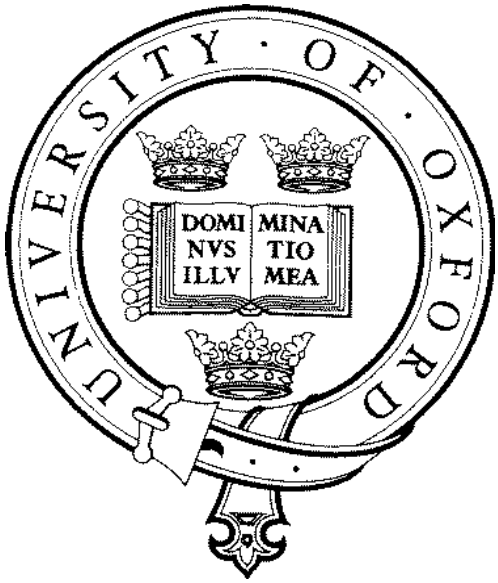
PEDUNCULOPONTINE NUCLEUS STIMULATION FOR GAIT AND POSTURAL DISORDERS IN PARKINSON'S DISEASE



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ABSTRACT

The pedunculopontine nucleus (PPN) is a reticular collection of neurons at the junction of midbrain and pons. The PPN in animal models appears topographically organised and functionally related to locomotion and arousal. In Parkinson's disease, the PPN degenerates and is susceptible to abnormal basal ganglia output. In patients with Parkinson's disease, low frequency PPN stimulation is proposed to improve gait freezing and postural instability. However, the therapeutic mechanisms, optimal clinical application and precise effects on gait and posture of PPN stimulation are unclear.

Here, a topographic arrangement of the PPN was supported by local field potential recordings in parkinsonian patients. In the PPN region, beta oscillations were recorded rostrally and alpha oscillations caudally. Alpha oscillations, consistent with their putative role in allocating attention, correlated with gait performance and attenuated with gait freezing. Thus the caudal PPN subregion may be the most relevant target for gait disorders.

Accordingly, an unblinded clinical study suggested that stimulation of the caudal PPN subregion was beneficial for gait freezing, postural instability and falls. In a double-blinded study using spatiotemporal gait analysis, caudal PPN stimulation reduced triggered gait freezing, with bilateral stimulation more effective than unilateral. However, akinesia including akinetic gait did not improve with PPN stimulation. Accordingly, dopaminergic medication requirements did not change.

Mechanisms underlying gait freezing and PPN stimulation were explored with reaction time experiments. Parkinsonian patients with severe gait freezing and postural instability

demonstrated a ‘block’ to pre-programmed movement. This was evidenced by prolonged simple reaction times and the absence of ‘StartReact’, whereby pre-prepared responses are normally accelerated by loud acoustic stimuli. PPN stimulation improved simple reaction time and restored Startreact. The relief of this ‘motor block’ with PPN stimulation may therefore explain the associated improvement in gait freezing and postural instability, as these tend to occur in circumstances requiring triggered, pre-prepared adjustments.

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PUBLICATIONS

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Alpha oscillations in the pedunculopontine nucleus correlate with gait performance in Parkinsonism
Brain, In Press

Chapter 4:

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Pedunculopontine nucleus stimulation improves gait freezing in Parkinson's disease
Neurosurgery, In Press

Chapter 5:

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Chapter 6:

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Chapter 7:

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

ABBREVIATIONS

ACC	Accelerometer
AChE	Acetylcholinesterase
ANOVA	Analysis of Variance
ASR	Acoustic startle reflex
ChAT	Choline acetyl transferase
COMT	Catechol-O-Methyl-Transferase
CRT	Choice reaction RT
CT	Computed tomography scan
DBS	Deep brain stimulation
DTI	Diffusion tensor imaging
EEG	Electroencephalography
EMG	Electromyography
FallsQ	Falls Question (item of the Gait and Falls Questionnaire)
FLAIR	Fluid attenuation inversion recovery
FOG	Freezing of Gait
FOGQ	Freezing of Gait Questionnaire
GABA	γ-aminobutyric acid
GFQ	Gait and Falls Questionnaire
GPi	Globus pallidus interna
Hz	Hertz
IT27-30	Items 27-30 of the Unified Parkinson's disease Rating Scale
LAS	Loud auditory stimuli
LDT	Laterodorsal tegmental nucleus
LFP	Local field potential
MAO	Monoamine Oxidase

MLR	Mesencephalic locomotor region
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging scan
OHDA	6-Hydroxydopamine
PD	Parkinson's disease
PGO	Ponto-geniculo-occipital
PI	Postural instability
PM	Pontomesencephalic
PPN	Pedunculopontine nucleus
PPNc	Pedunculopontine nucleus pars compacta
PPNd	Pedunculopontine nucleus pars dissipata
PSP	Progressive supranuclear palsy
RAS	Reticular activating system
REM sleep	Rapid eye movement sleep
rGS	Relative gait speed
RT	Reaction time
SCM	Sternocleidomastoid muscle
SD	Standard deviation
SEM	Standard error of the mean
SMA	Supplementary motor area
SNPc	Substantia nigra pars compacta
SNPr	Substantia nigra pars reticulata
SRT	Simple reaction time
STN	Subthalamic nucleus
UPDRS	Unified Parkinson's disease Rating Scale
VIGRT	Vigilance reaction time
ZI	Zona incerta

Chapter 1: Introduction

1.1 PARKINSON'S DISEASE

Parkinson's disease (PD) is the second most common neurodegenerative condition after Alzheimer's disease.(de Lau and Breteler 2006) The lifetime risk of developing PD is 1.5%.(de Rijk, Breteler et al. 1995) The major risk factor is age.(Lees, Hardy et al. 2009)

Many of the core features of Parkinson's disease were described in James Parkinson's 'An essay of the shaking palsy' in 1817.(Parkinson 1817) Charcot later gave credit to Parkinson and suggested the condition, previously known as ''paralysis agitans'' be referred to as ''maladie de Parkinson".(Kempster, Hurwitz et al. 2007)

1.1.1 Pathology and aetiology

The pathological hallmark of Parkinson's disease is the presence of Lewy neurites and Lewy bodies inside neurons.(Lewy 1912) These deposits consist of the naturally occurring protein, alpha-synuclein, which undergoes a pathological change in conformation to become insoluble amyloid.(Spillantini, Crowther et al. 1998) Neurons containing these deposits become dysfunctional and ultimately degenerate. The cause of this cascade is unknown.

Certain neurons are at increased risk of developing Lewy pathology. More vulnerable neurons become involved earlier in the disease course and are consequently more severely affected. This gradient of vulnerability results in a predictable 'stereotyped' ascending progression of neurodegeneration, split into six stages by Braak.(Braak, Del Tredici et al.

2003; Braak, Ghebremedhin et al. 2004) These pathological stages correlate well with clinical progression. Initially there is involvement of the olfactory and vagal nuclei which is usually asymptomatic, but in retrospect patients may recall anosmia.(Ansari and Johnson 1975) At this stage, some patients also develop REM sleep behavioural disturbance.(Schenck, Bundlie et al. 1986; Schenck, Bundlie et al. 1996) Later the process ascends to involve the dopaminergic neurons in the substantia nigra pars compacta (SNPc) causing striatal dopamine deficiency.(Brissaud 1895; Tre´tiakoff 1919; Ehringer and Hornykiewicz 1960; Anden, Carlsson et al. 1964) Reserve and compensation mean that up to 50-80% of SNPc dopaminergic neurons are lost before the typical motor features of Parkinson's disease emerge.(Bernheimer, Birkmayer et al. 1973) Ultimately, Lewy pathology ascends to involve the cortex. This can manifest with visual hallucinations and cognitive impairment. Neurodegeneration is highly selective and involves only certain neuronal populations, whilst sparing others that may even lie directly adjacent.(Braak, Ghebremedhin et al. 2004)

Vulnerable neuronal populations share common features that may provide clues to the cause of PD. Involved neurons tend to have very long projections and relatively small cell bodies. Their axons are typically unmyelinated or thinly myelinated.(Braak, Ghebremedhin et al. 2004) Many of these cells are continuously firing in a pacemaker or 'gain setting' fashion.(Bean 2007) All of these properties result in high metabolic demands that make these neurons sensitive to perturbations in energy supply. Mitochondrial dysfunction has therefore been suggested to be a prominent factor in the cascade leading to neurodegeneration in PD.(Schapira 2008) Intravenous drug users in San Francisco who mistakenly injected 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an inhibitor of a mitochondrial enzyme 'complex 1', developed severe

Parkinsonism due to selective degeneration of the substantia nigra.(Langston, Ballard et al. 1983) In patients with true idiopathic Parkinson's disease, complex 1 deficiency has been detected in-vivo in platelets and post-mortem in the substantia nigra.(Parker, Boyson et al. 1989; Schapira 2008) Intravenous MPTP is used experimentally to create nonhuman primate animal models of Parkinson's disease. Another inhibitor of the mitochondrial respiratory chain is 6-Hydroxydopamine (OHDA), often used to create rodent models of PD via direct microinjection into the substantia nigra.(Uretsky and Iversen 1970)

Both genetic and environmental factors have been implicated in susceptibility to developing PD. The single most important risk factor for sporadic PD is age.(de Rijk, Breteler et al. 1995; Lees, Hardy et al. 2009) Although single gene causes of Parkinsonism are described, the contribution of these genes to sporadic PD appears minimal.(Lees, Hardy et al. 2009) Sporadic PD is likely to involve polygenetic susceptibility factors interacting with aging and environmental factors.(de Lau and Breteler 2006) However, so far only environmental factors weakly linked to PD have been identified, such as rural upbringing and insecticide exposure.(Lees, Hardy et al. 2009) Interestingly, a sensation seeking personality, smoking history and caffeine use is associated with reduced risk of PD.(Hernan, Zhang et al. 2001; Evans, Lawrence et al. 2006) Rather than being protective, such factors are considered as reflecting lower reward seeking behaviour from prodromal dopamine depletion.(Evans, Lawrence et al. 2006)

1.1.2 Clinical features

Symptoms of PD can be classified as motor and non-motor.

The cardinal motor features of PD are considered to be akinesia, rigidity, tremor and postural instability.(Hughes, Daniel et al. 1992) Of these akinesia is the most essential element. Unlike slowness of movement from depression, motor neuron disease and even dystonia, the akinesia of parkinsonian disorders decrements in speed and amplitude.(Berardelli, Rothwell et al. 2001) Tremor is present in only around 75% of patients over the disease course.(Hughes, Daniel et al. 1993) Asymmetry of signs is usual, reflecting asymmetry of SNPC degeneration. Freezing of gait and postural instability tend occur at later disease stages.(Giladi, McDermott et al. 2001)

Cognitive deficits are usual after a prolonged disease course e.g. >80% prevalence after 15 years.(Hely, Reid et al. 2008) However, the biggest risk factor for dementia is age rather than disease duration.(Kempster, Williams et al. 2007; Hely, Reid et al. 2008) Cognitive domains most prominently involved are frontal (e.g. attention and executive) and occipital (e.g. spatial organisation). Hallucinations are usually visual, initially occurring at night and illusory but later becoming formed and occurring during daytime.(Fenelon, Mahieux et al. 2000) REM sleep behavioural disorder is common even in early PD and later narcolepsy-like symptoms and disrupted nocturnal sleep architecture can occur.(Schenck, Bundlie et al. 1996; Arnulf, Leu et al. 2008) Depression and anxiety are common.(Aarsland, Bronnick et al. 2007) Autonomic dysfunction, including impotence, postural hypotension and constipation tend to occur later in the disease course.(Chaudhuri, Healy et al. 2006) Although not considered a sensory disorder, sensory symptoms are common in PD, including back and limb pain relating to rigidity.

The diagnosis of PD is usually made on clinical grounds. The Queen square Brain bank criteria is a useful guide.(Hughes, Daniel et al. 1992) However, the rate of misdiagnoses (as defined by dopamine transporter scanning or pathology) is around 5-10% even in

specialist centres.(Hughes, Ben-Shlomo et al. 1992; Hughes, Daniel et al. 1992) PD is the one of a group of disorders characterised by 'Parkinsonism' which have in common the feature of decrementing bradykinesia. The other Parkinsonian disorders are far less common, are less dopamine responsive and have poorer prognosis. These include progressive supranuclear palsy, multiple system atrophy, corticobasal ganglionic degeneration and Lewy body dementia. In addition, several non-parkinsonian disorders can mimic PD, although without true decrementing bradykinesia. For example, dystonic tremor can mimic PD in what has been termed 'SWEDDS' (scans without evidence of dopamine deficiency).(Schneider, Edwards et al. 2007) As implied by this term, a dopamine transporter (DAT) SPECT scan can distinguish Parkinsonian disorders from non-parkinsonian mimics such as tremulous dystonia, essential tremor and drug induced parkinsonism.(Scherfler, Schwarz et al. 2007) However DAT scanning cannot distinguish between the parkinsonian disorders which require post-mortem pathology for definitive diagnosis.

Disease severity assessment can be quantified in clinical practice using rating scales. These include the Hoehn and Yahr scale, rating disease stage from 1-5.(Hoehn and Yahr 1967) More detailed is the Unified Parkinson's disease rating scale (UPDRS) which is a combination of questionnaire and examination findings.(Fahn S 1987)

1.1.3 Treatment

The treatment of PD is symptomatic. No treatment has been convincingly demonstrated to alter the underlying neurodegenerative process in clinical practice.

Dopamine replacement therapy is the mainstay of treatment for PD.(Birkmayer and Hornykiewicz 1961; Barbeau, Sourkes et al. 1962; Cotzias, Van Woert et al. 1967) Dopaminergic medication includes levodopa and the dopamine agonists (including ergot and non-ergot oral drugs and apomorphine injections/infusions). Of these, levodopa is the most effective. It is the motor symptoms of PD that are generally dopamine responsive, although gait freezing and postural instability often poorly so.(Bloem 1992; Hughes, Daniel et al. 1993; Ferraye, Debu et al. 2008) Motor fluctuations develop during dopaminergic therapy. This refers to the alternation of "off medication" symptoms and dyskinesias in the 'on' state. Catechol-O-Methyl-Transferase (COMT) inhibition and dopamine agonists including apomorphine infusions are options to smooth serum dopaminergic levels and thereby lessen fluctuations. Dopamine agonists are used to spare levodopa, higher doses and longer durations of which are associated with dyskinesias.(Rascol, Brooks et al. 2000) However, the risk of dyskinesias from levodopa can be overstated leading to inappropriate under-medication. Amantadine can be employed to reduce dyskinesias and Monoamine Oxidase (MAO) inhibitors for early symptoms.(Olanow, Rascol et al. 2009) Non-dopaminergic drugs are also used to treat tremor, such as beta blockers and anticholinergics.

Neuropsychiatric complications of medications are extremely common and a compromise is often struck between optimising motor versus neuropsychiatric disturbance. Dopamine agonists commonly cause impulse control disorders including obsessions and compulsions regarding hobbies, objects, sex, eating and gambling – and even with their own treatment.(Weintraub 2008) Dopamine dysregulation is where there is an addiction to dopaminergic medication so that escalating doses are taken.(Evans and Lees 2004) All dopaminergic medication and also anticholinergic drugs can worsen hallucinations and

precipitate psychosis. Dopamine agonists are also particularly prone to causing excessive daytime sleepiness.

For those with troublesome motor fluctuations, deep brain stimulation of the subthalamic nucleus and internal pallidum are therapeutic options.(Deuschl, Schade-Brittinger et al. 2006) However, these offer only to improve motor function. The effect of deep brain stimulation on non-motor symptoms is variable (and sometimes detrimental).(Thevathasan and Gregory 2010) Additionally, with the exception of tremor, these treatments are only effective for motor symptoms that respond to levodopa and percentage improvement of the motor subsection of the UPDRS predicts outcome from surgery.(Charles, Van Blercom et al. 2002) Tremor, even when medication resistant, can respond to subthalamic nucleus stimulation or more reliably, to thalamic stimulation. However thalamic stimulation does not help bradykinesia or rigidity. Lesioning (as opposed to stimulation) is still performed in many centres for discrete indications. For example, unilateral thalamic lesioning remains a compelling option for elderly patients with PD and predominantly unilateral tremor.(Thevathasan and Gregory 2010)

Many experimental therapies are under investigation including dietary modification with ketone esters to enhance mitochondrial function as well as stem cell treatments.(Kashiwaya, Takeshima et al. 2000)

1.1.4 Prognosis

Although dopaminergic medication is not known to alter the underlying neurodegenerative process, survival has increased to around 15 years compared with 9 years in the pre-

levodopa era (although this may also relate to improved overall healthcare). (Hoehn and Yahr 1967; Hely, Reid et al. 2008)

In the longest prospective follow up study of PD, 130 patients were followed from first diagnosis for 20 years.(Hely, Reid et al. 2008) There were 30 survivors, only one of whom was living independently. Before death, over 70% of patients developed dementia, hallucinations, motor fluctuations, falls and urinary incontinence. However, the biggest determinant for many of these endpoints is age, rather than duration of disease.(Kempster, Williams et al. 2007)

1.1.5 Pathophysiology mechanisms of deficits and mechanisms of therapy

Dopamine depletion underpins the pathogenesis of the dopamine responsive symptoms of akinesia, rigidity and tremor. The rate model of the basal ganglia predicts that striatal dopamine deletion abnormally increases activity in the indirect pathway of the basal ganglia.(DeLong 1990; Kravitz, Freeze et al. 2010) This is in turn 'brakes' thalamo-cortical excitation and abnormally modulates the pedunculopontine nucleus.(Nauta and Mehler 1966; Kravitz, Freeze et al. 2010) However, the pattern of neural activity within the basal ganglia-cortical loops is also critical. Abnormal synchronisation in the beta band reduces information coding assessed by entropy measures.(Cruz, Mallet et al. 2009) The degree of synchronisation in the beta band correlates with akinesia and rigidity (but not tremor) and is suppressed by dopaminergic medication and deep brain stimulation.(Kuhn, Kupsch et al. 2006; Ray, Jenkinson et al. 2008; Eusebio, Thevathasan et al. 2010) Tremor meanwhile is related to synchronisation at tremor frequencies and first harmonic in addition to gamma band synchronisation.(Timmermann, Gross et al. 2003; Weinberger,

Hutchison et al. 2009) Dyskinesias are associated with synchronisation at low frequencies.(Alonso-Frech, Zamarbide et al. 2006)

The degree of motor severity of akinesia and rigidity correlates with the degree of contralateral dopamine denervation.(Bernheimer, Birkmayer et al. 1973) With the progressive loss of nigral neurons, there is a loss of buffering of dopamine levels which become more reflective of serum pharmacokinetics contributing to motor fluctuations.(Jankovic 2005) Dyskinesias may also be promoted by unregulated release of dopamine as a 'false neurotransmitter' by relatively intact serotonergic cells.(Tanaka, Kannari et al. 1999; Carlsson, Carta et al. 2007) Abnormal plastic changes also contribute to dyskinesias and thus certain alleles of Brain Derived Neurotrophic Factor (BDNF) have been identified as a susceptibility factor.(Picconi, Centonze et al. 2003; Foltynie, Cheeran et al. 2009)

Although current treatments are thought to have no direct impact on neurodegeneration, it has been noted that dopamine modulating treatments including levodopa/COMT inhibitor combinations, dopamine agonists and MAO inhibitors may all modestly reduce the rate of decline in UPDRS scores.(Olanow, Rascol et al. 2009) This non-specific effect may be mediated by reducing stress on under-functioning neurons or by reducing harmful compensatory mechanisms.

Other neurotransmitter defined systems are also disrupted in Parkinson's disease including cholinergic, noradrenergic and serotonergic systems. These have been implicated in the non-dopamine responsive features of PD such as sleep disturbance, attention, mood and autonomic dysfunction.(Fox, Brotchie et al. 2008)

1.2 GAIT DISTURBANCE IN PARKINSON'S DISEASE

James Parkinson's recognised gait disturbance as a prominent feature of the disorder, including gait akinesia ("the legs are not raised to that height, or with that promptitude which the will directs"), reduced automaticity ("walking becomes a task which cannot be performed without considerable attention") and festination ("…irresistibly impelled to take much quicker and shorter steps, and thereby to adopt unwillingly a running pace").(Parkinson 1817) However, Parkinson did not describe gait freezing.(Buzzard 1882)

Three major categories of parkinsonian gait disturbance will be discussed here: Akinesia, festination and gait freezing.

1.2.1 Akinetic gait:

Similar to akinesia of other body parts, gait is affected by a reduction in speed and amplitude of movement, thereby reducing overall velocity.(Jankovic 2008) Step length is reduced in PD whilst cadence is relatively conserved and used as a compensatory measure.(Morris, Iansek et al. 1994) The ability of modulate step length is also abnormal, reflected in abnormally increased variability.(Morris, Iansek et al. 1994) Consistent with other akinesia, step length is liable to decrement.(Morris, Iansek et al. 1994; Chee, Murphy et al. 2009) Step length deficiencies can respond to focussing attention on gait and to visual cues.(Morris, Iansek et al. 1996) Akinesia of gait and indeed akinesia of any movement, can be overcome by intense arousing stimuli – a phenomenon termed 'paradoxical kinesis'.(Souques 1921; Glickstein and Stein 1991) This is proposed to act via phasic arousal recruiting processing resources and can occur independently of

dopaminergic mechanisms.(Keefe, Salamone et al. 1989; Anzak, Tan et al. 2011) Spinal cord stimulation administered intermittently and at startling intensities, was recently demonstrated to alleviate gait akinesia and restore locomotion in parkinsonian rodents.(Fuentes, Petersson et al. 2009) However this has been attributed to paradoxical kinesis and was not replicated by chronic sub-threshold stimulation in patients.(Nicolelis, Fuentes et al. 2010; Thevathasan, Mazzone et al. 2010) Deficits in step length, step length variability and velocity are responsive to dopaminergic medication and subthalamic nucleus stimulation.(Faist, Xie et al. 2001)

1.2.2 Festination:

As Parkinson observed, festination is the need to take quicker, smaller steps eventually transforming walking into uncontrolled running. Festination has been interpreted as an attempt to maintain centre of gravity between the feet to prevent falling forwards. It may thus relate to a deficit of postural reflexes whereby instead of a single, appropriately scaled corrective step, the patient makes a series of hypometric steps leaving the centre of gravity forward of the feet.(Giladi, Shabtai et al. 2001) Festination is associated with increased disease duration, gait freezing as well as festination of speech.(Giladi, Shabtai et al. 2001; Moreau, Ozsancak et al. 2007) Like postural instability and gait freezing, festination responds poorly to dopaminergic medication and causes falls.(Giladi, Shabtai et al. 2001)

1.2.3 Gait freezing:

Freezing of gait (FOG) is an episodic arrest of forward progress in locomotion due to an inability to take normal steps.(Giladi and Nieuwboer 2008) Patients often describe the

feeling of ‘feet glued to the floor’.(Snijders, Nijkrake et al. 2008) Freezing can also affect other movements such as writing.(Nieuwboer, Vercruysse et al. 2009) Freezing of gait is classically episodic. However, episodes can be so brief as to be clinically covert or so continuous as to prevent all locomotion. Gait freezing causes falls and diminishes quality of life.(Moore, Peretz et al. 2007; Kerr, Worringham et al. 2010; Thevathasan and Aziz 2010)

In PD, gait freezing becomes commoner and tends to be less medication responsive as the disease progresses.(Giladi, McDermott et al. 2001; Bloem, Hausdorff et al. 2004) The overall prevalence of gait freezing in PD is approximately 50%.(Macht, Kaussner et al. 2007) However severe “on medication” gait freezing as the predominant issue is unusual in PD and raises the question of atypical pathologies.(Factor 2008; Jankovic 2008) Such atypical pathologies include progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration, Lewy body dementia, vascular Parkinsonism, normal pressure hydrocephalus and two distinct syndromes; pure akinesia and primary progressive gait freezing.(Factor, Higgins et al. 2006; Factor 2008)

In PD, gait freezing is associated with speech disturbance and postural instability – the axial and proximal deficits of Parkinson’s disease.(Giladi, McDermott et al. 2001) Indeed, the syndrome of FOG and postural instability is considered a subtype of PD.(Selikhova, Williams et al. 2009) Together, these are the major causes of falls in PD.(Kerr, Worringham et al. 2010) However a factor analysis found that, whilst gait freezing and postural instability often occur together, they are distinct, having different risk factors.(van Rooden, Visser et al. 2009) Attentional deficits and psychosis are more common in FOG.(Amboni, Cozzolino et al. 2008; Yogev-Seligmann, Hausdorff et al. 2008; Factor, Steenland et al. 2011) A relationship between FOG and bradykinesia after adjusting for

disease duration has not been found using clinical scales but has been found using gait analysis (see below).(Giladi, McDermott et al. 2001; Bartels, Balash et al. 2003) FOG is negatively associated with tremor, perhaps relating to the more benign disease course reported for tremulous PD.(Giladi, McDermott et al. 2001; Selikhova, Williams et al. 2009; Factor, Steenland et al. 2011)

Certain environmental and internal contingencies are recognised to trigger episodes of gait freezing. These include the need to adjust gait such as starting off, turning, avoiding obstacles and negotiating tight spaces.(Chee, Murphy et al. 2009; Spildooren, Vercruysse et al. 2010; Almeida and Lebold 2010) Other triggers seem to have in common the potential to distract attention away from gait such as dual tasking, for example 'walking whilst talking'.(Spildooren, Vercruysse et al. 2010) For unclear reasons, time demanding situations can also trigger FOG.(Fahn 1995) Relieving factors for FOG are also described. One example is external cues, including visual (such as horizontal lines along a path), auditory (such as a metronome) and somatosensory cues (e.g. wristband pulsed vibrations).(Nieuwboer, Kwakkel et al. 2007) The provision of such sensory cues improves activity in the posterior parietal cortex and cerebellum, presumably relating to sensory integration.(Hanakawa, Fukuyama et al. 1999) Such cues also improve activity in the lateral premotor cortex.(Hanakawa, Fukuyama et al. 1999) The premotor cortex is involved in externally generated movement and thus sensory cues are thought to promote gait by using a relatively intact 'route to action' as an alternative to internally generated movement involving the supplementary motor area.(Jenkins, Fernandez et al. 1992; Hanakawa, Fukuyama et al. 1999) . The relief of freezing due to external cues is distinct from 'paradoxical kinesis' which is mediated by arousal and non-specifically relieves both akinesia and gait freezing.(Asmus, Huber et al. 2008; Robottom, Weiner et al. 2009)

Confusingly, some environmental factors can both trigger and relieve freezing at different times. One example is emotional arousal, the net effect of which could be considered to either distract or recruit attention from walking. Another curious observation is the conserved ability in many patients incapacitated by freezing to ride a bicycle. This perhaps reflects the distinct pathways involved in these two motor acts or, alternatively, the flow of cues, both visual and somatosensory (pedalling), during cycling.(Glickstein and Stein 1991; Snijders and Bloem 2010; Snijders, Toni et al. 2011)

Although considered an episodic deficit, patients with gait freezing also have abnormalities of background gait compared to disease matched patients without gait freezing, namely reduced stride length and increased stride length variability.(Hausdorff, Schaafsma et al. 2003; Chee, Murphy et al. 2009) This suggests that a susceptibility to freezing may be contributed to by lower limb akinesia. Indeed, gait freezing episodes are commonly preceded by a sequential reduction in step length and small steps, deliberately taken, can trigger freezing.(Chee, Murphy et al. 2009) This may in part explain the freezing that occurs with turning, as this requires a reduction in steps length particularly of the inner foot. During freezing episodes, there is a shift of cadence away from the usual 'locomotor frequency' of 2Hz towards higher frequency attempts at stepping of between 3-8Hz with a complex multi-peak appearance on spectral analysis (i.e. it is not a tremor).(Hausdorff, Balash et al. 2003) This observation has been used to detect subtle freezing episodes in the lab.(Delval, Snijders et al. 2010)

Quantifying freezing severity is difficult. Freezing is notorious for disappearing during single session assessments, possibly due to the non-automatic nature of walking when under observation.(Giladi and Nieuwboer 2008) Thus clinical examination and gait lab assessments can bias towards underestimating the disorder. The UPDRS dedicates only

one item to gait freezing, rated by patients out of four.(Fahn S 1987) More detailed is the validated "Freezing of Gait Questionnaire", rated out of 16, which estimates the impact of freezing in patients' usual environments and medication states.(Giladi, Shabtai et al. 2000)

Treatment of gait freezing is often unsatisfactory. Gait freezing is only partially and often poorly responsive to levodopa and subthalamic nucleus stimulation.(Bloem, Hausdorff et al. 2004; Ferraye, Debu et al. 2008) In PD, gait freezing is often classified according to its' response to levodopa – as "off" freezing (that responds to levodopa) or "on" freezing (which persists even in the optimally medicated state). 'On freezing' tends to develop later in the disease course. Occasionally, high doses of dopaminergic medication may even worsen freezing.(Ambani and Van Woert 1973) It has even been suggested, that given the paucity of freezing descriptions in the pre-levodopa era, dopaminergic medication may be a substantial contributor to the pathogenesis of the disorder.(Garcia-Ruiz 2011) Similar to levodopa, subthalamic nucleus (and to a lesser extent, pallidal stimulation) can improve "off" freezing.(Ferraye, Debu et al. 2008) However, STN and pallidal stimulation can also worsen freezing, particularly 'on freezing".(Ferraye, Debu et al. 2008; Adams, Keep et al. 2011) It is reported that this detrimental effect on gait can be minimised by reducing the frequency of subthalamic stimulation from the usual 130Hz to 60Hz (although these lower frequencies are often ineffective for tremor).(Moreau, Defebvre et al. 2008) Pedunculopontine nucleus stimulation proposes to improve freezing, even where resistant to these other treatments. Modest gains are reported with non-pharmacological methods. A common example is providing an ambulatory method of external cueing with a 'laser stick'.(Nieuwboer, Kwakkel et al. 2007) In rehabilitation, patients can be trained to use strategies that optimise cues and step length under challenging circumstances.(Morris, Iansek et al. 2008)

The anatomy of neural networks involved in gait freezing has been investigated by clinical, pathological and imaging studies. Amongst the parkinsonian disorders associated with gait freezing, the distribution of neurodegeneration varies. However, in common, all involve dysfunction of cortico-basal ganglia loops and brainstem structures. In progressive supranuclear palsy, a condition where freezing is common and severe, patients have abnormalities of blink and startle reflexes, implicating dysfunction of midbrain and pons.(Vidailhet, Rothwell et al. 1992) In In PD, patients with gait freezing compared to disease matched controls without freezing had atrophy in the PPN region on voxel based morphometry.(Snijders, Leunissen et al. 2011) In this same study using fMRI during imagined gait, PD patients with freezing compared to controls had abnormally reduced activity in the pontine 'mesencephalic locomotor region' which correlated with FOGQ scores and of the supplementary motor area which correlated with step length. Additionally, there was reduced activation of the posterior parietal cortex, implicated in sensory integration.(Snijders, Leunissen et al. 2011) Implicating attentional deficits, another study using SPECT found reduced blood flow in bilateral orbitofrontal regions in PD patients with gait freezing compared to those without.(Matsui, Udaka et al. 2005)

These observations suggest several common themes of potential relevance for the pathophysiology of gait freezing, listed as follows. 1) Abnormally reduced internally generated movement involving the SMA.(Hallett 2008) SMA dysfunction is associated with reduced step length.(Snijders, Leunissen et al. 2011) Interestingly, deficits in SMA function and step length are potentially responsive to levodopa and subthalamic nucleus stimulation. Some external cues can relieve freezing by offering an alternative route to action via the relatively spared externally generated pathway involving the premotor cortex.(Hanakawa, Fukuyama et al. 1999) 2) Perceptual integration with motor function.

This is implicated by abnormal under-activity of the superior parietal lobule in gait freezing. Of further support is the observation that freezing improves with certain visual cues (such as transverse lines) but is impaired by others (such as doorways).(Hanakawa, Fukuyama et al. 1999; Almeida and Lebold 2010) The cerebellum may also be involved in this integration. 3) Brainstem (particularly pontine) dysfunction in the region of the mesencephalic locomotor region including the pedunculopontine nucleus. However, the exact functional contribution of pontine dysfunction to gait freezing is unclear. 4) Attention and the effective allocation of processing resources that flows from it. In PD, attentional deficits are common and there is also impaired automaticity of movement so that processing demands are higher.(Wu and Hallett 2005; Wu and Hallett 2008) Parkinsonian patients with gait freezing are reported to have even more attentional deficits than those without gait freezing.(Amboni, Cozzolino et al. 2008; Yogev-Seligmann, Hausdorff et al. 2008) Attentional distractors can precipitate gait freezing episodes. Patients with PD have a deficit in the ability to suppress distraction.(Lee, Cowan et al. 2010)

1.3 POSTURAL DISTURBANCE IN PARKINSON'S DISEASE

Postural disturbances in PD include deformity and impaired postural reflexes.

1.3.1 Postural deformity

Prominent postural deformities in PD include camptocormia (flexed trunk), Pisa syndrome (lateral flexion of the trunk) and anterocollis (flexed neck).(Doherty, van de Warrenburg et al. 2011) These disorders typically have in common the property of being present upon

standing and walking and, unless contractures, resolution with recumbency.(Azher and Jankovic 2005) They are usually resistant to dopaminergic medication and pallidal and subthalamic nucleus stimulation.(Azher and Jankovic 2005) The pathophysiology is poorly understood and several factors have been implicated including axial dystonia, rigidity and disordered sensory processing.(Doherty, van de Warrenburg et al. 2011)

1.3.2 Postural instability

Impaired postural reflexes are a cardinal feature of PD and a major cause of falls.(Hughes, Daniel et al. 1992; Kerr, Worringham et al. 2010; Thevathasan and Aziz 2010) The emergence of postural instability is a milestone in disease, defining Hoehn and Yahr stage III.(Hoehn and Yahr 1967)

The common co-existence of postural instability and gait freezing suggests at least some common disease mechanisms. For example, dual tasking also appears to worsen postural instability.(Morris, Iansek et al. 2000) In PD, postural instability is associated with depression.(Factor, Steenland et al. 2011) Like gait freezing, postural instability is less likely in tremulous PD.(Factor, Steenland et al. 2011)

In clinical practice, postural instability is examined by assessing response to the 'pull test'.(Goetz 1986; Munhoz, Li et al. 2004) This makes use of the fact that in postural instability there a pronounced deficit in reflexic adjustments to backwards perturbation compared with forwards and laterally.(Horak, Dimitrova et al. 2005) Upon pulling, patients can appear to 'fall like a tree trunk' with little evidence of any attempt to correct position. Objectively, this has been detected as a lack of compensatory knee bending.(Horak, Dimitrova et al. 2005)

Similar to gait freezing, postural instability is associated with clinically covert continuous deficits. Centre of pressure regulation recorded using posturography in PD reportedly demonstrates increased displacement as measured by sway parameters (e.g. C90 area) and path length/velocity.(Rocchi, Chiari et al. 2002) Displacement may be partly constrained by axial tone in PD.(Maurer, Mergner et al. 2003) This may explain why levodopa and STN and pallidal stimulation all cause displacement to become more abnormal - as they relieve axial rigidity and may thereby unmask further displacement.(Rocchi, Chiari et al. 2002; Maurer, Mergner et al. 2003) Velocity is reduced towards normal by these treatments.(Rocchi, Chiari et al. 2002; Maurer, Mergner et al. 2003)

Postural instability responds poorly to dopaminergic medication. Subthalamic nucleus stimulation only improves postural instability that responds to levodopa.(Visser, Allum et al. 2008) It is reported that pallidal stimulation may improve postural instability more than subthalamic nucleus stimulation.(St George, Nutt et al. 2010) Pedunculopontine nucleus stimulation is proposed to improve postural instability even where resistant to these therapies. Rehabilitation, for example repetitively applying the pull test and encouraging compensatory steps, has shown some modest efficacy.(Jobges, Heuschkel et al. 2004)

1.4 THE PEDUNCULOPONTINE NUCLEUS IN HEALTH

1.4.1 Normal Structure

1.4.1.1. What and where is the PPN?

The Pedunculopontine “nucleus” is a reticular collection of neurons, at the junction of midbrain and pons. (Mesulam, Geula et al. 1989) It is relatively conserved in structure and neurochemistry across species.(Grillner, Wallen et al. 2008) The PPN is phylogenetically

ancient, being present in amphibians and Teleost fish.(Brantley and Bass 1988; Marin, Smeets et al. 1998)

Some brainstem ‘nuclei’ such as cranial nerve nuclei are classified as ‘closed’, with homogenous cellular appearance and confined within demarcated boundaries. However the PPN is an ‘open’ reticular nucleus exhibiting cytological heterogeneity with indistinct boundaries and cells encroaching neighbouring structures. (Mannen 1960; Ramon-Moliner and Nauta 1966)

The PPN was first described by Jacobsohn in 1911 lateral to the decussation of the SCP.(Jacobsohn 1911) Its identity has evolved along with cellular techniques.(Usunoff, Itzev et al. 2003) Olszewski and Baxter identified the PPN based on cytoarchitectural identification of dark staining, large (>20 micron) neurons.(Olszewski and Baxter 1954) Paxinos and Huang employed immunohistochemistry using labelled acetylcholinesterase (AChE).(Paxinos, Tork et al. 1990) However AChE is also expressed by noncholinergic cells. Specific to cholinergic cells is Choline-Acetyltransferase (ChAT), an enzyme in the pathway producing acetylcholine. Immunolabelled ChAT was employed to define the PPN by Mesulam and Manaye.(Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999) Thus although PPN neurons express a variety of neurotransmitters, it has come to be identifiable by the location of its cholinergic neurons.(Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999)

The PPN and laterodorsal tegmental nucleus (LDT) form part of a complex of cholinergic neurons extending from the midbrain to mid pons. (Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999) PPN neurons are nominated as ChAT5 and LDT neurons as ChAT6. This nomenclature extends to involve other components of the cholinergic system

including the basal forebrain (Chat1-4) and other brainstem nuclei.(Mesulam and Geula 1988)

The exact location of the PPN is controversial. Given the implications for surgical targeting, this is covered in detail. PPN neurons are densely packed together at the 'pars compacta' (PPNc). The PPNc, as defined by ChAT staining, is located along the dorsolateral border of the SCP as the "SCP ascends towards the dorsolateral pons towards its' decussation".(Manaye, Zweig et al. 1999) This suggests a rostrocaudal level below the inferior colliculus (depending on the axial plane taken). The PPNc extends for about 2-3mm.(Manaye, Zweig et al. 1999) The PPNc is surrounded along its' rostro-caudal extent ventrally by the 'pars dissipata' (PPNd) which is wedged between the superior cerebellar peduncle and the lemniscal system. The PPNd is a reticular network of neurons with cells that invade neighbouring structures including the cuneiform, superior cerebellar peduncle and locus coeruleus.(Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999) PPNd neurons are more numerous than PPNc neurons rostrally, which is also where the majority of non-cholinergic neurons are located. The PPNd extends for a rostrocaudal distance of about 7mm.(Manaye, Zweig et al. 1999) The entire ChAT5 complex extends rostrally from the substantia nigra ventrally and red nucleus dorsally to its' caudal pole bordered ventrally by the pontine reticular formation and locus coeruleus and dorsally by the cuneiform nucleus. (Pahapill and Lozano 2000)The LDT is located medial and caudal to the PPN, but intermingling neurons means there is no clear PPN-LDT margin.(Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999) The entire PPN-LDT complex extends for a rostrocaudal distance of around 10mm.(Manaye, Zweig et al. 1999)

The majority of ChAT positive neurons are actually in the PPNd not PPNc, just less compactly organised. However, cholinergic neurons comprise the greater proportion of

neurons in the PPNc than PPNd. The LDT accounts for only around 13% of cholinergic neurons in the complex. The point identified as containing the highest population of cholinergic cell bodies, whether in PPN pars compacta or dissipata, is between 2-6mm below the 'point of the midbrain'.(Manaye, Zweig et al. 1999)

Several issues have complicated the accurate surgical localisation of the PPN. The reticular 'open' nature of the PPN means it can't be seen on conventional MRI.(Zrinzo, Zrinzo et al. 2008) The PPNc can possibly be visualised on specialised 3Tesla proton density scans and its relative location aided by diffusion weighted tractography.(Aravamuthan, Stein et al. 2008; Zrinzo, Zrinzo et al. 2008) The PPN is not mentioned in some commonly used atlases (e.g. Schaltenbrand). In others its' location is defined only by cytoarchitectural methods.(Plaha and Gill 2005; Zrinzo, Zrinzo et al. 2008; Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) A stereotactic atlas that identifies the PPN with ChAT staining has not been developed. It is possible that the reliance on cytoarchitecture by Olszewski and Baxter has caused the caudal extent of the nucleus to be underestimated although this requires further research.(Zrinzo, Zrinzo et al. 2008) Adding further confusion are the various terminologies used to denote the PPN such as 'PPTg'. This may explain the erroneously implantation of electrodes in the peripeduncular nucleus by one group.(Stefani, Lozano et al. 2007; Yelnik 2007; Zrinzo, Zrinzo et al. 2007; Zrinzo, Zrinzo et al. 2007) Variability of brainstem position, size and shape between individuals means that indirect targeting using a distant reference such as the commissure is inappropriate. Direct targeting of the region is necessary and this requires expertise.

1.4.1.2 Neurotransmitter expression:

Although the PPN is defined by its' cholinergic cells, noncholinergic PPN neurons are more numerous in the human.(Manaye, Zweig et al. 1999) Cholinergic cells are the predominant cell type in PPNc, whereas noncholinergic cells predominate in the PPNd.(Manaye, Zweig et al. 1999) At least in rodents, cholinergic PPN neurons do not co-express other neurotransmitters (contrary to previous held notions).(Wang and Morales 2009) PPN cholinergic neurons are heterogeneous, displaying more than one firing characteristic and engage in different phases of cortical oscillations (see below). (Takakusaki, Shiroyama et al. 1997; Mena-Segovia, Sims et al. 2008)

The major noncholinergic neurotransmitter types are Glutamate and γ-aminobutyric acid (GABA), which are expressed by both PPN and LDT.(Wang and Morales 2009) Glutamatergic cells (identified by expression of glutamate transporters – GLUT) are found throughout the rostro-caudal extent of the PPN. GABAergic cells (identified by GAD antibodies) are clustered most densely in the rostral PPN.(Mena-Segovia, Micklem et al. 2009; Martinez-Gonzalez, Bolam et al. 2011) PPN neurons are also reported to express substance P and nitrous oxide.(Reese, Garcia-Rill et al. 1995) Noncholinergic cells also exhibit a range of firing characteristics including bursting and random firing (see below).(Kang and Kitai 1990) Non cholinergic cells are also classified according to expression of differing Calcium binding proteins (calbindin and calretinin).(Martinez-Gonzalez, Bolam et al. 2011)

1.4.1.3 Connectivity:

An important constraint when interpreting connectivity data is that the size of a pathway does not necessarily reflect its functional significance. For example, the relatively modest

pallidostriatal pathway is of substantial functional significance because it modulates striatal interneurons.(Bevan, Booth et al. 1998) Lesioning and activation studies coupled with neuronal recordings provide support for functional significance. The techniques used to assess connectivity each have their limitations. Anterograde and retrograde tracer studies can inform about both the presence and directionality of pathways. However, tracer studies are performed in animal models and are prone to error if tracer spreads into neighbouring regions and their pathways.(Kobbert, Apps et al. 2000) DTI can be used in vivo in humans, however it provides no information regarding directionality and it similarly prone to errors of 'seeding' neighbouring pathways.(Johansen-Berg and Behrens 2006)

The major connections of the PPN are with the Basal ganglia, thalamus, motor cortical areas as well as brainstem nuclei and spinal cord – and to the other PPN.(Jenkinson, Nandi et al. 2008) Ascending connectivity with basal ganglia, thalamus and cortex is more prominent ipsilaterally than contralaterally, raising the possibility than each PPN may be more relevant in contralateral movement.(Grofova and Keane 1991; Jenkinson, Nandi et al. 2008)

Afferents to the PPN are as follows. The PPN receives fast 'unprocessed' auditory sensory input from the cochlear nucleus.(Reese, Garcia-Rill et al. 1995; Reese, Garcia-Rill et al. 1995) From the indirect pathways of the basal ganglia, the PPN receives prominent GABAergic input from the GPi and also SNr and a small glutamatergic projection from the STN.(Nauta and Mehler 1966; Smith, Hazrati et al. 1990; Rye, Turner et al. 1996) These pathways provide a mechanism by which the basal ganglia could control movement separate to its impact on the thalamo-cortical circuit.(Forman and Ward 1957) Connectivity between the PPN and internal pallidum appears important, with a majority of

GPi neurons being antidromically activated by PPN stimulation in the monkey.(Harnois and Filion 1980) Cortical motor areas send afferents directly to PPN in the monkey which appear to be somatotopically organised.(Matsumura, Nambu et al. 2000) Connectivity between the PPN and deep cerebellar nuclei raises the possibility that the PPN is where the basal ganglia and cerebellum can interact.(Hazrati and Parent 1992; Jenkinson, Nandi et al. 2008) Afferent from the monoaminergic brainstem nuclei (raphe and locus coeruleus) are thought to have inhibitory influences on the PPN which may help regulate of sleep and wakefulness (see below).(Garcia-Rill, Charlesworth et al. 2008)

Efferents from the PPN are as follows. PPN cholinergic and glutamatergic neurons project to the SNc and the LDT projects especially to the ventral tegmental area, providing pathways by which the PPN-LDT complex can influence motor behaviour through motivation and reward.(Mesulam, Mash et al. 1992; Lavoie and Parent 1994; Mena-Segovia, Winn et al. 2008) The PPN send reciprocal projection back to the STN (cholinergic, glutamatergic and GABAergic) and reciprocal projections back to the GPi, thus potentially modulating activity in the indirect pathway of the basal ganglia.(DeVito, Anderson et al. 1980; Lavoie and Parent 1994; Bevan and Bolam 1995) PPN projects to striatum (both caudate and putamen).(Lavoie and Parent 1994) Cholinergic PPN neurons project to the nonspecific midline and intralaminar thalamic nuclei, providing the major cholinergic innervation of this structure consistent with the role of the PPN in the Reticular activating system (RAS).(Steriade, Pare et al. 1988)Reciprocal connections from the PPN project to motor cortical areas and frontal eye fields.(Matsumura, Nambu et al. 2000) The major descending projections from the PPN are to the reticular formation including the gigantocellular nucleus, nucleus reticularis pontis caudalis and oralis. These connections are stronger ipsilaterally than contralaterally.(Mitani, Ito et al. 1988; Grofova and Keane

1991) The PPN also projects to the spinal cord, providing a direct mechanism for control of spinal central pattern generators.(Skinner, Kinjo et al. 1990)

DTI has supported the existence of such pathways in vivo in man. (Aravamuthan, Muthusamy et al. 2007; Muthusamy, Aravamuthan et al. 2007)

1.4.1.4 Neuronal firing patterns:

PPN neurons differ in their intrinsic membrane properties and firing characteristics demonstrated by in-vitro slice preparations using intracellular recordings.(Kang and Kitai 1990; Takakusaki and Kitai 1997; Takakusaki, Shiroyama et al. 1997) Burst non-rhythmic firing neurons (type 1) are noncholinergic (ChAT negative).(Kang and Kitai 1990) Non-burst rhythmic firing neurons (type 2) are both ChAT positive and negative.(Kang and Kitai 1990) Based on intracellular recordings, non-burst type 2 neurons were divided into 2 groups, both of which were about 50% ChAT positive: Short duration action potentials that fire frequently and long duration action potentials with less frequent tonic firing.(Takakusaki, Shiroyama et al. 1997) Burst and non-burst neurons have membrane potentials that oscillate at about 11 and 8-9Hz respectively. (Takakusaki and Kitai 1997) All PPN neuron types, at least in rodents, tend to fire at gamma frequencies. (Simon, Kezunovic et al. 2010; Kezunovic, Urbano et al. 2011)

In healthy monkeys and also in patients in patients with Parkinson's disease, in-vivo extracellular single unit recordings have also demonstrated burst (the minority) and as well as non-burst (the majority) random firing neuron types.(Matsumura, Watanabe et al. 1997; Weinberger, Hamani et al. 2008) In humans, non-burst random firing neurons with the longest action potential duration and lowest firing rate were presumed to be cholinergic

and tended to be recorded at and below the PPN region (as defined by the investigators).(Weinberger, Hamani et al. 2008) Within the investigator defined PPN region, beta oscillations were recorded. All neuron types responded to movement.(Weinberger, Hamani et al. 2008)

In rodents, an interaction between single cell firing and cortical oscillations has been investigated with the juxtacellular technique in a series of experiments in rodents. Cholinergic neurons fired rhythmically at low frequency in time with phases of cortical slow wave oscillations, most prominently during the peaks but some neurons fired in the trough.(Mena-Segovia, Sims et al. 2008) Noncholinergic neurons were classified based on their in-vivo firing characteristics in relation to slow wave cortical oscillations as quiescent, tonic and irregular firing.(Ros, Magill et al. 2010) Some noncholinergic cells fired in time with cortical slow waves (particularly during troughs) but the majority were uncorrelated.(Mena-Segovia, Sims et al. 2008; Ros, Magill et al. 2010) In accordance with these findings, cholinergic neurons were found to arborize incredibly widely including to cortex.(Mena-Segovia, Sims et al. 2008) Noncholinergic cells had more restricted local connectivity, for example with the basal ganglia.(Mena-Segovia, Sims et al. 2008; Ros, Magill et al. 2010)

1.4.1.5 PPN internal architecture: Is there topographical organisation?

The PPN is clearly contains a heterogeneous group of cells. Even cells which express the same neurotransmitter, display a diverse array of connectivity, intrinsic firing properties and engagement with oscillations.

However, a cumulative body of data from animal models suggests that there may be a topographical organisation to these elements that helps make sense of this complexity.(Martinez-Gonzalez, Bolam et al. 2011) GABAergic PPN neurons have been found most densely in the rostral PPN.(Ros, Magill et al. 2010) Glutamatergic neurons are located along the entire rostrocaudal extent of the nucleus.(Martinez-Gonzalez, Bolam et al. 2011) Cholinergic neurons are also located along the nucleus but particularly at the caudal extent.(Martinez-Gonzalez, Bolam et al. 2011) The noncholinergic cells have restricted connectivity, particularly with the basal ganglia.(Mena-Segovia, Sims et al. 2008; Ros, Magill et al. 2010) Cholinergic neurons arborize widely, to basal ganglia, thalamus, cortex as well as descending to locomotor centres.(Mena-Segovia, Sims et al. 2008; Martinez-Gonzalez, Bolam et al. 2011) However, it is unknown if such topography exists in humans, or if this would have implications for where stimulation should be applied for gait and postural disorders in Parkinson's disease.

1.4.2 Normal Function

The PPN is implicated in diverse (and seemingly incongruous) functions. It is considered part of the reticular activating system modulating states of wakefulness and sleep. It is also a component of the mesencephalic locomotor region, modulating spinal central pattern generators that produce rhythmical limb movements.

1.4.2.1 Modulation of states of wakefulness and sleep

Magoun recognised that stimulation of the tegmentum desynchronised the electroencephalogram (EEG).(Moruzzi and Magoun 1949) This property defines

membership of the reticular activating system which includes the PPN, LDT, locus coeruleus and raphe nucleus and is likely mediated via influences on the intralaminar nuclei of the thalamus and thalamocortical projections.(Pahapill and Lozano 2000; Garcia-Rill, Charlesworth et al. 2008) EEG desynchronisation is a feature of both wakefulness and REM sleep. The PPN is thought to have additional roles in REM sleep including production of REM sleep atonia via influences on the pontine reticular formation as well as generation of ponto-geniculo-occipital (PGO) waves. (Mitler and Dement 1974) Consistent with the above, lesions to cholinergic cells in the PPN region in rodents diminishes REM sleep, REM sleep atonia and PGO waves.(Jones 1991)

Consistent with these ideas, in patients with PD, stimulation of PPN increases REM sleep and can even acutely precipitate it.(Romigi, Placidi et al. 2008; Lim, Moro et al. 2009; Arnulf, Ferraye et al. 2010) No study has yet addressed if PPN stimulation may improve REM sleep atonia and REM sleep behavioural disturbance – or cognitive effects though to be promoted by REM sleep such as motor learning.

That the PPN may increase both wakefulness and REM sleep seems paradoxical. However, the answer may lie in the circuitry involving the PPN, locus coeruleus and raphe nucleus.(Garcia-Rill, Charlesworth et al. 2008) These monoaminergic nuclei have inhibitory effects on the PPN during wakefulness but not sleep.(Garcia-Rill, Charlesworth et al. 2008) It is therefore proposed that REM sleep reflects full expression of PPN activity whereas some features of PPN activity such as PGO waves and atonia are suppressed during wakefulness.(Garcia-Rill, Charlesworth et al. 2008)

1.4.2.2 Motor control including locomotion

A role in motor function is suggested by the connectivity of the PPN. The PPN modulates pontine nuclei that give rise to reticulospinal pathways which primarily innervate proximal and axial musculature.(Mitani, Ito et al. 1988; Grofova and Keane 1991) The PPN also send descending projections to the spinal cord which can influence spinal central pattern generators.(Skinner, Kinjo et al. 1990) Descending projections from the basal ganglia to the PPN provide a potential conduit to spinal motor neurons separate to the thalamocortical loop.(Jenkinson, Nandi et al. 2008)

PPN neuronal firing responds to ipsilateral and contralateral voluntary limb movements in monkeys and patients with PD.(Matsumura, Watanabe et al. 1997; Weinberger, Hamani et al. 2008) Local field potentials recorded from PPN electrodes in PD patients are also responsive to self-paced joystick movements. Event related synchronisation occurred in the alpha band many seconds prior to movement. This interval is too long for immediate motor preparation, rather it suggests attentional recruitment.(Androulidakis, Mazzone et al. 2008; Tsang, Hamani et al. 2010) As found in STN local field potentials, beta desynchronisation also occurs in PPN local field potentials just before and during movement.(Tsang, Hamani et al. 2010)

The PPN is a component of the ‘mesencephalic locomotor region’, a region defined simply by the ability of electrical stimulation to produce stepping movements. (Orlovskii, Severin et al. 1966) This appears to be a relatively nonspecific feature of any region with connectivity, orthodromic or antidromic, with central pattern generators in the spinal cord.(Reese, Garcia-Rill et al. 1995) Some of these MLR areas may not actually have any direct role in the control of locomotion. However, PPN neurons are responsive to locomotion, even ‘fictive’ locomotion (which excludes sensory afference) indicating it is

functionally relevant to gait.(Garcia-Rill and Skinner 1988; Karachi, Grabli et al. 2010) Recordings dorsal to the PPN, a region consistent with the cuneiform region, have demonstrated burst neuronal firing in time with stepping, both in animals during fictive locomotion and PD patients during microelectrode recordings and mimicked stepping.(Garcia-Rill and Skinner 1988; Piallat, Chabardes et al. 2009) Thus cuneiform neurons are proposed to regulate cadence. Recordings from the PPN in rodents have demonstrated two neuronal types according to gait – neurons that fire tonically during gait ('on' cells) and those that respond when locomotion is initiated or terminated ('off' cells).(Garcia-Rill and Skinner 1988) 'Off' cells may thus have a role in the adjustment of gait in response to the environment.

The PPN is also proposed to influence postural control via modulation of pontine tegmental areas which increase or decrease postural tone.(Mori, Kawahara et al. 1982) This may also be relevant to the production of REM sleep atonia by the PPN.(Jones 1991)

1.4.2.3 Modulation of acoustic reflexes

The PPN has been identified as a likely effector of prepulse inhibition of the acoustic startle and blink reflexes. For example, in rats PPN lesions increase acoustic startle reflex amplitude and abolish prepulse inhibition without affecting long term habituation.(Swerdlow and Geyer 1993) This circuit is likely to involve PPN cholinergic inputs to the Nucleus reticularis pontis caudalis.(Bosch and Schmid 2006)

The PPN is also implicated as the source or in gating of the auditory P50 (P1) evoked response. A distinguishing feature of the P1 apart from its' latency is that it habituates with auditory stimuli over 1Hz.(Erwin and Buchwald 1986) Several features suggest that the

P50 is modulated by the PPN. It is state dependant – occurring only during wakefulness and REM sleep.(Erwin and Buchwald 1986) It is abolished by intravenous injection of the anticholinergic scopolamine.(Buchwald, Rubinstein et al. 1991) The P50 equivalent can be recorded in the reticular formation and intralaminar thalamus in single unit recordings in animals.(Hinman and Buchwald 1983) In cats, the P50 equivalent correlated in amplitude with the degree of cholinergic cell loss in the PPN.(Harrison, Woolf et al. 1990) This raises the possibility that the P50 in humans could be a biomarker of cholinergic PPN activity. However a paradox is that the drug Modafanil, which suppresses REM sleep, increases rather than decreases p50 amplitudes.(Garcia-Rill, Simon et al. 2010)

1.4.2.4 Motivation and Reward

Cholinergic and glutamatergic projections from PPN to the SNc and LDT to the ventral tegmental area provide a potential "interface between brainstem motor systems and reward".(Pahapill and Lozano 2000) The potential modulation of dopaminergic systems by the PPN and its' relationship to other PPN functions needs further exploration.(Alderson, Latimer et al. 2004) For example, one proposal is that this would provide a mechanism by which arousing auditory stimuli can trigger motivation that my accompany (or even partly be responsible for) paradoxical kinesis.(Pahapill and Lozano 2000)

1.4.3 What does the PPN do? An attempt at integrating structure and function

That the PPN is a phylogenetically ancient structure and conserved over species suggests a fundamental role for survival. Its' connectivity suggests that it is not just a relay but in a position to modify behaviour. The PPN receives rapid, unprocessed sensory information,

modulates startle and blink responses, can arouse and motivate the animal as well as activate movement via reticulospinal pathways and recruit locomotion. One could therefore speculate a role in the fright/flight reaction and externally triggered movement. The reciprocal connectivity with the basal ganglia suggests that activity produced by the PPN is coordinated with action selection processes in cortico-basal ganglia-thalamic loops (mediated largely through corticospinal pathways).

1.5 THE PEDUNCULOPONTINE NUCLEUS IN PARKINSON'S DISEASE

1.5.1 Pathology

PPN neurons, both cholinergic and non-cholinergic, degenerate in PD and even more so in PSP.(Hirsch, Graybiel et al. 1987; Zweig, Whitehouse et al. 1987; Jellinger 1988; Zweig, Jankel et al. 1989; Rinne, Ma et al. 2008; Karachi, Grabli et al. 2010) Cholinergic PPN neuron degeneration is relatively specific to PD and PSP compared to aged controls and Alzheimer's disease (where cholinergic degeneration is focussed on the forebrain cholinergic system).(Zweig, Whitehouse et al. 1987; Jellinger 1988; Zweig, Jankel et al. 1989) Cholinergic cells loss in PD is most prominent in the caudal PPN.(Rinne, Ma et al. 2008) Cholinergic cells loss in the PPN in PD correlates with degeneration of the SNPc and with Hoehn and Yahr disease staging, of which gait and balance is an important determinant.(Rinne, Ma et al. 2008) However, in man, there is no convincing evidence that PPN cholinergic cell loss in PD is related specifically to gait, balance and falls after controlling for disease stage.(Karachi, Grabli et al. 2010) However, PPN cholinergic cells loss in normal and parkinsonian monkeys has been associated with balance and gait disturbance. (Karachi, Grabli et al. 2010)

1.5.2 Abnormal influences from afferent pathways

The PPN is likely to be influenced by abnormal activity in the Parkinsonian basal ganglia through their reciprocal connections. Increased activity in the indirect pathway in PD would be predicted to cause excessive GABAergic inhibitory influences from the GPi and SNr and increased glutamatergic influences from the STN.(Aziz, Davies et al. 1998) However, the net effect from these inhibitory and excitatory influences on the PPN is unclear and it is argued whether the PPN is 'overactive' or 'underactive' in PD. (Garcia-Rill, Simon et al. 2010) Connectivity with the parkinsonian basal ganglia could also cause the PPN to become engaged in abnormal beta band synchronisation.(Hammond, Bergman et al. 2007)

In the MPTP primate model, 2-deoxyglucose (2DG) autoradiograph studies found increased uptake in the PPN.(Mitchell, Boyce et al. 1992; Gnanalingham, Milkowski et al. 1995) However, 2DG activity is reflects synaptic activity, not discriminating between excitatory or inhibitory.(Schwartz, Smith et al. 1979; Mata, Fink et al. 1980; Bezard, Crossman et al. 2001) This increased PPN activity in PD was corroborated by metabolic markers in OHDA lesioned rats.(Orieux, Francois et al. 2000) In OHDA lesioned rats, the firing rate of PPN neurons was abnormally increased and this normalised with STN lesioning – suggesting that the glutamatergic STN influences on the PPN predominate over GABAergic pallidofugal influences. However, the firing pattern of PPN neurons in OHDA lesioned rats, whilst increased, was also abnormal with a greater proportion of random rather than tonic firing.(Breit, Bouali-Benazzouz et al. 2001) Thus it is not clear if the increased but abnormal firing of PPN neurons would translate into an increase or decrease of information coding and PPN function.

1.5.3 Evidence of functional consequences of abnormal PPN activity in patients with PD

Several in vivo studies have suggested that abnormal PPN activity is functionally relevant to motor deficits in patients with PD. Neurophysiological studies have demonstrated that habituation of the p50 is abnormally *decreased* in PD, with less habituation with greater disease stage.(Teo, Rasco et al. 1997) Furthermore, there was a trend suggesting increased amplitude of p50 in PD compared with healthy controls.(Teo, Rasco et al. 1997) This has been taken to suggest that PPN activity is abnormally *increased* in PD.(Garcia-Rill, Simon et al. 2010) However, it is unclear if the subgroup of PD with severe FOG might behave differently to other PD. Diminished prepulse inhibition of the acoustic blink reflex in has been a variable finding in PD.(Morton, Chaudauri et al. 1995; Valls-Sole, Munoz et al. 2004; Costa, Valls-Sole et al. 2006)

A PET study in PD using labelled anticholinesterase found a reduction in thalamic cholinergic activity (which is largely derived from PPN projections) which correlated with falls.(Bohnen, Muller et al. 2009) Interestingly dopaminergic depletion did not, after controlling for cofactors of disease stage and cholinergic activity, correlate with falls frequency. As freezing of gait and PI are the major causes of falls in PD this is one of the few studies that have related these deficits to cholinergic PPN function in patients.(Kerr, Worringham et al. 2010)

1.6 PPN STIMULATION FOR PARKINSON'S DISEASE

1.6.1 Development as a therapeutic target in PD

The possibility that motor symptoms in PD may partly be due to PPN dysfunction that may be reversible through disinhibition, suggested it as a therapeutic target.(Aziz, Davies et al. 1998) This was explored through a series of experiments in the nonhuman primate, which to summarise, suggested that reversal of an underactive PPN could improve motor symptoms.

Radiofrequency PPN lesions in healthy monkeys caused motor deficits, termed 'akinesia'.(Aziz, Davies et al. 1998) However, this finding was curious in several respects. Firstly, the akinesia persisted only when lesions were bilateral. 'Akinesia' was reported largely on the basis of reduced movement counts which can have many causes including reduced volition. Indeed, the monkeys were observed to move normally in reflexic fashion to noxious stimuli but lacked goal directed motor activity. Additionally, the radiofrequency lesions extended into structures including the cuneiform.(Aziz, Davies et al. 1998) However, similar findings were replicated in further studies that lesioned the PPN in normal moneys using excitotoxic lesions with kainic acid, GABA agonism with muscimol and high frequency stimulation.(Matsumura and Kojima 2001; Nandi, Liu et al. 2002) A more recent study has offered more detail on the type of motor deficits seen in monkeys from PPN lesions, reporting deficits predominantly of posture and ambulation in addition to limb rigidity.(Karachi, Grabli et al. 2010)

In the MPTP primate model of PD, infusion of bicuculline (a GABA antagonist) into the PPN improved movement counts, postural stoop but not tremor.(Nandi, Aziz et al. 2002) Interestingly, unilateral bicuculline appeared to have bilateral effects. (Nandi, Aziz et al. 2002) The effects of bicuculline were mimicked by low frequency PPN stimulation of around 5-10Hz.(Jenkinson, Nandi et al. 2004) Dopamine produced additional benefits to

low frequency PPN stimulation and together they restored normal movement to the MPTP monkey.(Jenkinson, Nandi et al. 2006)

A further observation that deserves comment is that monkeys with PPN lesions experienced less SNPc degeneration following MPTP administration.(Matsumura and Kojima 2001) If MPTP SNc destruction is an activity dependant process, then this could be explained through reduced glutamatergic inputs from the PPN to the SNPc. However, It is unclear if this finding has relevance for patients with progressive neurodegenerative Parkinson's disease.

1.6.2 PPN stimulation as a therapy for patients with Parkinsonian disorders

The finding that PPN stimulation alleviated movement abnormalities in the MPTP primate was translated extremely rapidly to treat parkinsonian patients by groups in Bristol and Rome.(Mazzone, Lozano et al. 2005; Plaha and Gill 2005) Low frequencies of stimulation were employed around 20-30Hz. These early studies noted an effect of PPN stimulation that mimicked those found in the nonhuman primate – an improvement in akinesia in addition to gait and posture.

This was later followed by a series of six patients with dual STN and PPN stimulators.(Stefani, Lozano et al. 2007) This study suggested modest improvements to akinesia and more marked benefit for FOG and PI. The benefits of STN and PPN DBS were said to be complementary. However a major issue arose regarding the target which appeared to lie in the neighbouring peripeduncular nucleus. This prompted debate about the exact location of the PPN and this remains a controversial issue.(Yelnik 2007; Zrinzo and Hariz 2007; Zrinzo, Zrinzo et al. 2007; Zrinzo, Zrinzo et al. 2008)

Two further clinical series have reported PPN stimulation in two differing scenarios - dual bilateral STN and PPN stimulation and single target unilateral PPN stimulation.(Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) These studies have reported very modest therapeutic effects from PPN stimulation, largely limited to gait freezing and postural instability. One study found very limited effects even on gait freezing and questioned the clinical utility of this treatment.(Ferraye, Debu et al. 2009) However, several aspects to the clinical application of PPN stimulation in these studies could have affected efficacy. Some patients were selected for PPN stimulation with severe motor fluctuations requiring STN stimulation and variable degrees of gait disturbance.(Stefani, Lozano et al. 2007; Ferraye, Debu et al. 2009) For example, patients were selected for PPN stimulation to treat FOG that developed during STN stimulation. Other patients had been selected for PPN stimulation who had not experienced gait freezing that persisted 'on medication' or recurrent falls.(Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) Furthermore, it is possible that co-stimulation of the STN could influence the efficacy of PPN stimulation particularly given their substantial reciprocal connections.(Jenkinson, Nandi et al. 2008) In this regard, it should be noted that high frequencies required for STN stimulation (eg 130Hz) appear to worsen gait when delivered to the PPN. The PPN was targeted above the pontomesencephalic junction, which ChAT5 staining studies in humans suggests could have missed the caudal extent of the nucleus, which is most degenerate in PD.(Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999; Rinne, Ma et al. 2008) It is unclear if unilateral stimulation is as effective as bilateral stimulation. Finally, outcomes measured with the Unified Parkinson's Disease Rating Scale may lack sensitivity for gait and posture. Indeed, the precise effects of PPN stimulation on motor function in PD including gait is not established.(Peppe, Pierantozzi et al. 2010)

1.7 RESEARCH QUESTIONS

This research has three major aims:

1. Assess the structure and function of the PPN in Parkinsonian patients, particularly that which may be relevant to the clinical application of PPN stimulation. One critical issue is whether the PPN is topographically organised in man and whether a particular subregion is most relevant for gait, as the dorsolateral STN subregion is for akinesia. Another question is whether any oscillatory activity in the PPN correlates with gait and may thus be potentially useful as a biomarker to guide electrode placement.

2. Address key controversies regarding the clinical application of PPN stimulation in PD. These controversies are; nature of the effects of PPN stimulation on parkinsonian symptoms including precise effects on gait which may guide patients selection, optimal target location along the rostro-caudal long axis of the nucleus, relative efficacy of unilateral and bilateral stimulation, frequency dependant effects of stimulation and therapeutic outcome assessment using objective measures.

3. Assess the pathophysiological mechanisms of gait freezing and postural instability in PD and the therapeutic mechanisms of PPN stimulation. A key question regards the functional nature of the deficit in the pons in patients with gait freezing and postural instability and its' reversal by low frequency stimulation of the PPN region.

Chapter 2: Methods

2.1 Patients implanted with PPN stimulators

The dominant symptomatic issue in patients implanted with PPN stimulators at all study centres was severe FOG and PI persisting even "on medication", causing frequent falls. In PD, FOG/PI becomes commoner and tends to be less medication responsive as the disease progresses.(Bloem et al. , 2004, Giladi et al. , 2001) The overall prevalence of FOG/PI in PD is approximately 50%.(Macht et al. , 2007) However severe "on medication" FOG/PI as the predominant issue is unusual in PD 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 PD is presumptive.

2.2 Clinical assessments

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 of dopaminergic therapy. These clinical assessments at all centres (in two countries) were performed unblinded by the same neurologist specialised in movement disorders. UPDRS was segmented into items 27-30 (IT27/30, 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 FOG, festination and falls.(Giladi et al. , 2000) The Freezing of

Gait Questionnaire (FOGQ, score/24) and Falls Question (FallsQ, score/4) are components of GFQ.(Giladi et al. , 2000, Giladi et al. , 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.

2.3 Localisation of electrode contacts and stimulation sites

Sites of stimulation were localised as being midway between active contacts for bipolar stimulation and cathodes for monopolar stimulation. Contacts were identified on post-operative computerised tomography fused with pre-operative magnetic resonance images and transformed onto Montreal Neurological Institute (MNI) space using the fMRIB Software Library by a fellow researcher.(Smith, Jenkinson et al. 2004) Coordinates were calculated in; millimetres from midline (laterality), ventrodorsal distance (d) from floor of the fourth ventricle and rostro-caudal distance (h) from a pontomesencephalic (PM) line connecting the pontomesencephalic junction to the caudal end of the inferior colliculi, as described previously.(Ferraye, Debu et al. 2009) The relative location/extent of the PPN is outlined in figures, based on choline-acetyltransferase immunohistochemical (ChAT5) staining in the human.(Mesulam, Geula et al. 1989)

Chapter 3:

Alpha oscillations in the pedunculopontine nucleus correlate with gait performance in Parkinsonism

3.1 Abstract

The pedunculopontine nucleus, a component of the reticular formation, is topographically organised in animal models and implicated in locomotor control. In Parkinson's disease, pedunculopontine nucleus stimulation is an emerging treatment for gait freezing. Local field potentials recorded from pedunculopontine nucleus electrodes in such patients have demonstrated oscillations in the alpha and beta frequency bands, reactive to self-paced movement. Whether these oscillations are topographically organised or relevant to locomotion is unknown. Here, we recorded local field potentials from the pedunculopontine nucleus in parkinsonian patients during rest and unconstrained walking. Relative gait speed was assessed with trunk accelerometry. Peaks of alpha power were present at rest and during gait, when they correlated with gait speed. Gait freezing was associated with attenuation of alpha activity. Beta peaks were less consistently observed across rest and gait, and did not correlate with gait speed. Alpha power was maximal in the caudal pedunculopontine nucleus region and beta power was maximal rostrally. These results indicate a topographic distribution of neuronal activity in the pedunculopontine nucleus region and concur with animal data suggesting that the caudal subregion has particularly relevance to gait. Alpha synchronisation, proposed to suppress 'task irrelevant' distraction, has previously been demonstrated to correlate with performance of cognitive

tasks. Here we demonstrate a correlation between alpha oscillations and improved gait performance. The results raise the possibility that it is the caudal pedunculopontine nucleus region that may be the optimal surgical target for gait disturbances in Parkinson's disease, and offer a candidate functional intra-operative marker of this region.

3.2 Introduction

The pedunculopontine nucleus (PPN) is a reticular collection of neurons, located at the junction of midbrain and pons.(Jacobsohn 1911; Olszewski and Baxter 1954) The PPN, at least in animal models, appears to be topographically organised, with γ-aminobutyric acid (GABA) expressing neurons predominating rostrally and cholinergic and glutamatergic neurons caudally.(Martinez-Gonzalez, Bolam et al. 2011) In Parkinson's disease (PD), cholinergic PPN neurons degenerate and this cell loss has been associated with gait dysfunction.(Hirsch, Graybiel et al. 1987; Zweig, Jankel et al. 1989; Rinne, Ma et al. 2008; Karachi, Grabli et al. 2010) PPN neurones in PD may also be disrupted through their reciprocal connectivity with the basal ganglia.(Breit, Bouali-Benazzouz et al. 2001; Mena-Segovia, Bolam et al. 2004) In patients with PD, deep brain stimulation of the PPN at low frequencies is an emerging treatment for postural instability and gait freezing.(Mazzone, Lozano et al. 2005; Plaha and Gill 2005; Stefani, Lozano et al. 2007; Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010; Thevathasan, Coyne et al. 2011)

The control of locomotion likely involves coordination between spatially segregated nervous system regions in order to modulate the rhythmic activity produced by spinal central pattern generators.(Grillner, Wallen et al. 2008) Synchronised oscillatory neuronal activity is proposed to bind together such neuronal assemblies and enhance information

representation – whilst also suppressing task irrelevant or competing processes.(Fries 2005; Schoffelen, Oostenveld et al. 2005; Jensen and Mazaheri 2010) Local field potential recordings from DBS electrodes implanted in the PPN in parkinsonian patients have variably been reported to demonstrate alpha or beta band oscillations, reactive to self-paced movement.(Androulidakis, Khan et al. 2008; Androulidakis, Mazzone et al. 2008; Tsang, Hamani et al. 2010) Whether these different oscillatory patterns conform to any topographic distribution or are relevant to locomotion is unknown.

In this study, we assessed local field potentials from Parkinsonian patients implanted with PPN electrodes and assessed the spatial pattern of oscillatory activity in the region and its relationship to the performance of gait.

3.3 Methods

3.3.1 Subjects and clinical assessments

Seven patients with PD implanted with DBS electrodes in the PPN region were assessed (table). Following their initial implantation, electrodes were attached to special extension cables ‘externalised’ through the scalp to permit connection with recording equipment. Final implantation of DBS hardware was therefore deferred for the period of ‘externalisation’ (3-7 days). Eleven PPN electrodes were recorded from the seven patients. Patients were recruited from hospitals in Oxford and London, UK. Local ethics committee approval was obtained from both centres and participants gave written informed consent. Reaction time results from one of the patients have been reported in previous publications.(Thevathasan, Silburn et al. 2010; Thevathasan, Pogosyan et al. 2011)

The indication for PPN stimulation was severe gait freezing and postural instability, persisting even 'on medication' and either causing frequent falls or precluding walking.

Patient	Age (years)	PD Duration (years)	UPDRS III off/on meds (score/108)	R-UPDRS off/on meds (score/92)	IT27-30 off/on meds (score/16)	GFQ (score/64)	FOGQ (score/24)	FallsQ (score/4)	Ldopa dose equivalent (mg/day)	Supportive for UK Brain bank criteria*
1	55	14	35/24	28/18	7/6	55	22	4	1600	A, P
2	76	16	34/25	25/16	9/9	38	20	1	600	D, A, P
3	55	25	33/22	27/17	6/5	36	15	3	300	D, A, P
4	68	9	40/26	29/18	11/8	49	24	2	1650	A, P
5	70	20	35/22	29/17	6/5	36	13	3	900	D, A,T,P
6	71	20	37/19	27/14	10/5	na	na	2	1450	D,A,T, P
7	54	20	53/19	47/14	6/5	38	14	4	800	D,A,T, P

Table. Clinical details of the study participants. All patients were operated in Oxford except patient six (London). All patients were male. NA = not assessed. For all motor scales, higher scores indicate worse function. Key to UK Brain bank criteria; D=dyskinesias, A = asymmetry persistent, T=tremor at rest, P=progressive disease course. *Additional to disease duration and levodopa response as documented elsewhere in the table.

Techniques to target and implant electrodes in the PPN have been described previously.(Pereira, Muthusamy et al. 2008; Zrinzo, Zrinzo et al. 2008; Foltynie and Hariz 2010) Electrodes (Medtronic, Minneapolis, USA) were configured with four active contacts each 1.5mm in diameter. Electrodes in six patients were model 3389 (0.5 mm spacing between contacts) and in one patient were model 3387 (1.5mm spacing between contacts). Electrode locations are summarised in Fig 1a.

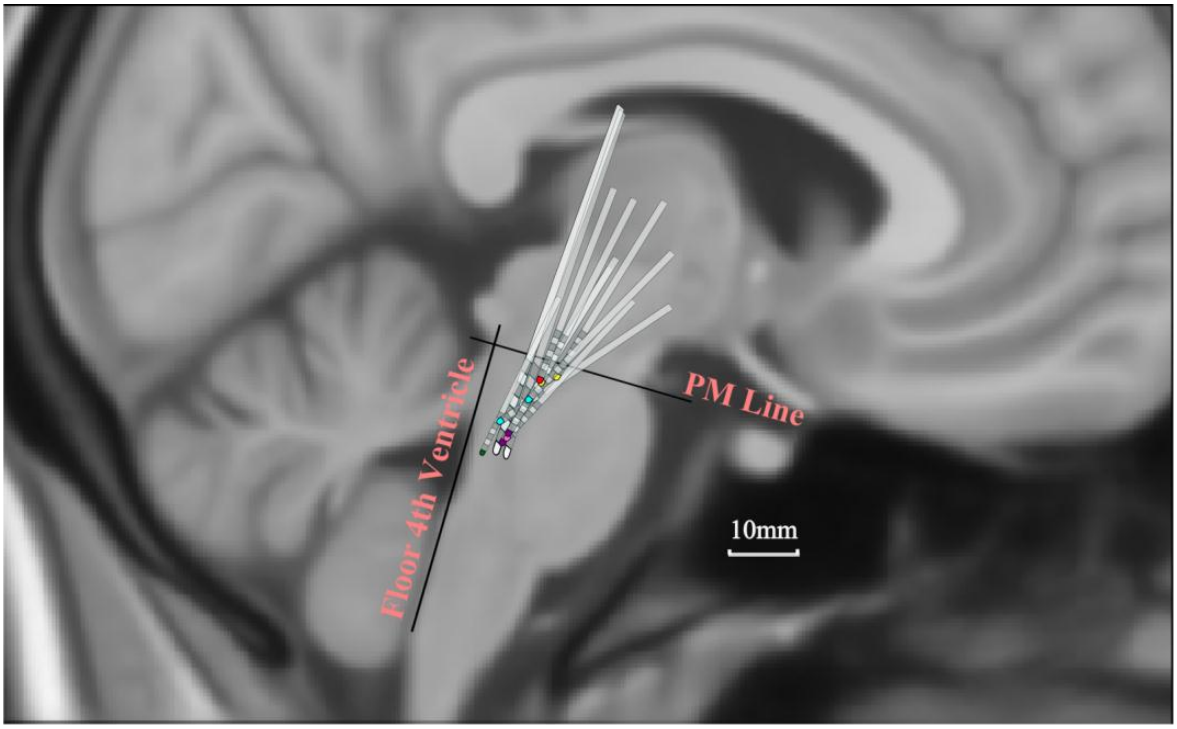

Figure 1. Localisation of electrodes and contact locations represented in Montreal Neurological Institute (MNI) space (sagittal view). The colouring of electrode tips identifies the electrodes of individual patients. PM = Ponto-Mesencephalic line connecting the pontomesencephalic junction to the caudal end of the inferior colliculi.

3.3.2 Experiments and recordings

Experiments took place 2-6 days after electrode implantation. Assessments were performed ‘off medication’ (after overnight withdrawal of dopaminergic medication) to limit variance from fluctuating dopaminergic state and to maximise gait disturbance.

There were two conditions; rest and gait. For rest recordings, patients sat comfortably with eyes open for 2-3 minutes. For gait recordings, patients walked at their preferred speed along an unobstructed path 10 meters long, 10-30 times (depending on speed and fatigue).

Local field potentials (LFPs) were recorded in bipolar configuration from consecutive contacts (01, 12, 23) of each electrode (Fig 2a). LFPs were band-pass filtered between 0.5 and 500 Hz. Single channel EEG was recorded from FzCz. In patients 1-6, a triaxial accelerometer (TMSI, Enschede, NL) was firmly fixed with tape over the spinous processes at the upper thoracic level to record trunk acceleration. A portable battery powered amplifier (Porti amplifier, TMSI, Enschede, NL) was attached with a waist-belt. Data were sampled at 2048 Hz and recorded on a laptop (Porti32 software) via a long fibre-optic cable.

3.3.3 Data analysis and parameters

Data were analysed in Spike 2 (Cambridge electronic design, Cambridge, UK) using routines therein. Unless stated otherwise, all frequency spectral analyses employed non-overlapping blocks of 4 s duration that afforded a resolution of 0.25 Hz (fast Fourier transform, Hanning window). The mean (±SEM) duration of recordings at rest was 222 ± 38 s and for gait was 253 ± 68 s.

3.3.3.1 Gait assessment with trunk accelerometer

Three dimensional trunk accelerometry is a validated method to assess spatiotemporal parameters of gait in healthy subjects and patients with Parkinson's disease.(Dijkstra, Zijlstra et al. 2008; Lord, Rochester et al. 2008; Senden, Grimm et al. 2009) Acceleration in the anteroposterior (AP) plane is predicted by an inverted pendulum model.(MacKinnon and Winter 1993) During single support after mid stance, forward acceleration increases as the body falls forwards and downwards.(Zijlstra 2004) Acceleration peaks at the point of

foot contact after which there is sharp deceleration and ultimately reversal of body motion to upwards and backwards along with contralateral foot elevation. Thus the biggest transients in AP trunk acceleration oscillate in time with stepping.

Gait speed can be reliably estimated from the amplitudes of AP acceleration related to stepping.(Cappozzo 1982; Moe-Nilssen 1998; Zijlstra and Hof 2003; Zijlstra 2004) The predominant frequency of stepping (cadence) within four second blocks is identifiable as the peak in spectral power over 1-3 Hz, corresponding to the 'locomotor frequency band'.(Ichinoseki-Sekine, Kuwae et al. 2006) This frequency band captures the typical ranges of cadences reported during unconstrained walking in healthy and Parkinsonian subjects.(MacDougall and Moore 2005; Sofuwa, Nieuwboer et al. 2005; Mirelman, Gurevich et al. 2011) Relative gait speed can be derived from the power of the spectral peaks indicating cadence, as gait speed has a quadratic relationship with amplitudes of AP acceleration.(Moe-Nilssen 1998) The estimation of relative gait speed (rGS) was considered sufficient for our purposes as we sought correlations between this parameter and LFP activity within subjects. Estimation of absolute gait speed requires further assumptions, such as leg length helping predict step length. Such accelerometer based methods have been validated in both healthy subjects and Parkinsonian patients and are substantially more accurate than the threshold crossing algorithms employed by pedometers.(Ichinoseki-Sekine, Kuwae et al. 2006; Dijkstra, Zijlstra et al. 2008; Lord, Rochester et al. 2008; Speelman, van Nimwegen et al. 2011)

In accordance with the above, rGS was computed as follows. The accelerometer channel detecting acceleration in the anterior-posterior axis was selected for analysis. Periods marked during experiments as other than unconstrained walking (e.g. turning, standing) were spliced out. Cadence was identified for every non-overlapping 4s block as the peak

frequency in acceleration over 1-3 Hz. In each patient, depending on the variability of cadence, a span of 4-6 spectral bins (1.0-1.5 Hz) was identified to cover the predominant spectrum of cadences over all blocks. Power within this individualised frequency band computed for every four second block of walking yielded the rGS.

3.3.3.2 Local field potential analysis

LFPs during rest and gait: LFP frequency spectra from each bipolar contact pair (01, 12 and 23) were derived for the periods of sitting and walking (Fig 2b and c). The frequency and power of spectral peaks was assessed. Peaks were identified as local elevations in power spanning at least five contiguous 0.25 Hz bins with a minimum rise of two and fall of three adjacent bins (the asymmetry in criteria necessary given the background (1/f) decrease of LFP power with frequency, f). Where several peaks occurred within a broader based power elevation, the peak frequency was defined as the midpoint of this activity. Spectral peaks were sought in the alpha (defined here as 7-12 Hz) and beta (13-30 Hz) bands. Where more than one discrete peak occurred within a given frequency band, the most prominent peak was selected for analyses. Power in each spectral peak was calculated as the sum of power around the centre of the peak, spanning 4 Hz for alpha and 2 Hz for beta (the differing ranges reflecting the relative sizes and skirt-widths of these peaks). For rest recordings, log power of LFP peaks was used in analyses. To limit the effects of any movement artefact, LFP power during walking was normalised (as was rest LFP power when compared with walking LFP power). Alpha power was expressed as a % of total power over 5-40 Hz and beta power expressed as a % of total power over 15-40 Hz. The more restricted range for beta aimed to exclude reciprocal effects from higher amplitude alpha activity, whereby the generally larger changes in the alpha band might

have obscured changes in the beta band. This approach was considered reasonable as no direct comparison was made between frequency bands.

Localisation of LFP power: The centre of each bipolar recording site was calculated from the coordinates derived for electrode contacts (described above). For rest recordings, the mean un-normalised LFP spectral log power in the alpha band could be assessed over 4 mm subregions spanning the entire rostro-caudal extent of recordings. For recordings during gait (including all beta band analyses, as fewer beta peaks necessitated collapsing data at rest and during gait), the smaller gradients afforded by normalising LFP power meant that power in spectral peaks was simply compared between sites below and above a point 2mm below the pontomesencephalic line, used to define the 'caudal PPN' and 'rostral PPN' regions.

3.3.3.3 Coherence between LFP and EEG

Coherence was sought between LFPs and single channel EEG at FzCz (Fig 2d). This was estimated using non-overlapping data blocks of 1 s, affording 1 Hz resolution, using standard techniques.[15,16] Peaks in coherence were sought at the frequency of the LFP spectral peaks, at the contact pairs expressing the highest power at these peaks. Coherence was considered relevant where there was significant coherence spanning at least two contiguous bins in a 4 Hz or 2 Hz band corresponding to the LFP spectral peak in the alpha and beta bands, respectively.

3.3.3.4 Correlation between LFP and relative gait speed

LFP power spectra from each bipolar contact pair were derived from consecutive, non-overlapping 4 s blocks during walking. Cadence varied insufficiently to assess for correlations with LFP. Correlations between the rGS and LFP were assessed as follows:

Spectral Peak LFP correlation with rGS: Normalised LFP power at spectral peaks was assessed (from the bipolar pair expressing the highest power in each electrode) for each 4 s block during walking and correlated with the rGS from corresponding sections (Fig 2e).

Across frequency LFP correlation with rGS: Normalised LFP power across different frequencies was correlated with rGS yielding correlation spectra. Thereby, the frequency of LFP that correlated most strongly with the gait index could be identified (Fig 2f). We could therefore test the assumption that correlations were strongest at the frequency of the peak in LFP power spectra.

3.3.3.5 LFP power averaged to gait freezing episodes

In patient 6, discrete freezing episodes were sufficient in number to allow LFP activity to be averaged to the onset of gait freezing. Freezing onset was determined by thresholding the root mean square of the AP accelerometer signal over 1.0-3.0Hz. LFP power was derived from non-overlapping data blocks of 1 s, affording a 1Hz spectral resolution. Power at the alpha peak and flanking two spectral bins at the contact pair expressing highest alpha power was aligned to freezing onset according to change-point analysis, using commercial software (Change-Point Analyser 2.0 shareware program, Taylor Enterprises Inc., Illinois, USA, http://www.variation.com) and techniques described previously.(Cassidy and Brown 2002) Change-point analysis iteratively uses time varying cumulative sum charts (cusums) and bootstrapping to detect changes in time series.(Taylor

2000) For this analysis, cusums were determined by plotting the sequentially summed deviation of each spectrum from the average determined for the whole record (total of 11s). 10,000 bootstraps were performed and only changes with probabilities of >95% were highlighted.

3.3.4 Statistics

The Kolmogorov-Smirnov Test demonstrated that the distribution of normalised peak LFP power and rGS was not different from the normal. Normalised LFP power in specific frequency bands was compared between conditions and/or recording sites using paired t-tests. Differences in normalised LFP power across a range of frequencies were assessed with serial t tests performed for each frequency. Correlations (Pearson's) were sought between peak LFP frequencies across conditions and between normalised LFP power and rGS. Level of significance was $P<0.05$.

3.4 Results

3.4.1 Gait assessment

Following electrode implantation and without stimulation, all patients experienced a reduction in gait freezing which persisted up to six weeks – a time course consistent with 'stun effect'.(Koop, Andrzejewski et al. 2006) Preoperatively, when 'off medication', every patient experienced frequent, long duration freezing. However, post-operatively when 'off medication' during experiments, freezing in five patients was infrequent and then typically brief (< 3 seconds). In two patients (who were both implanted unilaterally),

freezing had improved but remained clinically severe. In one of these patients (patient 6) discrete freezing episodes were identifiable whilst in the other (patient 2) freezing was almost continuous. Festination was not observed during experiments. The mean cadence (±SD) across patients for every 4 second block of walking was 1.81 Hz (±0.28). rGS coefficients of variation ranged from 40-308% between patients, consistent with some degree of gait disturbance, despite the stun effect. Cadence was less variable, with coefficients of variation ranging from 6–21 % between patients.

3.4.2 Local field potentials and their relationship to EEG and gait

Spectral analysis during rest and gait

A discrete peak in the alpha band was present in all electrode recordings during rest and gait (see Fig 2b and c for examples). A discrete peak in the beta band was observed in 6/11 electrodes (5/7 patients). Beta peaks occurred inconsistently across rest and gait, being present across both conditions in only two electrodes.

3.4.2.1 Alpha band activity

Activity during rest and gait

The frequencies of alpha peaks during rest (mean 8.2 Hz, range 7-10 Hz) and gait (mean 8.4 Hz, range 7-10 Hz) correlated strongly ($r=0.90$, $p<0.001$) across patients (Fig 2A-C; Fig 3A), suggesting that the peaks were homologous across the two states. Alpha peaks were relatively focal to one bipolar contact pair. *Normalised* peak alpha (±SEM) power dropped in the remaining two contact pairs by 15.6 (±2.5) % at rest and 14.2 (±2.8) %

during gait. Even so, these values likely underestimate the gradient across contacts due to the normalisation procedure. At rest, where there was no potential contamination by movement artefact, the *un-normalised* peak alpha (±SEM) power dropped in the remaining two contact pairs by 42.7 (±9.0) %. In all but one electrode, the normalised alpha power during rest and gait was maximal at the same (7/11 electrodes) or an adjacent (3/11 electrodes) contact pair. Normalised peak alpha power did not significantly differ between rest and gait.

Localisation

Normalised alpha peak power during gait was significantly greater at caudal (deeper than 2mm below the pontomesencephalic junction) compared with rostral recording sites ($T(31)=4.183$, $P<0.001$) (Fig 4a). During rest, normalised alpha peak power was also significantly greater in caudal compared with rostral recording sites ($T(31)=3.359$, $P<0.001$).

For rest recordings, which did not require normalisation, we were able to follow *un-normalised* log alpha peak power across recording depth (Fig 4b). The resting *un-normalised* log alpha peak power was divided according to the depth of each recording site into subregions spanning 4 mm along a rostro-caudal axis. An ANOVA revealed a significant difference in log alpha peak power across these subregions ($F(4,28)=7.873$, $P<0.001$) (Fig 4b). Resting u*n-normalised* log alpha peak power was maximal in the caudal PPN subregion between 2 and 6 mm below the pontomesencephalic junction, falling significantly at rostral subregions (-2 to -6 mm Vs. -2 to +2 mm, $t(10)=3.177$, $P=0.010$ and -2 to -6 mm Vs. +2 to +6 mm $t(8)=4.793$, $P=0.003$) and caudal subregions (-2 to-6 mm Vs. -6 to -10 mm, $t(14)=2.845$, $P=0.013$ and -2 to -6mm Vs. -10 to -14mm $t(11)=2.667$, $P=0.022$). These subregions along the rostro-caudal axis are represented in

figure 1b in standard MNI space. For patient 3 left electrode (used as the individual example for LFP's in figure 2) the highest resting peak alpha power was at 2.5 and 4.2mm below the pontomesencephalic line – reflecting findings from the grouped analysis. For this patient, to give an example of the anatomy of the region expressing highest alpha power, axial MRI slices across this and flanking regions are displayed in figure 5.

Coherence with EEG

In 7/11 electrodes, significant LFP EEG coherence spanning at least two consecutive 1 Hz bins was observed within the alpha peak range during rest and/or walking (Fig 2D).

R*elationship with relative gait speed*

In eight of nine electrodes where LFP and trunk acceleration were simultaneously recorded, normalised peak alpha power correlated significantly with the rGS. In these electrodes, the mean correlation was $r=0.433$, $p<0.01$ (see Fig 2E for an example from a single electrode). LFP rGS correlation spectra in all electrodes revealed that the correlation peaked in the alpha band (see Fig 2F for an example from a single electrode). The frequency of this peak correlated strongly with the peak LFP during gait ($r=0.782$, $p=0.013$) and peak LFP at rest ($r=0.779$, $p=0.013$; Fig 3B). When averaged across recordings, spectra of the Fisher transformed correlations between LFP power and rGS demonstrated a discrete positive peak in correlation at 7-10 Hz, regardless of whether LFP power was raw (Fig 3C) or normalised (Fig 3D). Note that normalisation inevitably induced a negative correlation at frequencies below 7 Hz.

Averaged to gait freezing episodes

Patient 6 had 24 discrete freezing episodes identifiable during recordings and an LFP alpha peak during gait at 8Hz. LFP power over 7-9 Hz averaged to the onset of the freezing

episodes is represented in figure 6. There is a significant associated attenuation in alpha power which begins around 1s prior to the onset of freezing, and continues for over 2s thereafter.

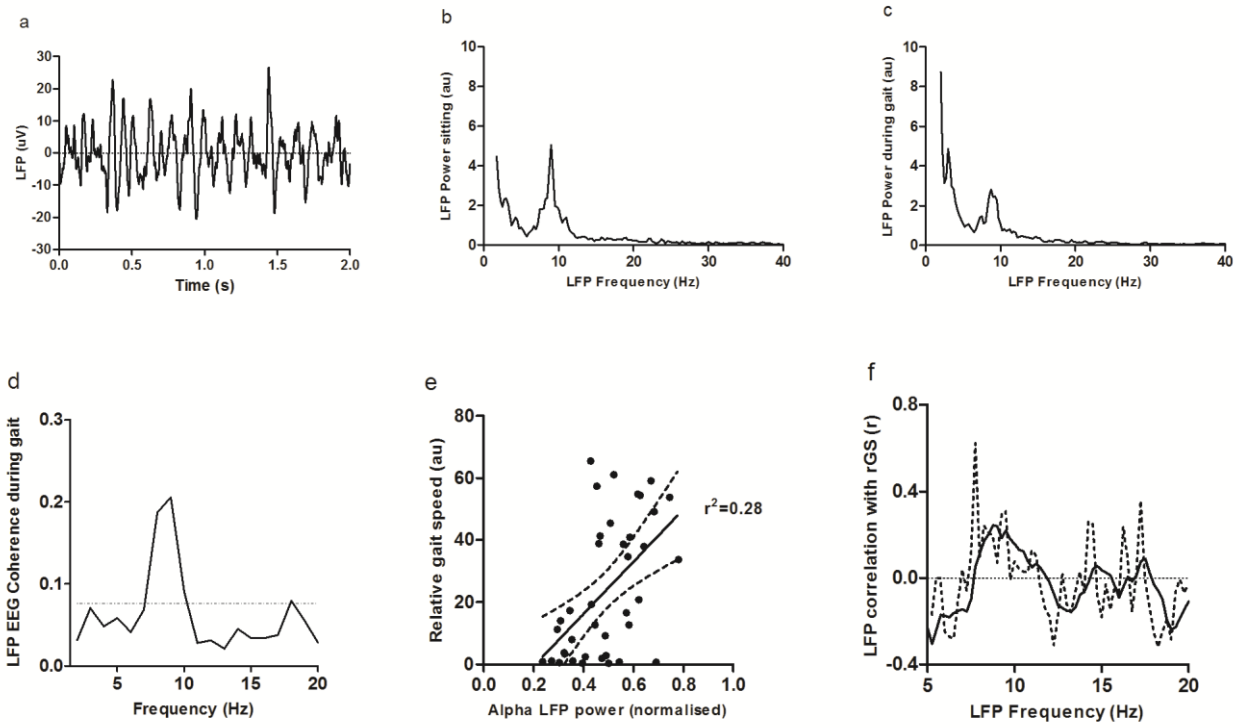

Figure 2. Example data from patient 3, left PPN electrode at contact pair 01. a: Raw LFP during rest. b: LFP autospectrum averaged over the period (127s) of rest. Note peak at 9Hz ('peak rest LFP'). c: Autospectrum of LFP averaged over the period of unconstrained walking. Note peak at 8Hz ('peak gait LFP'). d: Spectra of coherence between the LFP and EEG (FzCz) during the period of walking. Horizontal line is the 95 % confidence limit (CL). Note the peak in coherence at 8-9Hz. e: Peak alpha power (sum of normalised power over 6-10Hz) correlation with rGS. Linear regression line and its 95 % CL are shown. f: Cross frequency LFP (normalised) correlation with rGS. Dotted line is the raw correlation and continuous line is the 6 point (backwards) moving-average. Note the peak in correlation in the alpha band.

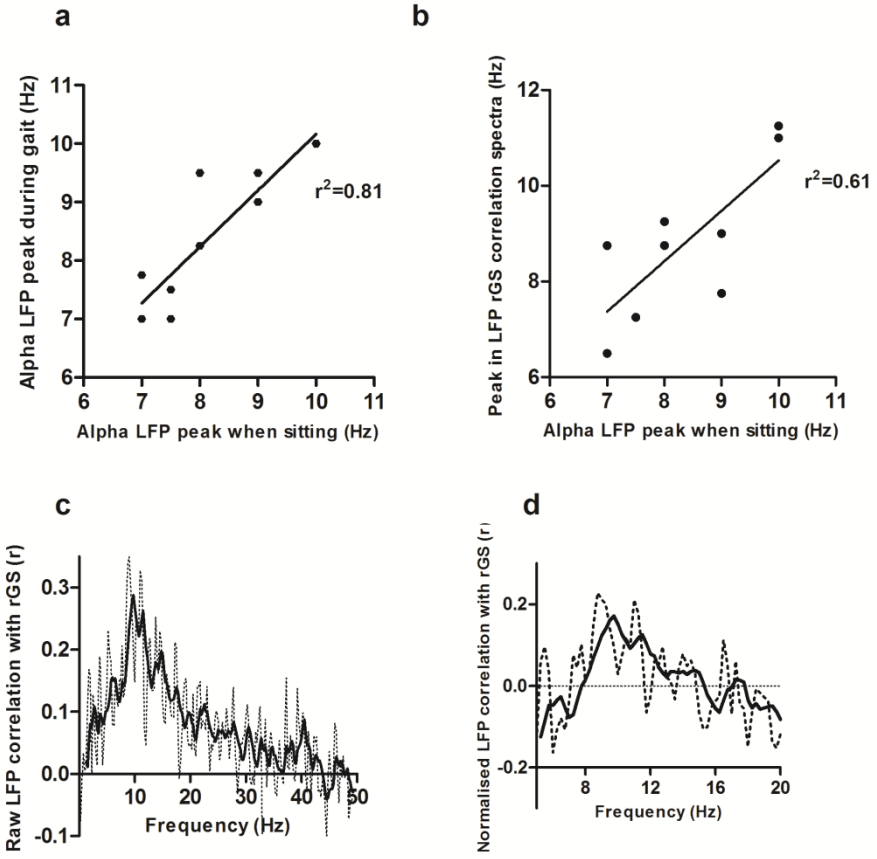

Figure 3. a: Relationship between the peak rest LFP and peak gait LFP. The strong correlation [r=0.90, p<0.001] suggests that the two LFP peaks may be related. b: Relationship between the peak rest LFP and the frequency of the peak in the LFP-rGS correlation spectra. The strong correlation [r=0.779, p=0.013] suggests that the peak rest LFP may be relevant to the performance of gait. c: Group average un-normalised LFP and rGS correlation spectra demonstrating a peak in correlation in the alpha band. Dotted line is the average correlation and continuous line is the 6 point (backwards) moving-average. d: Group averaged normalised (across 5-40Hz) LFP and rGS correlation spectra demonstrating persistence of the peak in correlation in the alpha band. Dotted line is the average correlation and continuous line is the 6 point (backwards) moving-average. Correlations were Fisher transformed prior to averaging in c and d.

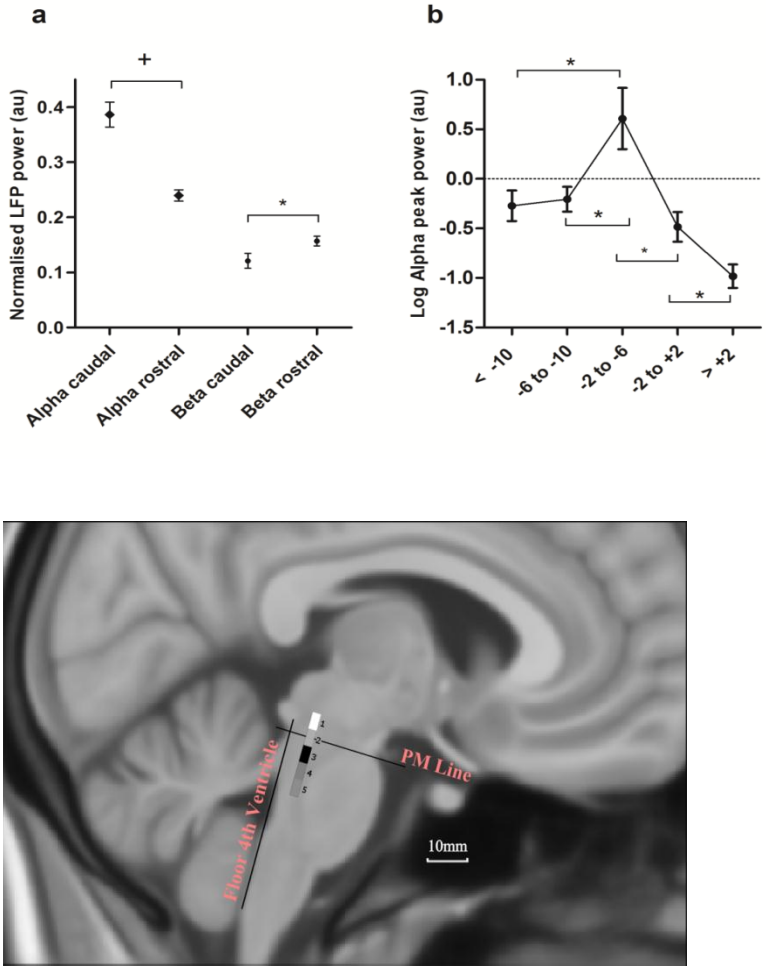

Figure 4. a. Normalised power (Mean ± SEM) of alpha peaks during gait and beta peaks during rest or gait grouped according to caudal (deeper than 2mm below the pontomesencephalic junction) or rostral recording site. Alpha peak power was greater caudally and beta power was greater rostrally. b. Log alpha peak power at rest (Mean ± SEM) divided according to recording site depth into 4mm subregions (denoted on the x axis in millimetres relative to the PM junction). Log alpha peak power is maximal in the -2 to -6mm region, falling significantly at surrounding sites. * $P<0.05$. c. Representation of the rostro-caudal location of peak un-normalised alpha power at rest - which correlated strongly in frequency and location with peak alpha power that correlated with gait (but which required normalisation to remove movement artefact thereby also diminishing power gradients). Relative log alpha power is represented in grey-scale intensity whereby black is highest and white lowest beta power. Regions relative to the PM line are numbered as follows; 1) +2 to +6mm 2) -2 to +2mm, 3) -6 to -2mm, 4) -10 to -6mm, 5) -14 to -10mm. Alpha power was maximal at location 3 (-6 to -2mm below the PM line) Beta power was highest in regions 1 and 2 combined. Note: No inference is made regarding location in ventrodorsal or mediolateral planes.

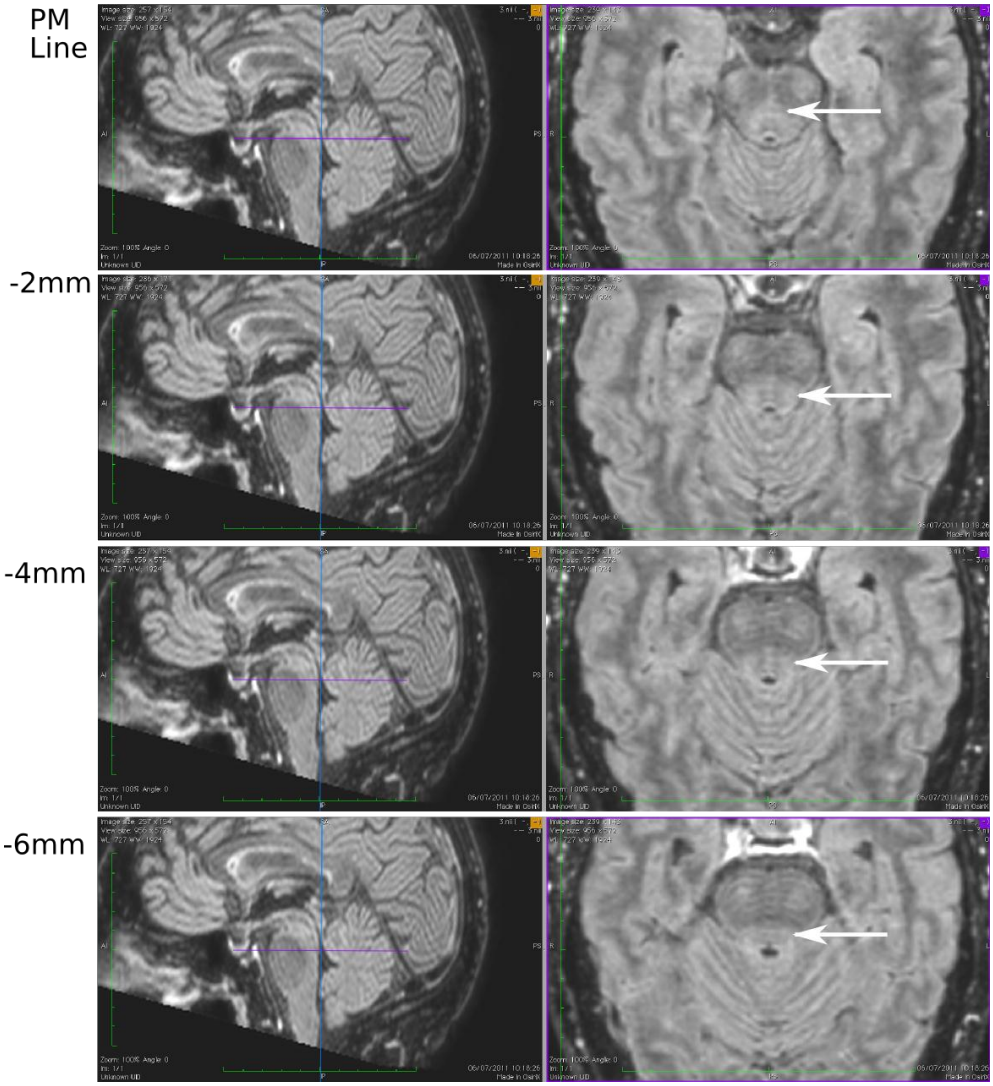

Figure 5. Example brainstem FLAIR MRI of patient 3 (whose results also appear as the individual LFP example in figure 2). Axial slices have been rendered parallel to the pontomesencephalic (PM) line, at that level and 2mm, 4mm and 6mm below it. The region 2-6mm below the PM line is where resting un-normalised alpha peak power was found to be maximal in the group analysis. For this individual patient left electrode, un-normalised log peak alpha power at rest was highest almost equally (within 5%) at the bottom (01) and middle (12) contact pairs, centred at 2.5mm and 4.2mm below the PM line respectively. This alpha power fell by >97% at the remaining rostral contact pair (23) centred at 0.8mm below the PM line reflecting the focal nature of alpha power in this region. Note; the rostrocaudal distance between bipoles depends on the angulation of the electrode trajectories. White arrow indicates the superior cerebellar peduncle and its' decussation. The PM line and axis parallel to the floor of the fourth ventricle are outlined on the sagittal views.

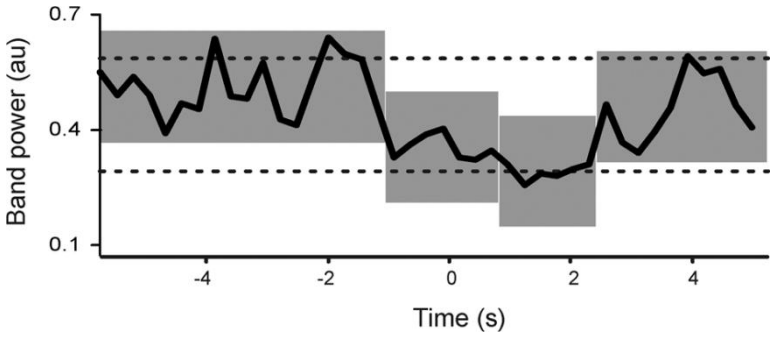

Figure 6. Change point analysis of the time series of mean LFP power over 7-9 Hz averaged to onset (0 s) of freezing episodes (n = 24) in case 6. Horizontal dotted lines are the 95 % confidence limits of the whole record and the grey blocks represent stable periods between changes in power as defined by change point analysis. There is a significant drop in 7-9 Hz power about 1 s before the onset of freezing, and 7-9 Hz activity continues to be attenuated for just over 2 s thereafter.

3.4.2.2 Beta band activity

Activity during rest or gait

Beta peaks (mean 21.1Hz, range 17.3-28.5) were relatively focal to one bipolar contact pair, with *normalised* peak beta (±SEM) power dropping by 15.4 (±2.4) % at the remaining two contact pairs of each electrode. At rest, the *un-normalised* peak beta (±SEM) power dropped in the remaining two contact pairs by 45.6 (±11.1) %.

Localisation

In the six electrodes demonstrating a beta peak (during rest or gait), *normalised* beta peak power was significantly greater in rostral (higher than 2mm beneath the pontomesencephalic junction) than caudal contacts ($t(16)=-2.232$, $p=0.040$; Fig 4). There

were insufficient beta peaks at rest to compare *un-normalised* beta peak power with location.

Coherence with EEG

In only one of the six electrodes demonstrating a beta peak, was there significant coherence spanning at least 2 consecutive 1 Hz bins between the LFP at beta peak frequency and FzCz.

Relationship with relative gait speed

Normalised beta peak power did not significantly correlate with rGS. Similarly, spectra of correlations between normalised LFP at each frequency and rGS did not reveal discrete peaks in correlation at the frequencies corresponding to beta peaks.

3.5 Discussion

In parkinsonian patients, we found alpha oscillations in a network involving the caudal subregion of the PPN and the cerebral cortex. Synchronisation of alpha activity in the caudal PPN region correlated with the performance of gait. Beta oscillations were found in the rostral PPN subregion but this activity did not correlate with gait.

A rostro-caudal topographical organisation of the PPN is supported by extensive data from animal research.(Martinez-Gonzalez, Bolam et al. 2011) Rostral PPN neurons predominantly express GABA and are strongly interconnected with the basal ganglia including subthalamic nucleus and internal pallidum,(Mena-Segovia, Bolam et al. 2004;

Ros, Magill et al. 2010; Martinez-Gonzalez, Bolam et al. 2011) which exhibit beta activity in untreated PD.(Hammond, Bergman et al. 2007) Caudal PPN neurons express predominantly acetylcholine and glutamate.(Martinez-Gonzalez, Bolam et al. 2011) Cholinergic neurons arborize widely, including to cortex and locomotor centres.(Skinner, Kinjo et al. 1990; Mena-Segovia, Sims et al. 2008) It is therefore congruent that we found beta oscillations in the rostral PPN region and a distinct oscillatory activity in the caudal PPN region, alpha activity, coherent with cortex and associated with locomotion.

Such topography could explain why different centres have reported different dominant oscillatory patterns, in the alpha or beta frequency bands, from PPN recordings – as this would be determined by the depth of the implanted electrodes.(Androulidakis, Khan et al. 2008; Androulidakis, Mazzone et al. 2008; Weinberger, Hamani et al. 2008; Tsang, Hamani et al.) That such topography of the PPN region seems to exist in man may be clinically relevant as it raises the tentative prospect that the caudal PPN region could be the most relevant target for gait in PD and that alpha activity could provide a functional biomarker for this subregion. PPN region alpha activity was focal, evidenced by un-normalised alpha peak power at rest dropping by an average of 42% across bipolar recording sites. This is similar to the gradients of beta activity reported across the dorsolateral subthalamic nucleus which can be detected in intraoperative and post-operative recordings and used to guide deep brain stimulation electrode implantation.(Chen, Pogosyan et al. 2006)

The origin of the focal alpha activity in the PPN region needs consideration. The distribution of electrode contacts in this study was broad – and this facilitated the relationship between oscillatory power and location to be recognised. Some contacts lie more caudal than any described location of the PPN but these were not the ones expressing

alpha at highest power across patients. Across patients, un-normalised peak alpha power at rest (which correlated strongly in frequency and location with alpha power during gait) was maximal between 2mm to 6mm beneath the pontomesencephalic junction. The spatial accuracy of this finding is limited by the bipolar nature of the LFP recordings and the averaging of data across patients. However, with this limitation in mind, a key question is whether this region corresponds to the PPN. The PPN comprises a well-defined 'pars compacta' subregion (PPNc) but also a reticular 'pars dissipata' (PPNd) with indistinct boundaries.(Olszewski and Baxter 1954) The stereotactic atlas of Olszewski and Baxter identifies the PPN based on cytoarchitectural methods. (Olszewski and Baxter 1982) Later, cholinergic PPN neurons were identified in humans by immunohistochemical labelling of choline-acetyltransferase (ChAT) (Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999) Although these latter studies do not provide stereotactic coordinates, several observations are helpful. Whilst cholinergic PPN neurons were clustered most densely in the PPNc, the PPNd accounts for the greater proportion of cholinergic neurons .(Manaye, Zweig et al. 1999) Cholinergic PPN neurons span at least 7mm in the rostro-caudal plane being at highest density 2.5mm under the rostral pole (as defined by ChAT staining, so potentially missing any rostral component not expressing ChAT; see(Manaye, Zweig et al. 1999). Cholinergic PPN neurons were noted to lie where the "superior cerebellar peduncle ascends towards the dorsolateral pons towards its decussation" with the PPNc located at the level of the decussation .(Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999) Identifying these anatomical landmarks on the axial images of patient 3 suggests that the levels 2-4mm below the pontomesencephalic line would therefore correspond to the caudal PPN region. Of course other gait related entities also exist around this region including the cuneiform, albeit centred more rostrally. Anatomical considerations aside, stimulation of this caudal region has been reported to be beneficial for gait freezing and postural

instability in PD .(Thevathasan, Coyne et al. 2011) The relative efficacy of rostral versus caudal PPN stimulation warrants further investigation.

The distribution of PPN oscillatory activity also concurs with previous work in PD suggesting that alpha and beta oscillatory networks are segregated.(Litvak, Jha et al.) The functional nature of alpha and beta oscillations in PD also appears distinct. Beta band activity is pathologically increased in PD, is suppressed by levodopa and high frequency subthalamic stimulation and correlates with deficits of bradykinesia and rigidity.(Eusebio, Thevathasan et al. ; Silberstein, Pogosyan et al. 2005; Kuhn, Kupsch et al. 2006) Alpha power in the PPN tends to increase with levodopa, suggesting that it could be pathologically attenuated in PD.(Androulidakis, Khan et al. 2008; Androulidakis, Mazzone et al. 2008) It now also appears that increases in PPN alpha power in PD may correlate with an improvement in gait.

The potentially contrasting effects of beta and alpha activity may have implications for the interpretation of the post-operative 'stun effect' we observed. In the STN, the 'stun' or 'microlesion' effect of surgery is usually attributed to suppression of local beta activity in the target nucleus by acute tissue disruption from electrode implantation.(Chen, Pogosyan et al. 2006; Koop, Andrzejewski et al. 2006) However, in the caudal PPN we found evidence of alpha activity that persisted despite a clinically evident stun effect and correlated with improved gait performance. Accordingly, we speculate that stun effects in PPN surgery might predominately arise from microlesioning inhibitory supraspinal influences along the electrode trajectory. Excessive inhibition of the PPN (for example from the internal pallidum) has been considered a pathophysiological factor causing gait and postural disturbance in PD, in addition to PPN neuronal degeneration(Aziz, Davies et al. 1998). Consistent with this reasoning, in the 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP) primate model of Parkinson's disease, the microinjection of a GABA agonist into the PPN improved mobility.(Nandi, Aziz et al. 2002) Microlesioning inhibitory afferents to the PPN would be expected to have analogous effects.

However, before further discussion, a potential confound needs consideration; that the correlation between alpha power and gait speed could be due to movement artefact. Several factors militate against this. First, our LFP recordings were bipolar, so a signal common across contacts should have been subtracted out. Second, the rGS correlated maximally with alpha oscillations (7-10 Hz) which differed in frequency from the accelerometer frequencies used to derive gait speed (1-3 Hz). Third, correlation with the rGS was relatively *specific* to alpha LFP activity, evidenced by the peak in correlation spectra at alpha frequencies and the lack of any correlation with beta power. Fourth, correlations were present even when LFP power was normalised to broad band activity which effectively eliminated any common artefact from movement.

An important qualification is that the correlation between gait speed and alpha power does not necessarily imply causation and the relationship could be epiphenomenal. For example, alpha power may be permissive for higher gait speeds or reactive to it. However, correlations were found within a brain area that is functionally relevant to gait disturbance, as evidenced by the potential for improvement upon PPN stimulation. Furthermore, the positive nature of the correlation between alpha synchronisation and increasing gait speed, concurs with the synchronisation of alpha power observed with Levodopa,(Androulidakis, Khan et al. 2008; Androulidakis, Mazzone et al. 2008) which also tends to improve gait. Yet, even if PPN alpha oscillations are mechanistically important in gait disturbance, the relationship does not appear obligatory or exclusive. The mean correlation was 0.43, so that fluctuations in alpha power only predicted around 20 % ($r^2 = 0.19$) of the variance in

rGS. A further question is whether this alpha activity also relates specifically to gait freezing. Due to the reduction in freezing with stun effect, we could only compare freezing episodes with alpha power in a single patient (who was implanted unilaterally). This demonstrated an attenuation of alpha activity related to the onset of freezing. This suggests that higher gait speeds could have, at least partly, resulted from relief of freezing related deficits even if discrete freezing episodes did not frequently occur. For example, patients with gait freezing are reported to have continuous background deficits in gait, including reduced gait velocity, step length and increased spatiotemporal variability.(Hausdorff, Schaafsma et al. 2003; Chee, Murphy et al. 2009; Snijders, Leunissen et al. 2011) It is notable that attenuation of alpha LFP power preceded freezing onset. However, this latter finding should be interpreted cautiously given the limited accuracy in determining when exactly freezing begins.

How might alpha activity in the caudal subregion of the PPN relate to gait performance? Gait speed reduces in healthy subjects, elderly fallers and in Parkinson's disease during the performance of a second, unrelated task ('dual tasking').(Hausdorff, Balash et al. 2003; Springer, Giladi et al. 2006; Lamoth, van Deudekom et al. 2011) In elderly subjects, an inability to 'walk whilst talking' predicts falls.(Lundin-Olsson, Nyberg et al. 1997) Such findings implicate 'attention' and the effective allocation of processing resources that flows from it, as a potentially important factor influencing gait speed. In PD, attentional deficits are common and there is also impaired automaticity of movement so that processing demands are higher.(Wu and Hallett 2005; Wu and Hallett 2008) Parkinsonian patients with gait freezing are reported to have even more attentional deficits than those without gait freezing.(Amboni, Cozzolino et al. 2008; Yogev-Seligmann, Hausdorff et al.

2008) Dual tasking can worsen gait freezing, as can other precipitants that are thought to 'distract' attention away from gait.(Giladi and Hausdorff 2006)

There is increasing evidence that alpha activity has an important role in attention and the allocation of processing resources. The synchronisation of occipital alpha with eye closure was once interpreted to reflect passive 'idling'.(Berger 1929; Pfurtscheller, Stancak et al. 1996) However, alpha activity is now considered to support active suppression of task irrelevant processes.(Jensen and Mazaheri 2010) For example, during working memory tasks, cortical alpha power in visual and motor-sensory areas increases and the degree of synchronisation correlates with the number of items recalled.(Jensen, Gelfand et al. 2002; Haegens, Osipova et al. 2010) Correlation of performance with oscillatory power in task irrelevant regions suggests suppression rather than mere idling. It has been proposed that within the motor system, suppression of competing processes with alpha could aid the smooth execution of motor programs.(Pfurtscheller and Neuper 1994; Suffczynski, Kalitzin et al. 2001) Regions like the STN and caudal PPN that have distributed functional connectivity, including cerebral cortical areas, would be placed to operationalize this putative role. Consistent with this, here, we provide evidence of an association between alpha power and improved motor performance with respect to gait.

Chapter 4:

PPN stimulation for gait freezing and postural instability in Parkinson's disease: An open label clinical study

4.1 Abstract

Background: Pedunculopontine nucleus (PPN) stimulation is a novel therapy for Parkinson's disease. However, controversies remain regarding the clinical application of this new therapy, including patient selection, electrode positioning and how best to assess outcomes.

Objective: To clarify the clinical application of PPN stimulation in Parkinson's disease.

Methods: Five consecutive patients with Parkinson's disease complicated by severe gait freezing, postural instability and frequent falls (all persisting even 'on-medication') received bilateral stimulation of the mid-lower PPN, without co-stimulation of other brain targets. Outcomes were prospectively assessed over two years using gait specific questionnaires and the Unified Parkinson's Disease Rating Scale (part III).

Results: The primary outcome, Gait and Falls Questionnaire score, improved significantly with stimulation. Benefits were maintained over two years. Unified Parkinson's disease Rating Scale (part III) items assessing gait and posture were relatively insensitive to these treatment effects. Beneficial effects often appeared to outlast stimulation for hours or longer. Thus, single session 'on' versus 'off' stimulation assessments may be susceptible

to ‘delayed washout effects’. PPN stimulation did not change akinesia scores or dopaminergic medication requirements.

Conclusions: Bilateral stimulation of the mid-lower PPN (more caudal than previous reports), without co-stimulation of other brain targets, may be beneficial for the subgroup of patients with Parkinson’s disease who experience severe gait freezing and postural instability with frequent falls, which persist even ‘on medication’. Choosing appropriate outcome measures and accounting for the possibility of prolonged stimulation washout effects appear important for detecting the clinical benefits.

4.2 Introduction

Pedunculopontine nucleus (PPN) stimulation is a novel therapy for Parkinson’s disease (PD).(Jenkinson, Nandi et al. 2005) In the non-human primate model of PD, low frequency PPN stimulation mimicked local disinhibition and improved movement counts, posture and balance.(Nandi, Aziz et al. 2002; Jenkinson, Nandi et al. 2004; Jenkinson, Nandi et al. 2006) Several small series have now reported that low frequency PPN stimulation in patients with PD appears to selectively improve freezing of gait (FOG), postural instability and falls.(Stefani, Lozano et al. 2007; Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) However, controversies remain regarding the clinical application of this new therapy, including patient selection, electrode positioning and how best to assess outcomes. Consequently, the clinical utility and efficacy of PPN stimulation has been questioned.

In this study, we implanted bilateral PPN stimulators in five patients with PD complicated by severe gait freezing and frequent falls. Stimulation was delivered to the mid-lower PPN,

without co-stimulation of other brain targets. Outcomes were prospectively assessed over two years using validated gait specific questionnaires and the Unified Parkinson's Disease Rating Scale (UPDRS).

4.3 Methods

4.3.1 Participants

Five patients with PD (meeting UK Brain Bank Criteria) were implanted consecutively with bilateral PPN stimulators in Brisbane, Australia (table). Patients gave written informed consent.

Patient	Age/Sex	PD Duration (years)	LDopa equivalent dose (mg)	Total UPDRS (score/108) Off/On meds	R- UPDRS (score/92) Off/On meds	IT27-30 (score/16) Off/On meds	GFQ (score/64) Usual meds	FOGQ (score/24) Usual meds	FallsQ (score/4) Usual meds
1	70F	8	1200	39/24	30/17	9/7	48	22	4
2	70M	16	1900	30/25*	22/18*	8/7*	30	14	4
3	74M	5	400	24/13	16/6	8/7	51	22	3
4	60F	8	800	49/20	38/14	11/6	61	24	4
5	73M	18	2400	41/12	32/11	9/1	46	21	4

Table: Preoperative clinical features of the study participants. LevoDopa equivalent dose reflects dopaminergic medication requirements expressed as equivalent to LevoDopa dose (mg). Usual meds = usual medication state. *In this patient, 'off meds' UPDRS may be underestimated due to use of long acting dopamine agonist.

It is currently unknown whether bilateral PPN stimulation is more beneficial than unilateral. The PPN have bilateral connectivity, perhaps suggesting that unilateral PPN stimulation may be sufficient. Other basal ganglia targets also have bilateral connectivity, such as the STN. However, ipsilateral effects from subthalamic nucleus stimulation are minimal and often temporary.(Walker, Watts et al. 2009) Additionally, in animal models, it is reported that activation of cholinergic projections from one PPN may cause inhibition of the other.(Reese, Garcia-Rill et al. 1995) Given such theoretical uncertainties, we elected to implant and stimulate bilaterally.

The indication for PPN stimulation was severe gait freezing, postural instability and falls, persisting even 'on medication'. For these patients, freezing and falls were the predominant sources of morbidity, rather than motor fluctuations. Patients were therefore implanted in the PPN only and did not, for example, receive co-stimulation of the subthalamic nucleus (STN).

Mean (SD) baseline characteristics were; age 69.4(5.6) years, disease duration 11.0(5.7) years, Unified Parkinson's Disease Rating Scale part III (UPDRS III, score/108) off/on medication 36.6(9.8)/18.8(6.1), Mini Mental State Examination (score/30) 28.8(1.1). Four patients were falling ≥daily and one patient ≥weekly.

Major cognitive and psychiatric impairment were excluded preoperatively by formal assessment with a neuropsychiatrist.

4.3.2 Surgery and Stimulation

The mid-lower PPN was targeted. The PPN region was located directly on 3T T2-FLAIR MRI lateral to the superior cerebellar peduncle and its' decussation, medial to the region of the medial lemniscus below the level of the inferior colliculus (figure 1). Trajectories passed through the subthalamic region, into the PPN along its long axis. Dopaminergic medication was ceased the night before surgery. Stereotactic CT and MRI were volumetrically fused (Stealth, Medtronic). Electrodes (model 3387, Medtronic) were implanted with a CRW frame.

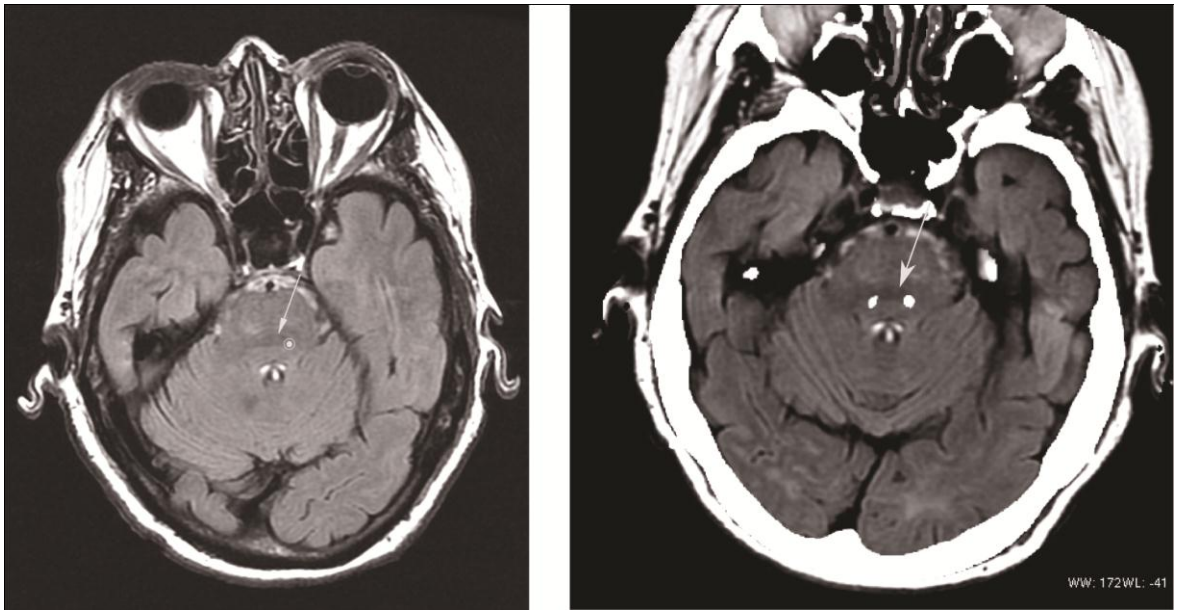

Figure 1: FLAIR MRI images in patient 1 demonstrating the preoperative targeting and postoperative electrode placement (at the level of the lowest contact). Arrows indicate the superior cerebellar peduncle/decussation (hypointense). On the preoperative scan, the targeted PPN region is indicated by a target symbol.

Microelectrode recordings were collected for later analysis, but were not used to guide electrode placement. Awake intraoperative clinical assessments allowed stimulation side effects to be assessed (as described previously).(Ferraye, Debu et al. 2009; Ferraye, Gerardin et al. 2009; Moro, Hamani et al. 2010) Beneficial effects of stimulation on gait and posture were not testable on table.

Monopolar stimulation commenced at 35Hz and 60μs with titration of voltage. Choice of initial contact was based on results of postoperative imaging (aiming to stimulate the mid-lower PPN). The choice of frequency was based on early reports of PPN stimulation in humans.(Stefani, Lozano et al. 2007) Lower frequencies (including 10Hz stimulation used in the primate experiments) were found to be less effective.(Thevathasan, Silburn et al. 2010) High frequency stimulation at 130Hz appeared to worsen motor function including gait. Voltage was initially titrated on each electrode in turn, against bedside assessment of gait and postural stability – including the 'pull test' for postural stability and turning on the spot for gait freezing. Both electrodes were then activated together and comparisons made between on versus off bilateral stimulation. Improvements in gait and postural control were often not immediately apparent and titration would sometimes require several visits over the course of the day or days. Similarly, turning off stimulation often did not result in acute rebound of gait and postural disturbance to pre-stimulation levels. We observed that this apparent prolonged effect of stimulation could sometimes last up to several days. Frequently, fine oscillopsia (as described previously) created a ceiling to voltages.(Ferraye, Gerardin et al. 2009) However, this could usually be overcome by slow voltage escalation over hours to weeks. All five patients experienced this oscillopsia which we eventually regarded as a marker reassuring of correct stimulation location.

4.3.3 Assessments: Stimulation clinical effects

Questionnaires were prospectively administered preoperatively and 6 months and 2 years postoperatively (except patient 2; preoperatively and postoperatively at 12 months and 2.5 years).

UPDRS assessments occurred preoperatively and two years postoperatively (except patient 2; preoperatively and 2.5 years postoperatively), off and on both medication and simulation. Off medication assessments were performed following overnight (≥ 12 hours) withdrawal of dopaminergic medication. On medication assessments occurred in a 'practically defined on state' following levodopa administration.(Defer, Widner et al. 1999) On/off stimulation assessments occurred after a minimum 1 hour stimulation washout – a time period previously employed by other studies investigating the effects of subthalamic nucleus and PPN stimulation on gait and postural control.(Moreau, Defebvre et al. 2008; Ferraye, Debu et al. 2009) This one hour washout was also chosen to reflect what would be expected to occur realistically in routine clinical practice, over a single visit.

4.3.4 Data analysis

The primary endpoint was GFQ score, considered to best capture Parkinsonian gait disturbance in patients' usual environments and medication states. GFQ scores were compared between time-points with Friedman's test and posthoc Wilcoxon signed ranks test. Given that there were only five study participants, we have otherwise favoured expressing individual outcomes rather than performing grouped statistical analysis. Where we have compared means, the Wilcoxon signed ranks test was employed for rating scales

and paired t-tests for medication doses. Significance level was $p<0.05$. Statistical analysis was performed with the SPSS statistical package, version 19.0 (SPSS Inc, Chicago).

4.4 Results

4.4.1 Surgery and Stimulation

On immediate postoperative imaging, one electrode was found to be mal-positioned too ventrally and was replaced two days after the initial surgery. There were no other surgical complications. Positioning of active contacts in four patients are represented in figure 2.

Colour	Pt/Side	*h* from PM Line (mm)	*d* from IVth (mm)	Laterality (mm)
Pink	1 L	-4.79	5.89	8
Pink	1 R	-2.95	4.05	6.67
Yellow	2 L	-2.21	7.37	8.67
Yellow	2 R	-4.05	5.52	6
Black	3 L	-3.31	6.26	8
Black	3 R	-6.26	4.05	7.33
Green	4 L	-4.79	5.16	8
Green	4 R	-7	4.42	6

Figure 2: Localisation of active contacts in MNI space. In the legend, negative h values reflect positioning below the PM line. The PPN region is highlighted in dark grey. SC=superior colliculus, IC= inferior colliculus. Imaging for one subject was missing.

Stimulation side effects were as described previously.(Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) At two years, stimulation parameters were monopolar 35hz, 60usec and mean(SD) 3.5V(0.79).

4.4.2 Clinical assessments

GFQ scores for all five patients were improved at 6 months and 2 years compared with preoperatively (figure 3a). Accordingly, GFQ scores differed significantly between time-points [χ^2=7.60, p=0.022] and were significantly better at 6 months [mean 23.0 Vs 47.2, Z=-2.023, p=0.043] and 2 years [mean 24.8 vs 47.2, Z=-2.023, p=0.043] compared with preoperatively. GFQ scores at 6 months and 2 years were not significantly different [23.0 Vs 24.8, Z=0.674, p=0.50].

Similarly, FOGQ scores of all five patients were improved at six months and two years compared with preoperatively (figure 3b). In three patients, improvements of FOGQ scores at six months were sustained or further improved at two years.

Patients reported fewer falls with PPN stimulation. In all patients, FallsQ scores at 6 months were improved compared with preoperatively (figure 3c). Four of five patients (not patient 3) had improved Falls Question scores at 2 years compared with preoperatively.

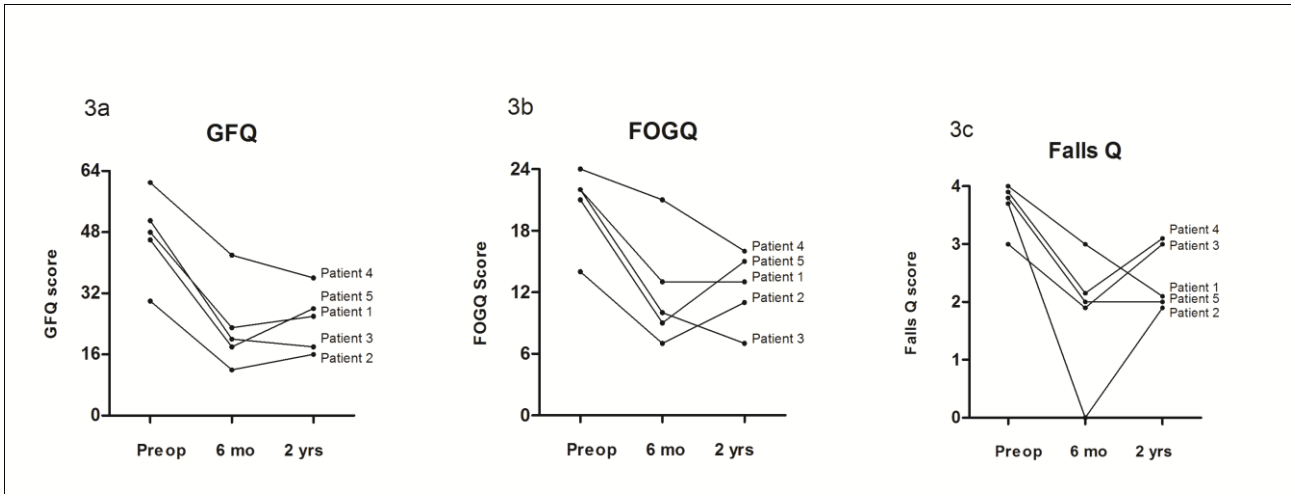

Figure 3: Individual questionnaire score outcomes - assessed preoperatively and postoperatively at 6 months and 2 years (except patient 2; preoperatively and postoperatively at 1 year and 2.5 years).

'Off medication' IT27-30 scores were improved in all patients when 'on stimulation' at two years compared with preoperatively (figure 4a). Intriguingly, 'off medication' IT27-30 scores at 2 years when 'off stimulation' were also better than preoperatively in four patients, despite washout periods of at least an hour. In only two patients at 2 years were 'off medication' IT27-30 scores better when 'on stimulation' compared with 'off stimulation'.

'On medication' IT27-30 scores were improved in three patients when 'on stimulation' at two years compared with preoperatively (figure 4b). In two patients, 'on medication' IT27-30 scores at 2 years when 'off stimulation' were better than preoperatively. In only two

patients did ‘on medication’ IT27-30 scores at 2 years improve when ‘on stimulation’ compared with ‘off stimulation’.

Residual UPDRS scores both off and on medication did not consistently change with stimulation in any patient. Residual UPDRS scores at two years ‘on stimulation’ were not different to preoperative scores, either ‘off medication’ [Z=-0.735, P=0.46] or ‘on medication’ [Z=-0.674, P=0.500]. Two years postoperatively, Residual UPDRS scores did not differ on versus off stimulation, either ‘off medication’ [mean 25.2 Vs 25.6, Z=-1.0, p=0.32] or ‘on medication’ [mean 14.6 Vs 14.6, Z=0.0, p=1.0].

Dopaminergic medication requirements were not different at two years compared with preoperatively [mean 1370mg Vs 1340mg, t=-0.17, p=0.87].

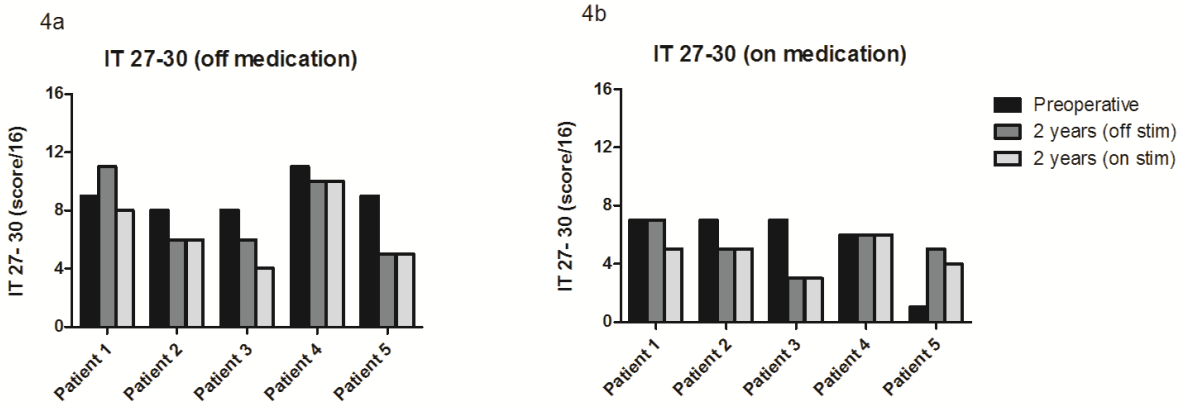

Figure 4: Individual outcomes of IT27-30 (items 27-30 UPDRS, score/16) off and on medication and stimulation. Time-points are preoperatively and 2 years postoperatively (except patient 2; preoperatively and 2.5 years postoperatively).

4.5 Discussion

In five patients with PD complicated by severe and medically refractory FOG, postural instability and falls, we found that PPN stimulation improved questionnaire scores measuring Parkinsonian gait and balance disturbance in patients' usual environments and medication states. Benefits persisted over two years. However, PPN stimulation did not improve UPDRS items assessing akinesia, rigidity and tremor. Accordingly, dopaminergic medication requirements did not change.

We selected patients for PPN stimulation whose predominant symptomatic issues were severe FOG and postural instability persisting even 'on medication' and frequent falls, rather than motor fluctuations. In these patients, the therapeutic impact of PPN stimulation appeared substantial. However, this subgroup of patients is relatively uncommon. In two previous studies, patients were selected for PPN stimulation who had severe motor fluctuations requiring STN stimulation and variable degrees of gait disturbance.(Stefani, Lozano et al. 2007; Ferraye, Debu et al. 2009) For example, some patients were selected for PPN stimulation to treat FOG that developed during STN stimulation. Other patients have been selected for PPN stimulation who had not experienced gait freezing that persisted 'on medication' or recurrent falls.(Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) In such patients, the results of PPN stimulation have been disappointing.(Ferraye, Debu et al. 2009) Furthermore, it is possible that co-stimulation of the STN could influence the efficacy of PPN stimulation particularly given their substantial reciprocal connections.(Jenkinson, Nandi et al. 2008) In this regard, it should be noted that high frequencies required for STN stimulation (eg 130Hz) appear to worsen gait when delivered to the PPN.

The optimal site for PPN stimulation remains to be determined. Whilst the bulk of the PPN appears to be at the rostral level, cholinergic neurons are reported to be clustered most densely in the caudal 'pars compacta' PPN.(Jenkinson, Nandi et al. 2008) Degeneration of cholinergic PPN neurons in parkinsonism has been associated with disturbances of gait and posture as well as falls.(Bohnen, Muller et al. 2009; Karachi, Grabli et al. 2010) We therefore targeted the mid-lower PPN, more caudal than previous reports in humans but similar to the target employed in the nonhuman primate by two of our investigators.(Jenkinson, Nandi et al. 2004; Ferraye, Debu et al. 2009) However, we acknowledge that the beneficial effects of stimulation cannot necessarily be attributed to the target itself, but may, for example, reflect current spread to neighbouring nuclei or to afferent or efferent projections.(Gradinaru, Mogri et al. 2009)

Outcome measures that are sensitive and practical will be needed for detecting the beneficial effects of PPN stimulation in routine clinical practice. For STN stimulation, it is usual to assess patients on and off stimulation at a single session, using the UPDRS. However, such methods may be inappropriate for PPN stimulation. Indeed, we found that comparing IT27-30 scores on and off PPN stimulation during a single session with one hour washout periods was insensitive. One issue is that gait freezing is notorious for disappearing during medical assessments, possibly due to arousal mechanisms.(Chee, Murphy et al. 2009) Consequently, any single session assessment of parkinsonian gait, even formal gait analysis, may not accurately reflect function in patient's usual circumstances.(Chee, Murphy et al. 2009; Peppe, Pierantozzi et al. 2010) Of further relevance is our finding that IT27-30 scores often did not rebound to preoperative levels upon ceasing stimulation for an hour. This may be due to a delayed washout effect of PPN stimulation which, during titration, we observed could outlast stimulation for up to several

days. This phenomenon has also recently been reported by others.(Ostrem, Christine et al. 2010) However, several previous studies of PPN stimulation have, like us, employed the IT27-30 with washout periods of an hour or less– an approach our findings suggest may cause therapeutic effects to be underestimated.(Ferraye, Debu et al. 2009) Questionnaires have the advantage of reflecting function over prolonged periods and in patients' usual environments and medication states. The GFQ and FOGQ are specifically designed to assess parkinsonian gait disturbance and appeared sensitive to the impact of PPN stimulation. Ideally however, an objective and ambulatory method to assess the effects of PPN stimulation would be preferable, the development of which may be informed by emerging evidence from gait analysis.(Peppe, Pierantozzi et al. 2010) We acknowledge that our reliance on the FallsQ as the sole method of assessing falls frequency was not ideal. Falls diaries would likely have better captured this important endpoint.

4.6 Conclusion

PPN stimulation is a novel treatment. We have performed a small prospective open label study with unblinded outcome assessments. Our patient selection and specific PPN target differs from previous studies – and our findings suggest that bilateral stimulation of the mid-lower PPN, without co-stimulation of the STN may be beneficial for the specific subgroup of PD patients who experience severe gait freezing, postural instability and falls, which all persist even 'on medication'. Choosing appropriate outcome measures and accounting for the possibility that therapeutic effects may persist long after PPN stimulation is switched off (delayed washout effects) appear important for accurately detecting therapeutic efficacy. These issues would be worth considering in the planning of any future randomised trial.

Chapter 5:

A spatiotemporal analysis of gait freezing and the impact of pedunculopontine nucleus stimulation

5.1 Abstract

Objective: To assess the impact of unilateral and bilateral pedunculopontine nucleus stimulation at a level beneath the pontomesencephalic junction on triggered episodes of gait freezing and on background deficits of unconstrained gait in Parkinson's disease.

Methods: In a double blinded study, spatiotemporal parameters of gait were assessed in Parkinsonian patients with severe gait freezing implanted with pedunculopontine nucleus stimulators. Patients were assessed 'off medication' during three conditions; off stimulation, unilateral stimulation and bilateral stimulation. Results were compared to parkinsonian patients without gait freezing matched for disease severity and healthy controls. Episodes of freezing were triggered by turning in a tight space and quantified by task duration and frequency of discrete steps. Spatiotemporal parameters of gait were also assessed during unconstrained walking.

Results: Pedunculopontine nucleus stimulation improved objective measures of gait freezing, with bilateral stimulation more effective than unilateral. During unconstrained walking, Parkinsonian patients who experience gait freezing had reduced step length and increased step length variability compared to patients without gait freezing. However, these deficits were unchanged by pedunculopontine nucleus stimulation. Objective

measures of gait freezing correlated strongly with Freezing of Gait Questionnaire scores, and the latter also improved with chronic bilateral pedunculopontine nucleus stimulation.

Interpretation: Pedunculopontine nucleus stimulation beneath the pontomesencephalic junction improved gait freezing, although not step length. Bilateral stimulation was more effective than unilateral. Stimulation of the caudal pedunculopontine nucleus region can be therapeutic for gait freezing.

5.2 Introduction

Gait freezing is an episodic arrest of forward progress in locomotion due to an inability to take normal steps.(Giladi and Nieuwboer 2008) It is a common, intrusive feature of Parkinsonian disorders that causes falls and diminishes quality of life.(Giladi, McDermott et al. 2001; Moore, Peretz et al. 2007; Kerr, Worringham et al. 2010) Gait freezing is only partially and often poorly responsive to levodopa and subthalamic nucleus stimulation.(Bloem, Hausdorff et al. 2004; Ferraye, Debu et al. 2008) Pedunculopontine nucleus (PPN) stimulation is proposed to improve gait freezing, even when resistant to medication.(Mazzone, Lozano et al. 2005; Plaha and Gill 2005) However, the precise effects of PPN stimulation on Parkinsonian gait disturbance are not yet established.(Peppe, Pierantozzi et al. 2010) The clinical application of this new treatment is controversial and basic questions remain regarding patient selection, targeting and whether bilateral stimulation is better than unilateral.(Stefani, Lozano et al. 2007; Zrinzo, Zrinzo et al. 2007; Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010; Thevathasan, Coyne et al. 2011)

In this double blinded study, we assessed spatiotemporal aspects of gait in Parkinsonian patients with severe gait freezing implanted with PPN stimulators and compared results to

those of parkinsonian patients without gait freezing and healthy controls. We assessed the impact of unilateral and bilateral PPN stimulation on triggered episodes of gait freezing as well as on background deficits of gait.

5.3 Methods

5.3.1 Subjects and clinical assessments

Three subject groups were assessed; i) seven patients with Parkinson's disease (PD) complicated by severe freezing of gait (FOG), chronically implanted with bilateral PPN stimulators (PD FOG group), ii) eight PD patients of akinetic/rigid subtype without significant gait freezing (PD control group) and iii) nine age matched healthy controls. PD patients were matched for age, disease duration, motor severity and cognitive status. Subjects were recruited from centres in Oxford, England and Brisbane, Australia. Ethics committee approval was obtained from both centres and participants gave written informed consent.

Seventeen patients with PD had received bilateral PPN stimulators from the study centres at the time of experiments; eleven were recruited and seven completed the study protocol. We sought to capture gait disturbance reflective of the untreated state in the PD FOG patients. Experiments were therefore performed 'off medication' and with PPN stimulation ceased for at least 12 hours. Furthermore, only PD FOG patients who exhibited freezing at baseline were included. This was necessary given reports that therapeutic effects can outlast PPN stimulation for hours to days and also to avoid a floor effect.(Ostrem, Christine et al. 2010; Thevathasan, Coyne et al. 2011) Thus two PD FOG patients were excluded when freezing could not be provoked 'off medication' and 'off stimulation'. Two

additional PD FOG patients were excluded as they could not perform experimental tasks when ‘off medication’ (either off or on PPN stimulation) due to severe akinesia. Six patients implanted with PPN stimulators from the centres were not recruited due to; death (1), living overseas or out of state (2), unilateral stimulation (1), stimulation under titration (1), DBS system explanted due to therapeutic failure (1). Of the seven PD FOG patients ultimately assessed, clinical outcomes of four and reaction times of six are previously reported.(Thevathasan, Coyne et al. 2011; Thevathasan, Pogosyan et al. 2011) Nine PD control patients were recruited and one rejected when (unexpected) gait freezing emerged during experiments.

PD FOG patients were receiving bilateral stimulation to the caudal PPN region for severe gait freezing and postural instability persisting even “on medication”, causing frequent falls. One patient was also receiving subthalamic nucleus stimulation (switched off for experiments). No other patients had received surgery to any other brain target. Surgical implantation of the PPN from both centres is described previously.(Pereira, Muthusamy et al. 2008; Thevathasan, Coyne et al. 2011) Figure 1 demonstrates the stimulation locations (midpoint between active contacts for bipolar stimulation and cathodes for monopolar). Stimulation parameters were: Frequency range 30-40 Hz, voltage range 2.2-4.3Vand pulse width 60 µs.

Clinical details of the study participants are shown in Tables 1 and 2. PD FOG, PD controls and healthy controls were not significantly different in age [$F(2,21)=0.317$, $P=0.732$]. PD FOG and PD control patients did not differ with respect to disease duration [$t(13)=-0.053$, $P=0.958$], R-UPDRS subscore [$t(12)=0.570$, $P=0.579$] or MMSE [$t(11)=-0.416$, $P=0.686$]. PD FOG patients had higher scores in IT27/30 [$t(12)=-5.543$, $P<0.001$], GFQ [$t(13)=-9.212$, $P<0.001$] and FOGQ [$t(12)=-10.240$, $P=0.001$]].

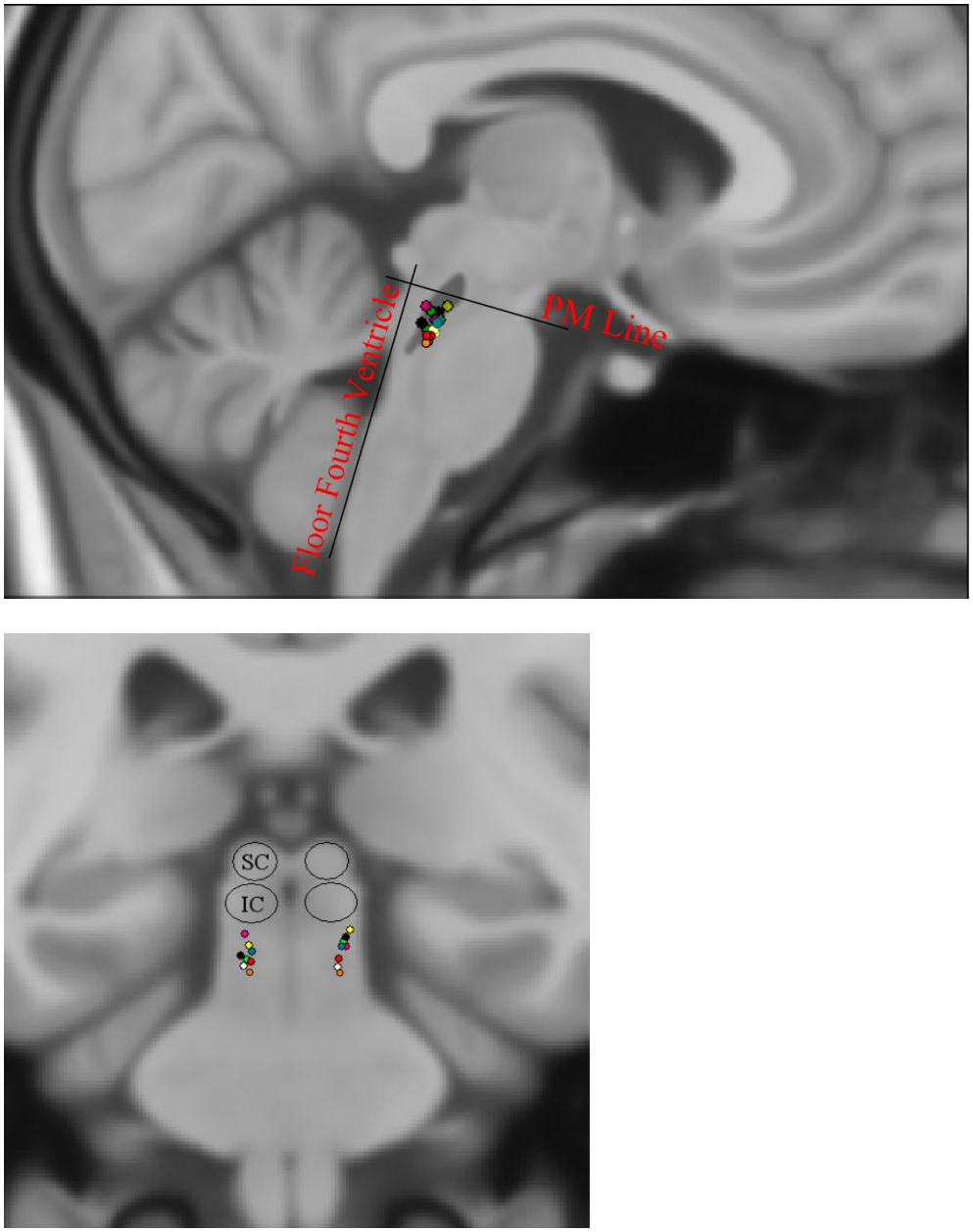

Figure 1. Localisation of stimulation locations represented in Montreal Neurological Institute (MNI) space (sagittal and coronal views). PM = Ponto-Mesencephalic line connecting the pontomesencephalic junction to the caudal end of the inferior colliculi. SC = Superior Colliculus. IC = Inferior Colliculus. The relative location/extent of the PPN has been outlined on the sagittal view, based on choline-acetyltransferase immunohistochemical (ChAT5) staining in the human (see methods).

	Age (years)	Sex	PD Duration (years)	MMSE	R-UPDRS Off meds/stim	IT27-30 Off meds/stim	GFQ	FOGQ
Healthy controls	67.3 (8.3)	7M, 2F	\	\	\	\	\	\
PD controls	64.4 (6.1)	5M, 3F	11.9 (3.4)	29.2 (1.0)	29.4 (9.5)	2.9 (1.6)	3.8 (3.7)	1.9 (1.9)
PD FOG	66.9 (9.6)	5M, 2F	12.0 (5.5)	28.9 (1.6)	26.7 (8.3)	8.3 (2.1)*	44.9 (12.0)*	19.7 (4.5)*

Table 1. Baseline characteristics; Means (SD). Questionnaire scores for PD FOG patients are from preoperatively. *Different from PD controls, P≤0.001. R-UPDRS = items 1-26 For one PD FOG patient, preoperative FOGQ scores were missing (see table 2). For PD controls, UPDRS in one and MMSE in two were not tested.

Patient	Age/Sex	PD duration (years)	Postop duration (years, months)	LDopa dose equivalent (mg/day)	UPDRS III off/on meds (off stim)	IT27-30 off/on stim (off meds)	GFQ pre/postop	FOGQ pre/postop	Supportive for UK brain bank criteria*
1	61F	10	2	800	40/23	10/9	61/36	24/16	D,A, P
2	72M	18	2,5	2500	25/17	6/6	30/16	14/11	D,A,T, P
3	76M	6	2	600	26/14	6/4	51/18	22/7	A, P
4	72F	10	2	950	38/22	11/8	48/26	22/13	D,A,T, P
5	77M	6	0,6	1400	31/17	10/10	31/14	^/6	A, P
6	55M	20	1	850	51/19	8/6	38/40	14/15	D,A,T, P
7	55M	14	0,2	1650	34/24	7/4	55/37	22/16	A,P

Table 2. PD FOG patients. Postoperative clinical assessments were performed on the same day as gait analysis. Patients 6 and 7 were from Oxford, other patients from Brisbane. Patient 6 also had STN stimulators which were turned off during experiments. ^ = not known. Key to UK Brain bank criteria; D=dyskinesias, A = asymmetry persistent, T=tremor at rest, P=progressive disease course. *Additional to disease duration and levodopa response as documented elsewhere in the table.

5.3.2 Experiments

Assessments were performed after overnight withdrawal of dopaminergic medication and after 12 hours PPN stimulation washout.

PD FOG patients were blinded to four conditions presented in counterbalanced order: Off PPN stimulation, bilateral PPN stimulation, left PPN stimulation and right PPN stimulation. The mean of left and right unilateral stimulation results was used in analyses. Choice of contacts and stimulation parameters were as employed for chronic therapy. After changing stimulation, thirty minutes wash-in was enforced between conditions.

Data were acquired with an 8.3m long electronic walkway (GAITRite, CIR Systems Inc. NJ) which detected footsteps through embedded pressure sensors.(Bilney, Morris et al. 2003) GAITRite has been validated to assess spatiotemporal parameters of gait in health and PD.(Bilney, Morris et al. 2003; Chien, Lin et al. 2006)

All participants performed two tasks in counterbalanced order:

1) Turn task: This aimed to capture gait freezing, known to be precipitated by turning and tight spaces.(Okuma 2006; Almeida and Lebold 2010) Subjects walked to a central marker placed two thirds down the walkway on its' surface, turned 180° around this marker and returned to the starting position. In sequential trials, patients alternately turned left and right. Patients were confined to a turning arc limited by the width of the electronic walkway (70cm) which was placed in a narrow corridor 1.4m wide (either pre-existing or created by a movable screen).

2) Straight task: This aimed to capture background deficits of gait. Subjects walked at self-selected speed down the centre of the walkway. Distractions were minimised and

subjects were requested not to talk. The walkway was positioned to record established walking and not gait initiation or slowing down towards destination.

Patients performed four trials per task and the mean result used in analyses. Trials with falls were discarded and repeated.

5.3.3 Parameters and data analysis

The primary outcome measure was gait freezing severity as quantified by task duration (seconds) and cadence (steps per minute) during turning. These turn task parameters were assessed manually by researchers, blinded to condition, as follows. The 180° arc of the turn was selected for assessment by the appearance of footsteps at the marker region. Foot-strike was visually identified, frame by frame, so that task duration and cadence could be derived for every trial. This method did not allow detection of any high frequency attempts at stepping that didn't alter foot position as reported previously in gait freezing.(Hausdorff, Balash et al. 2003; Spildooren, Vercruysse et al. 2010) Here cadence pertained to successful stepping and reflected a fundamental feature of gait freezing – a deficiency in steps that alter position.(Giladi and Nieuwboer 2008) Turn task duration was considered a global measure of functional limitation from freezing when compared to control subjects.

For the straight task, mean cadence, mean step length and step length standard deviation were computed automatically by GAITRite software.[25,26] Step length coefficients of variation (CoV) were then calculated.

5.3.4 Statistics

The Kolmogorov-Smirnov Test demonstrated that parameters during turning were unlikely to be normally distributed. Log transformed data of all parameters were normally distributed and used in analyses.

Differences between subject groups were assessed with ANOVA and post-hoc independent samples t-tests. Two such ANOVAs were performed, one with PD FOG patients off stimulation and one with PD FOG patients on bilateral stimulation. In PD FOG, differences between stimulation conditions were assessed with repeated measures ANOVA and posthoc paired t-tests. Posthoc tests were corrected for multiple comparisons using the False Discovery Rate Procedure.(Bejamini and Hochberg 1995)

In PD FOG, off and on stimulation results were considered together and correlations (Pearson's) sought between turn task duration and cadence and an independent clinical measure of gait freezing, the FOGQ.

Level of significance was $P<0.05$.

5.4 Results

PD FOG patients 'off medication' and 'off stimulation' experienced clinically evident gait freezing episodes when turning but not when straight walking. Patients were unable to identify the conditions of stimulation.

5.4.1 Primary outcome: Gait freezing during turning

Turn task duration

Turn task duration differed between PD FOG patients off stimulation, PD controls and healthy subjects [$F(2,21)=61.213$, $P<0.001$]. Posthoc tests revealed that turn task duration was greater in PD FOG patients off stimulation than in PD controls [mean 31.1s PD FOG Vs. 2.7s PD controls, $t(13)=7.223$, $P<0.001$] and healthy controls [2.3s, $t(14)=7.627$, $P<0.001$]. DBS did not return this measure to normal, so that turn task duration in PD FOG patients on bilateral stimulation (mean 11s), although improved, remained different to the other subject groups [$F(2,21)=29.066$, $P<0.001$].

In PD FOG, turn task duration differed between stimulation conditions [$F(1,6)=16.825$, $P<0.001$; Fig 2a]. Posthoc tests revealed that compared with off stimulation, turn task duration reduced with bilateral stimulation [mean 31.1s to 11.0s, $t(6)=-5.053$, $P=0.006$] and unilateral stimulation [to 17.5s, $t(6)=3.068$, $P=0.022$]. Bilateral stimulation reduced turn task duration more than unilateral stimulation [$t(6)=-3.308$, $P=0.032$]. Percentage improvement of turn task duration with bilateral stimulation was also greater than unilateral stimulation [57.9% Vs. 35.5%, $t(6)=2.924$, $P=0.026$].The impact of unilateral stimulation was not influenced by the direction of turning that provoked freezing [19.2s ipsilateral turning Vs. 14.4s contralateral turning $t(6)=0.729$, $P=0.494$].

Cadence

Cadence during turning differed between PD FOG patients off stimulation, PD controls and healthy subjects [$F(2,21)=9.885$, $P=0.001$]. Posthoc tests revealed a deficit in cadence during turning in PD FOG patients off stimulation compared with PD controls [mean

cadence 77.6 steps/minute (spm) PD FOG Vs. 105.9spm PD controls, t(13)=-3.093, P=0.032] and healthy controls [106.1spm, t(14)=-3.132, P=0.032]. With PD FOG patients on bilateral stimulation, cadence during turning no longer differed between subject groups [F(2,21)=0.126, p=0.882].

In PD FOG, cadence during turning differed between stimulation conditions [F(2,12)=16.599, P<0.001; Fig 2b]. Posthoc tests revealed that compared with off stimulation, turning cadence increased with bilateral stimulation [77.6spm to 110.1spm, t(6)=-4.633, P=0.012] and unilateral stimulation [to 91.9spm, t(6)=-3.987, P=0.014]. Bilateral stimulation increased cadence during turning more than unilateral stimulation [t(6)=3.050, P=0.023]. Percentage improvements of cadence were also greater with bilateral than unilateral stimulation [47.4% Vs. 19.7%, t(6)=2.590, P=0.041].The impact of unilateral stimulation on turning cadence was not influenced by the direction of turning that provoked freezing [89.4spm ipsilateral turning Vs. 97.2spm contralateral turning, t(6)=-1.215, P=0.270].

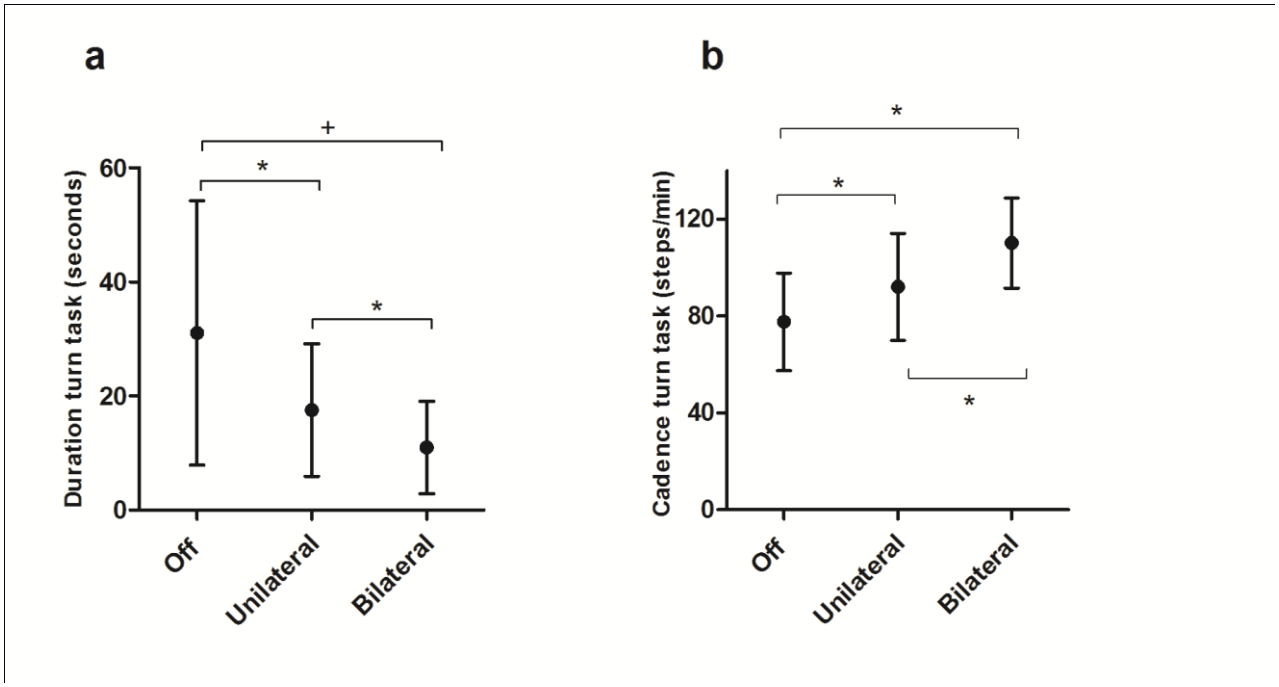

Figure 2. Gait analysis parameters (Means±SD) recorded from PD FOG patients when turning in a tight space, a task which precipitated gait freezing. Results are grouped according to stimulation condition: Off stimulation, unilateral stimulation and bilateral stimulation. Unilateral stimulation results are the grand averages of the means of stimulating each side in each patient. a. Turn task duration (seconds). b. Turn task cadence (steps/minute). Compared to the off stimulation state, bilateral stimulation improved both parameters more than unilateral stimulation. Differences between groups: +P<0.01. *P<0.05.

5.4.2 Secondary outcomes

Unconstrained walking: Straight task parameters

With PD FOG patients off stimulation, there were significant differences between subject groups in step length [F(2,21)=31.190, P<0.001] and step length CoV [F(2,21)=15.298, P<0.001]. Cadence did not differ between subject groups [F(2,21)=0.229, P=0.797]. Posthoc tests revealed a deficit in step length in PD FOG off stimulation compared with PD controls [mean 34.9cm PD FOG Vs. 59.8cm PD controls, t(13)=4.987, P=0.002] and healthy controls [65.5cm, t(14)=5.874, P=0.002]. Step length CoV was greater in PD FOG patients off stimulation than PD controls [mean 0.09cm PD FOG Vs 0.03cm PD controls, t(13)=-3.509, P=0.004] and healthy controls [0.02cm, t(14)=5.947, P=0.009]. These group differences remained with PD FOG patients on stimulation (data not shown).
In PD FOG, a multivariate ANOVA revealed no differences between stimulation conditions during the straight task in step length [F(2,12)=1.074, P=0.372], step length CoV [F(2,12)=0.215, P=0.810] or cadence [F(2,12)=1.589, P=0.244].

Falls during recordings

In PD FOG, falls were recorded in only one patient when turning; five times when off stimulation and a mean of 3.5 times for left and right unilateral stimulation. No falls occurred during bilateral stimulation or in the straight task.

Pre and post-operative clinical scores

In PD FOG patients, chronic PPN stimulation improved scores of GFQ [t(6)=4.422, P=0.012] and FOGQ [t(5)=2.988, P=0.031] compared with preoperatively (table 2). FOGQ scores correlated (Fig 3) with turn task duration [r=0.727, P=0.010] and turning cadence [r=-0.681, P=0.010].

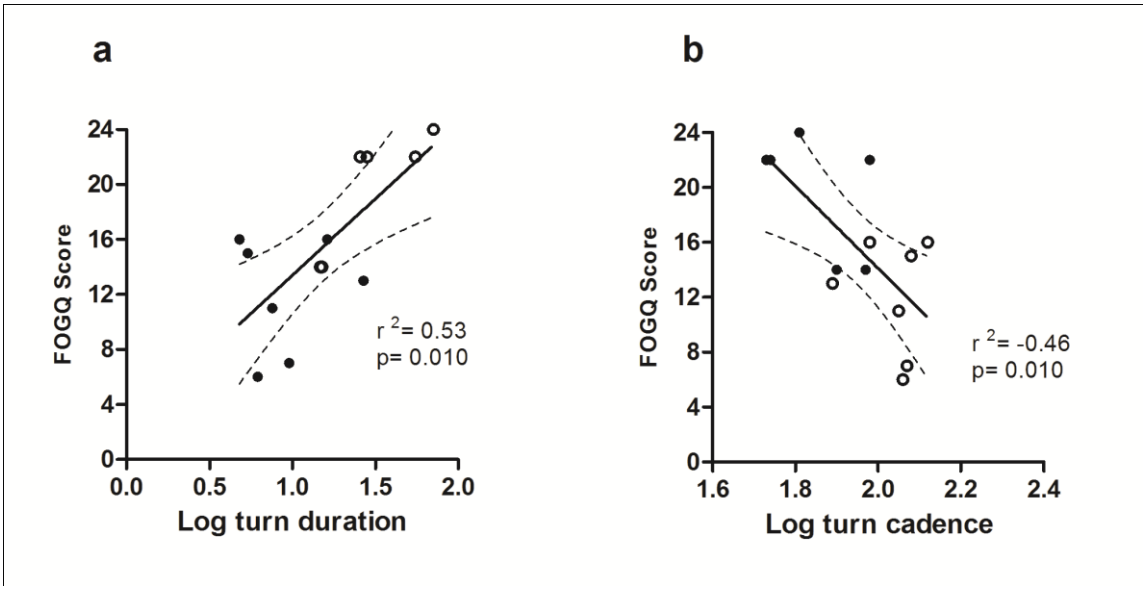

Figure 3. Correlation of Freezing of Gait Questionnaire scores (FOGQ, Score/24) and parameters recorded from PD FOG patients when turning in a tight space, a task that precipitated gait freezing. Linear regression (solid lines) and 95% confidence intervals (dotted lines) are shown. Open circles are from off stimulation and filled circles are from bilateral stimulation a. FOGQ correlation with turn task duration. b. FOGQ correlation with turn task cadence. The log transformation of these gait analysis parameters were normally distributed and used in analyses. The significant correlations suggest that task duration and cadence during tight quarters turning in the PD FOG patients relate to the intrusion of gait freezing in the patients' usual environments and medication states.

5.5 Discussion

Our primary outcome measure was the severity of gait freezing triggered by turning in a tight space under objective, double blinded experimental conditions. PPN stimulation reduced gait freezing, with bilateral stimulation more effective than unilateral stimulation. During unconstrained walking, Parkinsonian patients who experienced gait freezing had reduced step length and increased step length variability compared to patients without gait freezing, but these deficits were unchanged by PPN stimulation.

Before further discussion, the validity of our measures to quantify gait freezing needs consideration. Freezing is notorious for disappearing during single session assessments, which are therefore prone to underestimating the disorder.(Giladi and Nieuwboer 2008) For this reason, we assessed patients 'off medication' and employed a strong trigger of freezing; turning in a tight space. Although patients were assessed 'off medication' and under experimental conditions, our measures of gait freezing were strongly correlated with FOGQ scores which measure freezing in patients' usual environments and medication states.(Giladi, Shabtai et al. 2000) Accordingly, FOGQ scores also improved with chronic PPN stimulation. Our turn task measures aimed to quantify rather than characterise gait freezing. For example, we could not assess high frequency attempts at stepping that did not substantially displace the feet.(Spildooren, Vercruysse et al. 2010) Rather, we sought to provide objective measures of functional impairment from gait freezing (turn task duration) and of stepping that could progress position (turn task cadence).

This study contributes objective, double-blinded evidence that PPN stimulation can be therapeutic for gait freezing in PD. To limit ascertainment bias, we recruited all eleven patients receiving established bilateral PPN stimulation from the study centres living within a reasonable distance. Of these, two patients could not perform the tasks due to

severe ‘off medication’ akinesia, a deficit that appears unresponsive to PPN stimulation.(Moro, Hamani et al. 2010) Two patients, apparently successfully treated with PPN stimulation, had persistent remission of freezing despite having ceased stimulation for over twelve hours. Although we excluded such patients, washout effects could still have influenced our results, persisting from either chronic therapy or over the thirty minute interval which could reasonably be provided between conditions. This would tend to bias towards underestimating the impact of PPN stimulation. Furthermore, wash-in effects (e.g. delays to reach optimal treatment effects) may also have limited the measured impact of PPN stimulation. Even on bilateral stimulation, PD FOG patients remained substantially impaired during turning relative to controls. It is not clear if continuous PPN stimulation for longer than thirty minutes might yield further benefits or if such improvements found experimentally would enhance quality of life. Such questions call for a randomised clinical trial.

An important constraint is that the outcomes presented here reflect the specific selection criteria, target location and stimulation strategies employed for PPN stimulation in this study, which differ in some respects from previous reports.(Stefani, Lozano et al. 2007; Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010) Selected patients were an uncommon subgroup of PD who experience extremely severe gait freezing, postural instability and falls, persisting even ‘on medication’. Severe motor fluctuations were absent, although these later developed in one patient who was then implanted with subthalamic nucleus stimulators, inactivated during experiments. Thus our results reflect lone PPN stimulation, excluding interference from stimulation elsewhere. Furthermore, stimulation was specifically applied more caudally than previous reports, beneath the pontomesencephalic junction. This target was chosen based on the experience of two authors (NJ,TZA) in

applying PPN stimulation in the nonhuman primate model of PD and on the distribution of cholinergic cells in humans identified by ChAT5 immunohistochemistry.(Olszewski and Baxter 1954; Mesulam, Geula et al. 1989; Manaye, Zweig et al. 1999; Nandi, Aziz et al. 2002; Jenkinson, Nandi et al. 2004) However, it is important to add that although stimulation of this region appears beneficial, we cannot be certain that these effects arose from stimulating the PPN as opposed to its' projections or neighbouring structures also implicated in locomotor control.(Orlovskii, Severin et al. 1966; Zrinzo, Zrinzo et al. 2007; Piallat, Chabardes et al. 2009)

The relative efficacy of unilateral versus bilateral PPN stimulation has been controversial. Given the state of equipoise, some have elected to implant unilaterally, given the greater risks inherent in bilateral implantation.(Moro, Hamani et al. 2010) However, in an experimental setting, we found that bilateral PPN stimulation improved 'off medication' gait freezing approximately twice as much as unilateral stimulation (in terms of percentage improvements). We did not find that the effectiveness of unilateral PPN stimulation was influenced by the direction of turning that triggered freezing. Thus we cannot explain the greater impact of bilateral stimulation by a unilateral effect of unilateral stimulation.

During unconstrained walking, patients with PD FOG had reduced step length and increased step length variability compared to well-matched PD controls without gait freezing. Thus these background deficits, unless due to the PPN electrodes or failed stimulation washout, appear associated with gait freezing and corroborate findings from previous studies.(Hausdorff, Schaafsma et al. 2003; Chee, Murphy et al. 2009; Snijders, Leunissen et al. 2011) Although we did not clinically observe gait freezing during straight task trials, the abnormalities of step length could still reflect covert freezing interrupting the smooth execution of gait. Against this, cadence was not abnormal in PD FOG patients

during straight walking and the step length deficits did not improve with PPN stimulation despite improvements in triggered freezing. The step length deficits could simply be epiphenomenal to gait freezing. However, a previous study found that gait freezing episodes are commonly preceded by a sequential reduction in step length – a deficit that would account for the increased step length variability in our PD FOG patients.(Chee, Murphy et al. 2009) Furthermore, the same previous study found that small steps, deliberately taken, can trigger freezing.(Chee, Murphy et al. 2009) Step length along with other manifestations of akinesia, are potentially responsive to levodopa and subthalamic nucleus stimulation – suggesting a potential mechanism by which these therapies can improve 'off medication freezing'.(Faist, Xie et al. 2001) However we found that PPN stimulation did not improve step length or its variability, supporting the proposition that PPN stimulation may improve gait freezing through alternative, potentially complementary, pathways.(Jenkinson, Nandi et al. 2006; Thevathasan, Pogosyan et al. 2011)

Chapter 6:

The impact of low frequency stimulation of the pedunculopontine nucleus region on reaction time in Parkinsonism

6.1 Abstract

Objectives**:** Attentional augmentation and enhanced motor function are potential mechanisms by which stimulation of the region of the pedunculopontine nucleus (PPN) may improve gait in parkinsonism. Here we assess the impact of stimulation of this region on attentional and motor aspects of reaction task performance in patients with parkinsonism.

Methods**:** Eleven patients implanted with PPN stimulators underwent computerised assessment of simple, choice and digit vigilance reaction tasks. Patients were assessed 'off medication' during stimulation at different frequencies (0Hz, 5Hz, 10Hz and 'therapeutic' 20-35Hz). There were two primary endpoints: 'Speed of Reaction' (sum of the mean task reaction times) and 'Accuracy of Reaction' (reflecting omissions and percentage of correct responses). Baseline performance was compared to aged and sex matched healthy controls. Clinical effects of stimulation were assessed using the Unified Parkinson's Disease Rating Scale and a falls frequency scale.

Results**:** Compared with healthy controls, subjects had significant deficits in 'Speed of Reaction' and in all mean task reaction times. 'Accuracy of Reaction' was not different to

healthy controls and did not improve with stimulation. ‘Speed of Reaction’ significantly improved with stimulation at therapeutic frequencies (20-35Hz). Of the individual tasks, only simple reaction time significantly improved. Simple reaction time distribution analysis revealed a general speeding of responses rather than a selective reduction in outliers. Acute PPN stimulation improved gait and balance but not akinesia scores. Chronic PPN stimulation significantly improved falls frequency. Falls score improvement significantly correlated with changes to simple reaction time with therapeutic stimulation.

Conclusion**:** The pattern of reaction time improvement with stimulation of the PPN area suggests a benefit on motor performance, rather than augmentation of attention.

6.2 Introduction

Pedunculopontine nucleus (PPN) stimulation is a novel therapy for freezing of gait and postural instability (FOG/PI) in Parkinsonian disorders.(Stefani, Lozano et al. 2007; Weinberger, Hamani et al. 2008; Moro, Hamani et al. 2009) The mechanisms of PPN stimulation for FOG/PI are unknown, but potentially involve augmentation of attention or improved motor control.

In FOG/PI, attentional deficits may contribute to the final motor dysfunction (Giladi and Hausdorff 2006; Yogev-Seligmann, Hausdorff et al. 2008). For example, in PD, episodes of freezing can be triggered by external ‘distracters’ and gait disturbance can emerge during dual task performance (Camicioli, Oken et al. 1998; Morris, Iansek et al. 2008). The PPN is considered a component of the ‘reticular activating system’ and may modulate states of arousal and attention.(Mesulam, Geula et al. 1989; Winn 2006; Mena-Segovia,

Sims et al. 2008) Consistent with such a role, PPN stimulation in patients with Parkinson's disease (PD) increases rapid eye movement sleep and there is PPN-cortical coherence in the alpha band during wakefulness.(Androulidakis, Mazzone et al. 2008; Romigi, Placidi et al. 2008) However, the PPN is also proposed to be part of the basal ganglia and appears important to motor control.(Mena-Segovia, Winn et al. 2008) For example, in the nonhuman primate, the microinjection of a γ-aminobutyric acid (GABA) antagonist into the PPN partially reverses the akinesia induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).(Nandi, Aziz et al. 2002)

The aim of this study was to assess the impact of stimulation of the PPN region on attentional and motor aspects of reaction time (RT) performance, in patients with parkinsonism and FOG/PI.

6.3 Methods

6.3.1 Subjects and clinical evaluation

Twelve patients implanted with PPN stimulators were recruited from centres in Brisbane, Bristol and Oxford. Local ethics committee approval was obtained and participants gave informed consent. One patient had significant cognitive impairment (Mini Mental State Examination Score = 23) and could not reliably follow the study protocol so was excluded from further analyses. Clinical details of the 11 final study participants are shown in Table 1. Three patients also had electrodes implanted in Zona Incerta (ZI).

Subject number	Centre	Age (years)	MMSE	Disease duration (years)	UPDRS Off/On Meds (Off Stim)	L-Dopa equivalent dose(mg)	DBS targets	PPN stimulation: Frequency (Hz), Voltage (V), Pulse width (usec)	Postop. duration (months)
1	Brisbane	71	30	17	26†/22	1700	BilateralPPN	30/3.3/60	13
2	Brisbane	74	29	5	37/21	300	BilateralPPN	30/2.2/60	8
3	Brisbane	60	30	8	42/26	800	BilateralPPN	30/3.1/60	5
4	Brisbane	69	26	4	56†/47	1300	BilateralPPN	30/3.7/60	3
5	Brisbane	71	28	8	37/20	1200	BilateralPPN	30/3.3/60	6
6	Oxford	54	30	12	46/29	1800	BilateralPPN	20/2.3/60	13
7	Oxford	63	27	12	75/29	2100	BilateralPPN	20/2.0/60	12
8	Oxford	54	30	20	53/19	800	BilateralPPN	20/2.5/60	2
9	Bristol	61	27	12	56/20	1300	BilateralPPN/ZI	35/2.5/60	4
10	Bristol	67	28	15	29/24	1200	Unilat PPN/Bilat ZI	30/3.3/60	36
11	Bristol	65	29	15	54/43	1000	BilateralPPN/ZI	20/3.5/60	38

Table 1: Baseline clinical characteristics of patients. †Off medication UPDRS likely underestimated due to use of long acting ergot dopamine agonists.

All patients were diagnosed with Parkinson's disease and had PPN stimulators implanted for severe FOG/PI that persisted in the "on medication" state, causing falls. In all cases, the electrodes implanted in the PPN region were model 3387 (Medtronic, Minneapolis, USA). This electrode is configured with four active contacts, each 1.5mm in diameter and separated from the adjacent contact by 1.5mm. The surgical technique used to implant the PPN has been described previously.(Plaha and Gill 2005; Stefani, Lozano et al. 2007; Pereira, Muthusamy et al. 2008) Targeting the PPN has been controversial.(Yelnik 2007; Zrinzo and Hariz 2007; Zrinzo, Zrinzo et al. 2007) We therefore adopt the conservative position that 'PPN electrodes' in this study lie within the region of the PPN. The optimal parameters for therapeutic PPN stimulation (without confounding ZI stimulation, if

present) established by the usual clinical teams prior to our study were as follows: frequency range 20-35 Hz, voltage range 2.0-3.7 V and pulse width 60 µs.

The acute motor effects of stimulation of the PPN area with clinical stimulation parameters were assessed with the Unified Parkinson's Disease Rating Scale (UPDRS) and compared to the unstimulated state on the same day as RT testing. Clinical evaluations were performed both off and on dopaminergic medication. After changing stimulation parameters, there was a minimum 30 minutes "washout" period before clinical assessment. Off medication assessments occurred after overnight withdrawal (>12 hours) of dopaminergic therapy. The chronic efficacy of stimulation of the PPN area was judged by comparing the frequency of falling before and after PPN surgery. Falls frequency was determined using an item of the 'gait and falls questionnaire' constructed by Giladi et al.(Giladi, Shabtai et al. 2000) This meant that the frequency of falls was estimated to be either very often (daily or more), often (about weekly), rarely (about monthly), very rarely (about yearly) or never. Patient responses were recorded prospectively (patients 1-8) or assigned by the assessor based on falls diaries (patients 9-11). For statistical analysis, responses were converted to scores as follows; daily = 3, weekly = 2, monthly = 1, less than monthly = 0.

6.3.2 Tasks

Reaction times were assessed using components of the Cognitive Drug Research Ltd (CDR) Computerised Assessment System.(Simpson P 1991; Emre, Aarsland et al. 2004; Wesnes, McKeith et al. 2005; Molloy, Rowan et al. 2006; Collerton, Collerton et al. 2007; Allcock, Rowan et al. 2009) Three RT tasks were administered. In every task, the

presentation of an un-warned imperative visual stimulus in the centre of a computer screen signalled the need for a speeded response. RTs were recorded via a two button (YES/NO) response box. At the start of every task, the right index finger rested upon the right "Yes" button and left index finger upon the left "No" button. The block of three tasks required approximately seven minutes for completion. The three RT tasks were as follows:

1. *Simple Reaction Time Task*: Serial presentation of 50 imperative stimuli consisting of the word "Yes" occurring with a variable inter-stimulus interval (range 1-3.5 s). Subjects were instructed to respond only with the right "Yes" button. Minimum response time = 0.1 s. Measured variable: SRT = Simple reaction time.

2. *Digit Vigilance Task*: A continuous performance task. Serial presentation of 450 stimuli (random digits from 1 to 9) in the centre of the screen at a rate of 150 per minute. One digit (any from 1-9) was assigned as the imperative stimulus requiring a speeded response with the right hand "Yes" button. The imperative stimulus occurred randomly 45 times per task. As a reminder, the identity of the imperative stimulus was displayed constantly on the right side of the screen. Measured variables: VIGR = Vigilance reaction time, VIGACC = accuracy (% of imperative stimuli detected), VIGFA = false alarms (absolute number of false alarms).

3. *Choice Reaction Time Task*: Serial presentation of 50 imperative stimuli consisting of either the word "Yes" or "No" (occurring randomly; each with a 50% chance of occurrence). Inter-stimulus interval was variable (range 1-3.5 s). Subjects were instructed to respond with the relevant "Yes" or "No" button. Minimum response time = 0.15 s. Measured variables: CRT = Choice reaction time, CRT accuracy (% correct responses).

6.3.3 Experiments

Experiments were conducted after overnight withdrawal of dopaminergic medication. Stimulation of ZI was switched off, if present. A block of RT tasks was performed for each the following four conditions: No stimulation, 5 Hz, 10 Hz and usual therapeutic frequency stimulation (20-35 Hz). The optimal frequency of PPN stimulation in PD is uncertain. In contrast to the high frequencies (usually about 130 Hz) delivered to the subthalamic nucleus (STN) and Globus pallidus interna (GPi), low frequency stimulation is employed for the PPN. In the MPTP treated nonhuman primate, 5-10 Hz PPN stimulation was effective at improving movement counts.(Jenkinson, Nandi et al. 2004) However, in clinical reports of stimulation of the PPN area in patients with PD and FOG/PI, the choice of stimulation frequency has been 20-35Hz for bilateral PPN stimulation and up to 70Hz for unilateral PPN stimulation.(Plaha and Gill 2005; Stefani, Lozano et al. 2007; Moro, Hamani et al. 2009) Thus a range of low frequencies was tested to establish any frequency selectivity for stimulation effects on RT performance.

Across conditions, pulse width and voltage remained constant (at their usual therapeutic levels). Stimulation was delivered bilaterally (except in one case with a unilateral deep brain stimulation electrode) through the usual contacts employed for therapy. There was a minimum 30 minutes “washout period” between the changing of stimulation frequency and RT testing. Patients were blinded to the parameters of stimulation. Transient parasthesia occurred occasionally when stimulation was adjusted. However, persistent symptoms allowing identification of the presence or type of stimulation did not occur. The ordering of conditions was pseudo randomised across patients.

To minimise learning effects, two blocks ("practice blocks") were performed on the day prior to the experiment. These practice blocks were administered in the on medication state and whilst both on and then off therapeutic PPN stimulation. In addition, prior to every block, an abbreviated practice CRT task was performed. Within each block, the three tasks were presented in a fixed order (SRT task, Digit vigilance task then CRT task).

6.3.4 Data analysis

Surgery in the PPN area is in its infancy and we were only able to recruit 12 patients, despite including three surgical centres. Thus to limit multiple testing which, after correction, would have demanded prohibitive p values from our small sample we used two composite RT scores as our primary endpoints:

1) Speed of Reaction = mean SRT + mean VIGRT + mean CRT

2) Accuracy of Reaction = (VIGRT accuracy x 0.45) + (CRT accuracy x 0.5) - VIGRT false alarms

These composite scores have been widely used as measures of reaction task performance in patients with parkinsonism and have also been termed "Power of Attention" and "Continuity of Attention", respectively.(Emre, Aarsland et al. 2004; Wesnes, McKeith et al. 2005; Collerton, Collerton et al. 2007) The rationale for calculation of these endpoints is based on principle components and Varimax factor analysis of the various RT measures, as described previously.(Wesnes, Ward et al. 2000) The two primary endpoints were compared between conditions using Friedman's test as the Shapiro Wilks test indicated that both primary outcome measures were very unlikely to be normally distributed ("Speed of Reaction' $p=0.004$ and 'Accuracy of Reaction' $p<0.001$). Where appropriate, posthoc

Wilcoxon signed ranks tests were conducted to determine differences on task performance between conditions. Task performance was compared with aged and sex matched healthy controls using the Mann Whitney U test. Each healthy control data set represented the mean results of >80 healthy subjects within a five year range of the patient's age (available from the CDR database). P values < 0.05 were considered to be significant after Bonferroni correction for multiple comparisons.

RT distribution analysis was performed to assess the impact of PPN stimulation on the entire population of reaction times. RT distribution curves were obtained using the "Vincentization" method.(Ratcliff 1979; Rouder and Speckman 2004) This involves ranking RTs for individual patients in order of duration, from fastest to slowest, and computing the percentiles. The mean RTs across patients are calculated for each quantile and plotted against cumulative percentile. This yields the cumulative distribution of the grouped mean RTs. Using this method, an average of all the patients' RT distributions is produced that retains characteristics of an individual RT distribution curve. Wilcoxon signed ranks test was used to determine differences in the grouped mean RTs for each quantile between different conditions.

RT distribution curves have well described properties.(Ratcliff 1979; Whelan 2008) The curves have an 'ex-Gaussian' appearance, which results from the convolution of a Gaussian and an exponential distribution. 'Mu' refers to the mean of the Gaussian component. 'Tau' refers to the mean of the exponential component. The tau component contains the slowest outliers that are likely to represent attentional lapses.(Leth-Steensen, Elbaz et al. 2000) Mu and tau in this study were calculated using a validated ex-Gaussian curve fitting function of a toolbox available for Matlab (The Mathworks Inc, USA).(Lacouture and Cousineau 2008)

Individual RT distribution curves were also assessed by calculating ‘skew’. Skew measures the degree of asymmetry around the mean. After ‘Vincentization’ of an individual’s RTs, skew was assessed using a function of the SPSS statistical package, version 17.0 (SPSS Inc, Chicago). Skew of individual RT distributions was compared between conditions using Wilcoxon signed ranks test.

Clinical scores at different timepoints were compared using Wilcoxon signed ranks test. Correlations between clinical scores and RT results were assessed using Spearman’s rank test.

6.4 Results

The acute and chronic clinical motor responses to stimulation of the PPN region are summarised in Table 2. After a 30 minute washout period, switching on therapeutic stimulation significantly improved IT27-30 (8.2 ± 1.3 off vs 7.2 ± 1.3 on, Z = -2.460, p = 0.04). However, the residual UPDRS did not change with acute therapeutic stimulation (38.3 ± 3.7 off vs 37.7 ± 3.9 on, Z= -1.532, p = 0.38). Chronic stimulation of the PPN region reduced the frequency of falls (fall scores of 2.7 ± 0.2 off vs 1.3 ± 0.2 on, Z = -2.873, p = 0.01).

Subject number	Total Motor UPDRS Off/On PPN stim. (Off medication)	IT27-30 Off/On PPN Stim. (Off medication)	Residual UPDRS Off/On PPN stim. (Off medication)	Falls frequency Preoperative	Falls frequency Postoperative
1	26/26	6/6	20/20	Daily	Never
2	37/32	9/6	28/26	Weekly	Monthly
3	42/42	13/12	29/30	Daily	Monthly
4	56/59	10/10	46/49	Daily	Weekly
5	37/37	2/2	35/35	Daily	Weekly
6	46/36	10/7	36/29	Daily	Weekly
7	75/74	15/15	60/59	Monthly	Monthly
8	53/52	4/3	49/49	Daily	Weekly
9	56/54	12/11	44/43	Daily	Weekly
10	29/28	4/3	25/25	Daily	Monthly
11	54/54	5/4	49/50	Daily	Never

Table 2: Acute and chronic clinical motor outcomes of stimulation of the PPN region using clinically selected frequencies (20-35 Hz).

Baseline (no stimulation) reaction times from the study participants were compared to age and sex matched healthy control data. 'Speed of Reaction' was significantly worse in study participants (1691.8 ± 189.7) compared with healthy controls (1193.1 ± 8.0, $p < 0.001$). However, there was no deficit in 'Accuracy of Reaction' in patients (82.2 ± 5.2) compared with controls (90.6 ± 0.1, $p = 0.56$). Mean baseline reaction times of all three individual tasks were worse in study participants compared with controls (SRT; 415.9 ± 35.9 vs 282.8 ± 2.9, $p < 0.001$, VIGRT; 531.5 ± 20.7 vs 428.3 ± 1.50, $p = 0.001$, CRT; 744.4 ± 149.0 vs 481.6 ± 3.7, $p = 0.002$).

Amongst stimulation conditions, analysis of the primary endpoints revealed a significant difference for 'Speed of Reaction' (Chi-square = 9.66, $p = 0.02$; Fig 1A) but not 'Accuracy of Reaction' (Chi-square = 2.27, $p = 0.52$; Fig 1 B). Post hoc analysis revealed that only the therapeutic frequency condition significantly improved 'Speed of Reaction' compared

with baseline (Z = - 2.756, p = 0.02; Fig 1 A). Analysis of the subscores contributing to the 'Speed of Reaction' revealed a significant improvement for only SRT during therapeutic stimulation (SRT; Z = -2.401, p = 0.048, VIGRT; Z = 0.00, p = 1.0, CRT; Z = -2.045, p = 0.12, Fig 2). There was a trend suggesting a greater percentage improvement of SRT compared with VIGRT (Z = -2.134, p = 0.07). However, the percentage improvements of SRT and CRT were not significantly different (Z = -0.622, p = 0.53).

Figure 1A

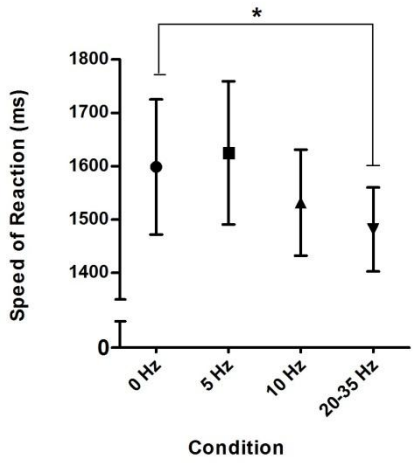

Figure 1B

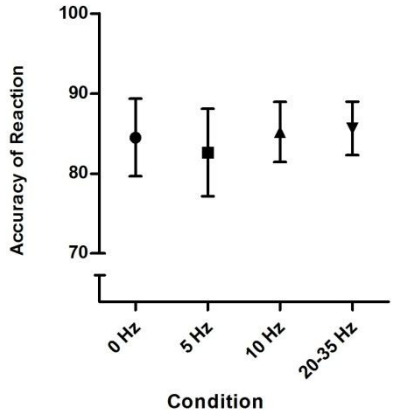

Fig 1A and 1B: Mean ± SEM of (A) 'Speed of Reaction' and (B) 'Accuracy of Reaction' in different conditions. * Significant difference between 0 Hz and 20-35 Hz (P = 0.02)

Figure 2

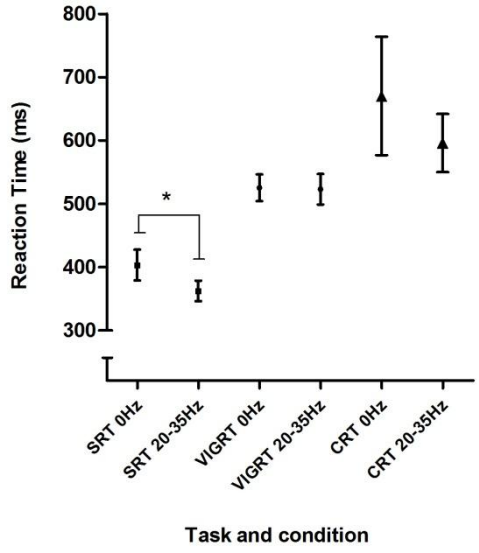

Fig 2: Mean ± SEM of SRT, VIGRT and CRT off stimulation and during clinically effective (20-35 Hz) stimulation of PPN. * P=0.048

We therefore analysed the RT distributions of the SRT task for the therapeutic frequency and baseline conditions. There was a significant difference in the per-quantile grouped mean RTs between conditions ($Z = -5.671$, $p < 0.001$). For every quantile, the mean grouped RT for the therapeutic frequency condition was faster than for the baseline condition. This is evident as a shift of the entire SRT distribution curve to the left (Figure 3). The mu and tau components of the SRT distribution curve were similarly reduced by therapeutic stimulation (by 9.3 % and 9.0 %, respectively). There was also no significant difference in skewness of SRT distributions of individual patients between 0Hz and therapeutic stimulation conditions ($Z=-0.889$, $p=0.374$).

Further exploratory analysis revealed that the improvement in fall scores significantly correlated with percentage improvement in SRT (Spearman's rho = 0.638, p=0.035) and trended towards correlation with percentage improvement in 'Speed of Reaction' (Spearman's rho = 0.558, p=0.074) with therapeutic stimulation. Changes in IT27-30 did not significantly correlate with changes in SRT or 'Speed of Reaction' with therapeutic stimulation.

Figure 3

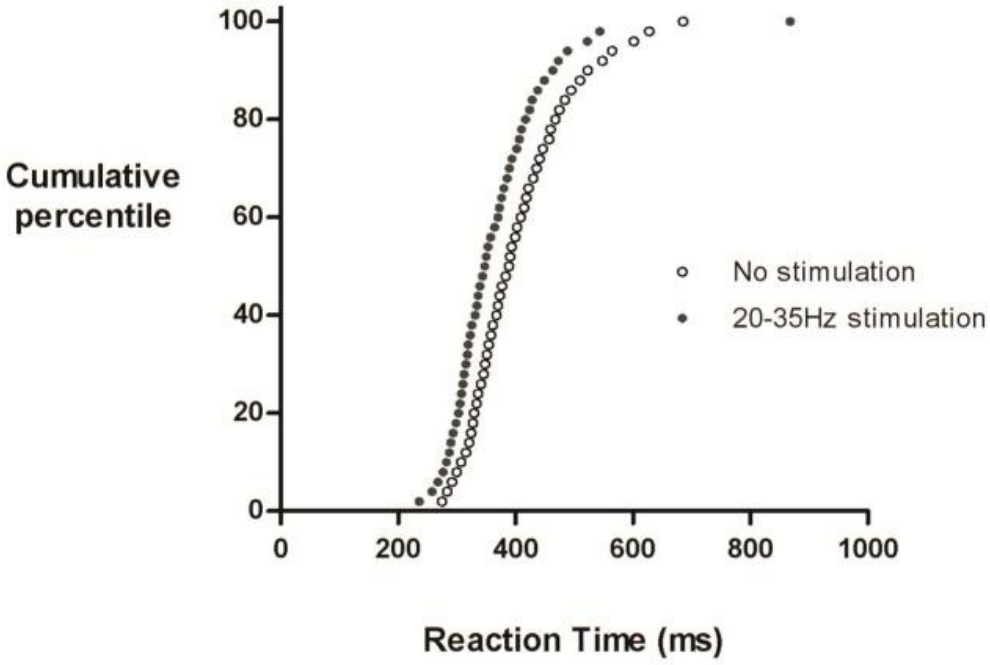

Figure 3: SRT distribution curves for 0 Hz and therapeutic frequency (20-35 Hz) conditions

6.5 Discussion

The major findings of this study may be summarised as follows. Stimulation of the PPN region at therapeutic frequencies improved ‘Speed of Reaction’. ‘Accuracy of Reaction’ did not improve, but subjects did not have a deficit in this measure at baseline compared with healthy controls. Therapeutic frequency stimulation yielded a significant improvement in SRT but not CRT or VIGRT. SRT reaction time distribution analysis revealed a general quickening of all SRTs rather than a selective effect on outliers. Therapeutic frequency stimulation acutely improved the IT27-30 subscore of the UPDRS (assessing gait and balance) but not the residual motor UPDRS (assessing akinesia, rigidity and tremor).Chronic PPN stimulation significantly improved frequency of falls. Improvement in SRT with therapeutic stimulation significantly correlated with improvement in fall scores.

We acknowledge several limitations of this study. The available cohort was small. However the non parametric analysis limited the impact of any single subject on results. Neurosurgical targeting of the PPN is controversial and was not the focus of this study. We consequently take the position that stimulation was delivered within the PPN region and not necessarily directly into the PPN itself. This also respects the fact that volume conduction can occur to neighbouring structures.(Butson, Cooper et al. 2007) Patients were blinded to the stimulation condition in RT experiments but the clinical data were collected by an unblinded researcher. RT assessments were performed in only the off medication state to limit variance from dopaminergic fluctuation. Although the order of conditions was pseudorandomised, the order of tasks within each condition was fixed. We addressed practice effects by rehearsal on the day prior to experiments and before every block. However, it remains conceivable that order effects may have contributed to the

differential results across tasks. Low frequency stimulation of the PPN region improved SRT in patients with parkinsonism and FOG/PI, but only at therapeutic frequencies (20-35 Hz). Ten Hz stimulation, which might have mimicked the alpha activity found in the PPN region following dopaminergic therapy,(Androulidakis, Mazzone et al. 2008) was not beneficial.

Bilateral PPN lesions in rats and the microinjection of a GABA agonist into the PPN in monkeys cause slowing of RTs.(Florio, Capozzo et al. 1999; Inglis, Olmstead et al. 2001; Matsumura and Kojima 2001) Improved RTs with PPN stimulation therefore is consistent with the proposition that the PPN in parkinsonism is underactive and that low frequency stimulation may somehow enhance or mimic normal PPN activity.(Pahapill and Lozano 2000; Jenkinson, Nandi et al. 2004) This does not necessarily imply activation of the nucleus *per se*, but may, for example, arise from modulation of surrounding structures including afferent pathways.(Gradinaru, Mogri et al. 2009)

Might the selective improvement of SRT reflect augmentation of attention or improved motor control? All reaction tasks in this study comprised multiple processing stages including, at minimum, alertness and attention, perception, motor preparation and the final movement itself. A general alerting effect is unlikely to account for our results, as it should have applied similarly across all tasks. In particular, tasks of continuous performance (such as the Digit Vigilance task) are sensitive to alterations in alertness such as due to sleep deprivation,(Drummond, Bischoff-Grethe et al. 2005; Haskell, Kennedy et al. 2005; Oken, Salinsky et al. 2006) and yet did not change. Slow outliers in RT tests (lying in the tau segment of the RT distribution curve) are likely to represent lapses in attention.(Ratcliff 1993; West, Murphy et al. 2002; Weissman, Roberts et al. 2006) However, with therapeutic stimulation there was a shift of the entire SRT distribution curve, without a

specific effect on tau. Correspondingly, the degree of skew for individual SRT distributions also did not change. These observations suggest improved motor performance rather than augmentation of alertness or general attention.

The question then arises whether any improvement in motor performance involved 'central' motor processing prior to movement initiation or a speeding of the movement once initiated. Although we did not record EMG there is one helpful observation; the SRT and VIGRT tasks required identical movements and yet only the SRT improved. The implication is that it must have been some element of 'central' motor processing that differed between tasks and sped up in the SRT during stimulation. One notable difference between tasks is the degree of motor preparation that could occur before the imperative stimulus. In the VRT, only one in ten stimuli was salient so that responses were under tonic inhibition (a Go/NoGo paradigm). (Carlsen, Chua et al. 2008) Only in the SRT task, could the motor response be fully anticipated and therefore "pre programmed" and stored for release.(Queralt, Weerdesteyn et al. 2008) PPN stimulation could therefore act by improving anticipatory motor preparation or by facilitating the release of the pre-prepared response as is believed to occur in the startle-react phenomenon.(Valls-Sole, Rothwell et al. 1999) Our RT results do not necessarily reflect the underlying therapeutic mechanism of PPN stimulation. However, it is notable that poorer 'Speed of Reaction' assessed with the same computerised tool used in this study, has been found to correlate with a higher frequency of falls.(Allcock, Rowan et al. 2009) Correspondingly, we found that with therapeutic PPN stimulation, improved falls scores significantly correlated with percentage improvements to SRT. Moreover, adjustments to locomotion (e.g. obstacle avoidance) can be facilitated by startle, raising the possibility that some aspects of gait may be "pre

programmed" and potentially subject to the same facilitatory effects of stimulation of the PPN area as the SRT.(Camicioli, Oken et al. 1998; Queralt, Weerdesteyn et al. 2008)

Finally, the effects of PPN stimulation appear to differ from those of dopaminergic medication and subthalamic nucleus (STN) stimulation. In a study using the same computerised tasks, acute and chronic dopaminergic therapy did not improve RTs in non-demented PD patients.(Molloy, Rowan et al. 2006) In studies using different computerised tasks, STN stimulation significantly improved not just SRT but also CRT and Go/NoGo RT.(Brown, Dowsey et al. 1999; Temel, Blokland et al. 2006) Furthermore, unlike dopaminergic medication and STN stimulation, we found a clinical benefit of PPN stimulation for only gait and balance. PPN stimulation did not improve akinesia as had been suggested by the MPTP primate model and early reports in humans.(Jenkinson, Nandi et al. 2004; Stefani, Lozano et al. 2007) The lack of effect on akinesia has recently been corroborated by others.(Moro, Hamani et al. 2009) It is also notable that acute on/off stimulation assessments of posture and gait (with IT27-30) revealed only modest effects compared with the substantial chronic impact on falls. This suggests that longer term outcome measures (such as questionnaires and ambulatory monitoring) may be more appropriate assessments for this target.

Chapter 7:

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

7.1 Abstract

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.

7.2 Introduction

Freezing of gait (FOG) and postural instability (PI) are the major causes of falls in Parkinsonian disorders, including Parkinson's disease (PD).(Factor 2008; Kerr,

Worringham et al. 2010) FOG and PI are often poorly responsive to dopaminergic medication.(Bloem, Hausdorff et al. 2004) Informed by experimental studies in animal models (Nandi, Aziz et al. 2002; Jenkinson, Nandi et al. 2004; Jenkinson, Nandi et al. 2006), deep brain stimulation (DBS) of the pedunculopontine nucleus (PPN) has emerged as a novel therapy for FOG/PI .(Mazzone, Lozano et al. 2005; Plaha and Gill 2005; Ferraye, Debu et al. 2009; Moro, Hamani et al. 2010)

The pathophysiology of FOG and PI is poorly understood – but their frequent coexistence raises the possibility of shared mechanisms.(Giladi, McDermott et al. 2001; Karachi, Grabli et al. 2010) Both conditions are considered deficits of axial and proximal musculature.(Jankovic 2008) In PI, postural reflexes that adjust for body sway and environmental perturbations are diminished.(Bloem 1992) Similarly, FOG 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, Murphy 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, Bissig et al. 2007; Reynolds and Day 2007; Queralt, Weerdesteyn 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, Sole et al. 1995; Valls-Sole, Rothwell et al. 1999) StartReact occurs when the relevant motor response can be prepared in advance, as seen experimentally in simple reaction time (SRT) tasks.(Valls-Sole, Rothwell et al. 1999; Carlsen, Chua et al. 2008) The assumption is that some motor programs 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, Chua et al. 2004) The triggering by loud auditory stimuli has further prompted speculation that StartReact responses may utilise the same efferent pathway as the startle reflex - the reticulospinal tract.(Valls-Sole, Sole et al. 1995; Valls-Sole, Kumru et al. 2008) The reticulospinal tract appears to predominantly innervate proximal and axial musculature, as supported by early lesioning studies in primates (Lawrence and Kuypers 1968; Lawrence and Kuypers 1968) Accordingly, StartReact has been shown to be preferentially expressed in proximal compared to distal muscles.(Carlsen, Chua et al. 2009)

Here we explore the hypothesis that in FOG/PI there is impairment of the system supporting the reflexic release of pre-prepared motor programs. We therefore predicted that Parkinsonian patients with FOG/PI will have a deficit in StartReact in proximal muscles, and, importantly, that this deficit would be reversed by PPN stimulation.

7.3 Subjects and Methods

7.3.1 Subjects and clinical assessments

Three subject groups were assessed; i) eight patients with PD complicated by severe FOG/PI, chronically implanted with bilateral PPN stimulators (PD FOG/PI group), ii) eight PD patients of akinetic/rigid subtype without significant FOG/PI (PD NoFOG/PI group) and iii) ten age matched healthy controls. PD patients 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.

PD FOG/PI patients were receiving chronic bilateral PPN 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. PPN electrodes were model 3387 (Medtronic, Minneapolis, USA), configured with four active contacts, each 1.5 mm in diameter with 1.5 mm spacing between adjacent contacts. Surgical implantation of the PPN has been described previously.(Pereira, Muthusamy et al. 2008) The lower PPN region was targeted, below the level of the inferior colliculus. Localisation of stimulation sites (midpoint between active contacts for bipolar stimulation and cathodes for monopolar stimulation) is represented in figure 1. Parameters employed for chronic therapeutic stimulation were as follows: frequency range 30 or 35 Hz, voltage range 2.5-4.3V and pulse width 60 µs.

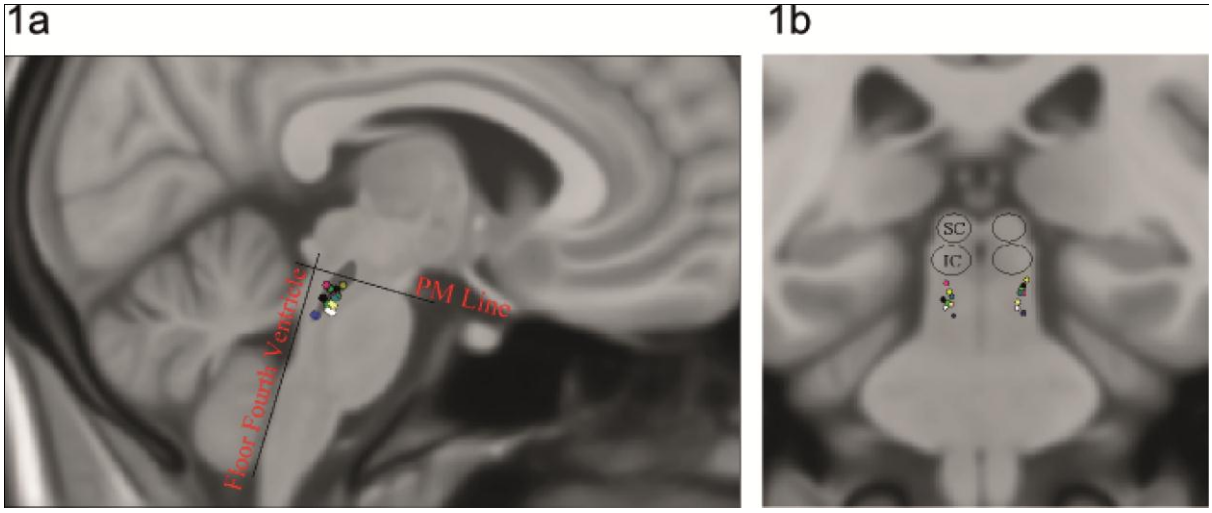

Figure 1: Localisation of the sites of stimulation - represented in Montreal Neurological Institute (MNI) space on sagittal (figure 1a) and coronal (figure 1b) views. In figure 1a, the relative location/extent of the PPN has been outlined in dark grey, based on choline-acetyltransferase immunohistochemical (ChAT5) staining in the human (see text). PM = Ponto-Mesencephalic line connecting the pontomesencephalic junction to the caudal end of the inferior colliculi. SC = Superior Colliculus. IC = Inferior Colliculus.

Clinical details of the study participants are shown in Tables 1 and 2. Healthy controls, PD NoFOG/PI and PD FOG/PI patients did not significantly differ with respect to age [F(2.23)=0.365, P=0.698]. PD No FOG/PI and PD FOG/PI 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 MMSE [t(12)=-0.408, P=0.690]. PD FOG/PI patients had significantly higher scores in IT27/30 [t(14)=-4.743, P<0.001], GFQ [t(14)=-5.281, P<0.001], FOGQ [t(14)=-5.967, P=0.001] and FallsQ [t(14)=-6.325, P<0.001].

	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)	4 M, 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)†	26.9 (11.6)†	11.8 (4.2)†	2.9 (0.8)†

Table 1: Baseline characteristics; Mean (SD). For PD FOG/PI, scores are from postoperatively. †Different from PD/NoFOG, P≤0.001.

Patient	Age/Sex	PD duration (years)	Postop 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/postop	FOGQ pre/postop	FallsQ pre/postop	Supportive for UK brain bank criteria*
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

Table 2; PD FOG/PI patients. Postoperative clinical assessments were performed on the same day as RT assessment. Patients 7 and 8 were recruited from Oxford, other patients from Brisbane. Patient 7 also had STN stimulators which were turned off one hour prior to and during experiments. ^ = not known. Key to UK Brain bank criteria; D=dyskinesias, A = asymmetry persistent, T=tremor at rest, P=progressive disease course. *Additional to disease duration and levodopa response as documented elsewhere in the table.

7.3.2 Tasks

Three tasks were administered.

4. *Auditory Blink and Startle Reflex task:* Patients were presented with 10 trials of loud auditory stimuli (LAS: 122 dB, 40 ms duration, 1000 Hz). Inter-trial 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.

5. *Proximal SRT task*: A warned SRT 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 5 trials were 'practice' normal trials, followed by 20 normal and 10 LAS trials randomly intermixed. Warning periods (1-3.5 s) and inter-trial (6-10 s) intervals were variable. Patients were instructed to react as quickly as possible with ballistic elbow flexion.

6. *Distal SRT task:* The same task as the proximal SRT except patients responded with ballistic abduction of the fore-finger. 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, Cambridge, UK). 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, Denmark) via an artificial ear and headphone adaptor.

Bipolar surface electromyographic (EMG) activity was recorded using 9 mm diameter silver-silver chloride electrodes. EMG electrodes were tapped to skin overlying Orbicularis Oculi (OOc) and Sternocleidomastoid (SCM) contralateral to the limb to be moved in reaction time (RT) assessments. RTs were assessed with both EMG and a triaxial accelerometer (ACC). For the proximal SRT task, EMG was applied to biceps and ACC tapped to the radial styloid. For the distal SRT task, EMG was applied over the first dorsal interosseous and ACC tapped to the tip of index finger. In one healthy subject, distal EMG RT was not assessable due to misplaced EMG electrodes. Data were sampled (or down

sampled to) 256 Hz (Porti amplifier, TMSI, Enschede, NL). EMG recordings were amplified and low pass filtered at 70 Hz. ACC (TMSI, Enschede, NL) was band pass filtered between 2 and 60 Hz.

7.3.3 Experiments

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 minimise habituation effects on this task. The order of proximal and distal SRT tasks was counterbalanced.

In PD patients, experiments were conducted after overnight withdrawal of dopaminergic medication to limit variance from fluctuating dopaminergic state. For PD FOG/PI patients, there were two conditions – on and off therapeutic bilateral PPN stimulation. Ordering of conditions was counterbalanced, with a minimum one hour washout period between conditions.

Subjects were blinded to condition and to experimental hypotheses.

7.3.4 Parameters and Data analysis

Two RT parameters were assessed; accelerometer (ACC RT) and electromyography (EMG RT). ACC analysis was automated by a script developed for Matlab (The Mathworks Inc, Natick, MA, USA). Priority is therefore given to this data set. ACC RTs were computed for every trial before averaging to yield the task ACC RT. EMG RTs 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 RT was therefore assessed from averages of the trials for a given condition in a given patient in Spike 2 (Cambridge electronic design, Cambridge, UK). 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 SRT task (always normal trials) were discarded as practice. Anticipatory responses (EMG response prior to the imperative cue) were discarded. The automated ACC analysis involved initial DC removal (time constant individualised for each trial from the average DC level 0.45ms prior to the imperative) before rectification. ACC response onset was defined as an amplitude rise exceeding the mean of the pre-stimulus (0.5s) baseline by 3 standard deviations. The EMG RT signal was first subject to DC removal, using a fixed averaging interval of 0.002s (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 RT onset was defined in the latter as the first data point exceeding the mean plus 3 standard deviations of the pre-stimulus (1s) baseline that had a steep (> 2uV/ms) rise in amplitude sustained for > 20 ms.

Auditory startle reflexes (ASR) were assessed during reflex and SRT tasks. An ASR was considered present if there was a short latency (<130 ms in healthy subjects and <150 ms in PD subjects) SCM response following a loud stimulus, sustained above the background, which in SRT tasks was required to precede the limb EMG response (determined from the native un-rectified signals). Orbicularis and SCM EMG were then subject to DC removal (individualised for each recording) then rectification. Amplitude and latency of the first occurring ASR was assessed. For auditory blink reflexes, amplitude and latency of the averaged Orbicularis response was assessed in the Auditory Blink and Startle Reflex task.

7.3.5 Statistics

The Kolmogorov-Smirnov Test demonstrated that the distribution of all measures was not different from the normal.

Within each group, RTs for each joint (proximal and distal) and stimulus (normal and LAS) were compared using repeated measures ANOVA with factors 'Joint' and 'Stimulus'. PD FOG/PI patients had an additional factor 'DBS' (on and off). We report Joint effects only where they interact significantly with other factors (eg Joint*Stimulus). Posthoc tests were performed with paired t-tests. StartReact benefit (Normal RT – LAS RT) was compared between groups with ANOVA and post hoc independent samples t-tests.

In PD FOG/PI, off and on stimulation results were considered together and correlations (Pearson's) sought between LAS RTs and two independent clinical measures of Parkinsonian gait and balance disturbance – the GFQ (contains both the FOGQ and FallsQ) and IT27/30.

Reflex latencies and amplitudes were compared between groups using single factor ANOVA and posthoc independent samples t-tests. The frequency of individuals recording a recognisable ASR was compared between groups using Pearson's Chi Square.

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

7.4 Results

7.4.1 ACC Reaction time (figure 2)

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

In PD NoFOG/PI, there was again a significant effect of Stimulus [$F(1,7)=9.2$, $p=0.019$]. Posthoc tests revealed the presence of StartReact with LAS trials significantly faster than normal trials [mean Normal RT 164.8 ms Vs Loud RT 130.2 ms, $t(15)=3.755$, $P=0.002$]. The Joint*Stimulus interaction was not significant.

In contrast, in PD FOG/PI, the effect of Stimulus was absent [$F(1,7)=3.7$, $P=0.097$]. There was no main effect of DBS nor a DBS*Joint interaction. There was a trend towards a DBS*Stimulus interaction [$F(1,7)=5.0$, $P=0.060$]. However, there was a significant DBS*Joint*Stimulus interaction [$F(1,7)=7.2$, $P=0.031$]. Posthoc tests revealed that this was due to a selective improvement in Proximal LAS RT with PPN stimulation [mean LAS RT 125.0 ms off stimulation Vs 101.5 ms on stimulation, $t(7)=3.6$, $P=0.036$]. PPN stimulation also meant that Proximal LAS RTs became faster than Proximal Normal RTs, so that PPN stimulation restored Proximal StartReact [mean Normal RT 139.4 ms Vs LAS RT 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 (with PD FOG/PI patients off stimulation) [$F(2,23)=4.729$, $P=0.019$]. Posthoc tests revealed Proximal StartReact benefit to be

significantly less in PD FOG/PI patients (off stimulation) compared with PD NoFOG/PI [-0.08 ms Vs 41.3 ms, t(14)=-2.639, P=0.040] and healthy controls [-0.08 ms Vs 26.0 ms, t(16)=-2.600, P=0.019]. However, with PD FOG/PI patients on stimulation, Proximal StartReact did not differ between groups [F(2,23)=0.615, P=0.549].

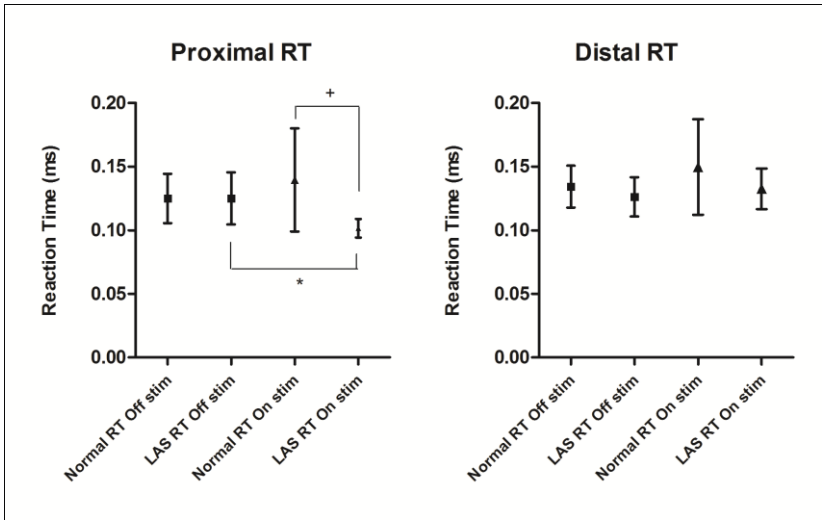

Figure 2: ACC Reaction times [Means and SD] for PD FOG/PI patients, on and off bilateral PPN stimulation. * P=0.036. + P=0.040. LAS = Loud Auditory Stimuli. Increased variance of Normal RT (reflected in widened SD) is noted in the 'on stimulation' condition. This is mostly attributable to two patients who recorded more RT outliers 'on stimulation', suggestive of attentional lapses/fatigue.

7.4.2 EMG RT (figure 3)

EMG RT results followed a similar pattern as ACC RT.

In healthy subjects, there was a significant effect of Stimulus [F(1,8)=16.7, p=0.003].

Posthoc tests revealed the presence of StartReact, with LAS trials significantly faster than

normal trials [mean Normal RT, averaged across proximal and distal muscles 86.4 ms Vs LAS RT, averaged across proximal and distal muscles 77.2ms, t(18)=3.860, P=0.001]. The Joint*Stimulus interaction was not significant.

In PD NoFOG/PI, there remained a significant effect of Stimulus [F(1,7)=8.7, p=0.021]. Posthoc tests revealed the presence of StartReact, with loud trials significantly faster than normal trials [mean Normal RT 114.3ms Vs LAS RT 92.1ms, t(15)=2.884, P=0.011]. The Joint*Stimulus interaction was not significant.

In PD FOG/PI, the effect of Stimulus was absent [F(1,7)=0.00, P=0.987]. There was no effect of DBS. There were significant interactions between DBS*Joint [F(1,7)=8.119, P=0.025], DBS*Stimulus [F(1,7)=8.312, P=0.024] and DBS*Joint*Stimulus [F(1,7)=5.669, P=0.049]. Posthoc tests revealed that this was due to a selective improvement in Proximal LAS RT with PPN stimulation [mean LAS RT 84.8ms off stimulation Vs 65.4ms on stimulation, t(7)=6.167, P<0.001]. PPN stimulation significantly increased Proximal StartReact benefit [Proximal StartReact off stimulation -10.4ms Vs on stimulation 15.5ms, t(7)=-3.363, P=0.012].

Accordingly, an ANOVA of Proximal StartReact benefit showed a significant difference between subject groups (with PD FOG/PI patients off stimulation) [F(2,23)=9.810, P=0.001]. Posthoc tests revealed Proximal StartReact to be significantly less in PD FOG/PI patients (off stimulation) compared with PD NoFOG/PI [-10.4ms Vs 36.3ms, t(14)=3.580, P=0.010] and healthy controls [-10.4ms Vs 10.9 ms, t(16)=3.469, P=0.006]. However, with PD FOG/PI patients on stimulation, Proximal StartReact did not differ between groups [F(2,23)=2.397, P=0.113].

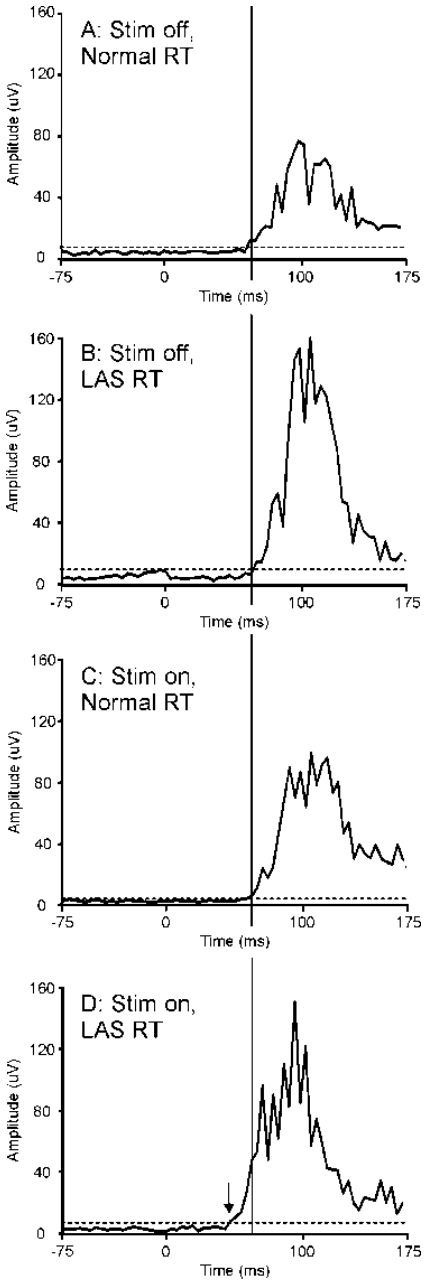

Figure 3: Proximal EMG RTs for a PD FOG/PI patient. Speeded responses (ballistic elbow flexion) were recorded to normal (89dB) auditory stimuli (Normal RT) and Loud (122dB) Auditory Stimuli (LAS RT), off and on PPN stimulation. Traces are the averaged biceps EMG waveforms from the proximal SRT task in PD FOG/PI patient 3. 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 Standard deviations of the prestimulus (1s) baseline. The solid vertical line transects all traces at the same time-point (and at the onset defined for the Stimulation On, Normal RT). When off stimulation, the averaged LAS RT is not faster than the Normal RT; StartReact is absent. PPN stimulation speeds the LAS RT but not the Normal RT. Thus PPN stimulation restored proximal StartReact.

7.4.3 Correlations of RT with clinical measures

In PD FOG/PI (with off and on stimulation results considered together), Proximal LAS ACC RT correlated with GFQ [r=0.60, P=0.036] and IT27/30 [r=0.52, P=0.039] (figure 4). Distal LAS ACC RT and proximal and distal EMG LAS RTs did not correlate with these measures.

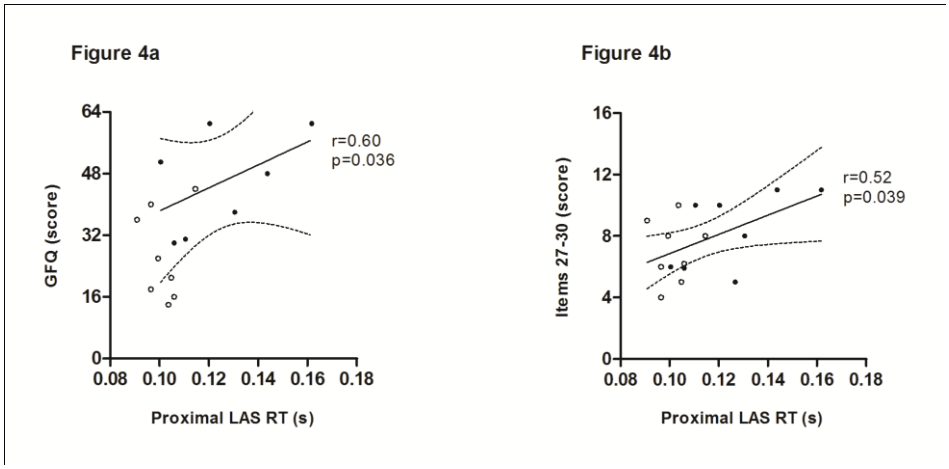

Figure 4: Correlation between Proximal LAS ACC RT and clinical measures in PD FOG/PI patients. Linear regression (solid line) and 95% confidence intervals (dotted lines) are shown. Data from on stimulation (open circles) and off stimulation (filled circles) are both included, affording 16 potential data points (2 results from each patient, except for one patient in figure 4a in whom preoperative off stimulation GFQ data were absent, as indicated in Table 2) 4a; Correlation between Proximal LAS ACC RT and GFQ (Gait and Falls Questionnaire). Off and on stimulation GFQ scores were prospectively obtained preoperatively (off stimulation) and postoperatively (on stimulation). 4b; Correlation between Proximal LAS ACC RT and IT27-30 (Items 27-30 of the Unified Parkinson's disease rating scale). IT27-30 scores were rated by the same examiner, off and on stimulation at the same postoperative visit with a minimum one hour washout period. LAS = Loud Auditory Stimuli.

7.4.4 Acoustic startle and blink reflexes (table 3)

ASR were identified in 7/10 healthy, 5/8 PD NoFOG/PI and 1/8 PD FOG/PI subjects. The frequency of individuals with ASR differed significantly between subject groups [χ^2 (2,26)=6.60, P=0.037). There were significantly fewer ASR in PD FOG/PI compared with PD NoFOG/PI [χ^2 (1,16)=4.267, P=0.030]. In all subjects, ASRs were infrequent, usually occurring with the first LAS trial then rapidly habituating. In PD FOG/PI, PPN DBS did not restore ASR in any patient. Comparing healthy subjects and PD NoFOG/PI, there were no significant differences in ASR amplitudes [321.3 uv Vs 238.5 uv, t(10)=0.456, P=0.658] or latencies [98.2 ms Vs 101.9 ms, t(10)=-0.19, P=0.854]. Insufficient PD FOG/PI patients had an ASR to make comparisons with this group.

An averaged auditory blink reflex in the *Auditory Blink and Startle Reflex task* was identifiable in all healthy and PD NoFOG/PI subjects and 7/8 PD FOG/PI subjects (in one PD FOG/PI patient, any auditory blink reflex was obscured by excessive blinking during recordings). Between subject groups (with PD FOG/PI 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 PD FOG/PI patients, PPN stimulation did not alter auditory blink reflex amplitudes [t(6)=0.141, P=0.892] or latencies [t(6)=0.385, P=0.714].

	Acoustic Blink Reflex			Acoustic Startle Reflex		
	Occurrence	Latency (ms)	Amplitude (uv)	Occurrence	Latency (ms)	Amplitude (uv)
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	_	_

Table 3: Summary data for Acoustic Blink and Startle Reflexes. Occurrence indicates proportion of patients demonstrating the response. Otherwise data are Means (SD).

7.5 Discussion

We found that Parkinsonian patients with FOG/PI can be distinguished from those without FOG/PI 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 PPN stimulation.

The scarcity of auditory startle responses in Parkinsonian patients with FOG/PI recalls the reduced frequency of startle in progressive supranuclear palsy (PSP).(Vidailhet, Rothwell et al. 1992; Kofler, Muller et al. 2001; Gironell, Kulisevsky et al. 2003) Severe "on medication" FOG/PI as a dominating complaint in PD is unusual and itself flags the possibility of PSP.(Jankovic 2008) In the absence of a definitive test in life, the diagnosis of PD in our PD FOG/PI patients should be considered presumptive, although the persistence of normal auditory blink reflexes differs from the absent or abnormal auditory

blink reflexes reported in PSP.(Vidailhet, Rothwell et al. 1992; Valldeoriola, Valls-Sole et al. 1998; Kofler, Muller et al. 2001; Gironell, Kulisevsky et al. 2003; Williams, Doyle et al. 2008)

Regardless of pathological type, attenuation of startle in Parkinsonian patients with FOG/PI 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 PPN stimulation.(Brown, Rothwell et al. 1991; Vidailhet, Rothwell et al. 1992; Valls-Sole, Kumru et al. 2008) Conversely, preservation of the auditory blink reflex suggests that the midbrain can be relatively spared in FOG/PI.(Hori, Yasuhara et al. 1986)

7.5.1 The PPN region and release of pre-programmed movement

StartReact is described to occur when the relevant motor response can be fully anticipated, "pre-programmed" and stored for release, as in SRT tasks.(Valls-Sole, Rothwell et al. 1999; Carlsen, Chua et al. 2008) In line with this task specificity, we previously found that PPN stimulation in PD FOG/PI selectively improved SRT, but not choice or Go-NoGo reaction times.(Thevathasan, Silburn et al. 2010) Taken together with the current findings, it appears that PPN stimulation corrects a deficit in FOG/PI 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 PPN or a pathway in the region of the PPN supports the release of pre-prepared motor programs in PD. However, given that we only studied the effects of PPN stimulation in patients with PD, one can only speculate about the relevance of this to the normal functioning of the PPN.

Some of our findings differ from those previously reported. We did not replicate our previous result that PPN stimulation improved SRT in trials without LAS ('Normal RT' in the present study).(Thevathasan, Silburn et al. 2010) However, the tasks of the two studies are different. In the present study, we aimed to optimise StartReact, by, for example, using warning cues and long inter-trial intervals. This contrasts with the rapidly occurring unwarned visual cues of unchanging intensity employed previously, which could have promoted more reflexic SRT responses. Furthermore, a small change in SRT may have been undetected in this study as the long inter-trial intervals meant that Normal RTs 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, Chua 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, Chua 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 RT tasks not only by virtue of short latency SCM EMG activity (e.g. <130ms) but also, unlike the aforementioned study, by appearance of such activity before the limb response. This latter criterion was necessary to exclude SCM activity due to accessory muscle activation. Otherwise, responses sped by loud sounds (which had limb EMG latencies around 70-90ms) 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 programs were postural reflexes and stepping.(MacKinnon, Bissig 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, Chua et al. 2004) Furthermore, unlike acoustic blink and startle reflexes, StartReact is not modified by prepulse inhibition.(Valls-Sole, Kofler et al. 2005) Such results are consistent with our finding that PPN stimulation restored StartReact without restoring startle in PD FOG/PI. As has been argued previously, such findings support that the StartReact phenomenon and startle reflex may be dissociated (Valls-Sole, Kofler et al. 2005).

7.5.2 Relevance to the pathophysiology of FOG/PI and the therapeutic mechanism of PPN stimulation

StartReact was absent in PD FOG/PI but conserved in disease matched PD NoFOG/PI controls and healthy subjects. Unless the PPN electrodes themselves caused the reversible deficit in StartReact, then this deficit appears associated with gait freezing and postural instability.

PPN stimulation sped proximal LAS RTs significantly more than distal LAS RTs – and proximal (and not distal) LAS ACC RTs 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 1968) (Drew, Prentice et al. 2004; Davidson, Schieber et al. 2007; Stapley and Drew 2009) Distal musculature,

particularly intrinsic hand muscles, receive predominantly corticospinal input.(Lawrence and Kuypers 1968; Riddle, Edgley et al. 2009) A lesser benefit of PPN stimulation on distal StartReact is therefore consistent with an effect mediated through reticulospinal pathways and also with the therapeutic role of PPN stimulation on the axial and proximal deficits of FOG/PI.

In this study, we did not directly demonstrate that FOG/PI 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 pre-programmed and potentially subject to the same triggered release we have shown is deficient in FOG/PI and restored by PPN stimulation.(Reynolds and Day 2007; Queralt, Weerdesteyn et al. 2008) This interpretation was supported by the presence of correlations between proximal LAS ACC RTs and two independent measures of Parkinsonian gait and balance disturbance – the GFQ and IT27/30. These correlations were found when proximal LAS RTs 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 ACC RTs differed. In this study, EMG RTs tended to reflect the fastest occurring responses whereas ACC RT's were more representative of performance across all trials.

Optimisation of motor systems releasing pre-programmed movement may not be the only mechanism by which PPN DBS 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 PPN 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, Mazzone et al. 2008; Arnulf, Ferraye et al.)

Our observation that StartReact is deficient in PD FOG/PI and restored by PPN stimulation offers insights into the pathophysiology of FOG/PI and the mechanisms of PPN stimulation. However, we have not, in this study, addressed whether these findings might help predict response to PPN DBS. 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.

7.6 Conclusion

Our findings suggest that Parkinsonian patients with FOG/PI have a deficit in the release of pre-programmed movement, when the latter should have been promoted by loud auditory stimuli. Delays in the release of pre-programmed movement correlated with the severity of the FOG/PI phenotype. PPN stimulation, while improving the phenotype, also restored the deficit in pre-programmed movement release. Accordingly, FOG/PI may, in part, involve impairment of a pontine system supporting the reflexic release of pre-prepared motor programs.

Chapter 8: General Discussion

A qualification of this research is that any effect of PPN stimulation could have arisen from neighbouring regions. This includes nuclei such as the cuneiform nucleus in addition to afferent and efferent pathways of the PPN.(Gradinaru, Mogri et al. 2009) For example, an average field of influence from monopolar stimulation (using parameters employed in the STN) extends for around $3mm^3$ – a large volume in the brainstem.(Maks, Butson et al. 2009) Another limitation is that results obtained from patients with Parkinson's disease may not necessarily reflect function in health.

Implications of the research presented here will be considered in three categories, as follows.

8.1 Structure and function of the PPN in Parkinsonian patients

The PPN is said to be highly conserved across species. The research here suggests that this extends to humans. As proposed recently in animal models, the PPN in patients with PD appears topographically organised.(Martinez-Gonzalez, Bolam et al. 2011) The rostral PPN exhibited beta band oscillations consistent with connectivity with basal ganglia nuclei where excessive beta band synchronisation is a feature in PD.(Hammond, Bergman et al. 2007) The caudal PPN region exhibited a distinct oscillatory pattern, alpha band activity which correlated with gait. This concurs with findings in animal models implicating the caudal PPN with locomotor function.(Martinez-Gonzalez, Bolam et al. 2011)

The above findings support a functional role of the PPN in locomotor control, independent of stimulation effects. The correlation of PPN alpha activity with gait suggests that the nature of this functional role may be partly mediated through attentional modulation.

The PPN also appears to affect movement outside of gait. PPN stimulation enhanced the release of pre-prepared motor programs particularly in muscles predominantly innervated by reticulospinal pathways. This suggests a more fundamental role of the PPN in motor function outside of gait; supporting automatic, stimulus-driven, reflexic movement. The connectivity of the PPN suggests that it would be well placed to operationalize such a role. For example, the PPN receives rapid unprocessed sensory information and projects to reticulospinal motor pathways and locomotor pattern generators. Moreover, connectivity with the cerebellum could permit engagement of systems that tune characteristics of pre-programmed movement once initiated. (Stein 1986; Miall, Weir et al. 1993; Stein 2009)

8.2 Pathophysiology of gait freezing and postural instability and potential therapeutic mechanisms of PPN stimulation

Patients implanted with PPN stimulators at the centres collaborating in this research were profoundly affected by gait freezing and postural instability which persisted even "on medication". This cohort facilitated the recognition of differences with controls unaffected by freezing. However, there was also a limitation – that baseline findings 'off stimulation' could have been influenced by PPN stimulation, including any lesion effects from the initial surgery, presence of the electrodes or persistent effects of stimulation.

In the introduction, several pathophysiological mechanisms of gait freezing were proposed based on previous findings, including deficits in pontine function, the attentional allocation

of processing resources and internally generated movement. This research supports the role of these factors in gait freezing

Patients with severe FOG and PI were characterised neurophysiologically as having a deficit in the startle reflex but conserved blink reflexes. This, together with the effects of PPN stimulation on gait freezing, implicates the pons but not the midbrain as a site of functional disturbance in gait freezing and postural instability. The nature of the pontine dysfunction was suggested by the reversal by PPN stimulation of the deficit in pre-programmed movement in FOG/PI. This effect was most evident in proximal movement suggesting an effect mediated via reticulospinal pathways, which originate in the pontine reticular formation.

Local field potential analysis and electroencephalography indicated that the pedunculopontine nucleus region was part of a coherent neural network which included frontal cortical regions. The correlation of PPN alpha activity with gait performance further implicates attention and the effective allocation of processing resources that flows from it in the pathophysiology of gait freezing. However, PPN stimulation was not found to improve alertness or global attention, as measured by reaction time distribution analysis or reaction times in a continuous performance task. However, this does not preclude that PPN stimulation could mediate an attentional effect confined within the motor system. For example, one could speculate that PPN stimulation could divert processing resources towards reflexic adjustments to gait and posture.

In accordance with previous findings, patients with gait freezing were found to have smaller step length compared with controls without freezing. The step length deficit in gait freezing has been shown to be related to abnormally reduced activity in the SMA.(Snijders, Leunissen et al. 2011) Both SMA activity and step length are improved by

dopaminergic medication and STN stimulation – suggesting a method by which these can improve “off medication” gait freezing.(Jenkins, Fernandez et al. 1992; Hammond, Bergman et al. 2007) However, PPN stimulation improved gait freezing without affecting step length. This concurs with the lack of any effect of PPN stimulation on akinesia scores or levodopa dose requirements and suggests that the mechanism of PPN stimulation for gait freezing and perhaps also postural instability occurs through non-dopaminergic mechanisms.

8.3 Clinical application of PPN stimulation in Parkinson’s disease

Objective blinded evidence reported here suggests that PPN stimulation has therapeutic efficacy for gait freezing in Parkinson’s disease. Unblinded results suggested that the benefit of PPN stimulation included gait freezing that occurs both off and on medication in addition to postural instability and falls. Festination was not assessed as a discrete endpoint. PPN stimulation did not improve the classical dopamine responsive motor features of PD such as akinesia, rigidity and tremor and levodopa dose requirements did not change. These findings suggest that patients should be selected for PPN stimulation who have the predominant symptomatic issue of FOG and PI, even that which persists on medication. The efficacy for ‘on medication’ gait freezing raises the possibility that PPN stimulation could improve gait freezing not only in Parkinson’s disease but also in atypical parkinsonian disorders such as progressive supranuclear palsy.

Spatiotemporal analysis suggested that the therapeutic impact of PPN stimulation on Parkinsonian gait disturbance was limited to amelioration of gait freezing – which was triggered in experiments by turning in tight spaces. PPN stimulation did not improve step

length deficits however these can be responsive to dopaminergic medication or subthalamic nucleus stimulation. Thus these findings raise the possibility that the effects of PPN stimulation could be complementary to dopaminergic medication and subthalamic nucleus stimulation.(Faist, Xie et al. 2001) However, the efficacy of dual subthalamic nucleus and PPN stimulation needs to be addressed in future clinical studies, given theoretical concerns that stimulation at different sites could interact.(Ferraye, Debu et al. 2011)

Objective blinded evidence presented here indicates that bilateral PPN stimulation is more effective than unilateral for gait freezing. In terms of percentage improvement in gait freezing measures, bilateral stimulation was approximately twice as effective as unilateral. However, unilateral stimulation still appeared to have bilateral effects. The number of electrodes to implant will ultimately be a risk versus benefit decision in individual patients. However given that substantial gait freezing persisted even with bilateral stimulation, the case for unilateral stimulation is even less appealing.

The optimal place to target stimulation in the PPN region remains unclear. The correlation of alpha activity in the caudal PPN with gait suggests that this subregion warrants special consideration. Objective blinded evidence provided here demonstrated that stimulation of the caudal PPN subregion was effective for gait freezing and non-blinded evidence suggested the same for postural instability. However whether this caudal subregion is more effective than rostral PPN stimulation for gait freezing and postural instability needs investigation.

The microlesion effect from implantation of the PPN appeared to improve gait freezing. This suggests that stimulation titration may be difficult in the days following surgery. It is unclear if this stun effect might predict beneficial effects from chronic stimulation. This

seems unlikely if stun effect is due to microlesioning descending inhibitory inputs to the PPN rather than reflecting accurate implantation of the nucleus per se.

Stimulation effects were objectively explored over a range of frequencies including 5Hz, 10Hz and 20-35Hz. The greatest improvements to reaction time occurred with 20-35Hz stimulation, corroborating anecdotal clinical experience for effects on gait and postural deficits. However, it remains unclear if higher frequency stimulation up to 60Hz employed in one previous study may be equally beneficial.

Assessing the outcomes of PPN stimulation requires different methods to those employed for STN stimulation. The UPDRS appeared inadequate for this target. Indeed, any single session assessments are prone to underestimate gait freezing. This may be compounded by the apparent persistent washout effects from PPN stimulation which were observed to outlast stimulation for hours or longer. It is unclear if the mechanism of this delayed washout effect is truly due to the stimulation or simply reflects the difficulty in reproducing gait freezing during medical assessments. A finding that supports a true stimulation related phenomenon is that persistent washout effects also appeared to also affect postural instability. One single session assessment method was developed to try and quantify gait freezing – the measurement of task duration and cadence during tight quarters turning. This method was validated against FOGQ scores. Although the method of analysis was too time consuming for this to be employed in routine clinical practice, it does provide a way to quantify freezing in future studies. The best performing assessment method for PPN stimulation outcomes for use in routine clinical practice appeared to be the GFQ and FOGQ scores.

This research has mainly focussed on assessing the impact on PPN stimulation on gait disturbance in PD. The impact of PPN stimulation on postural disorders in PD needs to be addressed using more objective measures than the clinical assessments employed here.

8.4 Questions for future research

Following on from this research the following questions could be explored:

Use of alpha as an intraoperative biomarker for PPN location

Is caudal PPN stimulation more effective than rostral?

The relative efficacy of 60Hz versus 30Hz PPN stimulation for gait and posture

Does PPN stimulation have an effect on attention within the motor system, for example suppressing distractor effects during gait?

Objectively defining the impact of PPN stimulation on postural disturbances in PD.

Clarifying if PPN stimulation improves festination of gait

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