

A block to pre-prepared movement in gait freezing, relieved by pedunculopontine nucleus stimulation

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Gait freezing and postural instability are disabling features of Parkinsonian disorders, treatable with pedunculopontine nucleus stimulation. Both features are considered deficits of proximal and axial musculature, innervated predominantly by reticulospinal pathways and tend to manifest when gait and posture require adjustment. Adjustments to gait and posture are amenable to pre-preparation and rapid triggered release. Experimentally, such accelerated release can be elicited by loud auditory stimuli—a phenomenon known as 'StartReact'. We observed StartReact in healthy and Parkinsonian controls. However, StartReact was absent in Parkinsonian patients with severe gait freezing and postural instability. Pedunculopontine nucleus stimulation restored StartReact proximally and proximal reaction times to loud stimuli correlated with gait and postural disturbance. These findings suggest a relative block to triggered, pre-prepared movement in gait freezing and postural instability, relieved by pedunculopontine nucleus stimulation.

Keywords: deep brain stimulation; gait freezing; Parkinson's disease; pedunculopontine nucleus; StartReact

Abbreviations: LAS = loud auditory stimuli; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Freezing of gait and postural instability are the major causes of falls in Parkinsonian disorders, including Parkinson's disease (Factor, 2008; Kerr *et al.*, 2010) and are often poorly responsive to dopaminergic medication (Bloem *et al.*, 2004). Informed by experimental studies in animal models (Nandi *et al.*, 2002; Jenkinson *et al.*, 2004, 2006), deep brain stimulation of the

pedunculopontine nucleus has emerged as a novel therapy for freezing of gait/postural instability (Mazzone *et al.*, 2005; Plaha and Gill, 2005; Ferraye *et al.*, 2009; Moro *et al.*, 2010).

The pathophysiology of freezing of gait and postural instability is poorly understood—but their frequent coexistence raises the possibility of shared mechanisms (Giladi *et al.*, 2001; Karachi *et al.*, 2010). Both conditions are considered deficits of axial and proximal musculature (Jankovic, 2008). In postural instability,

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postural reflexes that adjust for body sway and environmental perturbations are diminished (Bloem, 1992). Similarly, freezing of gait typically occurs when adjustments are required to the locomotor rhythm—for example, with gait initiation, turning, overcoming reduced stride length and negotiating tight spaces and obstacles (Okuma, 2006; Chee *et al.*, 2009; Almeida and Lebold, 2010).

Adjustments to posture and gait can be considerably accelerated (or even triggered involuntarily) by loud auditory stimuli of the type that may also elicit a startle reflex (MacKinnon *et al.*, 2007; Reynolds and Day, 2007; Queralt *et al.*, 2008). The speeding of responses when such a stimulus is delivered with the imperative cue is known as the 'StartReact' phenomenon (Valls-Sole *et al.*, 1995, 1999). StartReact occurs when the relevant motor response can be prepared in advance, as seen experimentally in simple reaction time tasks (Valls-Sole *et al.*, 1999; Carlsen *et al.*, 2008). The assumption is that some motor programmes can be stored in a pre-prepared state and are subject to triggered reflex-like release, such as by loud auditory stimuli. The short latencies of StartReact responses have been interpreted to reflect direct subcortical release (Carlsen *et al.*, 2004a). The triggering by loud auditory stimuli has further prompted speculation that StartReact responses may utilize the same efferent pathway as the startle reflex—the reticulospinal tract (Valls-Sole *et al.*, 1995, 2008). The reticulospinal tract appears to predominantly innervate proximal and axial musculature, as supported by early lesioning studies in primates (Lawrence and Kuypers, 1968a, b). Accordingly, StartReact has been shown to be preferentially expressed in proximal compared to distal muscles (Carlsen *et al.*, 2009).

Here, we explore the hypothesis that in freezing of gait/postural instability there is impairment of the system supporting the reflexic release of pre-prepared motor programmes. We therefore predicted that Parkinsonian patients with freezing of gait/postural instability will have a deficit in StartReact in proximal muscles, and, importantly, that this deficit would be reversed by pedunculopontine nucleus stimulation.

Subjects and methods

Subjects and clinical assessments

Three subject groups were assessed: (i) eight patients with Parkinson's disease complicated by severe freezing of gait/postural instability, chronically implanted with bilateral pedunculopontine nucleus stimulators (Parkinson's disease freezing of gait/postural instability group); (ii) eight patients with Parkinson's disease of akinetic/rigid subtype without significant freezing of gait/postural instability (Parkinson's disease no freezing of gait/postural instability group); and (iii) 10 age-matched healthy controls. Patients with Parkinson's disease were matched for age, disease duration, motor severity and cognitive status. Subjects were recruited from centres in Oxford, UK and Brisbane, Australia. Subjects with bilateral deafness were excluded. Local ethics committee approval was obtained from both centres and participants gave written informed consent.

Parkinsonian patients were clinically assessed with the motor subsection (Part III) of the Unified Parkinson's Disease Rating Scale (UPDRS, score/108). OFF medication assessments occurred after

overnight withdrawal (>12 h) of dopaminergic therapy. Patients with pedunculopontine nucleus stimulators were also assessed on and off stimulation with a minimum 1 h washout period. These clinical assessments at both centres (in two countries) were performed unblinded by the same neurologist specialized in movement disorders (W.T.). UPDRS was segmented into items 27–30 (score/16) assessing posture, gait and balance and residual items 1–26 (R-UPDRS, score/92) assessing bradykinesia, rigidity and tremor. Patients also prospectively completed the Gait and Falls Questionnaire (GFQ, score/64) which assesses Parkinsonian gait disturbance including freezing of gait, festination and falls (Giladi *et al.*, 2000). The Freezing of Gait Questionnaire (FOGQ, score/24) and Falls Questionnaire (FallsQ, score/4) are components of the Gait and Falls Questionnaire (Giladi *et al.*, 2000, 2009). For all motor scales, higher scores indicate worse function. Additionally, cognition was assessed with the Mini-Mental State Examination (MMSE, score/30), with lower scores indicating worse function.

The dominant symptomatic issue in the patients with Parkinson's disease with freezing of gait/postural instability was severe freezing of gait/postural instability persisting even 'ON medication', causing frequent falls. In Parkinson's disease, freezing of gait/postural instability becomes more common and tends to be less medication-responsive as the disease progresses (Giladi *et al.*, 2001; Bloem *et al.*, 2004). The overall prevalence of freezing of gait/postural instability in Parkinson's disease is ~50% (Macht *et al.*, 2007). However, severe 'ON medication' freezing of gait/postural instability as the predominant issue is unusual in Parkinson's disease and raises the question of atypical pathologies (Factor, 2008; Jankovic, 2008). In the absence of a definitive test in life, we stress that the diagnosis of Parkinson's disease is presumptive.

Patients with Parkinson's disease with freezing of gait/postural instability were receiving chronic bilateral pedunculopontine nucleus stimulation. One patient was also receiving subthalamic nucleus stimulation (switched off an hour prior to and during experiments). No other patient had received surgery to any other brain target. Pedunculopontine nucleus electrodes were model 3387 (Medtronic), configured with four active contacts, each 1.5 mm in diameter with 1.5 mm spacing between adjacent contacts. Surgical implantation of the pedunculopontine nucleus has been described previously (Pereira *et al.*, 2008). The lower pedunculopontine nucleus region was targeted, below the level of the inferior colliculus. Localization of stimulation sites (midpoint between active contacts for bipolar stimulation and cathodes for monopolar stimulation) is represented in Fig. 1. Contacts were identified on postoperative computerized tomography fused with preoperative magnetic resonance images and transformed onto Montreal Neurological Institute (MNI) space using the Functional Magnetic Resonance Imaging of the Brain (fMRIB) Software Library (Smith *et al.*, 2004). Coordinates were calculated in millimetres from midline (laterality), ventrodorsal distance (*d*) from floor of the fourth ventricle and rostrocaudal distance (*h*) from a pontomesencephalic line connecting the pontomesencephalic junction to the caudal end of the inferior colliculi, as described previously (Ferraye *et al.*, 2009). The relative location/extent of the pedunculopontine nucleus has been outlined, based on choline-acetyltransferase immunohistochemical (ChAT5) staining in the human (Mesulam *et al.*, 1989). Parameters employed for chronic therapeutic stimulation were as follows: frequency range 30 or 35 Hz, voltage range 2.5–4.3 V and pulse width 60 μ s.

Clinical details of the study participants are shown in Tables 1 and 2. Healthy controls, patients with Parkinson's disease with no freezing of gait/postural instability and patients with Parkinson's disease with freezing of gait/postural instability did not significantly differ with

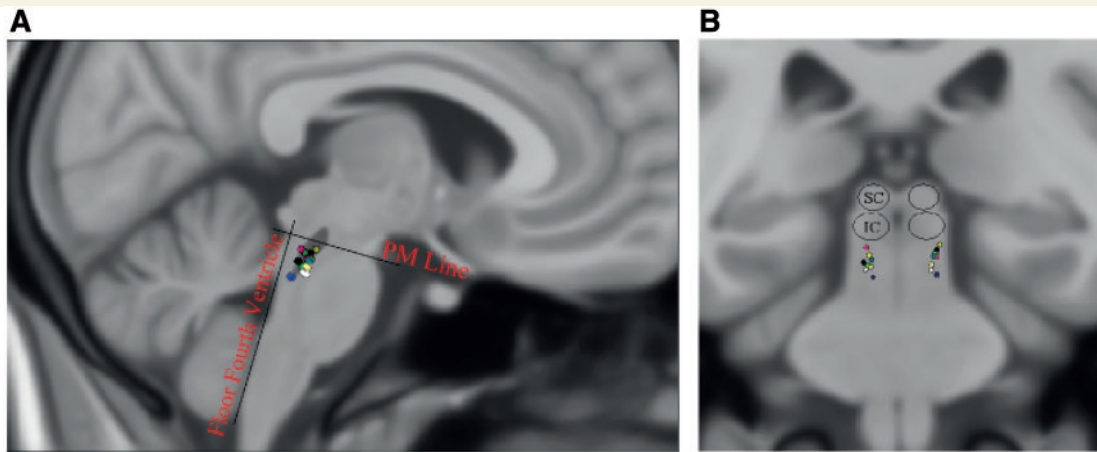


Figure 1 Localization of the sites of stimulation—represented in Montreal Neurological Institute (MNI) space on sagittal (A) and coronal (B) views. In (A), the relative location/extent of the pedunclopontine nucleus has been outlined in dark grey, based on choline-acetyltransferase immunohistochemical (ChAT5) staining in the human (see text). IC = inferior colliculus; PM = pontomesencephalic line connecting the pontomesencephalic junction to the caudal end of the inferior colliculi; SC = superior colliculus.

Table 1 Baseline characteristics

	Age (years)	Sex	PD Duration (years)	MMSE	R-UPDRS Off meds/ stim	IT27-30 Off meds/ stim	GFQ	FOGQ	FallsQ
Healthy controls	68.3 (7.6)	6M, 4F	\	\	\	\	\	\	\
PD NoFOG/PI	65.1 (7.6)	4M, 4F	11.5 (3.7)	29.2 (1.0)	28.1 (9.5)	3.3 (1.8)	4.0 (3.9)	2.0 (2.0)	0.4 (0.7)
PD FOG/PI	67.5 (8.8)	6M, 2F	11.3 (6.0)	29.4 (0.9)	26.8 (8.1)	8.4 (2.4) ^a	26.9 (11.6) ^a	11.8 (4.2) ^a	2.9 (0.8) ^a

Data are mean (SD).

^aDifferent from Parkinson's disease with no freezing of gait/postural instability $P \leq 0.001$.

Falls Q = Falls Questionnaire (score/4); FOG = freezing of gait; FOGQ = Freezing of Gait Questionnaire (score/24); GFQ = Gait and Falls Questionnaire (score/64); IT27-30 = items 27-30 Unified Parkinson's disease rating scale part III, assessing gait, posture and balance (score/16); MMSE = Mini-Mental State Examination (score/30); PD = Parkinson's disease; PI = postural instability; R-UPDRS = items 1-26 Unified Parkinson's disease rating scale part III (score/92).

For Parkinson's disease with freezing of gait/postural instability, scores are post-operative.

Table 2 Patients with Parkinson's disease with freezing of gait/postural instability

Patient	Age/Sex	PD duration (years)	Post-operative duration (years, months)	L-dopa dose equivalent (mg/day)	UPDRS III OFF/ON meds (off stim)	IT27-30 off/on stim (OFF meds)	GFQ pre-/post-operative	FOGQ pre-/post-operative	FallsQ pre-/post-operative	Supportive for UK brain bank criteria ^a
1	72F	10	2	950	38/22	11/8	48/26	22/13	4/2	D, A, T, P
2	72M	18	2,5	2500	25/17	6/6	30/16	14/11	4/2	D, A, T, P
3	76M	6	2	600	26/14	6/4	51/18	22/7	3/3	A, P
4	61F	10	2	800	40/23	10/9	61/36	24/16	4/3	D, A, P
5	77M	6	0,6	1400	31/17	10/10	31/14	^/6	^/2	A, P
6	71M	4	0,6	1550	27/18	5/5	^/21	^/9	^/3	P
7	55M	20	1	850	51/19	8/6	38/40	14/15	4/4	D, A, T, P
8	56M	16	2,10	1400	43/16	11/8	61/44	23/17	4/4	D, A, T, P

^aAdditional to disease duration and levodopa response as documented elsewhere in the table.

Post-operative clinical assessments were performed on the same day as reaction time assessment. Patients 7 and 8 were recruited from Oxford, UK, other patients from Brisbane, Australia. Patient 7 also had subthalamic nucleus stimulators that were turned off 1 h prior to and during experiments.

^ = not known; A = asymmetry persistent; D = dyskinesias; Falls Q = Falls Questionnaire (score/4); FOGQ = Freezing of Gait Questionnaire (score/24); GFQ = Gait and Falls Questionnaire (score/64); IT27-30 = items 27-30 Unified Parkinson's disease rating scale, assessing gait, posture and balance (score/16); MMSE = Mini-Mental State Examination (score/30); P = progressive disease course; PD = Parkinson's disease; T = tremor at rest; UPDRS III = part III (motor) Unified Parkinson's disease rating scale (score/108).

respect to age [$F(2,23) = 0.365$, $P = 0.698$]. Patients with Parkinson's disease with no freezing of gait/postural instability and patients with Parkinson's disease with freezing of gait/postural instability did not differ with respect to disease duration [$t(14) = 0.1$, $P = 0.92$], R-UPDRS subscore [$t(14) = 0.311$, $P = 0.761$] or Mini-Mental State Examination [$t(12) = -0.408$, $P = 0.690$]. Patients with Parkinson's disease with freezing of gait/postural instability had significantly higher scores in items 27/30 [$t(14) = -4.743$, $P < 0.001$], Gait and Falls Questionnaire [$t(14) = -5.281$, $P < 0.001$], Freezing of Gait Questionnaire [$t(14) = -5.967$, $P = 0.001$] and Falls Questionnaire [$t(14) = -6.325$, $P < 0.001$].

Experiments

Three tasks were administered:

- (i) Auditory blink and startle reflex task: patients were presented with 10 trials of loud auditory stimuli (LAS: 122 dB, 40 ms duration, 1000 Hz). Intertrial intervals were variable (10–15 s). Patients were advised that they would hear a series of sounds, some louder than others, and were requested to sit comfortably with eyes open, with no need to respond.
- (ii) Proximal simple reaction time task: a warned simple reaction time task. All stimuli were auditory, to eliminate the possibility of intersensory facilitation (Hershenson, 1962). Serial presentation of 35 trials, each consisting of a warning cue (92 dB, 40 ms duration, 300 Hz) followed by the imperative 'go' cue (40 ms duration, 1000 Hz). The imperative stimulus was either normal intensity (89 dB—normal trials) or loud (122 dB—LAS trials). The first five trials were 'practice' normal trials, followed by 20 normal and 10 LAS trials randomly intermixed. Warning periods (1–3.5 s) and intertrial (6–10 s) intervals were variable. Patients were instructed to react as quickly as possible with ballistic elbow flexion.
- (iii) Distal simple reaction time task: the same task as the proximal simple reaction time except patients responded with ballistic abduction of the forefinger. In this task, patients were seated with hand and forearm resting on a bench-top, flexed 90° at the elbow. The hand was positioned prone with digits adducted. Patients were instructed to react as quickly as possible with forefinger abduction then return to the resting hand position.

Stimuli were controlled through a digital to analogue converter (1401, Cambridge Electronic Design). Auditory tones were delivered binaurally through headphones (Audio Technica ATH-ES7). Sound pressure levels were assessed in a sound-proofed room with a modular precision sound analyser (Observer 2260, Bruel and Kjaer) via an artificial ear and headphone adaptor.

Bipolar surface EMG activity was recorded using 9 mm diameter silver–silver chloride electrodes. EMG electrodes were taped to skin overlying orbicularis oculi and sternocleidomastoid contralateral to the limb to be moved in reaction time assessments. Reaction times were assessed with both EMG and a triaxial accelerometer. For the proximal simple reaction time task, EMG was applied to biceps and accelerometer taped to the radial styloid. For the distal simple reaction time task, EMG was applied over the first dorsal interosseous and accelerometer taped to the tip of index finger. In one healthy subject, distal EMG reaction time was not assessable due to misplaced EMG electrodes. Data were sampled (or downsampled to) 256 Hz (Porti amplifier, TMSI). EMG recordings were amplified and low pass filtered at 500 Hz. Accelerometer (TMSI) was band pass filtered between 2 and 60 Hz.

Tasks were administered with subjects seated comfortably in a quiet, dimly lit room. The auditory blink and startle reflex task was always administered first, to minimize habituation effects on this task. The order of proximal and distal simple reaction time tasks was counterbalanced.

In patients with Parkinson's disease, experiments were conducted after overnight withdrawal of dopaminergic medication to limit variance from fluctuating dopaminergic state. For patients with Parkinson's disease with freezing of gait/postural instability, there were two conditions – on and off therapeutic bilateral pedunculopontine nucleus stimulation. Ordering of conditions was counterbalanced, with a minimum 1 h washout period between conditions. Subjects were blinded to condition and to experimental hypotheses.

Parameters and data analysis

Two reaction time parameters were assessed; accelerometer reaction time and EMG reaction time. Accelerometer analysis was automated by a script developed for Matlab (The Mathworks Inc). Priority is therefore given to this dataset. Accelerometer reaction times were computed for every trial before averaging to yield the task accelerometer reaction time. EMG reaction times for individual trials could not be reliably determined due to a poorer signal to noise ratio, partly due to resting EMG activity due to rigidity. For each task, EMG reaction time was therefore assessed from averages of the trials for a given condition in a given patient in Spike 2 (Cambridge Electronic Design). Note, however, that the onset of EMG activity in such averages tends to be dominated by trials with the shortest response times.

The first five trials in each simple reaction time task (always normal trials) were discarded as practice. Anticipatory responses (EMG response prior to the imperative cue) were discarded. The automated accelerometer analysis involved initial DC removal (time constant individualized for each trial from the average DC level 0.45 s prior to the imperative) before rectification. Accelerometer response onset was defined as an amplitude rise exceeding the mean of the prestimulus (0.5 s) baseline by 3 standard deviations (SDs). The EMG reaction time signal was first subject to DC removal using a fixed averaging interval of 0.002 s (the latter is defined as the time constant of the procedure in Spike 2). Thereafter the EMG signal was rectified and trials averaged across a given condition in each patient. EMG reaction time onset was defined in the latter as the first data point exceeding the mean plus 3 SD of the prestimulus (1 s) baseline that had a steep (>2 uV/ms) rise in amplitude sustained for >20 ms.

Auditory startle reflexes were assessed during reflex and simple reaction time tasks. An auditory startle reflex was considered present if there was a short latency (<130 ms in healthy subjects and <150 ms in subjects with Parkinson's disease) sternocleidomastoid response following a loud stimulus, sustained above the background, which in simple reaction time tasks was required to precede the limb EMG response (determined from the native unrectified signals). Orbicularis and sternocleidomastoid EMGs were then subject to DC removal (individualized for each recording) then rectification. Amplitude and latency of the first occurring auditory startle reflex was assessed. For auditory blink reflexes, amplitude and latency of the averaged orbicularis response were assessed in the auditory blink and startle reflex task.

Statistics

The Kolmogorov–Smirnov Test demonstrated that the distribution of all measures was not different from the normal. Within each group, reaction times for each joint (proximal and distal) and stimulus (normal

and LAS) were compared using repeated measures ANOVA with factors 'Joint' and 'Stimulus'. Patients with Parkinson's disease with freezing of gait/postural instability had an additional factor 'deep brain stimulation' (on and off). We report Joint effects only where they interact significantly with other factors (e.g. Joint \times Stimulus). *Post hoc* tests were performed with paired *t*-tests. StartReact benefit (normal reaction time–LAS reaction time) was compared between groups with ANOVA and *post hoc* independent samples *t*-tests.

In patients with Parkinson's disease with freezing of gait/postural instability, off and on stimulation results were considered together and correlations (Pearson's) sought between LAS reaction times and two independent clinical measures of Parkinsonian gait and balance disturbance—the Gait and Falls Questionnaire (contains both the Freezing of Gait Questionnaire and Falls Questionnaire) and items 27/30.

Reflex latencies and amplitudes were compared between groups using single-factor ANOVA and *post hoc* independent samples *t*-tests. The frequency of individuals recording a recognizable auditory startle reflex was compared between groups using Pearson's chi-square.

Post hoc tests were corrected for multiple comparisons (Bejamini and Hochberg, 1995). Level of significance was $P < 0.05$.

Results

Accelerometer reaction time

In healthy subjects, there was a significant main effect of Stimulus [$F(1,9) = 44.3$, $P < 0.001$]. *Post hoc* tests revealed the presence of StartReact with LAS trials significantly faster than normal trials [mean normal reaction time, averaged across proximal and distal muscles 135.3 ms versus LAS reaction time, averaged across proximal and distal muscles 113.4 ms, $t(19) = 6.057$, $P < 0.001$]. The Joint \times Stimulus interaction was not significant.

In Parkinson's disease with no freezing of gait/postural instability, there was again a significant effect of Stimulus [$F(1,7) = 9.2$, $P = 0.019$]. *Post hoc* tests revealed the presence of StartReact with LAS trials significantly faster than normal trials [mean normal reaction time 164.8 ms versus LAS reaction time 130.2 ms, $t(15) = 3.755$, $P = 0.002$]. The Joint \times Stimulus interaction was not significant.

In contrast, in patients with Parkinson's disease with freezing of gait/postural instability, the effect of Stimulus was absent [$F(1,7) = 3.7$, $P = 0.097$]. There was no main effect of deep brain stimulation nor a Deep brain stimulation \times Joint interaction. There was a trend towards a Deep brain stimulation \times Stimulus interaction [$F(1,7) = 5.0$, $P = 0.060$]. However, there was a significant Deep brain stimulation \times Joint \times Stimulus interaction [$F(1,7) = 7.2$, $P = 0.031$]. *Post hoc* tests revealed that this was due to a selective improvement in proximal LAS reaction time with pedunculopontine nucleus stimulation [mean LAS reaction time 125.0 ms off stimulation versus 101.5 ms on stimulation, $t(7) = 3.6$, $P = 0.036$] (Fig. 2). Pedunculopontine nucleus stimulation also meant that proximal LAS reaction times became faster than proximal normal reaction times, so that pedunculopontine nucleus stimulation restored proximal StartReact [mean normal

reaction time 139.4 ms versus LAS reaction time 101.5 ms, $t(7) = 3.0$, $P = 0.040$].

In line with the above, an ANOVA of proximal StartReact benefit showed a significant difference between subject groups (patients with Parkinson's disease with freezing of gait/postural instability off stimulation) [$F(2,23) = 4.729$, $P = 0.019$]. *Post hoc* tests revealed proximal StartReact benefit to be significantly less in patients with Parkinson's disease with freezing of gait/postural instability (off stimulation) compared with patients with Parkinson's disease with no freezing of gait/postural instability [-0.08 ms versus 41.3 ms, $t(14) = -2.639$, $P = 0.040$] and healthy controls [-0.08 ms versus 26.0 ms, $t(16) = -2.600$, $P = 0.019$]. However, with patients with Parkinson's disease with freezing of gait/postural instability on stimulation, proximal StartReact did not differ between groups [$F(2,23) = 0.615$, $P = 0.549$].

Electromyography reaction time

EMG reaction time results followed a similar pattern as accelerometer reaction time. In healthy subjects, there was a significant effect of Stimulus [$F(1,8) = 16.7$, $P = 0.003$]. *Post hoc* tests revealed the presence of StartReact, with LAS trials significantly faster than normal trials [mean normal reaction time, averaged across proximal and distal muscles 86.4 ms versus LAS reaction time, averaged across proximal and distal muscles 77.2 ms, $t(18) = 3.860$, $P = 0.001$]. The Joint \times Stimulus interaction was not significant.

In patients with Parkinson's disease with no freezing of gait/postural instability, there remained a significant effect of Stimulus [$F(1,7) = 8.7$, $P = 0.021$]. *Post hoc* tests revealed the presence of StartReact, with loud trials significantly faster than normal trials [mean normal reaction time 114.3 ms versus LAS reaction time 92.1 ms, $t(15) = 2.884$, $P = 0.011$]. The Joint \times Stimulus interaction was not significant.

In patients with Parkinson's disease with freezing of gait/postural instability, the effect of Stimulus was absent [$F(1,7) = 0.00$, $P = 0.987$]. There was no effect of deep brain stimulation. There were significant interactions between Deep brain stimulation \times Joint [$F(1,7) = 8.119$, $P = 0.025$], Deep brain stimulation \times Stimulus [$F(1,7) = 8.312$, $P = 0.024$] and Deep brain stimulation \times Joint \times Stimulus [$F(1,7) = 5.669$, $P = 0.049$]. *Post hoc* tests revealed that this was due to a selective improvement in proximal LAS reaction time with pedunculopontine nucleus stimulation [mean LAS reaction time 84.8 ms off stimulation versus 65.4 ms on stimulation, $t(7) = 6.167$, $P < 0.001$] (Fig. 3). Pedunculopontine nucleus stimulation significantly increased proximal StartReact benefit [proximal StartReact off stimulation -10.4 ms versus on stimulation 15.5 ms, $t(7) = -3.363$, $P = 0.012$].

Accordingly, an ANOVA of proximal StartReact benefit showed a significant difference between subject groups (with patients with Parkinson's disease with freezing of gait/postural instability off stimulation) [$F(2,23) = 9.810$, $P = 0.001$]. *Post hoc* tests revealed proximal StartReact to be significantly less in patients with Parkinson's disease with freezing of gait/postural instability (off stimulation) compared with patients with Parkinson's disease

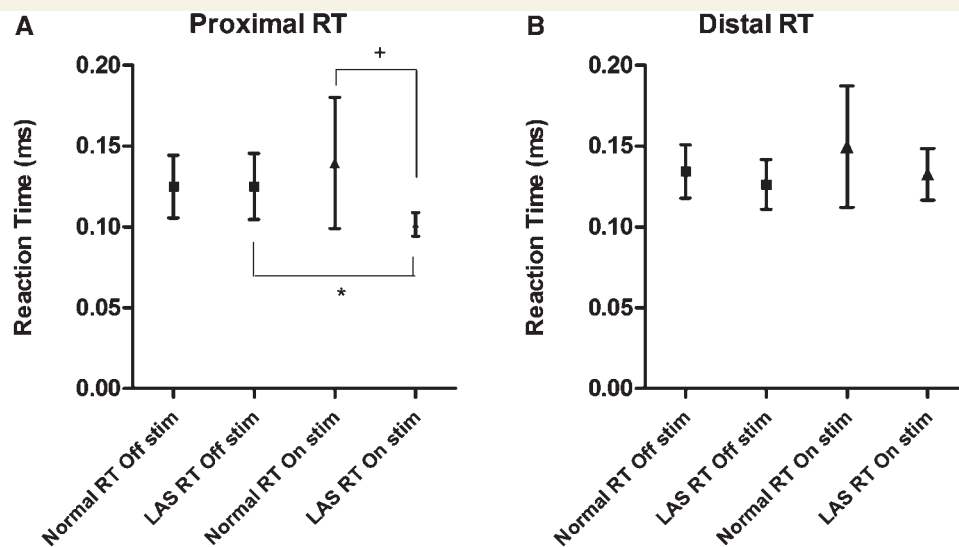


Figure 2 Proximal (A) and Distal (B) Accelerometer reaction times (means and SD) for patients with Parkinson's disease with freezing of gait/postural instability, on and off bilateral pedunculo-pontine nucleus stimulation. * $P = 0.036$; + $P = 0.040$. Increased variance of normal reaction time (reflected in widened SD) is noted in the 'on stimulation' condition. This is mostly attributable to two patients who recorded more reaction time outliers 'on stimulation', suggestive of attentional lapses/fatigue. This figure without the results of these two patients can be viewed in Supplementary material, but the two significant differences remain. RT = reaction time.

with no freezing of gait/postural instability [-10.4 ms versus 36.3 ms, $t(14) = 3.580$, $P = 0.010$] and healthy controls [-10.4 ms versus 10.9 ms, $t(16) = 3.469$, $P = 0.006$]. However, with patients with Parkinson's disease with freezing of gait/postural instability on stimulation, proximal StartReact did not differ between groups [$F(2,23) = 2.397$, $P = 0.113$].

Correlations of reaction time with clinical measures

In patients with Parkinson's disease with freezing of gait/postural instability (with off and on stimulation results considered together), proximal LAS accelerometer reaction time correlated with Gait and Falls Questionnaire ($r = 0.60$, $P = 0.036$) and items 27–30 ($r = 0.52$, $P = 0.039$) (Fig. 4). Distal LAS accelerometer reaction time and proximal and distal EMG LAS reaction times did not correlate with these measures.

Acoustic startle and blink reflexes

Auditory startle reflexes were identified in 7/10 healthy subjects, 5/8 subjects with Parkinson's disease with no freezing of gait/postural instability and 1/8 subjects with Parkinson's disease with freezing of gait/postural instability. The frequency of individuals with auditory startle reflex differed significantly between subject groups [$\chi^2(2,26) = 6.60$, $P = 0.037$] (Table 3). There were significantly fewer auditory startle reflex in patients with Parkinson's disease with freezing of gait/postural instability compared with patients with Parkinson's disease with no freezing of gait/postural instability [$\chi^2(1,16) = 4.267$, $P = 0.030$]. In all subjects, auditory startle reflexes were infrequent, usually occurring with the first LAS trial then rapidly habituating. In patients with Parkinson's disease with freezing of gait/postural instability, pedunculo-pontine nucleus deep brain stimulation did not restore auditory startle reflex in any patient. Comparing healthy subjects and patients with Parkinson's disease with no freezing of gait/postural

Table 3 Summary data for acoustic blink and startle reflexes

	Acoustic blink reflex			Acoustic startle reflex		
	Occurrence	Latency (ms)	Amplitude (μ V)	Occurrence	Latency (ms)	Amplitude (μ V)
Healthy controls	10/10	52.1 (7.8)	89.3 (127.6)	7/10	98.2 (29.7)	321.36 (354.3)
PD NoFOG/PI	8/8	42.4 (12.5)	84.2 (80.3)	5/8	101.9 (37.4)	238.5 (229.8)
PD FOG/PI off stim	7/7	48.5 (17.0)	44.0 (55.5)	1/8	–	–
PD FOG/PI on stim	7/7	47.3 (14.9)	43.5 (53.6)	0/8	–	–

Occurrence indicates proportion of patients demonstrating the response. Otherwise data are mean (SD). FOG = freezing of gait; PD = Parkinson's disease; PI = postural instability.

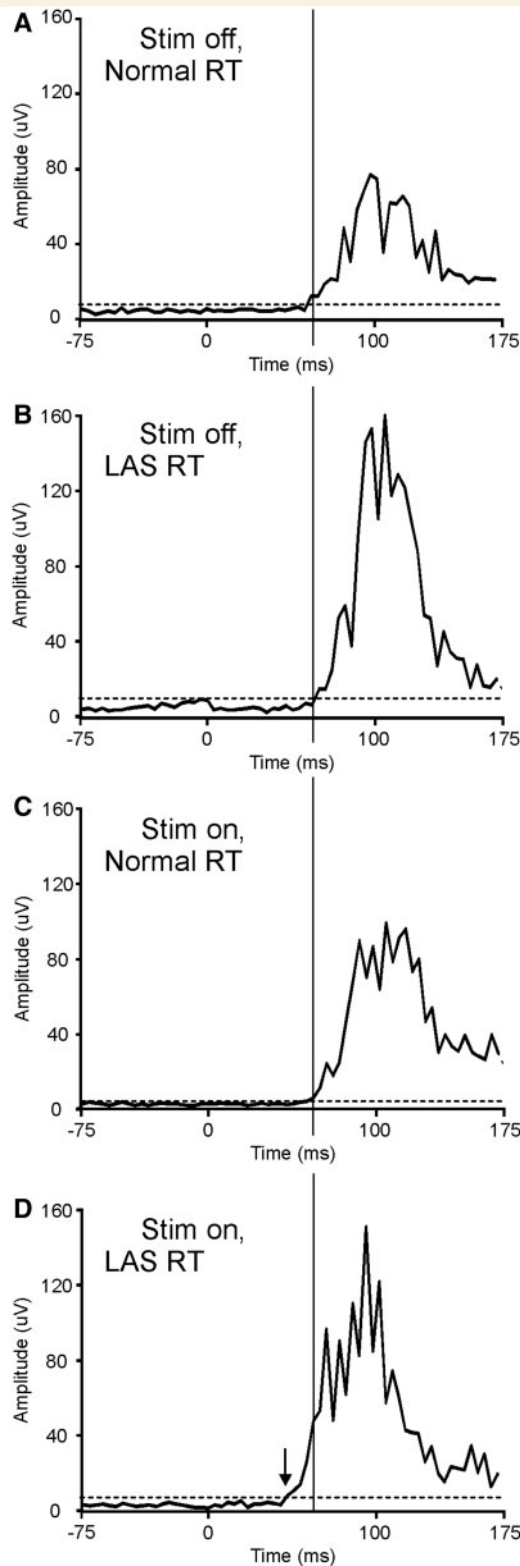


Figure 3 Proximal EMG reaction times for a patient with Parkinson's disease with freezing of gait/postural instability. Speeded responses (ballistic elbow flexion) were recorded to normal (89 dB) auditory stimuli (normal reaction time) and loud (122 dB) auditory stimuli (LAS reaction time), off and on pedunculopontine nucleus stimulation. Traces are the averaged biceps EMG waveforms from the proximal simple reaction time

instability, there were no significant differences in auditory startle reflex amplitudes [321.3 μ V versus 238.5 μ V, $t(10) = 0.456$, $P = 0.658$] or latencies [98.2 ms versus 101.9 ms, $t(10) = -0.19$, $P = 0.854$]. Insufficient patients with Parkinson's disease with freezing of gait/postural instability had an auditory startle reflex to make comparisons with this group.

An averaged auditory blink reflex in the auditory blink and startle reflex task was identifiable in all healthy subjects and patients with Parkinson's disease with no freezing of gait/postural instability subjects and 7/8 subjects with Parkinson's disease with freezing of gait/postural instability (in one patient with Parkinson's disease with freezing of gait/postural instability, any auditory blink reflex was obscured by excessive blinking during recordings). Between subject groups (with Parkinson's disease freezing of gait/postural instability off stimulation), no differences were found in auditory blink reflex amplitudes [$F(2,22) = 0.495$, $P = 0.616$] or latencies [$F(2,22) = 1.387$, $P = 0.271$]. In patients with Parkinson's disease with freezing of gait/postural instability, pedunculopontine nucleus stimulation did not alter auditory blink reflex amplitudes [$t(6) = 0.141$, $P = 0.892$] or latencies [$t(6) = 0.385$, $P = 0.714$].

Discussion

We found that Parkinsonian patients with freezing of gait/postural instability can be distinguished from those without freezing of gait/postural instability by attenuation of the StartReact phenomenon in a proximal muscle, the biceps, and through the scarcity of auditory startle responses. The deficit of StartReact, but not that of the auditory startle response, was reversed by pedunculopontine nucleus stimulation.

The scarcity of auditory startle responses in Parkinsonian patients with freezing of gait/postural instability recalls the reduced frequency of startle in progressive supranuclear palsy (Vidailhet *et al.*, 1992; Kofler *et al.*, 2001; Gironell *et al.*, 2003). Severe 'ON medication' freezing of gait/postural instability as a dominating complaint in Parkinson's disease is unusual and itself flags the possibility of progressive supranuclear palsy (Jankovic, 2008). In the absence of a definitive test in life, the diagnosis of Parkinson's disease in our patients with Parkinson's disease with freezing of gait/postural instability should be considered presumptive, although the persistence of normal auditory blink reflexes differs from the absent or abnormal auditory blink reflexes

task in Patient 3, with Parkinson's disease with freezing of gait/postural instability. The onsets of such averaged waveforms tend to reflect the fastest occurring responses during the task. The dotted horizontal line represents the mean plus 3 SD of the pre-stimulus (1 s) baseline. The solid vertical line transects all traces at the same time-point (and at the onset defined for the stimulation on, normal reaction time). When off stimulation, the averaged LAS reaction time (B) is not faster than the normal reaction time (A); StartReact is absent. Pedunculopontine nucleus stimulation speeds the LAS reaction time (D, onset indicated by the arrow) but not the normal reaction time (C). Thus pedunculopontine nucleus stimulation restored proximal StartReact. RT = reaction time.

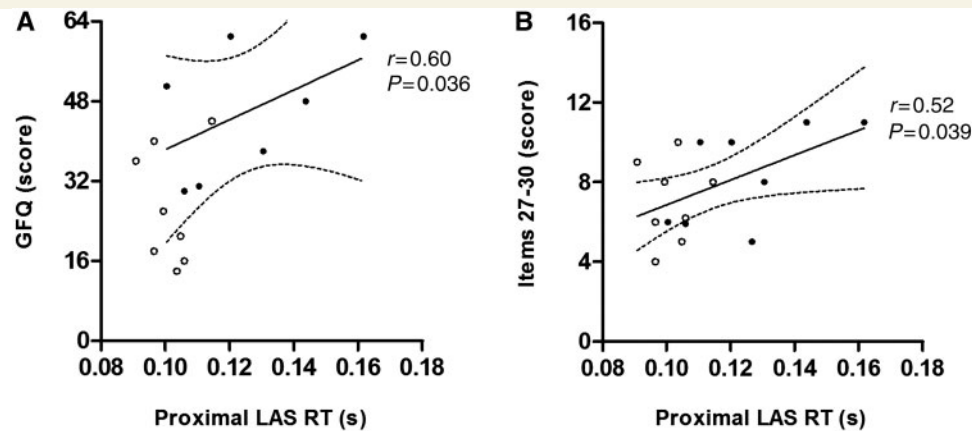


Figure 4 Correlation between proximal LAS accelerometer reaction time (RT) and clinical measures in patients with Parkinson's disease with freezing of gait/postural instability. Linear regression (solid line) and 95% confidence intervals (dotted lines) are shown. Data from on stimulation (open circles) and off stimulation (filled circles) are both included, affording 16 potential data points [two results from each patient, except for one patient (A) in whom preoperative off stimulation Gait and Falls Questionnaire (GFQ) data were absent, as indicated in Table 2]. (A) Correlation between proximal LAS accelerometer reaction time and Gait and Falls Questionnaire. Off and on stimulation Gait and Falls Questionnaire scores were prospectively obtained preoperatively (off stimulation) and postoperatively (on stimulation). (B) Correlation between proximal LAS accelerometer reaction time and items 27–30 of the UPDRS. Items 27–30 scores were rated by the same examiner, off and on stimulation at the same postoperative visit with a minimum 1 h washout period.

reported in progressive supranuclear palsy (Vidailhet *et al.*, 1992; Valldeoriola *et al.*, 1998; Kofler *et al.*, 2001; Gironell *et al.*, 2003; Williams *et al.*, 2008).

Regardless of pathological type, attenuation of startle in Parkinsonian patients with freezing of gait/postural instability implicates the pons as a site of significant functional disturbance in this phenotype, a conclusion further strengthened by the deficit in StartReact and its' reversal by pedunculopontine nucleus stimulation (Brown *et al.*, 1991; Vidailhet *et al.*, 1992; Valls-Sole *et al.*, 2008). Conversely, preservation of the auditory blink reflex suggests that the midbrain can be relatively spared in freezing of gait/postural instability (Hori *et al.*, 1986).

The pedunculopontine nucleus region and release of preprogrammed movement

StartReact is described to occur when the relevant motor response can be fully anticipated, 'preprogrammed' and stored for release, as in simple reaction time tasks (Valls-Sole *et al.*, 1999; Carlsen *et al.*, 2008). In line with this task specificity, we previously found that pedunculopontine nucleus stimulation in patients with Parkinson's disease with freezing of gait/postural instability selectively improved simple reaction time, but not choice or Go-NoGo reaction times (Thevathasan *et al.*, 2010). Taken together with the current findings, it appears that pedunculopontine nucleus stimulation corrects a deficit in freezing of gait/postural instability in the release of pre-prepared responses, both in response to simple cues, or more strikingly, when cues are accompanied or replaced by loud auditory stimuli. This suggests that tonic low frequency activity in the pedunculopontine nucleus or a pathway in the region of the pedunculopontine nucleus supports the release

of pre-prepared motor programmes in Parkinson's disease. However, given that we only studied the effects of pedunculopontine nucleus stimulation in patients with Parkinson's disease, one can only speculate about the relevance of this to the normal functioning of the pedunculopontine nucleus.

Some of our findings differ from those previously reported. We did not replicate our previous result that pedunculopontine nucleus stimulation improved simple reaction time in trials without LAS ('normal reaction time' in the present study) (Thevathasan *et al.*, 2010). However, the tasks of the two studies are different. In the present study, we aimed to optimize StartReact by, for example, using warning cues and long intertrial intervals. This contrasts with the rapidly occurring unwarned visual cues of unchanging intensity employed previously, which could have promoted more reflexic simple reaction time responses. Furthermore, a small change in simple reaction time may have been undetected in this study as the long intertrial intervals meant that normal reaction times were averaged over only 20 trials per task, compared with 50 previously.

A previous study in young healthy subjects demonstrated StartReact to be greater in proximal compared with distal movements (Carlsen *et al.*, 2009). Our study was not powered to demonstrate these differential effects, particularly in an elderly cohort. The same previous study in young healthy subjects suggested that an accompanying startle reflex might identify those responses with the greatest shortening of reaction time (Carlsen *et al.*, 2009). However, we found that in elderly healthy subjects, a startle reflex seldom accompanied the intended motor response, despite the use of loud auditory stimuli. Aside from the age difference of subjects, the differing results could be explained by the different criteria used to define the presence of a startle reflex. We defined startle during reaction time tasks not only by virtue of short

latency sternocleidomastoid EMG activity (e.g. <130 ms) but also, unlike the aforementioned study, by appearance of such activity before the limb response. This latter criterion was necessary to exclude sternocleidomastoid activity due to accessory muscle activation. Otherwise, responses sped by loud sounds (which had limb EMG latencies around 70–90 ms) might appear to be accompanied by startle. Other studies have similarly reported the absence of startle from trials where loud stimuli triggered rapid (sometimes even involuntary) responses – including where the released motor programmes were postural reflexes and stepping (MacKinnon *et al.*, 2007; Reynolds and Day, 2007). Further evidence for the separable nature of StartReact and the startle reflex is that in LAS trials, an accompanying startle reflex does not alter the triphasic EMG pattern of the intended motor response (Carlsen *et al.*, 2004b). Furthermore, unlike acoustic blink and startle reflexes, StartReact is not modified by prepulse inhibition (Valls-Sole *et al.*, 2005). Such results are consistent with our finding that pedunculopontine nucleus stimulation restored StartReact without restoring startle in patients with Parkinson's disease with freezing of gait/postural instability. As has been argued previously, such findings support that the StartReact phenomenon and startle reflex may be dissociated (Valls-Sole *et al.*, 2005).

Relevance to the pathophysiology of freezing of gait/postural instability and the therapeutic mechanism of pedunculopontine nucleus stimulation

StartReact was absent in patients with Parkinson's disease with freezing of gait/postural instability but conserved in disease matched patients with Parkinson's disease with no freezing of gait/postural instability controls and healthy subjects. Unless the pedunculopontine nucleus electrodes themselves caused the reversible deficit in StartReact, then this deficit appears associated with gait freezing and postural instability.

Pedunculopontine nucleus stimulation sped proximal LAS reaction times significantly more than distal LAS reaction times—and proximal (and not distal) LAS accelerometer reaction times correlated with clinical measures of gait and postural disturbance. Proximal and axial musculature is predominantly innervated by reticulospinal pathways—the likely conduit of modifications to spinal pattern generated locomotion as well as postural reflexes (Lawrence and Kuypers, 1968b; Drew *et al.*, 2004; Davidson *et al.*, 2007; Stapley and Drew, 2009). Distal musculatures, particularly intrinsic hand muscles, receive predominantly corticospinal input (Lawrence and Kuypers, 1968a; Riddle *et al.*, 2009). A lesser benefit of pedunculopontine nucleus stimulation on distal StartReact is therefore consistent with an effect mediated through reticulospinal pathways and also with the therapeutic role of pedunculopontine nucleus stimulation on the axial and proximal deficits of freezing of gait/postural instability.

In this study, we did not directly demonstrate that freezing of gait/postural instability involves a deficit in StartReact for gait and postural responses. However, adjustments to gait and posture are known to be amenable to StartReact—suggesting that some aspects of gait and posture are preprogrammed and potentially

subject to the same triggered release we have shown is deficient in freezing of gait/postural instability and restored by pedunculopontine nucleus stimulation (Reynolds and Day, 2007; Queralt *et al.*, 2008). This interpretation was supported by the presence of correlations between proximal LAS accelerometer reaction times and two independent measures of Parkinsonian gait and balance disturbance—the Gait and Falls Questionnaire and items 27–30. These correlations were found when proximal LAS reaction times were assessed with the accelerometer but not EMG. One explanation is that actual movement is more clinically relevant than the onset of motor recruitment. However, an additional consideration is that the methods of assessing EMG and accelerometer reaction times differed. In this study, EMG reaction times tended to reflect the fastest occurring responses whereas accelerometer reaction times were more representative of performance across all trials.

Optimization of motor systems releasing preprogrammed movement may not be the only mechanism by which pedunculopontine nucleus deep brain stimulation may improve gait as the strength of correlations suggested it accounts for only around a third of the variance in gait scores. In this regard, it is important to stress that the pedunculopontine nucleus has multiple functions. For example, local field potential studies and clinical observations have raised the possibility that attentional changes may also contribute to the effects of stimulation in this area (Androulidakis *et al.*, 2008; Arnulf *et al.*, 2010).

Our observation that StartReact is deficient in patients with Parkinson's disease with freezing of gait/postural instability and restored by pedunculopontine nucleus stimulation offers insights into the pathophysiology of freezing of gait/postural instability and the mechanisms of pedunculopontine nucleus stimulation. However, we have not, in this study, addressed whether these findings might help predict response to pedunculopontine nucleus deep brain stimulation. The clinical utility of our findings remains to be investigated, although the variability in StartReact benefit may preclude any inferences at the single subject level.

In conclusion, our findings suggest that Parkinsonian patients with freezing of gait/postural instability have a deficit in the release of preprogrammed movement, when the latter should have been promoted by loud auditory stimuli. Delays in the release of preprogrammed movement correlated with the severity of the freezing of gait/postural instability phenotype. Pedunculopontine nucleus stimulation, while improving the phenotype, also restored the deficit in preprogrammed movement release. Accordingly, freezing of gait/postural instability may, in part, involve impairment of a pontine system supporting the reflexic release of pre-prepared motor programmes.

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Supplementary material

Supplementary material is available at *Brain* online.

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