing is correlated to particular cognitive, motor and limbic features in effort to better understand their unique contributions and neural underpinnings. Thereby we further advance our

understanding of the whole-brain deficits that mediate symptom expression in Parkinson's disease.

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Supplementary material

Supplementary material is available at *Brain* online.

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