doi:10.1093/brain/awz325 BRAIN 2019: 0; 1–11 | 1

BRAIN
A JOURNAL OF NEUROLOGY

Hitting the brakes: pathological subthalamic nucleus activity in Parkinson's disease gait freezing

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Gait freezing is a complex and devastating paroxysmal motor arrest commonly suffered in Parkinson's disease that causes significant impairment to mobility, commonly resulting in falls and subsequent injury. The neurobiological basis of gait freezing in Parkinson's disease is poorly understood and thus, currently available therapies are partially effective at best. We used a validated virtual reality gait paradigm to elicit freezing behaviour intraoperatively in eight patients undergoing subthalamic nucleus deep brain stimulation surgery while microelectrode recordings were obtained. This allowed us to directly test the hypothesis that increases in pathological multi-unit activity in the subthalamic nucleus are associated with freezing onset in real time, manifest as dysfunctional firing of lower limb muscles typical of freezing that were detected by EMG. We present evidence that freezing is related to transient increases in pathological subthalamic nucleus activity. We performed time-frequency analysis to characterize the oscillatory dynamics of subthalamic nucleus activity coincident with freezing onset, demonstrating an increase in pathological beta and theta rhythms that are followed by a temporal chain of activity culminating in characteristically abnormal lower limb muscle firing detected by EMG. Finally, we interrogate the potential clinical utility of our findings by contrasting the subthalamic nucleus activity signature during pathological freezing against purposeful stopping. These results advance our understanding of the neurobiological basis of gait freezing in Parkinson's disease, highlighting the role of the subthalamic nucleus and emergent synchronous activity in basal ganglia circuits in driving non-purposeful motor arrests in individuals with Parkinson's disease. Pathological subthalamic nucleus activity identified in association with freezing is discernible from that of volitional stopping, paving the way towards more effective therapeutics such as adaptive closed-loop deep brain stimulation protocols.

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Keywords: freezing of gait; Parkinson's disease; subthalamic nucleus; deep brain stimulation; virtual reality

Abbreviations: DBS = deep brain stimulation; MUA = multi-unit activity

Received July 21, 2019. Accepted August 22, 2019

Introduction

Freezing of gait is a sudden, transient motor arrest prevalent in Parkinson's disease, causing frequent falls and a significant reduction in quality of life. Dopaminergic depletion in the midbrain is widely acknowledged as the characteristic pathology of Parkinson's disease (Braak *et al.*, 2004); however, the pathogenesis of freezing (a paroxysmal event within a chronically and progressively dopamine-deplete system) is poorly understood. This in turn, limits the efficacy of currently available therapies. Recent advances in rodent optogenetics have demonstrated that overwhelming pallidal-mediated inhibition of the pedunculopontine nucleus leads to transient, yet reversible cessation of locomotion as if a hand-brake was suddenly engaged (Roseberry *et al.*, 2016). However, although descending connections from the pedunculopontine nucleus to central pattern generators in the medulla and spinal cord that control gait dynamics are well characterized (Takakusaki, 2013), it is well known that a variety of cognitive and affective challenges can trigger freezing episodes in Parkinson's disease (Nutt *et al.*, 2011). This suggests a cortical contribution to the pathogenesis of freezing and a need to reconcile convergent cortico-subcortical circuitry, within the context of reduced striatal dopaminergic innervation in individuals with Parkinson's disease (Braak *et al.*, 2004), in order to bridge higher and lower centres within the pathophysiological mechanism of freezing (Lewis and Barker, 2009). Elucidating the precise mechanism generating paroxysmal pallidal-mediated inhibition triggered by the various behavioural contexts in which patients with Parkinson's disease suffer freezing, i.e. investigating the neural regions responsible for engaging the figurative hand-brake, remains critical to the development of novel and effective therapies.

Based on neuroimaging studies using a virtual reality gait task to elicit freezing episodes during functional MRI scanning (Shine *et al.*, 2013*a*, *b*), we hypothesized that emergent pathological activity in the subthalamic nucleus (Wilson, 2013), particularly in the theta and beta frequency bands (Frank, 2005; Little and Brown, 2014; Shine *et al.*, 2014), plays a contributory role in the pathogenesis of freezing (Shine *et al.*, 2013*c*). In our model (Fig. 1), conflict-mediated activity in the cortex activates the subthalamic nucleus (Zavala *et al.*, 2017), which via its strong glutamatergic output to the globus pallidus internus, triggers inhibition of the brainstem (including the pedunculopontine nucleus) (Shine *et al.*, 2013*c*), which in turn mediates the abnormal temporal coordination of paired agonist-antagonist lower limb muscles characteristic of gait freezing in Parkinson's disease (Nieuwboer *et al.*, 2004). While abnormal subthalamic nucleus dynamics have been demonstrated in individuals with freezing (Toledo *et al.*, 2014; Syrkin-Nikolau *et al.*, 2017; Hell *et al.*, 2018; Pozzi *et al.*, 2019), to date there is no direct evidence demonstrating abnormal neural activity in the subthalamic nucleus with the onset of motor arrests in humans in real time.

Materials and methods

Overview, participants and clinical assessments

To examine the neurophysiological basis of gait freezing, we collected microelectrode recordings of subthalamic nucleus multi-unit activity (MUA) from eight patients with idiopathic Parkinson's disease (see Supplementary Table 1 for demographic data) while they performed a virtual reality gait task intraoperatively during awake neurosurgical implantation of deep brain stimulation (DBS) electrodes. We obtained extracellular recordings of pooled cell body action potentials from multiple subthalamic nucleus neurons in the vicinity of our microelectrode (see below for details). Single cell microelectrode recording was not attempted because of the difficulty of maintaining prolonged stable recordings in an awake, active humans. Akin to placing a microphone in a room hosting a cocktail party, we were able to observe ongoing conversations (i.e. an ensemble of MUA), without concerning ourselves with precisely who said what (i.e. firing of individual subthalamic nucleus neurons).

Patients were recruited through the Parkinson's Disease Research Clinic at the Brain and Mind Centre, University of Sydney. Surgical procedures and recordings took place at Westmead Private Hospital, Sydney. The diagnosis of idiopathic Parkinson's disease satisfied United Kingdom Parkinson's Disease Society Brain Bank criteria (Gibb and Lees, 1988). The Human Ethics Research Committees of the University of Sydney and of Westmead Private Hospital approved this research, and written informed consent was obtained from all participants according to the Declaration of Helsinki.

All subjects underwent preoperative neurological assessment by a clinician consisting of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (UPDRS) including derivation of the motor subscore (UPDRS-III), (Goetz *et al.*, 2007). Assessment occurred both on medication (ON) and after overnight withdrawal (>12 h) of dopaminergic therapy (OFF). Patients also prospectively completed the Freezing of Gait Questionnaire (Giladi *et al.*, 2009), and their daily levodopa equivalent dose (mg/day) was calculated (Tomlinson *et al.*, 2010). For all motor scales, higher scores indicate worse function.

Intraoperative virtual reality gait task

Individuals lay supine on an operating table and navigated the virtual reality environment using a set of foot pedals held in place at their feet with a footboard while the virtual reality environment was presented on a 40-inch screen in front of their eyes. The screen was mounted on the operating theatre ceiling and lowered on an arm so that the virtual reality environment was in clear view at all times (Supplementary Fig. 1A). The virtual reality environment consisted of a realistic straight corridor (presented in first-person view) that subjects navigated with alternating left and right ankle movements on a set of fixed foot pedals mediated by coordinated dorsi- and plantarflexion of the ankle joint, using the tibialis anterior and gastrocnemius muscles, from which we also collected

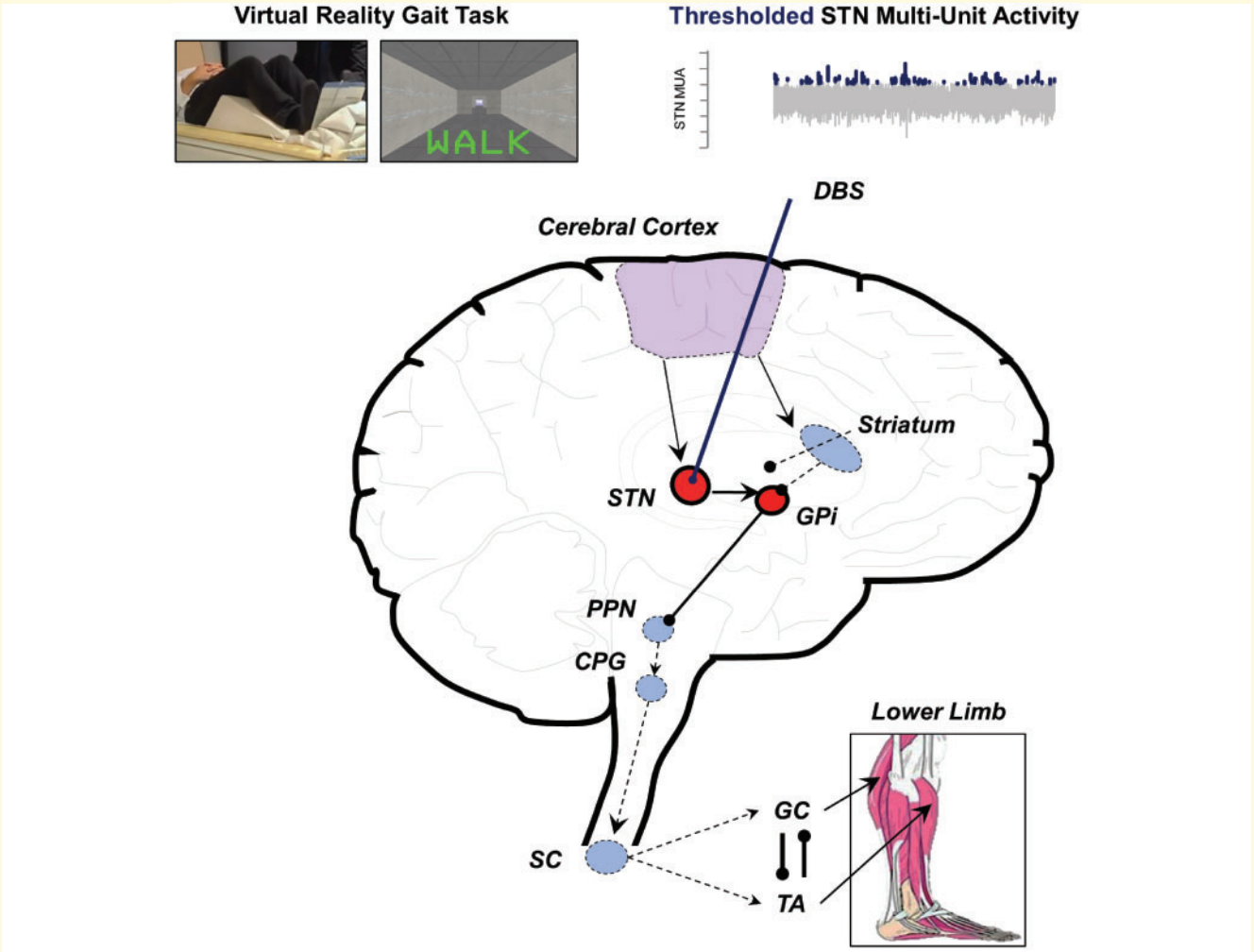

Figure 1 Pathophysiological model of freezing and experimental approach. Multi-unit data were collected from the subthalamic nucleus on the most affected side of eight individuals with Parkinson's disease while they performed an intraoperative virtual reality gait task designed to elicit freezing behaviour. Freezing is a symptom of Parkinson's disease during which paired agonist-antagonist lower limb muscles gastrocnemius (GC) and tibialis anterior (TA) undergo dysfunctional firing leading to a motor arrest. Given the impairments in striatal dopaminergic innervation in individuals with Parkinson's disease (Braak *et al.*, 2004), and the predominant GABAergic innervation of the glutamatergic pedunculopontine nucleus by the inhibitory internal segment of the globus pallidus, a plausible explanation for the transient inhibition of brainstem structures controlling gait during freezing is an overwhelming burst of inhibition from the basal ganglia (Lewis and Barker, 2009). Based on previous empirical and conceptual work, we thus hypothesized that freezing should occur secondary to increased MUA in the subthalamic nucleus (STN), driving the globus pallidus internus (GPi) to inhibit the pedunculopontine nucleus (PPN), and impairing the ability of the central pattern generators in the brainstem to coordinate motor activity in the spinal cord. CPG = central pattern generator; SC = spinal cord.

EMG data (Fig. 1) (Shine *et al.*, 2013*d*). Software configuration restricted forward progression and corresponding on-screen movement to only successful left-right alternating sequences where the pedal was depressed beyond a threshold position of 30° from the rest position. Consecutive pedal depressions by the same foot (e.g. left-left or right-right) did not produce forward progression; however, all foot pedal responses were recorded for further analysis. Although the virtual reality paradigm does not recreate all features of gait (such as balance and whole leg and trunk movement), it has previously been validated against actual gait metrics derived in a clinical setting (Matar *et al.*, 2013; Shine *et al.*, 2013*d*; Georgiades *et al.*, 2016; Ehgoetz Martens *et al.*, 2018).

The virtual reality task included environmentally salient features such as narrow doorways as well as written walk and stop cues of variable cognitive load that appeared in the bottom third of the screen (Supplementary Fig. 1B). In the low cognitive load condition, subjects responded to simple 'WALK' and 'STOP' cues that appeared on-screen in the colours green and red, respectively. In addition, participants were also trained to respond to high cognitive load cues involving congruent (e.g. 'BLUE' written in blue) and incongruent (e.g. 'BLUE' written in the colour green or red) colour-word pairs (Matar *et al.*, 2013; Shine *et al.*, 2013*d*). Subjects were instructed to keep walking for congruent colour-word cues. The task consisted of blocks of variable cognitive load with

complex cues presented in a pseudo-randomized fashion and counterbalanced with the presentation of simple cues throughout. After stopping, a 'WALK' cue would appear again within a variable period of 3–5 s, which did not allow anticipation by the subjects. Overall, the task took ~2 min to complete with each patient completing a minimum of 100 steps. All Parkinson's disease patients were tested in the practically defined OFF medication state, having withdrawn from their dopaminergic treatment overnight (>12 h since last dose).

The timing of participants' footsteps during the task was recorded as the onset of each sequential pedal depression. To derive a measure of typical latency between sequential virtual reality footsteps for each subject, the latency between each sequential pedal depression was collected and placed into 100 ms bins. From this output, the modal footstep latency for the duration of the protocol was calculated after removing all cognitive cues, freezing episodes and three steps immediately after a 'WALK' cue that would otherwise skew the variable (Shine *et al.*, 2013*d*).

Baseline virtual reality walking criteria

Periods of baseline walking in the virtual reality environment were carefully selected as blocks of at least eight consecutive footsteps that met the following criteria: (i) contained no motor arrests; (ii) contained no amplitude reduction; (iii) contained no out of sequence footsteps e.g. left-left; (iv) contained no cue presentations; and (v) occurring at least three steps after presentation of any cues. This yielded 49 individual epochs of baseline virtual reality walking. Windows taken for analyses were 2 s in duration centred on the midpoint of each segment so as to be as free as possible from potential contaminants.

Identification of virtual reality freezing

In keeping with established and validated definitions of virtual reality motor arrests previously shown to correlate with gait freezing severity experienced during overground walking (Matar *et al.*, 2013; Shine *et al.*, 2013*d*; Georgiades *et al.*, 2016), a freeze was defined as any footstep latency greater than twice the duration of a subject's virtual reality modal footstep latency. Periods of virtual reality motor arrests identified by this algorithm were scrutinized to increase the specificity of our temporal analyses, and the onset of each motor arrest was tagged at the precise moment of foot pedal velocity cessation (dA/dt $<$ 0.05 m/s). This yielded 19 individual epochs of virtual reality-elicited motor arrests. Data for these 19 freezing windows were extracted from 1 s prior to motor arrest onset to 1 s post motor arrest onset. The endpoints of freeze windows were tagged as the precise moment foot pedal movement resumed (velocity dA/dt $>$ 0.05 m/s).

Stopping

The derivation of virtual reality volitional stopping periods was made by identifying instances of successful motor inhibition following the presentation of either a simple 'STOP' cue (displayed in red) or complex incongruent colour-word stop cue. Events of unsuccessful stopping where subjects continued to make any virtual reality footsteps following stop cue presentation were excluded from the analyses. The endpoint of stopping windows was taken as the time point of subsequent 'WALK' cue presentation padded by 500 ms to avoid contamination of the signal. Fifteen such windows of successful responses to stop cues were extracted.

Surgical procedure and electrophysiological recording

A LeadPoint amplifier (1000 MΩ headstage impedance) and microTargeting^{TM} electrodes (FHC Inc., 25 kHz) were used to obtain extracellular microelectrode recordings of pooled cell body action potentials from multiple subthalamic nucleus neurons in the vicinity of our electrode (MUA) during electrode implantation in the subthalamic nucleus as part of routine DBS surgery. Data were collected from the subthalamic nucleus corresponding to the most affected side of each individual according to preoperative clinical assessments. Target location was determined from preoperative T_2-weighted MRI images co-registered to Brainlab navigation planning software, which was used for the trajectory planning. Intraoperatively, the desired recording site was identified based on its distance from the stereotactic coordinates of the target location along the implantation trajectory, and confirmed with assessment of intraoperative recordings by a neurologist (N.M.). During the surgery and intraoperative virtual reality gait task performance, EMG data from the gastrocnemius and tibialis anterior muscles in the lower limb contralateral to the side of subthalamic nucleus recording were simultaneously collected. Direct current component removal was achieved with an adaptive 50 Hz line filter in the LeadPoint amplifier. Recordings were bandpass filtered (subthalamic nucleus 200–5000 Hz, EMG 0.2–2000 Hz) and digitized at 25 kHz.

Electrophysiological data analyses

All data were then processed offline using custom routines in MATLAB R2017a (MathWorks, MA, USA). Individual subthalamic nucleus microelectrode recordings were high-pass filtered for MUA (150 Hz high-pass second order Butterworth filter passed forwards and backwards). To obtain a time series of increased (relative to baseline) MUA firing pooled from various subthalamic nucleus neurons within the vicinity of the microelectrode, a threshold value of two standard deviations above the mean signal value was set.

Multi-unit activity firing rate

The between-spike latency of consecutive supra-threshold spikes was computed. A time series of subthalamic nucleus MUA firing rate was derived by computing the inverse of the latency between supra-threshold data points and multiplying by the sample rate (25 kHz). To reduce noise in the data and aid permutation statistics, the signal was smoothed with a 1-ms sliding window. Individual smoothed signals were then standardized by scaling each data point to the respective signal range for each subject {[x – min(x)]/[max(x) – min(x)]} in order to permit grouped statistics. The standardized MUA firing rate timeseries was then aligned with 49 epochs of normal virtual

reality walking free of contaminants, 19 episodes of virtual reality-elicited motor arrests, and 15 successful responses to virtual reality stop cues as described above.

Time-frequency analysis: beta and theta modulation of multi-unit activity signal

Based on previous literature implicating abnormal oscillatory subthalamic nucleus dynamics in freezing (Toledo *et al.*, 2014; Syrkin-Nikolau *et al.*, 2017; Hell *et al.*, 2018), we isolated theta (3–8 Hz) and beta (13–30 Hz) modulation of the smoothed standardized MUA signal by computing the rectified bandpass filtered signal within the respective desired frequency ranges (second order Butterworth band-pass filter passed forwards and backwards). The envelope of beta and theta modulation was taken as the low pass filtered signal of these respective timeseries (second order Butterworth low-pass filter <4 Hz passed forwards and backwards).

Lower limb EMG

We calculated the freeze index from the lower limb gastrocnemius and tibialis anterior EMG signals contralateral to the side of subthalamic nucleus recording. The freeze index is a ratio between abnormal 'freeze band' 3–8 Hz EMG activity and 0.5–3 Hz 'locomotor band' EMG activity in the lower limbs (Moore *et al.*, 2008). Accordingly, the freeze index was calculated as the square of the area under the power spectra in the 'freeze' band (3–8 Hz components), divided by the square of the area under the spectra in the 'locomotor' band (0.5–3 Hz components). This index has previously been shown to increase with freezing during overground walking (Moore *et al.*, 2008). Freeze index was calculated separately for 2-s segments of data centred on freezing episodes and 2-s segments of data during normal baseline walking. Permutation statistical testing (described below) confirmed the freeze index was significantly higher during freezing than for baseline walking in the virtual reality task (walking = 2.7 ± 3.3; freeze = 10.0 ± 11.4; $P < 0.001$). This replicated previous findings (Moore *et al.*, 2008), and provided evidence of pathological lower limb muscle activation known to occur during freezing described as trembling in place (Supplementary Video 1) (Nieuwboer *et al.*, 2004). Thus, we used the 3–8 Hz activity as an *a priori* window to use Granger causality analysis (described below) to ask whether the beta and theta rhythms from the subthalamic nucleus temporally preceded emergent pathological oscillations in lower limb EMG patterns, and therefore suggest a plausible cascade of pathological firing associated with freezing behaviour.

Statistical analysis

Comparison of baseline virtual reality walking with freezing

We extracted 19 separate 2-s windows of processed subthalamic nucleus MUA data time-locked to the onset of each of the 19 virtual reality motor arrest episodes identified, as described above. We also extracted separate 2-s windows of baseline virtual reality walking data centred on the midpoint of each segment identified as described above, so as to be as free as possible from contaminants. Statistical significance was tested with a robust non-parametric permutation approach popularized by functional neuroimaging experiments (Nichols and Holmes, 2001). This approach compares the between-trial difference in means to a randomized dataset through 5000 permutations with a significance level of $P = 0.05$ testing the proportion of null permutations in which the randomized dataset had a greater between-trial mean than that of the experimental data (Supplementary Fig. 2). To interrogate the transient nature of these MUA changes with freezing the same permutation test was used to compare the MUA data within the freezing window and a 1-s window of data before freeze onset and a 1-s window extracted from the endpoint of the freeze interval.

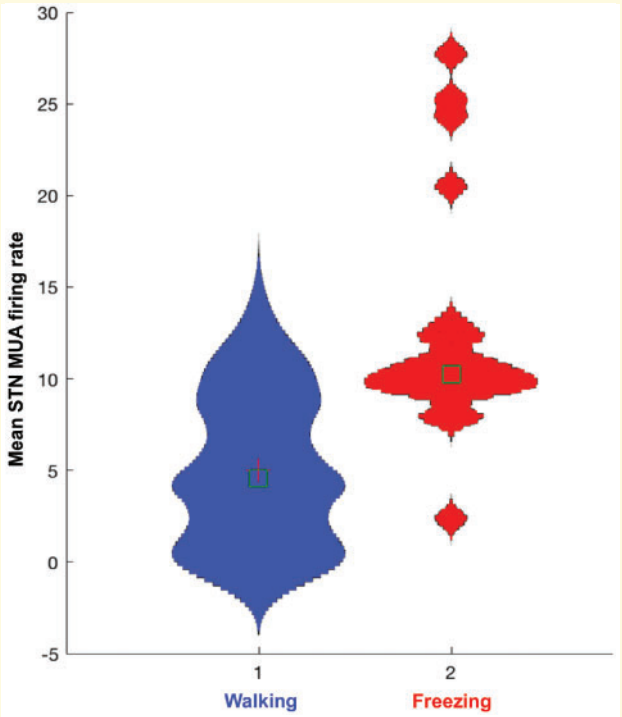

Figure 2 Comparison of subthalamic nucleus MUA firing rate. Virtual reality-elicited freezing episodes (red) were associated with a transient increase in subthalamic nucleus MUA firing rate in comparison to epochs of walking (blue); $P = 2.0 \times 10^{-5}$. STN = subthalamic nucleus.

Temporal dynamics of subthalamic nucleus multi-unit activity activity with freezing

We plotted the envelope of beta and theta modulation of the subthalamic nucleus MUA signal averaged across the standardized 2-s windows centred on each of the 19 freezing episodes (Fig. 3A). Statistically significant values of beta and theta modulation were determined as those greater than the 99th percentile from normal walking segments and plotted in the lower portion of the figure.

Granger causality analysis

We assessed Granger causality between the beta and theta modulation in the subthalamic nucleus MUA spiking signal

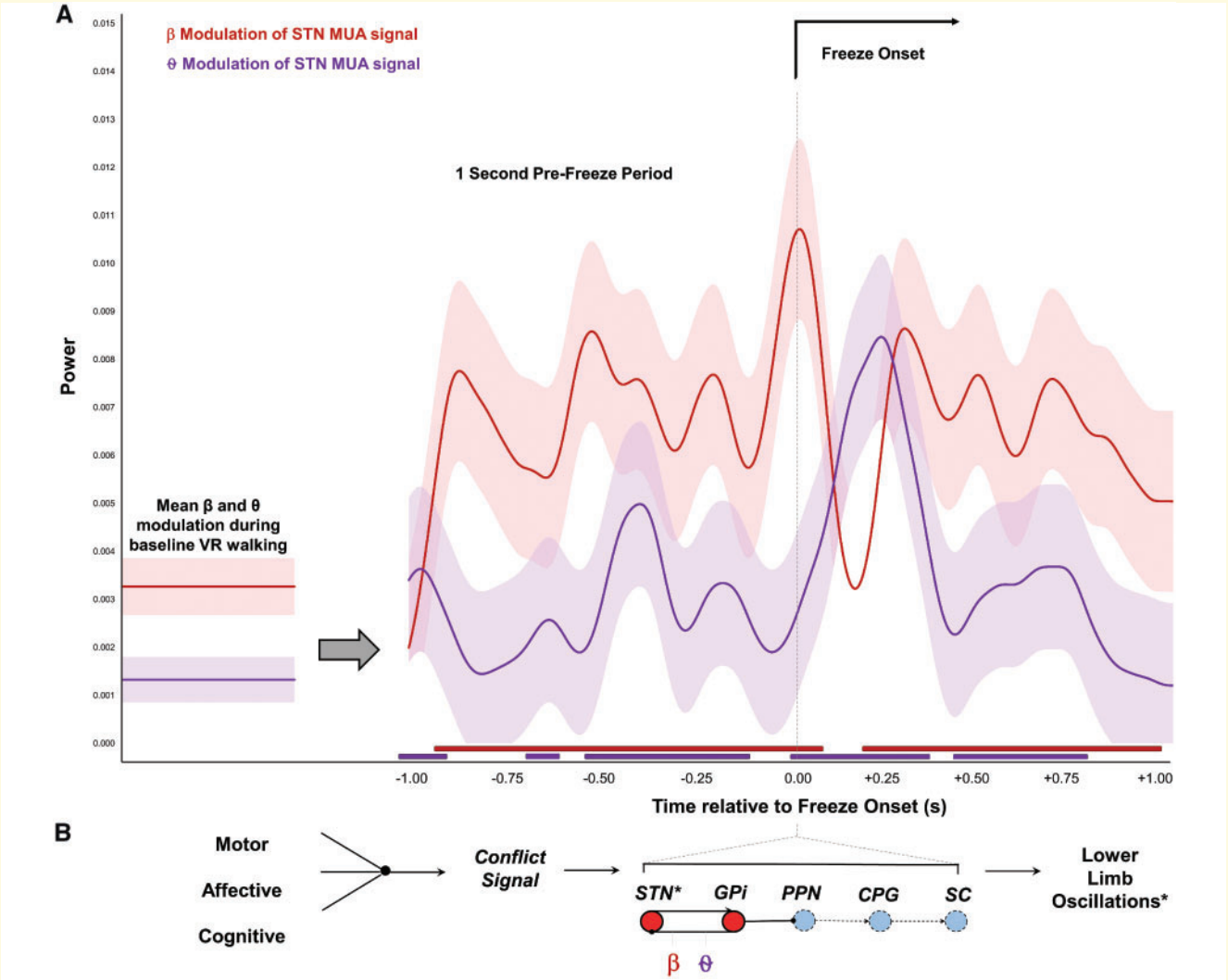

Figure 3 Oscillatory dynamics of subthalamic nucleus MUA with virtual reality-elicited freeze onset. (**A**) Observed beta activity (red) was prominent throughout motor arrests and peaked precisely at the moment of motor arrest onset. Theta activity (purple) was also increased during motor arrests but peaked shortly after (~200 ms) motor arrest onset. Red and purple bars below depict statistically significant values >99th percentile from normal walking segments. Shaded error bars are one standard deviation (1 SD) away from the mean. Results confirmed using a phase randomization null. (**B**) Schematic demonstrating pathological interactions in the hierarchy of brain centres that control gait during freezing episodes. Aberrant conflict processing in higher centres triggers overwhelming inhibition mediated by the effect of the subthalamic nucleus (via the globus pallidus internus, GPi) on lower centres in the brainstem and spinal cord (SC) manifest as pathological firing of lower limb muscles. We observed significant unidirectional Granger causality between subthalamic nucleus beta and theta activity (longest delay = 100 ms; $P < 0.001$), which in turn was unidirectionally linked with the 3–8 Hz trembling in place EMG activity that drives increased freeze index in the lower limb muscles that flex the ankle (longest delay = 100 ms; $P < 0.001$). Together, this suggests a temporal sequence of abnormal beta activity, then theta activity culminating in pathological antagonistic firing of lower limb muscles during freezing. *Measured activity; CPG = central pattern generator; PPN = pedunculopontine nucleus; STN = subthalamic nucleus; VR = virtual reality.

and the 3–8 Hz freeze band oscillations extracted from the contralateral gastrocnemius/tibialis anterior EMG signal at multiple lags (between 10–500 ms) using Granger's F-test at a significance level of $P = 0.05$ (Seth *et al.*, 2015).

Comparison of freezing with volitional stopping

First, non-parametric permutation testing was used to demonstrate higher mean subthalamic nucleus MUA firing rate associated with the 19 freezing windows compared to the 15 volitional stopping windows (Freeze = 12.6 ± 6.8; Stop = 7.6 ± 5.2; $P = 0.006$). Next, we compared the temporal dynamics of beta modulation of the subthalamic nucleus MUA signal in 2-s windows for freezing and volitional stopping. Again, extracted windows of data were centred on the precise moment of foot pedal cessation (dA/dt < 0.05 m/s) both in the spontaneous motor arrest and volitional stop (following stop cue presentation) conditions. The 99th percentile of beta modulation power in the 49 baseline walking segments was taken as a reference for comparison of the two conditions, plotted as the solid horizontal line in Fig. 4.

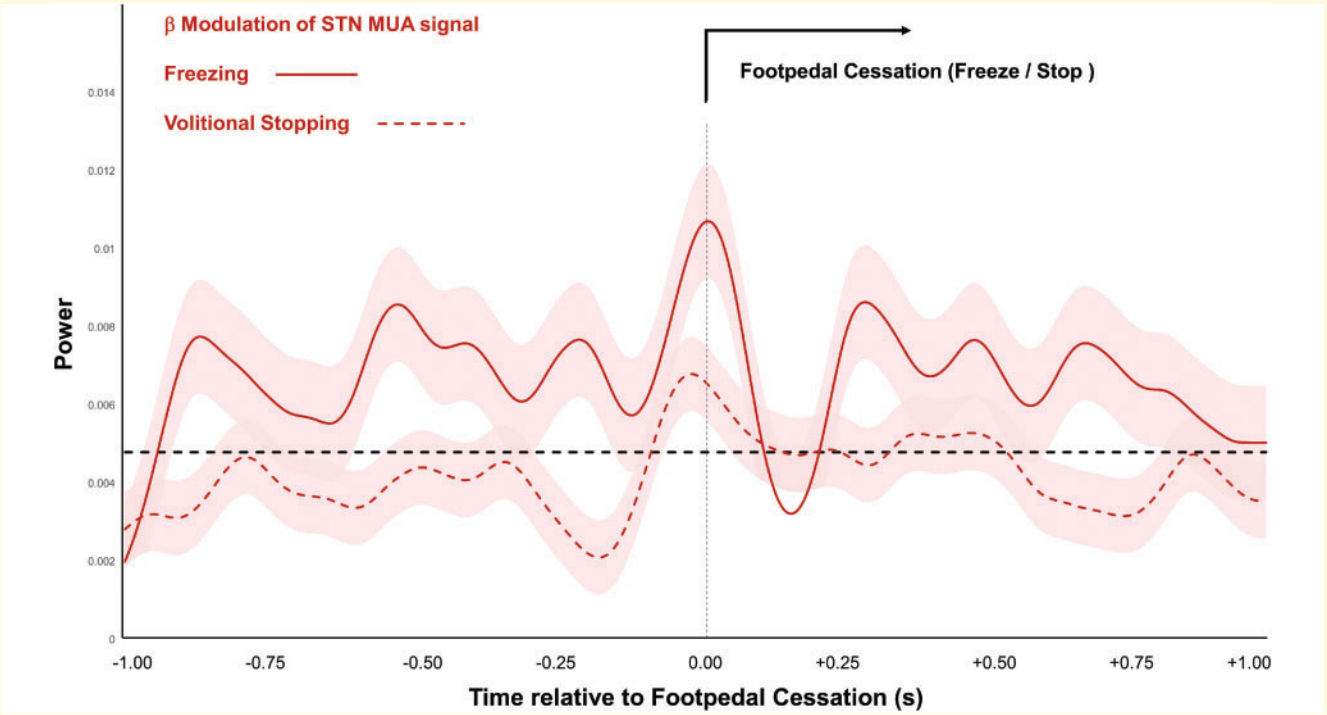

Figure 4 Beta modulation of subthalamic nucleus MUA signal in freezing versus volitional stopping. Comparison of beta modulation signature during temporal dynamics of virtual reality elicited paroxysmal motor arrests (solid) and volitional stopping (dashed) in response to stop cues. The vertical dotted line represents the precise moment of foot pedal cessation (dA/dt $<$ 0.05 m/s) in freeze onset and volitional stopping. The dotted horizontal line represents the 99th percentile of beta modulation in the subthalamic nucleus MUA signal of normal walking. Beta modulation during volitional stopping did not exceed this threshold until execution of stopping whereas in freezing, beta power is already beyond this threshold in the moments preceding a freezing event and peaks much higher with freeze onset than in volitional stopping. STN = subthalamic nucleus.

Data availability

The data that support the findings of this study and custom code used for analyses are available from the corresponding author upon reasonable request. The data have not been made publicly available as they contain information that could compromise the privacy of research participants.

Results

Our findings support our initial prediction (Shine *et al.*, 2013*c*), namely that motor arrests were associated with a marked elevation in mean subthalamic nucleus firing rate (Freeze = 12.6 ± 6.8; Walk = 6.5 ± 3.5; $P = 2.0 \times 10^{-5}$) (Fig. 2 and Supplementary Fig. 2). In addition, subthalamic nucleus activity was observed to increase transiently within the motor arrest period compared to equally-sized 1-s epochs prior to (9.4 ± 2.8; $P = 0.039$) and following (9.4 ± 2.7; $P = 0.041$) the episode, providing evidence for a paroxysmal increase in subthalamic nucleus spiking activity during freezing.

To refine the mechanistic role of subthalamic nucleus activity in the pathogenesis of gait freezing further, we performed time-frequency analysis to assess the dynamics of subthalamic nucleus theta (3–8 Hz) (Shine *et al.*, 2014; Zavala *et al.*, 2017) and beta (13–30 Hz) (Shine *et al.*, 2013*c*; Little and Brown, 2014; Zavala *et al.*, 2017) oscillatory activity relative to the motor arrests elicited by the task. Extraction and analysis of data from a 2-s window centred on motor arrest onset (i.e. the precise moment of foot pedal cessation, accurate to 1 ms) revealed a clear pattern (Fig. 3A): beta frequency modulation of the MUA was prominent throughout the 2-s period around the arrest and peaked precisely at the moment of arrest onset. Permutation testing demonstrated a statistically significant increase of peak subthalamic nucleus MUA beta modulation at freeze onset (0.010 ± 0.002) relative to mean beta values during walking segments (0.004 ± 0.001), $P = 0.0018$. In contrast, although theta activity was elevated both prior to and during the arrest, the peak in theta oscillatory activity occurred shortly after arrest onset (~200 ms). These results were substantiated using a phase randomization null. These frequency bands have long been implicated in the pathophysiology of Parkinson's disease (Frank, 2005; Wilson, 2013; Little and Brown, 2014; Shine *et al.*, 2014; Zavala *et al.*, 2017; Deffains *et al.*, 2018), and may indeed reflect emergent pathological synchronous oscillations in specific channels of the basal ganglia circuitry (Müller and Robinson, 2018).

In previous work (Shine *et al.*, 2013*c*), we also hypothesized that emergent oscillatory basal ganglia dynamics could drive the pedunculopontine nucleus at theta frequencies, providing a mechanistic explanation for the well-known phenomenon of 'trembling in place', in which the lower limbs oscillate abnormally during freezing (Supplementary Video 1) (Nieuwboer *et al.*, 2004). We replicated an increase in the freeze index (Moore *et al.*, 2008) in EMG recordings from gastrocnemius and tibialis anterior contralateral to the recording subthalamic nucleus during virtual reality elicited freezing episodes (walking = 2.7 ± 3.3; freeze = 10.0 ± 11.4; $P < 0.001$). Thus, we used the 'freeze band' 3–8 Hz EMG activity as an *a priori* window to use Granger causality analysis (Seth *et al.*, 2015) to ask whether emergent beta and theta rhythms from the subthalamic nucleus were temporally predictive of the pathological 'freeze band' (3–8 Hz) EMG oscillations recorded in the lower limb muscles. Our analysis demonstrated that subthalamic nucleus beta band activity was unidirectionally and selectively linked with subthalamic nucleus theta activity (longest delay = 100 ms; $P < 0.001$), which in turn was unidirectionally and selectively linked with the 3–8 Hz trembling in place EMG activity driving increased freeze index in the lower limb muscles that flex the ankle (longest delay = 100 ms; $P < 0.001$), thus completing a circuit mechanism potentially accounting for the manifestation of freezing of gait in Parkinson's disease (Fig. 3B). Subthalamic nucleus alpha and gamma frequencies did not yield the same temporal association (granger causality analysis not statistically significant)

Clinically, these findings raise the key question: What could stop this unwanted 'neural chain reaction'? Adaptive and closed-loop DBS protocols hold promise (Parastarfeizabadi and Kouzani, 2017; Deffains *et al.*, 2018). By acting like a neuronal defibrillator, adaptive DBS could limit stimulation to crucial epochs by sending a pulse of high-frequency activity to 'short-circuit' a freezing episode before pathological subthalamic nucleus dynamics can manifest as freezing. To this end, we contrasted the freezing MUA signature against that of purposeful stopping. We found that freezing episodes were associated with greater subthalamic nucleus MUA than volitional stopping in the virtual reality task (Freeze = 12.6 ± 6.8; Stop = 7.6 ± 5.2; $P = 0.006$). The same was found for subthalamic nucleus beta activity: during the 1-s period before motor arrest onset, beta modulation of the MUA signal was above the 99th percentile of that in walking, whereas in volitional stopping the power of beta modulation only exceeded this threshold at the moment of motor output cessation (Fig. 4).

Discussion

We provide robust evidence that abnormal subthalamic nucleus activity is associated with lower limb freezing and temporally precedes abnormal lower limb muscle activation characteristic of freezing of gait in Parkinson's disease. In 2013, a model of freezing behaviour was hypothesized implicating cortico-subthalamic decoupling and increases in subthalamic nucleus output activity, leading to an overwhelming inhibition (via the globus pallidus internus) of gait-controlling nuclei within the thalamus and brainstem (Shine *et al.*, 2013). Evidence of breakdown of cortico-subthalamic nucleus coupling in association with gait freezing in Parkinson's disease is emerging (Pozzi *et al.*, 2019), highlighting the role of deranged neural network dynamics in the pathogenesis of gait freezing; however, the identification of abnormal activity in the subthalamic nucleus with the onset of gait freezing in real time remained a challenge. In the present study, we demonstrate evidence of increases in subthalamic nucleus firing rate and pathological activity with the onset and evolution of lower limb freezing behaviour in humans with Parkinson's disease. Our approach represents a major advance over previous work interrogating the pathophysiology of freezing using the local field potential signal, which is generated by the pooled membrane currents of synaptic inputs (Pesaran *et al.*, 2018), and has yielded findings that are difficult to reconcile (Toledo *et al.*, 2014; Syrkin-Nikolau *et al.*, 2017; Hell *et al.*, 2018; Pozzi *et al.*, 2019). While studies examining local field potentials can interrogate impaired cortico-basal ganglia connections, the examination of subthalamic nucleus neuronal population output activity represented by the MUA signal is a superior means for directly testing the hypothesis that pathological increases in subthalamic nucleus output activity mediate freezing behaviour in Parkinson's disease (Burns *et al.*, 2010), providing more useful and interpretable insights into the mechanisms underlying freezing.

We demonstrate subthalamic nucleus MUA patterns in beta and theta frequency bands that characterize the onset of freezing events elicited during virtual reality gait task performance. To our knowledge, this is the first study to analyse subthalamic nucleus microelectrode recordings of MUA associated with motor arrests in real time in individuals with Parkinson's disease. Furthermore, we show that during freezing, emergent beta frequency activity precedes subthalamic nucleus theta activity, which in turn precedes oscillatory trembling in place within the lower limbs. This suggests a potential causal link between abnormal basal ganglia rhythmicity and abnormal lower limb dynamics. However, to appropriately investigate causality in this circuit, one would need to devise experiments in which the subthalamic nucleus was stimulated (or inhibited) and then freezing was evidenced in the lower limbs, likely requiring the involvement of an invasive animal model. Although the virtual reality paradigm fails to recreate all components of overground gait, it has been validated against gait metrics (Shine *et al.*, 2013*d*). There is correlation between behavioural aspects of overground freezing and virtual reality-elicited freezing including cognitive load effects (Matar *et al.*, 2013; Georgiades *et al.*, 2016), start hesitation freezing (Georgiades *et al.*, 2016), and

heterogeneity among freezers (Ehgoetz Martens *et al.*, 2018). Furthermore, by recording lower limb EMG during virtual reality-elicited freezing, we have reproduced an established metric of lower limb electrophysiology observed during overground freezing (freeze index) (Moore *et al.*, 2008). This suggests that pathological subthalamic nucleus electrophysiology associated with virtual reality-elicited freezing is not unjustifiably dissimilar from that associated with overground freezing. Together, our results refine the biological mechanism of freezing and provide a positive step towards the identification of clinically useful biomarkers for freezing events. Investigation of narrow band frequencies may assist in the identification of clinically useful biomarkers. Although subthalamic nucleus activity has not yet been examined during freezing behaviour before this study, a movement-induced power increase at 18 Hz upon movement initiation has been demonstrated in Parkinson's disease patients with freezing that was not prominent in non-freezers (Storzer *et al.*, 2017). Patients with dopamine-responsive freezing have also been shown to have increased high beta activity that reduced with dopamine administration, compared to non-freezers (Toledo *et al.*, 2014). Although difficult to reconcile, these findings suggest that frequencies within the beta range may associate with gait freezing more selectively than others. To this end, we analysed subthalamic nucleus MUA signals filtered separately for low beta (16–20 Hz) and high beta (25–35 Hz) modulation. Our data suggest that frequencies in the higher range of the beta band were more dramatically increased in amplitude and duration in association with freezing onset compared to low beta (Supplementary Fig. 3).

We also provide preliminary evidence that the signature of beta modulation during freezing may be discernible from that of volitional stopping in a manner that is of potentially considerable therapeutic benefit. In addition to the greater magnitude of beta modulation of the subthalamic nucleus MUA signal co-incident with freeze onset relative to volitional stopping, there was also a sustained increase of beta modulation preceding virtual reality motor arrest onset beyond the 99th percentile of beta modulation in normal walking (Figs 3 and 4). Given that the corresponding pattern of beta modulation associated with volitional stopping did not exceed this threshold in the same manner (Fig. 4), we suggest that this could represent a possible biomarker target for closed loop DBS systems. This would be suitable practically because these changes precede freeze onset and could therefore be detected before freeze onset. A data-driven approach in a larger cohort should define a threshold for magnitude and duration of pre-freeze beta modulation that may realize this potential. Although our data alone do not indicate that adaptive DBS systems could detect freezing with high specificity and sensitivity, we can speculate that it may be possible for adaptive DBS systems to detect and potentially prevent gait freezing without having to engage during purposeful stopping. Case studies in epilepsy suggest that the prediction of transient neural events is non-trivially difficult and offers numerous obstacles that need to be overcome including real time constant recording, transmission and processing of large amounts of MUA data, battery considerations and accuracy without providing undesirable effects (Ramgopal *et al.*, 2014). However, we predict that tracking data from multiple sources, such as lower limbs EMG (Yungher *et al.*, 2014), and scalp EEG (Handojoseno *et al.*, 2014), will ultimately confer clinical benefit to individuals with freezing of gait and DBS. Changes in cortico-subthalamic coupling have also recently been demonstrated in the moments prior to freeze onset, and not voluntary stopping (Pozzi *et al.*, 2019), evidencing further that pathological firing patterns preceding freezing could potentially be targeted by DBS of the subthalamic nucleus.

The challenge is thus to determine the sensitivity and specificity of the spiking activity in the subthalamic nucleus for freezing episodes (in combination with other modalities), and how this related activity could be modulated in a viable therapeutic option. While this is exciting, another practical concern is that current DBS macroelectrode systems are capable of recording local field potential activity. Although difficult, recent work suggests it may be possible using computational modelling approaches to characterize the coherence between certain features of MUA signals and local field potential signals further, which could overcome this challenge (Burns *et al.*, 2010; Müller and Robinson, 2018). Furthermore, the development of novel DBS devices may enable the recording of additional signal types and expand therapeutic targets (Roy *et al.*, 2018; Pozzi *et al.*, 2019). To this end, our results would also need to be confirmed in a larger cohort with a larger sample of freezing events.

Unfortunately, gait freezing remains at best only partially amenable to currently available therapies and knowledge of the causative neurobiology remains limited. We identified alterations in subthalamic nucleus activity during gait freezing elicited by an intraoperative virtual reality gait task during DBS surgery. By further characterizing the temporal dynamics of emergent beta and theta subthalamic nucleus activity and its contributory role in the evolution of pathological lower limb motor activity during freezing, we have refined our understanding of the neurobiological mechanism of freezing and highlighted the role of the subthalamic nucleus in driving pathological motor arrests in individuals with Parkinson's disease. This work offers a step towards the identification of clinically useful biomarkers for novel therapeutic interventions such as closed loop adaptive DBS protocols that will ultimately confer more effective relief of this devastating symptom of Parkinson's disease.

Acknowledgements

We would like to thank Professor Charles Wilson and A/Prof Brett Foster for their invaluable input. We also thank

the clinical and surgical staff of the Movement Disorders Team at Westmead Private Hospital. We thank Jason Hatvany for his technical expertise in the operating theatre. We would also like to thank Mark Pearson for his ongoing technical input. And most importantly, we would like to thank our patients for generously volunteering to be part of this study.

Funding

ForeFront is a collaborative research group at the Brain and Mind Centre, University of Sydney. We thank the support from NHMRC program (#1132524), Dementia Research Team (#1095127) and NeuroSleep Centre of Research Excellence (#1060992) grants. M.J.G is supported by the RA Money Postgraduate Research Scholarship in Neuroscience, Sydney Medical School at the University of Sydney; J.M.S. is supported by a National Medical Health and Research Council Fellowship; M.G. was funded by a University of Sydney International Scholarship and is currently funded by a Postdoctoral Mandate of Internal Funds KU Leuven; S.J.G.L. was supported by an NHMRC Practitioner Fellowship #1003007 and is currently supported by an NHMRC-ARC Dementia Fellowship #1110414. The funding sources had no role in study design, data collection, data analysis, data interpretation, or the writing of the report.

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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